Thermally Driven Fission of Protocells

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Abstract. We propose a simple mechanism for the self-replication of protocells. Our main hypothesis is that the amphiphilic molecules composing the membrane bilayer are synthesised inside the protocell through globally exothermic chemical reactions. The slow increase of the inner temperature forces the hottest molecules to move from the inner leaflet to the outer leaflet of the bilayer. This asymmetric translocation process makes the outer leaflet grow faster than the inner leaflet. This differential growth increases the mean curvature and amplifies any local shrinking of the protocell until it splits in two.

Contents

1. Protocells and metabolism

2. Hypotheses of our model

3. Flows, forces and energy dissipation
   3.1. Main irreversible processes
   3.2. Flows associated to each irreversible process
   3.3. Thermodynamical forces
   3.4. Conductance matrix
   3.5. Entropy production and stability

4. Membrane geometry and growth equation
   4.1. Conservation of matter and exponential growth
   4.2. Cylindrical growth in steady state

5. Thermal instability of cylindrical growth
   5.1. The Squeezed Sausage Theorem (SST)
   5.2. Fluctuations, translocation and heat transfer
   5.3. Thermal balance

6. Translocation between leaflets
   6.1. An effective potential for translocation
   6.2. Computation of $L_{m\theta}$
   6.3. Computation of $L_{mm}$
   6.4. Estimation of $L_{\theta\theta}$
   6.5. Destabilisation

7. Conclusions and perspectives

Appendix A. The mean curvature of the membrane

Appendix B. Solutions of the growth equation
   B.1. Case 1: $\eta \neq 1$ and $\tau > \tau_+ (\eta)$ or $\tau < \tau_- (\eta)$
   B.2. Case 2: $\eta \neq 1$ and $\tau \in \{ \tau_+ (\eta), \tau_- (\eta) \}$
   B.3. Case 3: $\tau_- (\eta) < \tau < \tau_+ (\eta)$

Appendix C. Smooth perturbation of cylindrical growth
   C.1. Isovolumic variation of the area
   C.2. Isovolumic variation of the total mean curvature

Appendix D. Asymptotic expansion of $F(a)$

References

1. Protocells and metabolism

The objects modeled in the present article are protocells, the putative ancestors of modern living cells [23, 34]. In the absence of fossils [38], we ignore their detailed properties. However, we can sketch a minimalist functional diagram of protocells (FIG. 1).
The protocell is a vesicle bounded by a bilayer made of amphiphilic molecules. Nutrient molecules (food) enter by mere diffusion, since they are consumed inside, where their concentration is lower than outside. Conversely, waste molecules have a larger concentration inside and therefore diffuse passively to the outside. The metabolism is a network of unknown chemical reactions taking place only inside the protocell. The net reaction is supposed to be exothermic, since living matter is hotter than abiotic matter (under the same external thermodynamical conditions).

Let us compare this scheme to actual evolved cells. The growth of bacteria in a nutrient rich medium follows a species dependent periodic process [5, 12]. At regular time intervals, each cell splits to form two daughter cells. This requires the synchronization of numerous biochemical and mechanical processes inside the cell, involving cytoskeletal structures positioned at the locus of the future cut (septum). However, in the history of life, such complex structures are a high-tech luxury and must have appeared much later than the ability to split. Protocells must have used a simple splitting mechanism to ensure their reproduction, before the appearance of genes, RNA, enzymes and all the complex organelles present today even in the most rudimentary forms of autonomous life [34].

In this article, we present a simple model for the growth and self-replication of a protocell, following the laws of irreversible thermodynamics near equilibrium. Our guide is the rate of entropy production, which is minimal in a steady state [32, 20]. A key point of our approach is that the heat produced by the metabolism of the protocell is approximately proportional to its volume, whereas the heat flow that it can lose is proportional to the area of its membrane. In a rod-shaped cell (bacillus) growing linearly, these two quantities are approximately proportional so that each increment of the membrane area should be sufficient, ideally, to evacuate the heat produced by the corresponding increment of the cell volume. However, the irreversible physical and chemical processes produce heat more quickly than the growing tubular membrane can dissipate to the outside. This increases slowly the inner temperature and enhances the fluctuations of the shape of the membrane, of the various concentrations and of the local electric field.

In a growing spherical protocell, the maximal heat flow that the membrane can expell to the outside without overheating the inside puts an upper limit to the radius of the protocell. Indeed, the formation of two small protocells from a big one releases work [33], so that large protocells are mechanically unstable. However, neither [33] nor [8] provides a path to follow to realise this deformation.

In our model, we start from a cylindrical shape to simplify the computations. As the inner temperature increases, the growth of the outer leaflet of the membrane becomes more probable than the growth of the inner leaflet. If a random thermal fluctuation lowers slightly the radius of this cylinder, then its area increases more quickly than during the steady state cylindrical growth (FIG. 2).
This reduction of the radius induces a loss of convexity of the membrane. This favors the outflow of heat and the ratio area/volume increases slightly, compared to a convex cylindrical shape.

The plan of the article goes as follows. In Section II, in order to formulate these ideas mathematically, we state all the physical hypotheses of our model of protocells. In Section III, we define the various flows of matter and energy and their associated thermodynamical forces. In the linear approximation, the rate of entropy production is the scalar product of these flows and forces and is minimal in a steady state [32]. In Section IV, we derive a differential equation for the evolution of the area and the integral of the mean curvature of the membrane, starting from the advancement of the chemical reaction for the synthesis of the membrane molecules. This linear equation admits a solution growing exponentially. In Section V, we use variational calculus [10] to prove that the local reduction of the radius of the cell increases its length and its area, if its volume is kept constant. This intuitive property implies that heat is more easily released when the protocell is squeezed. In Section VI, we propose a molecular mechanism for the increase of the mean curvature of the membrane associated to this squeezing. The position of each amphiphilic membrane molecule is reduced to a single degree of freedom: the distance from the polar head to the middle of the hydrophobic slice. We use a double well effective potential to describe the trapping of these molecules in the membrane. Due to the temperature difference between the inner and outer sides, the membrane molecules go from the inner leaflet to the outer leaflet more often than in the opposite direction. This asymmetry forces the membrane to curve and shrink around the middle of the protocell and initiates its splitting. Our main mathematical result (Proposition VI.1) states that a stability condition, \( L_{m\theta}^2 < L_{mm}L_{\theta\theta} \), can not be satisfied at high temperature, because the squared crossed conductance, \( L_{m\theta}^2 \), increases more quickly than the product of the diffusion coefficients, \( L_{mm} \) for membrane molecules and \( L_{\theta\theta} \) for heat. Hence, the cylindrical growth process is unstable when the temperature difference is sufficiently high. We conclude in Section VII with a proposition of an experimental test for our model. The appendices contain the detailed computations of our model. The mathematical notions involved are elementary (linear differential equations and geometry of surfaces).

2. Hypotheses of our model

Let us state more precisely the hypotheses underlying our model:

(1) Our protocells are made of a membrane of average thickness \( 2\varepsilon \), bounding a cytosol of finite volume \( V(t) \).

(2) The cytosol contains unknown specific molecules (reactants, catalysts, chromophores, ...) which participate to a network of chemical reactions. We suppose that the concentrations are constant and uniform in the volume \( V \).

(3) The protocell starts with a cylindrical shape closed by two hemispherical caps of fixed radius, \( R_0 \). The total length, \( \ell(t) + 2R_0 + 2\varepsilon \), increases with time due to the synthesis of membrane molecules (FIG. 3).
This may seem a rather drastic hypothesis, but the computations could be made for a generic, approximately spherical shape using an expansion in spherical harmonics. This would add to the model an unnecessary mathematical complexity that would hide the main physical phenomena. The use of cylindrical, rotation invariant shapes allows us to reduce the problem to one dimension. Moreover, this is a best case scenario for the release of heat in steady state, since the ratio volume/area can be held constant in a steady growth.

Due to the surface tension of the membrane, its mean curvature has an upper bound, $H_{\text{max}} = \frac{1}{R_0}$. Indeed, due to the attractive forces between the polar heads of the membrane molecules, and due to their geometry, they can not form structures arbitrarily small [27].

Food (nutrients and water) enters the protocell by mere passive diffusion through the membrane. Waste and heat also diffuse passively but in the opposite direction. Protocells did not use specialized membrane molecules for an active transport through the membrane.

The membrane molecules are synthesised inside the protocell in an unknown network of chemical reactions. It might use some encapsulated catalysts or chromophores trapped in the volume and catching part of the ambient light [23], but we will make no hypothesis on the details of this network.

These metabolic reactions generate heat to be evacuated and increase slowly the internal temperature, $T_1$, whereas the external temperature, $T_0$, remains fixed.

The characteristic time of the variations of $T_1(t)$ is much larger than the characteristic times of chemical reactions and diffusion processes across the membrane.

The cytosol is homogenous and contains no organelles, no cytoskeleton, no enzymes, no RNA/DNA. Just simple chemical reactants uniformly distributed. (Rashevsky’s model [33] allows for a slight radial variation of concentrations due to the diffusion of food and waste through the membrane).

The membrane is a bilayer made of unspecified amphiphilic molecules. We presume that their hydrophobic tails are long enough (10-12 carbon atoms) to form a stable bilayer, but not too bulky in order to allow flip-flop (or translocation) processes between the two leaflets. We do not include sterol molecules because they are the product of a long biochemical selection process [27], and a high-tech luxury for protocells.

The inner leaflet ($L_1$) is at temperature $T_1$ whereas the outer leaflet ($L_0$) is at temperature $T_0 < T_1$. This temperature drop allows the bilayer to undergo coupled transport phenomena (food and waste diffusion, including water leaks, heat diffusion, flip-flop, etc.).

The membrane may contain other molecules, in small concentrations, but we don’t need them to transport food, waste or any molecule through the membrane.

The validity of these hypotheses will depend on the agreement of their predictions with the results of future experiments made with real protocells.

### 3. Flows, forces and energy dissipation

In any living system, some processes release energy whereas other processes consume energy. Globally, the system takes usable energy from the outside and rejects unusable energy, in the form of heat and waste, that can be used by other living systems. In order to describe such a system, we must define the various flows of matter and energy and the forces causing these flows. Any gradient of concentration, pressure, temperature, etc. will cause a current of particles, fluid, heat, etc. These processes are generally irreversible and dissipate energy to inaccessible degrees of freedom. This dissipation of a conserved quantity is measured by the entropy function, which increases as time passes.

The study of irreversible thermodynamical processes near equilibrium [29, 30, 35, 20] is based on the rate of entropy production, represented by a bilinear function of flows (chemical reaction speed, thermal current, particle current, electric current, etc.) and forces (chemical affinity, temperature gradient, concentration gradient, electric tension, etc.). In a first approximation, flows and forces are related linearly, as in Ohm’s law:

\[
\text{electric current} = \text{conductivity} \times \text{electric field}
\]
and the power dissipated is a quadratic function of the tension:

\[ \text{power dissipated} = \text{tension} \times \text{current} = \text{conductance} \times \text{tension}^2. \]

Similarly, in viscous fluids:

\[ \text{power dissipated} = \text{friction coefficient} \times \text{velocity}^2. \]

We suppose that the protocell metabolism is in a steady state not too far from equilibrium, so that the various flows, \( J_i \), and the thermodynamic forces, \( X_k \), are linearly related:

\[ J_i = \sum_k L_{ik} X_k \]

and the entropy rate is a quadratic function of \( X \):

\[ \sigma := XJ = \sum_{ik} X_i L_{ik} X_k. \]

The coefficients \( L_{ik} \) are called phenomenological because their computation depends on the chosen model of microscopic dynamics (kinetic theory) and their numerical value has to be compared to a measurement in the real world to (in)validate this model and the linearity hypothesis. An important property of the phenomenological coefficients is provided by Onsager’s relations [29, 30, 20, 35]. Under the hypotheses of microscopic reversibility and parity of the variables under time reversal (in particular, in the absence of magnetic coupling and vorticity), the matrix \( L \) is symmetric:

\[ L_{ik} = L_{ki}. \]

This important law has been checked experimentally for various systems near equilibrium and is satisfied quite accurately in many cases.

### 3.1. Main irreversible processes

To each irreversible physical or chemical process are associated a flow of matter or energy and a thermodynamical force, just as an electric current and an electric tension correspond to each branch of an electric network. If we identify the main processes that take place during the growth of a protocell, we can compute the global rate of dissipation of energy, or entropy creation. According to Prigogine’s Theorem [32, 11], this rate reaches a minimum when the system is in a steady state.

In order to compute this dissipation, we need to define the various compartments containing energy. In the sequel of this article, the subscript 0 (resp. 1) will denote the variables outside (resp. inside) the protocell. The physical and chemical processes are grouped as follows:

- \( f_0 \rightarrow f_1 \) : food molecules (nutrients + water) diffuse into the protocell through the membrane.
- \( f \rightarrow m + c + w \) : food is transformed into membrane, cytosol and waste, inside the protocell. This is a global process, a superposition of catabolism and anabolism. Taking into account the stoichiometric coefficients, we can write more precisely:

\[
\sum_i \nu_{fi} f_i \longrightarrow \sum_j \nu_{mj} m_j + \sum_k \nu_{ck} c_k + \sum_l \nu_{wl} w_l
\]

where \( f_i \) denotes the food molecules of type \( i \), \( m_j \) the membrane molecules of type \( j \), \( c_k \) the cytosol molecules of type \( k \) and \( w_l \) the waste molecules of type \( l \). If \( N_\alpha \) is the number of molecules of type \( \alpha \), the advancement of this reaction, \( \xi \), is defined by:

\[
\frac{d\xi}{dt} = \sum_{\alpha} \frac{dN_\alpha}{\pm \nu_\alpha}
\]

where the stoichiometric coefficients, \( \nu_\alpha \), are counted positively for the products and negatively for the reactants. Note that our definition of \( \xi \) involves \( N_\alpha \) instead of the volumic concentration, \( C_\alpha = N_\alpha/V \), because the volume is not fixed.

- \( w_1 \rightarrow w_0 \) : waste molecules diffuse out of the protocell through the membrane.
- \( m_1 \equiv m_0 \) : molecules of the membrane bilayer go from one side to the other. In modern cells, this process is catalysed by enzymes (flippase for \( 0 \rightarrow 1 \) and floppase for \( 1 \rightarrow 0 \)), but in protocells such a complex machinery did not exist yet [34]. If we suppose that the first membranes were not as thick as today (most phospholipids in modern and healthy cell walls have hydrophobic chains made of \( \sim 16-22 \) atoms of carbon [27]), the exchange of molecules between the two leaflets could have been possible in a reasonable time to allow spontaneous splitting. Medium length lipids (10-14 atoms of carbon) could be good candidates to make stable, flippable and not too porous protocells. We isolate the process of translocation (\( \equiv \) [1]) because the ratio \( N_{m0}/N_{m1} \) of the numbers of membrane molecules on each side is related to the mean curvature of the bilayer, which is the geometric parameter monitoring the splitting process.

- \( q_1 \rightarrow q_0 \) : electric charges can be transferred from one side of the membrane to the other, by an ionic bound on the polar head of the membrane molecules. This electric current builds up an electric tension, \( U_{01} \), counteracted
by possible ionic leaks through the membrane. If we suppose that the membrane molecules are monovalent fatty acids, each one can carry a monocation ($\text{H}^+$, $\text{Na}^+$, $\text{K}^+$, ...). This cotransport process could be the ancestor of the modern sodium-potassium pump. Anions also can participate to this transmembrane electric current, by leaking through water pores [14].

3.2. Flows associated to each irreversible process. The main processes of our model are described by the following flows in the protocell (see FIG. 4):

$J_f$ : the flow of food entering the protocell through its membrane (molecules per unit time per unit area).
$J_w$ : the flow of waste exiting the protocell through its membrane (molecules per unit time per unit area).
$J_\theta$ : the heat flow exiting the protocell by diffusion through its membrane (energy per unit time per unit area).
$J_{mob}$ : the flow of membrane molecules from $a$ to $b$ (molecules per unit time per unit area). The possible values of $a$ and $b$ are:
- $c$ : the cytosol;
- $1$ : the inner leaflet of the membrane ($L_1$);
- $0$ : the outer leaflet of the membrane ($L_0$);
The net flow of membrane molecules is usually unidirectional, $C \rightarrow L_1 \rightarrow L_0$, hence $J_{mc1} > 0$ and $J_m := J_{m10} - J_{m01} > 0$.
$J_r$ : the speed of the synthesis reaction inside the cytosol (molecules per unit time per unit volume). $\xi$ being the advancement of the reaction $f \rightarrow m + c + w$, defined above, then $J_r$ is the time derivative of $\xi$:

$$J_r := \frac{d\xi}{dt}. \tag{9}$$

$J_q$ : some ions can be transported from one side to the other, bounded to the polar head of the membrane molecules.

\begin{align*}
\text{\textbf{Figure 4. Main flows of energy and matter in our model.}}
\end{align*}

We then have the following linear flow diagram for the synthesis and motion of membrane molecules:

$$\begin{align*}
E & \xrightarrow{J_f} C \xrightarrow{J_r} C \xrightarrow{J_{mc1}} L_1 \xrightarrow{J_{m10}} L_0.
\end{align*} \tag{10}$$

This picture is however slightly misleading. Indeed, the amphiphilic molecules being in a liquid phase, their positions fluctuate in each leaflet (transversal diffusion) and they undergo perpendicular motions (protrusion) and translocations from one leaflet to the other. The pictures obtained by molecular dynamics simulations [14, 15, 4, 1] give us a more precise representation of real world membranes.

3.3. Thermodynamical forces. The thermodynamical forces associated to these processes are defined as follows:

$X_\theta$ : the thermal force is the difference of the inverse temperatures inside and outside the protocell:

$$X_\theta := \frac{1}{T_0} - \frac{1}{T_1} > 0. \tag{11}$$

$X_{fi}$ : the chemical force driving the food molecules, $f_i$, is the difference of the ratios $-\mu_{fi}/T$ outside and inside the protocell:

$$X_{fi} := \frac{\mu_{fi0}}{T_0} - \frac{\mu_{fi1}}{T_1}. \tag{12}$$

The influx of food is guided by mere diffusion through the membrane (dedicated channel and intrinsic proteins did not exist yet in protocells). Since food is consumed inside the protocell, $[f_i]_1 < [f_i]_0$. For a spherical protocell, the profile of the concentration of each molecule (as a function of the distance to the center) can be
computed by solving the diffusion equation [33]. An important result of this computation is the existence of a discontinuity in the concentration of each molecule, \( f_i \), proportional to the radius, \( R \), of the protocell, to the rate of the reaction, \( q_i \) (concentration/time), and inversely proportional to the permeability, \( h_i \) (length/time), of the membrane for this molecule: 

\[
[f_i]_\Delta = [f_i]_0 \propto \frac{q_i R}{h_i}.
\]

**\( X_{w_j} \):** the force driving the waste molecules to the outside of the protocell is the difference of chemical potentials divided by the temperature:

\[
X_{w_j} := \frac{\mu_{w_j0}}{T_0} - \frac{\mu_{w_j1}}{T_1}.
\]

Note that \( X_{w_j} \) and \( X_{f_i} \) must have different signs for waste and food to go in opposite directions.

**\( X_r \):** the chemical force driving the synthesis reactions (metabolism) is the chemical reaction affinity, \( A_r \), of the global process \( f \rightarrow m + c + w \), divided by the inner temperature of the protocell:

\[
X_r := \frac{A_r}{T_1}.
\]

This affinity is a linear combination of the chemical potentials of the synthesis equation, weighted by the stoichiometric coefficients, counted positively for the reactants \((m, c, w)\):

\[
A_r = \sum_i \nu_{f_i} \mu_{f_i} - \sum_j \nu_{m_j} \mu_{m_j} - \sum_k \nu_{c_k} \mu_{c_k} - \sum_l \nu_{w_l} \mu_{w_l}.
\]

**\( X_{m'} \):** The membrane molecules are synthesised in the cytosol at temperature \( T_1 \). Their hydrophobic tail enforces the spontaneous organisation of these molecules into a bilayer. We suppose that the temperature varies only across the membrane. The driving force of this isothermal process is the affinity of the reaction \( m_c \rightarrow m_1 \), divided by the inner temperature, \( T_1 \):

\[
X_{m'} = \frac{A_{m1c}}{T_1} = \frac{\mu_{mc} - \mu_{m1}}{T_1}.
\]

Here, \( \mu_{mc} \) is the chemical potential of the free membrane molecules inside the cytosol and \( \mu_{m1} \) is their chemical potential in the inner leaflet. The heat released to the inner leaflet during this process is:

\[
Q_{m1c} = \mu_{mc} - \mu_{m1} = T_1 X_{m'}.
\]

**\( X_m \):** the membrane molecules are transferred from the inner layer, at temperature \( T_1 \), to the outer leaflet, at temperature \( T_0 < T_1 \), releasing the heat \( Q_{m10} \) into the environmental thermostat, at temperature \( T_0 \). The thermodynamical force of this process is:

\[
X_m = \frac{\mu_{m1}}{T_1} - \frac{\mu_{m0}}{T_0}.
\]

**\( X_q \):** the thermodynamical force driving the ions of species \( i \), of charge \( z_i e \), across the membrane is the difference of electrochemical potentials [2]:

\[
X_{qi} = \tilde{\mu}_{i1} - \tilde{\mu}_{i0} = (\mu_{i1} + z_i e \psi_1) - (\mu_{i0} + z_i e \psi_0) = \mu_{i1} - \mu_{i0} + z_i e U_{10}.
\]

where \( \psi \) denotes the electrostatic potential and \( U_{10} := \psi_1 - \psi_0 \) is the electric tension across the membrane.

Among these forces, only \( X_\theta \) is a linear function of the small temperature difference, \( \Delta T = T_1 - T_0 \). The others have, generically, a supplementary constant term, of order 0 in \( \Delta T \).

### 3.4 Conductance matrix.

The phenomenological coefficients, \( L_{ik} \), which couple all the irreversible processes of our linear model, can be put in a \( 7 \times 7 \) matrix:

\[
L = \begin{pmatrix}
L_{\theta0} & L_{\theta f} & L_{\theta w} & L_{\theta m} & L_{\theta m'} & L_{\theta q} & L_{\theta r} \\
L_{f 0} & L_{f f} & L_{f w} & L_{f m} & L_{f m'} & L_{f q} & L_{f r} \\
L_{w 0} & L_{w f} & L_{w w} & L_{w m} & L_{w m'} & L_{w q} & L_{w r} \\
L_{m 0} & L_{m f} & L_{m w} & L_{m m} & L_{m m'} & L_{m q} & L_{m r} \\
L_{m' 0} & L_{m' f} & L_{m' w} & L_{m' m} & L_{m' m'} & L_{m' q} & L_{m' r} \\
L_{q 0} & L_{q f} & L_{q w} & L_{q m} & L_{q m'} & L_{q q} & L_{q r} \\
L_{r 0} & L_{r f} & L_{r w} & L_{r m} & L_{r m'} & L_{r q} & L_{r r}
\end{pmatrix}
\]

In a first approximation, some coefficients can be set equal to zero:
The diagonal coefficients of \( L \) are positive but we let \( L_{\theta r} = 0 = L_{r \theta} \) because the synthesis reactions take place in the cytosol and are decoupled from the transport processes across the membrane. Similarly, we let \( L_{mm'} = 0 = L_{m'm} \), because the transfer of membrane molecules from the cytosol to the inner leaflet is decoupled from the other processes. Since the diffusion processes of different molecules (food, waste, ions or membrane constituents) across the membrane are supposed to be decoupled, we put \( L_{fw} = 0 = L_{wf}, L_{fm} = 0 = L_{mf} \) and \( L_{wm} = 0 = L_{mw} \).

\( L_{q\theta} \) is the thermal diffusion coefficient across the membrane. \( L_{m'm} \) is the diffusion coefficient for the transport of membrane molecules from the cytosol to the inner leaflet of the membrane. \( L_{ff}, L_{ww}, \) and \( L_{mm} \), are the conductance coefficients of food, waste and membrane molecules through the membrane. We suppose that all these diagonal coefficients are constant and uniform across the cytosol or the membrane, because protocells could not rely on local specialised channel molecules (intrinsic proteins, in evolved cells) to supply their food and evacuate their waste. We also suppose that food and waste molecules are electrically neutral and that the electric current is entirely due to the transport of small ions with the help of the translocation process and water pores.

The off-diagonal coefficients, \( L_{f\theta} = L_{\theta f}, L_{w\theta} = L_{\theta w}, L_{m\theta} = L_{\theta m} \) and \( L_{q\theta} = L_{\theta q} \), depend on the heat capacity of the molecules transported and on the rate constants of this transport. They couple the transport of matter and the heat flow. For our purpose, the most interesting off-diagonal coefficient is \( L_{\theta m} \). It can be viewed as the ratio of heat flow, \( J_\theta \), to the affinity \( X_m \) when \( T_0 = T_1 \) and in the absence of food and waste driving forces:

\[
L_{\theta m} = \left( \frac{J_\theta}{X_m} \right)_{(X_f, X_w, X_{m'})=0}.
\]

In this case, the thermal flow is due only to the asymmetry of the membrane, induced by its bending. This phenomenon is similar to the Dufour effect [20]. If one can prove experimentally that a bending of the membrane induces a heat flow through it, this means that \( L_{\theta m} \neq 0 \), hence, by Onsager’s reciprocity relations, \( L_{m\theta} \neq 0, \) i.e. a heat flow modifies the bending. Indeed, we also have the relation:

\[
L_{m\theta} = \left( \frac{J_m}{X_m} \right)_{(X_f, X_w, X_{m'})=0}.
\]

Hence, \( L_{m\theta} \) measures the effect of a slight temperature difference (between both sides of the membrane) on the induced flow of molecules between the leaflets, which implies a modification of its mean curvature. This phenomenon is similar to the thermodiffusion or Soret effect [20]. It is reciprocal to the previous effect and might be easier to observe and measure.

### 3.5. Entropy production and stability

Just as the power dissipated by Joule effect in an ohmic conductor is

\[
\text{Power dissipated} = \text{Current} \times \text{Voltage}
\]

\[
= \text{Conductance} \times \text{Voltage}^2,
\]

the rate of dissipation of energy, or entropy creation, in a general chemical system out of equilibrium is a quadratic function of the thermodynamical forces acting in the system [32, 20]:

\[
\text{Rate of entropy produced} = \text{Flows} \times \text{Forces} = \text{Forces} \times \text{Conductance matrix} \times \text{Forces}.
\]

This relation rests on a linearity hypothesis supposed to be valid only in the neighbourhood of an equilibrium state. The main difference between the ohmic conductor and the chemical system is that, in the latter, the conductance is not a single number but a matrix which, in the general case, couples all the currents. Taking into account the various thermodynamical forces defined previously, the rate of entropy production inside the protocell has to the following
expression:
\[
\sigma(X) = L_{ff}X_f^2 + L_{ww}X_w^2 + L_{mm}X_m^2 + L_{m'm'}X_{m'}^2 + L_{rr}X_r^2 + L_{\theta\theta}X_\theta^2 + 2X_\theta(L_{\theta f}X_f + L_{\theta w}X_w + L_{\theta m}X_m)
\]
\[
= L_{ff} \left( \frac{\mu_f}{T_0} - \frac{\mu_f}{T_1} \right)^2 + L_{ww} \left( \frac{\mu_w}{T_0} - \frac{\mu_w}{T_1} \right)^2 + L_{mm} \left( \frac{\mu_m}{T_0} - \frac{\mu_m}{T_1} \right)^2
\]
\[
+ L_{\theta\theta} \left( \frac{1}{T_0} - \frac{1}{T_1} \right)^2 + L_{m'm'} \left( \frac{\mu_{m'\mu} - \mu_{m''\mu}}{T_1} \right)^2 + L_{rr} \left( A_r \right)^2
\]
\[
+ 2 \left( \frac{1}{T_0} - \frac{1}{T_1} \right) L_{\theta f} \left( \frac{\mu_f}{T_0} - \frac{\mu_f}{T_1} \right) + L_{\theta w} \left( \frac{\mu_w}{T_0} - \frac{\mu_w}{T_1} \right) + L_{\theta m} \left( \frac{\mu_m}{T_0} - \frac{\mu_m}{T_1} \right).
\]

The stability of this steady state is equivalent to the positivity of the matrix \(L\), which is also the matrix of second order derivatives of \(\sigma\) in the coordinate system \(X = (X_\theta, X_f, X_w, X_m, X_{m'}, X_r)\):
\[
L_{ik} = \frac{1}{2} \frac{\partial^2 \sigma}{\partial X_i \partial X_k}.
\]
If \(P\) is an \(n \times n\) matrix with real coefficients, the positivity of \(P\), defined by:
\[
u_i^t P u > 0 \quad \forall u \in \mathbb{R}^n
\]
implies the following inequalities:
\[
Pi > 0 \quad \forall i \quad \text{and} \quad P_i P_j > \left( P_{ij} + \frac{P_{ii} + P_{jj}}{2} \right)^2 \quad \forall i, j.
\]
These conditions are necessary but not sufficient to ensure the positivity of \(P\). In the present case, \(L\) being symmetric, we have, in particular:
\[
L_{ii} > 0
\]
\[
L_{\theta\theta}L_{ff} > L_{\theta f}^2
\]
\[
L_{\theta\theta}L_{ww} > L_{\theta w}^2
\]
\[
L_{\theta\theta}L_{qq} > L_{\theta q}^2
\]
\[
L_{mm}L_{qq} > L_{mq}^2
\]
\[
L_{\theta\theta}L_{mm} > L_{\theta m}^2.
\]
If one of these inequalities is not satisfied, the growth process is destabilized. In Section VI, we will prove that the last one can be reversed as the inner temperature of the protocell increases. In order to prove this proposition, we must first write down evolution equations for the geometry of the cell.

4. Membrane geometry and growth equation

Just as the growth of a child depends on his diet, the evolution of the geometric parameters of a protocell depends on the flow of molecules to its membrane. This flow is determined by the food intake and by the rate of the synthesis of these structural molecules. In this section, we establish the differential equations governing the growth of the volume and area of a cylindrical protocell by relating them to the flows of matter.

4.1. Conservation of matter and exponential growth. The advancement, \(\xi\), of the overall synthesis reaction, \(f \rightarrow m + c + w\), is the internal clock of the protocell. The corresponding flow of matter, \(J_r = \frac{dL}{dt}\), is channeled to all the other processes in the protocell. In particular, it determines the flux of matter to the inner leaflet and the growth speed of the membrane. By writing the equations of conservation of matter, we can then determine the evolution of the size of the protocell.

Let \(a \in \{c, 1, 0\}\) denote the possible position of a membrane molecule: either in the cytosol (c), or the inner leaflet (1) or the outer leaflet (0). Let \(N_{na}\) be the number of membrane molecules in each of them. The time derivatives of these functions are related to the flows defined previously:
\[
\frac{dN_{na}}{dt} = -J_{mc1} A_1 + J_{mr} V
\]
\[
\frac{dN_{n1}}{dt} = J_{mc1} A_1 - J_{m10} A
\]
\[
\frac{dN_{n0}}{dt} = J_{m10} A.
\]
Similarly, the number of food (resp. cytosol and waste) molecules, $N_f$ (resp. $N_c$ and $N_w$), evolves according to the following relations:

\[
\frac{dN_f}{dt} = J_f A_0 - J_{rf} V \\
\frac{dN_c}{dt} = J_{rc} V \\
\frac{dN_w}{dt} = -J_w A_1 + J_{rw} V
\]

where the flows $J_{*,*}$ are defined by:

\[
J_{rm} := \nu_m \frac{d\xi}{dt} \\
J_{rf} := \nu_f \frac{d\xi}{dt} = \frac{\nu_f}{\nu_m} J_{rm} \\
J_{rc} := \nu_c \frac{d\xi}{dt} = \frac{\nu_c}{\nu_m} J_{rm} \\
J_{rw} := \nu_w \frac{d\xi}{dt} = \frac{\nu_w}{\nu_m} J_{rm}.
\]

In a steady state, the concentration of membrane molecules in the cytosol is constant:

\[
C_{mc} := \frac{N_{mc}}{V} = \text{cst.}
\]

Let $c_{m0}$ and $c_{m1}$ be the average number of membrane molecules per unit area in each leaflet:

\[
c_{m0} := \frac{N_{m0}}{A_0} \quad \text{and} \quad c_{m1} := \frac{N_{m1}}{A_1}.
\]

The conservation equations for $m$ imply the evolution equations of the geometry of the protocell:

\[
c_{m0} \frac{dA_0}{dt} = J_{m0} \left( \frac{A_0 + A_1}{2} \right) \\
c_{m1} \frac{dA_1}{dt} = J_{mc1} A_1 - c_{m0} \frac{dA_0}{dt} \\
c_{mc} \frac{dV}{dt} = J_{rm} V - J_{mc1} A_1.
\]

Let us introduce the following parameters:

\[
2\varepsilon := \text{average thickness of the membrane} \\
\eta := \frac{c_{m1}}{c_{m0}} \quad (\text{layer density ratio } \approx 1) \\
\tau := \frac{J_{m0}}{J_{mc1}} \quad (\text{transmission rate through the membrane}) \\
t_1 := \frac{J_{mc1}}{J_{mc1}} \quad (\text{inner leaflet characteristic time}) \\
\tau_c := \frac{J_{mc1}}{J_{rm}} \quad (\text{transmission rate to the membrane}) \\
t_c := \frac{c_{mc}}{J_{rm}} \quad (\text{cytosol characteristic time}).
\]

The transmission ratio, $\tau$, can be written in terms of thermodynamical forces:

\[
\tau := \frac{J_m}{J_{mc1}} = \frac{L_{mm} X_m + L_{m0} X_0 + \ldots}{L_{mm'} X_{m'}}.
\]

Let $U = V / \varepsilon$ and $X = t_1 \frac{dX}{dt}$. We obtain the following system of differential equations:

\[
\dot{A}_0 = \frac{\eta \tau}{2} (A_0 + A_1) = \eta \tau A_0 \\
\dot{A}_1 = -\frac{\tau}{2} A_0 + \left( 1 - \frac{\tau}{2} \right) A_1 = (1 - \tau) A_0 - B \\
\dot{U} = \frac{t_1}{t_c} U - \frac{c_{m1}}{c_{mc}} A_1.
\]
In matrix form:

\[
X = \begin{pmatrix} \dot{A}_0 \\ \dot{A}_1 \\ \dot{U} \end{pmatrix} = \begin{pmatrix} \frac{\alpha}{t} & \frac{\eta}{t} & 0 \\ \frac{\alpha}{t} & \frac{\eta}{t} & 0 \\ 0 & -\frac{\epsilon U}{\tau} & \frac{1}{\tau} \end{pmatrix} \begin{pmatrix} A_0 \\ A_1 \\ U \end{pmatrix} = MX
\]

(40)

\[
M := \begin{pmatrix} \frac{\alpha}{t} & \frac{\eta}{t} & 0 \\ \frac{\alpha}{t} & \frac{\eta}{t} & 0 \\ 0 & -\frac{\epsilon U}{\tau} & \frac{1}{\tau} \end{pmatrix} \quad \text{and} \quad X := \begin{pmatrix} A_0 \\ A_1 \\ U \end{pmatrix}.
\]

This growth equation is solved in Appendix B. The matrix \( M \) has a block diagonal form, hence \( A_0 \) and \( A_1 \) evolve independently of \( U \), whereas the equation for \( U \) contains terms linear in \( A_0 \) and \( A_1 \). The upper left 2 \times 2 block is not diagonal, hence \( A_0 \) and \( A_1 \) are linear combinations of exponential functions of time (multiplied by an affine function of \( t \) in the degenerate, non-diagonalisable case). The rates of growth of these exponential functions are the eigenvalues of this 2 \times 2 block, plus an exponential of growth rate \( \frac{\epsilon U}{\tau} \) for \( U \).

4.2. Cylindrical growth in steady state. When we meet an ordinary differential equation, describing the time evolution of a dynamical system, a first reflex is to search for constant solutions or at least steady state solutions, where the speed is constant. In the present case, we can look for a solution where the length increases steadily whereas the radius is constant. This corresponds to the observed growth of some bacterial species in difficult environments [28]. When the sludge content of wastewater is too high or when the composition is lopsided, a higher percentage of bacteria adopt a filamentous growth strategy which allows them to survive in harsher conditions, by catching food more easily.

If the protocell grows like a cylinder of radius \( R_0 \), we have \( \varepsilon A = R_0 B \), hence \( \frac{dA}{\varepsilon B} = \frac{dB}{\varepsilon} \) and

\[
x := \frac{R_0}{\varepsilon} = \frac{A}{B} = \frac{dA}{dB} = \frac{\dot{A}}{\dot{B}}
\]

(41)

\[
= \frac{(\eta - 1)\tau + 1)A - B}{(\eta + 1)\tau - 1)A + B}
\]

\[
= \frac{\alpha_+A - B}{\alpha_-A + B}
\]

where \( \alpha_{\pm} = (\eta \mp 1)\tau \pm 1 \). Therefore, \( x \) satisfies the fixed point equation:

\[
x = \frac{\alpha_+x - 1}{\alpha_-x + 1} \quad \text{i.e.} \quad \alpha_-x^2 - (\alpha_+ - 1)x + 1 = 0.
\]

The discriminant of this quadratic equation is

\[
(\alpha_+ - 1)^2 - 4\alpha_- = (\eta - 1)^2\tau^2 - 4(\eta + 1)\tau + 4 = 4\Delta(\eta, \tau)
\]

(cf. Appendix B) and its roots, \( x_{\pm} \), are related to the eigenvalues, \( \lambda_{\pm} \), of the matrix \( M \) (Eq. 40):

\[
x_{\pm} = \frac{1}{2\alpha_-} \left( \alpha_+ - 1 \pm \sqrt{(\alpha_+ - 1)^2 - 4\alpha_-} \right)
\]

(44)

\[
= \frac{(\alpha_+ - 1) \pm 2\sqrt{\Delta(\eta, \tau)}}{2\alpha_-}
\]

\[
= \frac{2\lambda_{\pm} - 1}{\alpha_-}
\]

Consequently, the radius, \( R_0 \), of the cylinder whose length increases in a steady state is determined by the flows \( (J_{mc1}, J_{m10}, J_{rm}) \) and the concentrations \( (C_{mc}, c_{m1}, c_{m0}) \), via the coefficients \( (\varepsilon, \eta, \tau) \):

\[
R_0 = \varepsilon x_{\pm} = \varepsilon \frac{\lambda_{\pm} - 1}{2\alpha_-} = \frac{\varepsilon ((\eta + 1)\tau \pm 2\sqrt{\Delta})}{2((\eta + 1)\tau - 1)}
\]

(45)

\[
= \frac{\varepsilon}{2} \frac{(\eta + 1)\tau \pm \sqrt{(\eta - 1)^2\tau^2 - (\eta + 1)\tau + 1}}{(\eta + 1)\tau - 1}
\]

5. Thermal instability of cylindrical growth

As long as the protocell grows by increasing only its length, keeping a cylindrical shape of fixed diameter, \( R_0 \), its volume and its membrane area grow proportionally, i.e. \( \dot{A} = \text{cst.} \times \dot{B} \). If the heat generated by the metabolic reactions were exactly proportional to the volume increment, the increase of the area of the membrane would be sufficient to evacuate steadily the heat generated by the chemical reactions taking place inside the newly created volume. However, the heat generated by all these irreversible processes adds up to that coming from the exothermic metabolic reactions.
and the inner temperature must therefore increase. This overheating generates larger fluctuations of all the physical parameters which destabilize the initial steady state of cylindrical growth. We will see below that the geometrical parameters \((A, B, V)\) can follow a path leading to a more efficient release of heat, by reducing the radius \(R_0\).

### 5.1. The Squeezed Sausage Theorem (SST)

When we squeeze a sausage, its length increases as well as its area. Indeed, the stuffing being incompressible, the squeezing is an isovolumic deformation. The stuffing is pushed longitudinally, away from the squeezed zone, and increases the length of the sausage, thanks to the elasticity of the gut. The area of the slice of reduced radius increases consequently to bound the same volume. Let us prove this mathematically.

A length \(\delta x\) of cylinder of radius \(R_0\) has volume \(\delta V\) and boundary area \(\delta A\) given by:

\[
\delta V = \pi R_0^2 \delta x \\
\delta A = 2\pi R_0 \delta x
\]

Let us suppose that this cylindrical growth is perturbed by a small, local radius variation, which can be positive (anevrism) or negative (stenosis). We study here a triangular perturbation and, in the appendix, a smooth (\(C^2\)), rotation invariant perturbation of the cylinder. To keep it simple, we suppose that this perturbation is piecewise linear and symmetric, with an extremum \(\delta R\) at \(x = 0\), and vanishes outside of the interval \([-\delta x', \delta x']\).

![Figure 5. Isovolumic variation of the area of a cylinder under a small triangular deformation.](image)

In the second and third pictures of FIG. 5, the Gaussian curvature is concentrated on the circular sections at \(x = 0\) and at \(x = \pm \frac{\delta x'}{2}\) (dotted lines), where the mean curvature has a finite discontinuity. The volume and lateral membrane area of this slice of thickness \(\delta x'\) (contained between the dotted lines) are therefore:

\[
\delta V' = \pi \left( R_0 + \frac{\delta R}{2} \right)^2 \delta x' \\
\delta A' = 2\pi \left( R_0 + \frac{\delta R}{2} \right) \delta x'.
\]

The straight slice and the deformed slice have equal volumes \((\delta V = \delta V')\) if their thicknesses satisfy:

\[
\frac{\delta x'}{\delta x} = \left(1 + \frac{\delta R}{2R_0}\right)^{-2} \approx \left(1 - \frac{\delta R}{R_0}\right).
\]

Hence the ratio of their areas is:

\[
\frac{\delta A'}{\delta A} \approx \left(1 + \frac{\delta R}{2R_0}\right) \left(1 - \frac{\delta R}{R_0}\right) \approx 1 - \frac{\delta R}{2R_0}.
\]

The heat flows through these surfaces are, respectively:

\[
\delta q = L_{\theta\theta} \left( \frac{1}{T_0} - \frac{1}{T_1} \right) \delta A \\
\delta q' = L_{\theta\theta} \left( \frac{1}{T_0} - \frac{1}{T_1} \right) \delta A'
\]
hence their ratio is the same as for the areas:

$$\frac{\delta q'}{\delta q} = \frac{\delta A'}{\delta A} = 1 - \frac{\delta R}{2R_0}.$$  

When $\delta R < 0$, this ratio is larger than 1. Consequently, the inner volume being held fixed, a small stenosis of a cylindrical protocell evacuates heat more efficiently than a small aneurism. This local reduction of the radius of the protocell increases its mean curvature. For this deformation to happen, the outer leaflet must grow more rapidly than the inner leaflet. Therefore, the equilibrium $m_1 \rightleftharpoons m_0$ must be shifted towards $m_0$ in order to have $\delta R < 0$.

This is possible if $T_1$ increases slightly and $m_1 \rightarrow m_0$ is exothermic. We propose that the translocation of membrane molecules to the outer leaflet [14, 15, 4, 1] can be triggered by the increase of the inner temperature, $T_1(t)$. The area of the outer leaflet then increases more quickly than the area of the inner leaflet, which leads to the bending of the membrane until the total splitting of the protocell into two daughters.

5.2. Fluctuations, translocation and heat transfer. In order to increase $L_{m\theta}$ and destabilise the cylindrical growth, the transfer coefficient, $\tau$, must also increase. In [14, 15], the authors present a detailed mechanism for the transfer of membrane molecules between the leaflets. Due to the fluctuations of ionic densities in the neighbourhood of the membranes, the local electric field fluctuates strongly enough to push molecules of water into the membrane, via the field-dipole interaction force (dielectrophoresis). When it is sufficiently strong, this force can create a transient water pore that is stable enough to let some membrane molecules dive into this water pore and join the other side.

The increase of the inner temperature can also enhance these ionic density fluctuations and favor this translocation process from the hot side to the cold side, since the hottest, most agitated molecules have a higher probability to dive into the water pore than the colder molecules. This asymmetric flow of hot molecules to the cold side enhances the outgoing heat flow and cools down the protocell.

During this process, the shape of the hydrophobic tails is not important, as long as they remain in the hydrophobic zone, surrounded by siblings. The only energetic cost is for the hydrophilic head surrounded by these aliphatic chains, and some clandestine water molecules forming the water pore (not represented below). The shape of the tail is irrelevant since the energy depends only on the position of the polar head (FIG. 6).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure6}
\caption{Translocation of a membrane molecule from one leaflet to the other.}
\end{figure}

5.3. Thermal balance. Let us make a thermal balance of the whole growth process. After heating its cold nutrient molecules from $T_0$ to $T_1$ and processing the isothermal inner chemical reactions ($J_e$), our protocell disposes of its hot waste (including some water flowing through the water pores) and loses heat by translocation of membrane molecules from the inside to the outside, and by diffusion ($J_\theta$) without mass transfer. Let $q_i$ be the heat exported out of the protocell by each molecule of type $i$. Cold entering molecules and hot outgoing molecules both have $q_i > 0$. Let $\kappa_i$ be the heat capacity of the molecules of type $i$. Let $J_e$ be the outgoing heat flow (energy/(time $\times$ area)). The heat flow exported by the cold entering food and water molecules is:

$$J_{f\theta} q_f = J_{f\kappa_f} (T_1 - T_0).$$

Similarly, the heat flow exported by the outgoing waste and water molecules is:

$$J_{w\theta} q_w = J_{w\kappa_w} (T_1 - T_0).$$

And the heat flow exported by the net translocation of membrane molecules is:

$$J_{m\theta} q_m = J_{m\kappa_m} (T_1 - T_0)$$

if we suppose that they immediately thermalise from $T_1$ to $T_0$ once they reach the outer leaflet. The contact of the hydrophobic tails inside the membrane allows for a diffusive heat flow:

$$J_\theta = \sum_i L_{\theta k} X_k.$$
The total heat flow is the sum of these terms:

\[ J_h := (J_f q_f + J_w q_w + J_m q_m) + J_\theta \]

\[ = (J_f \kappa_f + J_w \kappa_w)(T_1 - T_0) + (L_{m\theta} X_m + L_{\theta m} X_\theta + \ldots) \kappa_m(T_1 - T_0) + (L_{\theta \theta} X_\theta + L_{\theta m} X_m + \ldots). \]  

(56)

Since

\[ T_1 - T_0 = T_0 T_1 X_\theta = \frac{T_0^2 X_\theta}{1 - T_0 X_\theta} \]

(57)

\(L_{m\theta}\) appears as a factor of \(X_\theta^2\) in the convective term, \(J_m\), whereas \(L_{\theta m}\) is a factor of \(X_m\) in the diffusive term, \(J_\theta\). Moreover, \(X_m\) increases linearly with \(X_\theta\):

\[ X_m = \frac{\mu_{m0}}{T_0} - \frac{\mu_{m1}}{T_1} = \frac{\mu_0^\theta}{T_0} - \frac{\mu_1^\theta}{T_1} + k_B \ln \left( \frac{a_{m0}}{a_{m1}} \right) = \mu_0^\theta X_\theta + k_B \ln \left( \frac{a_{m0}}{a_{m1}} \right) \]

(58)

where \(\mu^0\) denotes the standard chemical potential, at temperature 298 K and pressure 1 atm [2]. The \(L_{m\theta}\)-dependent term in \(J_h\) becomes:

\[ J_h = L_{m\theta} \left( \kappa_m T_0^2 X_\theta^2 \right) \frac{1}{1 - T_0 X_\theta} + \mu_0^\theta X_\theta + \ldots \]  

(59)

Consequently, as the cytosol heats up, \(J_h\) increases more quickly by translocation (\(\kappa_m\) term) than by diffusion (\(\mu_0^\theta\) term). Translocation is a particular kind of heat convection and by analogy with the Rayleigh-Bénard instability [35], we conjecture the existence of a transition from a diffusive regime to a convective regime, where translocation overtakes diffusion and expells heat more efficiently.

6. Translocation between leaflets

The energetic barrier, of width 2\(c^\prime\) and height \(E^\star\), is difficult to penetrate for the hydrophilic head since this guarantees the stability of the bilayer under ordinary thermal fluctuations. When the ratio of concentrations, \(\eta = \frac{c_{m1}}{c_{m0}}\), becomes too large compared to unity, the mechanical constraint on the inner leaflet is released by pushing molecules to the outer leaflet. Conversely, when the outer leaflet is stretched and the inner leaflet compressed, \(\eta\) is slightly greater than unity (FIG. 7).

![Mechanical constraints modify the ratio, \(\eta\), of leaflet concentrations.](image)

To facilitate this process, some water molecules can leak through the hydrophobic zone and ease the passage of the hydrophilic head. This leakage of water lowers the activation energy, \(E^\star\), and realizes an aqueous catalysis of the translocation process [14, 15, 4, 1]. If we suppose that the density, \(n_p\), of water pores in the membrane is constant for fixed temperatures, \(T_0\) and \(T_1\), then \(J_{m10}\) depends only on this density and on the net number, \(J_{mp}\), of membrane molecules translocated from \(L_1\) to \(L_0\) during the lifetime of the pores:

\[ n_p := \text{number of water pores per unit area} \]

\[ j_{mp} := \text{net number of translocations through each water pore} \]

\[ J_m = n_p j_{mp}. \]

This first approximation is based on the hypothesis that the pores have the same size, the same lifetime and the same number of net translocations during their short life. However, to be more realistic, we must take into account the fact that larger pores live longer and leak more (over the same duration) than smaller short lived pores. We integrate over the interval of possible lifetimes \((t_p)\) the density of water pores of lifetime \(t_p\) created per unit time \((n_p(t_p))\) multiplied by the net number \((j_{mp}(t_p))\) of molecules each pore of lifetime \(t_p\) translocates from the inside to the outside during
THERMALLY DRIVEN FISSION OF PROTOCELLS

[201x791]THERMALLY DRIVEN FISSION OF PROTOCELLS 15

its existence:

\[ J_m = \int_0^\infty dt_p n_p(t_p) \nu_m(t_p). \]

The increase of \( X_\theta \) enhances at the same time the rate of formation of pores, hence \( n_p \), and the net number of translocated molecules, due to larger thermal fluctuations. Therefore, \( J_{m,10} \) increases more than linearly as a function of \( X_\theta \). Consequently, the crossed conductivity coefficient, \( L_{m\theta} \), increases with \( X_\theta \). On the other side of the inequality, \( L_{\theta\theta} \) and \( L_{mm} \) depend more weakly on the temperature. Indeed, the heat diffusion coefficient, \( L_{\theta\theta} \), involves the (temperature independent) number of interacting degrees of freedom between the hydrophobic tails inside the hydrophobic layer, and the molecular diffusion coefficient:

\[ L_{mm} = T_0 \left( \frac{J_m}{\mu_{m1} - \mu_{m0}} \right) \langle \chi_{s,x1,\chi_w,\chi_m'} \rangle = 0 \]

depends mainly on the ratio of concentrations between the two leaflets, i.e. on \( \eta \). In order to know if the initial inequality, \( L_{m\theta}^2 < L_{\theta\theta}L_{mm} \), can be reversed, the temperature dependance of the convective coefficient, \( L_{m\theta} \), must be computed and compared to that of the diffusion coefficients, \( L_{\theta\theta} \) and \( L_{mm} \). This necessitates a microscopic model of the interactions of membrane molecules and water and a precise description of the translocation process, to go beyond the linear response theory. In the sequel, we adopt a simple mean field approach where each molecule evolves in the same energetic landscape as the others.

6.1. An effective potential for translocation. The exact shape and position of each membrane molecule is described by dozens of parameters specifying the position of each atom and the orientation of each interatomic bond. It would be cumbersome to take them all into account to describe mathematically the evolution of a single molecule inside the membrane. However, we can make a simplifying approximation by remarking that the main energetic cost is in the displacement of the hydrophilic head into the hydrophobic layer or the protrusion of this head outside of the membrane, which forces the tail to go into the hydrophilic zone. We can make a mean-field approximation by considering only the position, \( z \), of the hydrophilic head as a dynamical variable, and defining an adequate effective potential energy, \( U(z) \), that traps the head inside the membrane. In the sequel of this article, we will use a double well effective potential to compute the net flow, \( J_m \), across a plane bilayer subject to a difference of temperatures. By differentiation, we obtain the coefficients \( L_{m\theta} \) and \( L_{mm} \) and, in particular, their dependence on temperature. This model suggests that the inequality \( L_{m\theta}^2 < L_{mm}L_{\theta\theta} \) can be reversed if the inner temperature increases sufficiently. Our hypotheses are the following ones:

1. The membrane molecules have length \( \varepsilon = \varepsilon' + \varepsilon'' \), where \( \varepsilon'' \) is the size of the hydrophilic head and \( \varepsilon' \) is the length of the hydrophobic tail.
2. The translocation process is described by only one parameter: the position of the center of mass of the hydrophilic head, varying between \(-\varepsilon\) and \(+\varepsilon\).
3. On each side of the membrane, the distribution of velocities of the heads follows a Maxwell-Boltzmann law [35]. The probability of finding a molecule with velocity \( v \) perpendicularly to the membrane is:

\[ p_i(v) = \frac{m}{\sqrt{2\pi k_B T_i}} \exp \left( -\frac{mv^2}{2k_B T_i} \right). \]

4. The translocation requires an energy \( E^* \) and the head of the molecule evolves in an effective double well potential (FIG. 8).
The hydrophilic heads trapped in the well \([-\varepsilon, -\varepsilon']\) have temperature \(T_1\), whereas those trapped in the well \([\varepsilon', \varepsilon]\) have temperature \(T_0\). The thermalisation processes for the motion along the \(z\) axis occur only once the head is trapped in the arrival well. This drastic hypothesis simplifies the computations and should be refined in a more realistic model. In reality, the motions of the hydrophobic tails between \(z = -\varepsilon'\) and \(z = +\varepsilon'\) can thermalise the molecule during the travel across the membrane and this affects the translocation time.

Only half of the molecules of kinetic energy \(E > E_*\) can escape from a well to the other side. The time it takes them to go through the barrier is given by:

\[
t_f = \int_{-\varepsilon'}^{+\varepsilon'} dz \sqrt{\frac{m}{2(E - E_*)}}
\]

\[
= 2\varepsilon' \sqrt{\frac{m}{2(E - E_*)}}
\]

\[
= 2\varepsilon' \sqrt{\frac{m}{m_0^2 - 2E_*}}.
\]

The flow of molecules of velocity belonging to the interval \([v, v + \text{dv}]\), with \(v > v_* := \sqrt{\frac{2E_*}{m}}\), going from side 1 to side 0, is proportional to the surface density of molecules, \(c_{m1}\), to the Maxwell-Boltzmann weight, \(p_1(v)\text{dv}\), of this velocity interval, and to the reciprocal of the translocation time:

\[
J_{m10} = \int_{v_*}^{+\infty} dv \frac{c_{m1}p_1(v)}{t_f}
\]

\[
= \int_{E_*}^{+\infty} \frac{dE}{\sqrt{2mE}} \frac{1}{2\varepsilon'} \sqrt{\frac{m}{2(E - E_*)}} \frac{c_{m1}e^{-E/k_B T_1}}{m} \sqrt{\frac{2\varepsilon' k_B T_1}{m}}
\]

\[
= \frac{1}{2\varepsilon' \sqrt{\pi}} \int_{E_*}^{+\infty} \frac{dE}{\sqrt{2mE}} \frac{k_B T_1}{c_{m1}} e^{-E/k_B T_1}.
\]

The net flow of molecules from leaflet 1 to leaflet 0 is:

\[
J_m := J_{m10} - J_{m01}
\]

\[
= \frac{1}{2\varepsilon' \sqrt{\pi}} \int_{E_*}^{+\infty} \frac{dE}{\sqrt{2mE}} \frac{E - E_*}{k_B T_1} c_{m1} e^{-E/k_B T_1}
\]

\[
- \frac{1}{2\varepsilon' \sqrt{\pi}} \int_{E_*}^{+\infty} \frac{dE}{\sqrt{2mE}} \frac{E - E_*}{k_B T_0} c_{m0} e^{-E/k_B T_0}.
\]
6.2. Computation of \( L_{m\theta} \). The temperature \( T_0 \) being fixed, we have:

\[
\begin{align*}
L_{m\theta} &= \frac{\partial J_m}{\partial X_\theta} - \frac{\partial J_m}{\partial T_1} \\
&= - \frac{c_{m1}}{2\varepsilon \sqrt{\pi}} \int_{E_*}^{+\infty} dE \sqrt{\frac{E - E_*}{2mE}} \frac{\partial}{\partial T_1} \left( \frac{e^{-E/k_BT_1}}{e^{E/k_BT_1}} \right) \\
&= \frac{c_{m1}}{2\varepsilon k_B 2\pi m k_BT_1} \int_{E_*}^{+\infty} dE \sqrt{\frac{E - E_*}{E}} \left( E - \frac{k_BT_1}{2} \right) e^{-E/k_BT_1}.
\end{align*}
\]

We set \( u_1 := \frac{E_*}{k_BT_1} \) and change the variable of integration from \( E \) to \( s := \frac{E}{E_*} \):

\[
L_{m\theta} = \frac{c_{m1}E_* \sqrt{k_BT_1}}{4\varepsilon k_B 2\pi m} \int_{1}^{+\infty} ds \sqrt{1 - \frac{1}{s}} (2su_1 - 1) e^{-su_1} \quad \alpha_1 := \frac{c_{m1}E_* \sqrt{k_BT_1}}{4\varepsilon k_B 2\pi m}
\]

where the function \( F \) is defined by:

\[
F(a) := \int_{1}^{+\infty} ds \sqrt{1 - \frac{1}{s}} (2as - 1)e^{-as}.
\]

We can now compute the relative variations of \( L_{m\theta} \) with respect to relative variations of temperature. Since \( L_{m\theta} \) depends on \( T_1 \) through \( E_* \) and \( F(u_1) \), we have:

\[
\frac{\partial \ln L_{m\theta}}{\partial \ln T_1} = \frac{\partial \ln \alpha_1}{\partial \ln T_1} + \frac{\partial \ln F}{\partial \ln T_1} = \frac{1}{2} \frac{\partial \ln E_*}{\partial \ln T_1} + \frac{\partial \ln \mu_{\theta}}{\partial \ln \mu_{\theta}} \frac{\partial \ln F}{\partial \ln \mu_{\theta}} \quad \alpha_1 := \frac{c_{m1}E_* \sqrt{k_BT_1}}{4\varepsilon k_B 2\pi m}
\]

\[
\frac{\partial \ln E_*}{\partial \ln T_1} \text{ can not be computed in the present model, because it depends on the microscopic details of the formation of water pores. However, we know that } E_* \text{ diminishes as } T_1 \text{ increases, since the water pores become more frequent (and, probably, larger and more durable) when the ionic density fluctuations increase [14, 15]. Consequently, we have :}
\]

\[
\frac{\partial \ln E_*}{\partial \ln T_1} < 0.
\]

In Appendix C, we prove that \( 1 + \frac{\partial \ln F}{\partial \ln u_1} \) is slightly negative at high temperature. Since \( \frac{\partial \ln E_*}{\partial \ln T_1} \) is also negative, we obtain the following estimate:

\[
\frac{\partial \ln L_{m\theta}}{\partial \ln T_1} \geq \frac{3}{2} \quad \text{at high temperature.}
\]

6.3. Computation of \( L_{mm} \). \( L_{mm} \) is obtained by differentiating \( J_m \) with respect to \( X_m = \frac{\mu_m - \mu_0}{T_0} \) while keeping the other thermodynamical forces equal to zero:

\[
L_{mm} = \left( \frac{\partial J_m}{\partial X_m} \right)_{(X_\theta,X_f,X_w,X_{m'})=0} = T_0 \left( \frac{\partial J_m}{\partial (\mu_m - \mu_0)} \right)_{(X_\theta,X_f,X_w,X_{m'})=0} = \frac{1}{k_B} \left( \frac{\partial J_m}{\partial (\ln a_1/a_0)} \right)_{(X_\theta,X_f,X_w,X_{m'})=0}.
\]

In our model, based on the double well effective potential, the activities of the membrane molecules in each leaflet are equal to their respective concentrations. A more accurate model, taking into account the attractive interactions inside each leaflet, is necessary to improve this first approximation. Replacing \( \frac{a_1}{a_0} \) by \( c_{m1}/c_{m0} = \eta \), we obtain:

\[
L_{mm} = \frac{1}{k_B} \left( \frac{\partial J_m}{\partial \ln \eta} \right)_{T_1=T_0}.
\]
\[ J_m = \zeta(T_1)c_{m1} - \zeta(T_0)c_{m0} \]

(75)

\[
\zeta(T) := \frac{E_* e^{-u_* \eta}}{2e^{\gamma}2\pi m k_B T} \int_0^{+\infty} dx \ e^{-u_* x} \sqrt{\frac{x}{x + 1}}
\]

If the temperatures of both leaflets are equal, then \( J_m \) is simply proportional to the difference of their concentrations :

(76)

\[
(J_m)_{T_0=T_1} = \zeta(T) (c_{m1} - c_{m0})
\]

and its derivative with respect to \( \ln \eta \), while \( c_{m0} \) is held fixed, is :

(77)

\[
\left( \frac{\partial J_m}{\partial \ln \eta} \right)_{c_{m0}=\text{const}} = \zeta(T_1)c_{m1} = k_B L_{mm}.
\]

Since

(78)

\[
\int_0^{+\infty} dx \ e^{-a x} \sqrt{\frac{x}{x + 1}} = \frac{1}{a} - \frac{\ln(a)}{2} + O(1) \quad (a \to 0^+)
\]

the high temperature expansion of \( L_{mm} \) gives :

(79)

\[
\left( \frac{\partial \ln L_{mm}}{\partial \ln T} \right)_{T_1=T_0} = \left( \frac{\partial \ln \zeta}{\partial \ln T} \right)_{T_1=T_0} = \frac{1}{2} + o(1).
\]

6.4 Estimation of \( L_{\theta \theta} \). The heat diffusion coefficient, \( L_{\theta \theta} \), depends only on the number of degrees of freedom that interact in the membrane bilayer. As long as the structure of the membrane is unchanged, the same hydrophobic tails interact similarly at any temperature. Therefore, we conjecture that \( L_{\theta \theta} \) is independent of the temperature in the liquid disordered phase [27]. Therefore :

(80)

\[
\frac{\partial \ln L_{\theta \theta}}{\partial \ln T} \simeq 0.
\]

6.5 Destabilisation. Putting together the scaling laws for \( L_{m\theta}, L_{mm} \) and \( L_{\theta \theta} \), we obtain :

(81)

\[
\frac{\partial}{\partial \ln T} \left( \frac{L_{m\theta}^2}{L_{mm}L_{\theta \theta}} \right) = 3 - \frac{1}{2} - 0 = \frac{5}{2}
\]

The main mathematical proposition of the present article is the following.

Proposition 6.1. Since \( \frac{L_{m\theta}^2}{L_{mm}L_{\theta \theta}} \) grows as \( T_1^{5/2} \), the stability condition, \( L_{m\theta}^2 < L_{mm}L_{\theta \theta} \), can not be satisfied at high temperature.

The exact value of \( T_1 \) for which this transition occurs can not be computed in our simple model, but the only characteristic temperature being \( \frac{E_*}{k_B} \), the critical temperature must be of this order of magnitude.

This destabilisation of the steady growth regime is comparable with the onset of heat convection in a fluid subject to a strong temperature gradient. In fact, the self-replication of protocols could be interpreted as a convective phenomenon inside their membrane, triggered by their metabolic activity.

7. Conclusions and perspectives

We have proposed a toy model of protocell growth, fission and reproduction. The scenario thus described can be viewed as the ancestor of mitosis. The main force driving this irreversible process is the temperature difference between the inside and the outside of the protocell, due to the inner chemical activity. We propose that the increase of the inner temperature, due to a rudimentary inner metabolism, enhances the transfer of membrane molecules from the inner leaflet to the outer leaflet, as described in *silico* by models of molecular dynamics [14, 15]. Due to this transfer of molecules, coupled to a heat transfer, the difference of their areas and the total mean curvature of the median surface increase. The cylindrical growth becomes unstable and any slight local reduction of the radius of the initial cylinder increases until the protocell is cut into two daughter protocells, each one containing reactants and catalysers to continue the growth and fission process. The cut occurs near the hottest zone, around the middle. This model is based on the idea [23] that the early forms of life were simple vesicles containing a particular network of chemical reactions, precursor of modern cellular metabolism :

Protolife = Cellularity + Inner Metabolism.

With a large supply of reactants in the so-called prebiotic soup [31, 16, 23], and with an optimal salinity and pH, these ingredients are sufficient to induce an exponential growth of prebiomass and make possible the exploration of a large number of chemical reactions in these miniature chemical factories. The possibility to synthesize complex molecules (sterols, RNA, DNA, proteins, etc.) comes later, once these factories self-replicate and thrive.
In order to test our model experimentally, we have to manipulate vesicles that can be heated from within in a controlled way. Let us imagine, in a solution maintained at temperature $T_0$, vesicles containing molecules of type $A$ able to absorb visible radiation, with which the surrounding molecules do not interact. Let us suppose that $A$ re-emits radiation in the near infrared. The heat thus generated inside the vesicle creates a controlled temperature difference, $T_1 - T_0 > 0$, between both sides of the membrane. If $L_{m0}$ is large enough, we should observe a bending of the membrane of the vesicles due to the transfer of the hottest molecules from the inner leaflet to the outer leaflet.

Another experimental test of our model can be made by observing eukaryotic cells, where the mitochondria are the main source of heat. It seems possible to measure their temperature variations using fluorescent molecules [3]. Although the very notion of temperature at this scale and far from a thermodynamical equilibrium is not clear, the measurement of the temperature variations inside the cell during its life cycle could be correlated with the onset of mitosis and with the shape of mitochondrial network [21].

Our model is obviously oversimplified since the polar heads of membrane molecules are treated as an ideal gas in a box. In particular, we haven’t taken into account the interaction between these molecules and the surrounding solution. This calls for the development of a better model to treat the effect of these interactions on the temperature dependence of the conductance coefficients. The scaling law of the ratio $\frac{L_{\text{m}0}^2}{L_{\text{m}0} L_{\theta}}$ at temperatures higher than $\frac{E}{k_B}$ is the key argument that explains the splitting of the protocell. Future investigations and experiments will decide of the plausibility of this proposition.

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**Appendix A. The mean curvature of the membrane**

Let $\Sigma_t$ be a family of surfaces, indexed by a time parameter $t \in [t_0, +\infty]$. We suppose that each $\Sigma_t$ is a smooth, orientable and closed (compact, without boundary) hence diffeomorphic to the standard 2-sphere. At each point $P \in \Sigma_t$, the Taylor expansion of the distance from $Q \in \Sigma_t$ to the tangent plane, $T_P \Sigma_t$, defines a quadratic form whose eigenvalues (homogenous to the inverse of a length) do not depend on the coordinate system in the neighbourhood of $P$. We denote them $R_-$ and $R_+$. The mean curvature of $\Sigma_t$ at $P$ is the arithmetic mean of the principal curvatures:

$$H := \frac{1}{2} \left( \frac{1}{R_+} + \frac{1}{R_-} \right)$$

and the gaussian curvature is their product:

$$K := \frac{1}{R_+ R_-}.$$  

In the case of a cylinder, $R_+ = +\infty$ and $R_- = R_0 = \text{its radius}$, hence $H_{\text{cyl}} (P) = \frac{1}{2 R_0}$ and $K (P) = 0$ at every point $P \in \Sigma_t$ (except on the end hemispheres).

Let $\Sigma_{0\epsilon}$ and $\Sigma_{1\epsilon}$ be the surfaces obtained by shifting $\Sigma_t$ in the normal direction, over an infinitesimal distance $\epsilon$ on both sides of $\Sigma_t$. Let $A_0 (t)$ (resp. $A_1 (t)$) be the average area of the outer (resp. inner) layer of the membrane, measured at the hydrophilic heads, and $A = \frac{1}{2} (A_0 + A_1)$ the average area of the median surface, where the hydrophobic tails join. The difference of their areas, $A_1 - A_0$, is given by the first term of Weyl’s Tube Formula [13]:

$$A_0 - A_1 = 4 \epsilon \int_{\Sigma_t} H \, dA + O(\epsilon^2).$$

Let $B$ be the infinitesimal variation of area along the outer normal:

$$B := 2 \epsilon \int_{\Sigma_t} H \, dA.$$

$A_0$ and $A_1$ can also be written as functions of $A$ and $B$:

$$A_0 = A + B \quad \text{and} \quad A_1 = A - B.$$  

Our dynamical variables are the area of the median surface, $A (t) = \int_{\Sigma_t} dA$, the volume of the cytosol, $V (t)$, and the variation of area, $B (t) = 2 \epsilon \int_{\Sigma_t} H \, dA$. In the next section, we will establish their evolution equations as a consequence of the balance equations for the number of membrane molecules.

Remark: In the case of a cylinder of radius $R_0$, we have $H = \frac{1}{2 R_0}$ and

$$A_0 - A_1 = 2 B = 4 \epsilon H A = \frac{2 \epsilon A}{R_0}.$$
In general, the two leaflets of the membrane grow at different speeds. Indeed, the characteristic polynomial of \( \tau \) and has distinct roots:

\[
\text{det}(\begin{vmatrix} \eta & \tau \\ \frac{\eta}{2} & 0 \end{vmatrix} - \lambda \begin{vmatrix} \frac{\eta}{2} & \tau \\ 0 & \frac{\eta}{2} \end{vmatrix}) = 2 \text{det}(\begin{vmatrix} \eta & \tau \\ \frac{\eta}{2} & 0 \end{vmatrix}) = \frac{\eta^2}{2} \text{det}(1 - \lambda \begin{vmatrix} \eta & \tau \\ \frac{\eta}{2} & 0 \end{vmatrix}) = \frac{\eta^2}{2} \left( \lambda^2 - \lambda \left( \frac{(\eta - 1)\tau}{2} \right) + \frac{\eta^2}{2} \right) = \left( \lambda^2 - \lambda \left( 1 + \frac{(\eta - 1)\tau}{2} \right) + \frac{\eta^2}{2} \right) = 0.
\]

The roots of this polynomial are \( \lambda_\pm(\eta, \tau) = \frac{1}{2} \left( 1 \pm \sqrt{1 - 2\tau} \right) \) and \( \Delta(\eta, \tau) = \frac{\eta^2}{2} \left( \lambda^2 - \lambda \left( 1 + \frac{(\eta - 1)\tau}{2} \right) + \frac{\eta^2}{2} \right) = \frac{\eta^2}{2} \left( \lambda^2 - \lambda \left( 1 + \frac{(\eta - 1)\tau}{2} \right) + \frac{\eta^2}{2} \right) = 0 \).

If \( \eta = 1 \), i.e. if both leaflets have the same density, then \( \Delta \) is an affine function of \( \tau \):

\[
\Delta(1, \tau) = 1 - 2\tau \quad \text{and} \quad \lambda_\pm(1, \tau) = \frac{1}{2} \pm \sqrt{1 - 2\tau}.
\]

If, moreover, \( \tau = \frac{1}{2} \), i.e. the inner leaflet transmits half of the incoming membrane molecules to the outer leaflet, then

\[
\Delta \left( \frac{1}{2}, \frac{1}{2} \right) = 0 \quad \text{and} \quad \lambda_\pm \left( \frac{1}{2}, \frac{1}{2} \right) = \frac{1}{2}.
\]

\[\Delta > 0\quad \Delta = 0 \quad \Delta < 0\]

\[\tau_+ \approx \tau_- \quad \tau_+ \gg \tau_- \]

\[
\text{physical region} \quad \text{non-physical region}
\]

\[\text{Figure 9. Exponential growth necessitates to keep } \tau < \tau_- .\]
Mathematically, we have three possibilities:

1. \( \tau < \tau_-(\eta) \) or \( \tau > \tau_+(\eta) \) \( \Rightarrow \Delta(\eta, \tau) > 0 \)
   \[ \lambda_+(\eta, \tau) \neq \lambda_-(-\eta, \tau) \] (real numbers);
2. \( \tau = \tau_-(\eta) \) or \( \tau = \tau_+(\eta) \) \( \Rightarrow \Delta(\eta, \tau_\pm) = 0 \)
   \[ \lambda_+(\eta, \tau_\pm(\eta)) = \lambda_-(-\eta, \tau_\pm(\eta)) = \frac{\sqrt{\eta}}{\sqrt{\eta} + 1}; \]
3. \( \tau_-(\eta) < \tau < \tau_+(\eta) \) \( \Rightarrow \Delta(\eta, \tau) < 0 \)
   \[ \lambda_+(\eta, \tau) = \overline{\lambda_-(-\eta, \tau)} \] (complex numbers).

B.1. Case 1: \( \eta \neq 1 \) and \( \tau > \tau_+(\eta) \) or \( \tau < \tau_-(\eta) \). In these intervals, \( N \) is diagonalisable and a basis of eigenvectors of \( N \) is given by:

\[
\mathcal{B}_\pm = \left( \frac{1}{\eta^\tau} - \frac{1}{\eta^\tau} \right) \left( \begin{array}{c} \eta_\pm(\eta, \tau) \\ \eta_\mp(\eta, \tau) \end{array} \right) = \frac{A_0 - A_1}{\eta^\tau} + \frac{\lambda_\pm(\eta, \tau)}{\eta^\tau} A_1
\]

\[ \mathcal{B}_\pm = \mathcal{B}_\pm(0) \exp \left( \frac{\lambda_\pm(\eta, \tau)}{t_1} t \right). \]

The area of the inner leaflet is:

\[
A_1(t) = \frac{\mathcal{B}_+(t) - \mathcal{B}_-(t)}{\lambda_+ - \lambda_-} = \frac{\eta^\tau}{\sqrt{\Delta}} \left( \mathcal{B}_+(0) e^{t\lambda_+/t_1} - \mathcal{B}_-(0) e^{t\lambda_-/t_1} \right).
\]

The area of the outer leaflet is:

\[
A_0(t) = \frac{2\lambda_+ - \eta^\tau \mathcal{B}_-(t) - (2\lambda_- - \eta^\tau) \mathcal{B}_+(t)}{\lambda_+ - \lambda_-} = \frac{2\lambda_+ - \eta^\tau}{\sqrt{\Delta}} \mathcal{B}_-(0) e^{t\lambda_-/t_1} - \frac{2\lambda_- - \eta^\tau}{\sqrt{\Delta}} \mathcal{B}_+(0) e^{t\lambda_+/t_1}.
\]

And \( U(t) \) is obtained from \( A_1(t) \):

\[
e^{t_{\ll} / t_c} \frac{d}{dt} \left( U(t) e^{-t_{\ll} / t_c} \right) = -\frac{c m_1}{\epsilon \epsilon_{m, c}} A_1(t)
\]

\[
U(t) e^{-t_{\ll} / t_c} = -\frac{\eta^\tau c m_1 B_+(0)}{\epsilon \epsilon_{m, c} \sqrt{\Delta}} e^{t \left( \frac{\lambda_+}{t_1} - \frac{\lambda_-}{t_1} \right)}
\]

\[
+ \frac{\eta^\tau c m_1 B_-(0)}{\epsilon \epsilon_{m, c} \sqrt{\Delta}} e^{t \left( \frac{\lambda_-}{t_1} - \frac{\lambda_+}{t_1} \right)} + \text{cst.}
\]

\[
U(t) = \frac{\eta^\tau c m_1}{\epsilon \epsilon_{m, c} \sqrt{\Delta}} \left( e^{t\lambda_- / t_1} - e^{t\lambda_+/t_1} \right)
\]

\[
+ \text{cst.} e^{t_{\ll} / t_c}
\]

where the integration constant is determined by \( U(0) \).

B.2. Case 2: \( \eta \neq 1 \) and \( \tau \in \{ \tau_+(\eta), \tau_-(\eta) \} \). In this singular case, the upper-left \( 2 \times 2 \) submatrix is not diagonalisable but conjugate to a lower triangular matrix of Jordan form:

\[
N := \frac{1}{2} \begin{pmatrix} \eta^\tau & \eta^\tau \\ -\tau & -2 - \tau \end{pmatrix} = T \begin{pmatrix} A_\pm(\eta) & 0 \\ 1 & A_\pm(\eta) \end{pmatrix} T^{-1}
\]
where $\Lambda_{\pm}(\eta)$ is the single eigenvalue of $N$ when $\tau$ is fixed equal to $\tau_{\pm}(\eta)$ or $\tau_{-}(\eta)$:

$$\Lambda_{\pm}(\eta) := \lambda(\eta, \tau_{\pm}(\eta)) = \frac{2 + (\eta - 1)\tau_{\pm}(\eta)}{4}.$$  \hfill (106)

An easy computation gives us:

$$\frac{2\Lambda_{+}}{\eta^2} - 1 = \frac{1 - 3\sqrt{\eta}}{\eta + \sqrt{\eta}} \quad \text{and} \quad \frac{2\Lambda_{-}}{\eta^2} - 1 = \frac{1 + 3\sqrt{\eta}}{\eta - \sqrt{\eta}}$$  \hfill (107)

$N$ has a unique proper line, generated by the vector

$$B_{\pm} := \left( \frac{\Lambda_{\pm}}{\eta^2} - \frac{1}{2} \right) = \frac{1}{2} \left( \frac{1 - \Lambda_{\pm}}{\eta + \sqrt{\eta}} \right)$$  \hfill (108)

where the lower $\ast$ means that $\lambda_{+} = \lambda_{-}$, whereas the upper $\pm$ depends on the choice between $\tau = \tau_{+}(\eta)$ and $\tau = \tau_{-}(\eta)$. Since

$$B_{\pm}(t) = B_{\pm}(0) e^{t\Lambda_{\pm}/t}$$ we obtain:

$$A_{0}(t) + \left( \frac{3\eta \mp 1}{\eta \pm \sqrt{\eta}} \right) A_{1}(t) = 2 B_{\mp}(0) e^{t\Lambda_{\pm}/t}.$$  \hfill (109)

Since $B_{\pm} = T(0)$, the vector $B_{\pm}$ is the right column of $T$. The left column of $T$ is the vector $B_{\mp} = (0)$ which satisfies the equation $(N - \Lambda_{\pm})B_{\mp} = B_{\pm}$, or in extenso:

$$\left( \frac{\eta^2}{2} - \Lambda_{\pm} \right) x + \left( \frac{\eta^2}{2} \right) y = \frac{1}{2}$$ $$-\frac{\tau}{2} x + \frac{2 - \tau - 2\Lambda_{\pm}}{2} y = \frac{\Lambda_{\pm}}{\eta^2} - \frac{1}{2}.$$  \hfill (110)

Taking $x = 0$ and $y = \frac{1}{\eta^2}$ gives a solution:

$$(N - \Lambda_{\pm})B_{\mp} = \left( \frac{\eta^2}{2} - \Lambda_{\pm} \right) \begin{pmatrix} \frac{\eta^2}{2} \\ \frac{\eta^2}{2} \end{pmatrix} = \left( \frac{1}{2} \right) \begin{pmatrix} 0 \\ \frac{1}{\eta^2} \end{pmatrix}.$$  \hfill (111)

The matrix $T$ and its inverse, $T^{-1}$, are therefore:

$$T = \left( \begin{array}{cc} 0 & \frac{1}{2\eta^2} \\ \frac{1}{2\eta^2} & 0 \end{array} \right) \quad \text{and} \quad T^{-1} = \left( \begin{array}{cc} \frac{\eta^2}{2} & \eta^2 \\ \frac{1}{2} & 0 \end{array} \right).$$  \hfill (112)

Since $B_{\mp} = \frac{A_{1}}{\eta^2(\eta)}$, we have $B_{\pm}(t) = B_{\pm}(0) \frac{t}{t_{1}} e^{t\Lambda_{\pm}/t_{1}}$ and:

$$A_{1}(t) = \eta \tau_{\pm}(\eta) B_{\pm}(0) \frac{t}{t_{1}} e^{t\Lambda_{\pm}/t_{1}}.$$  \hfill (113)

\section*{B.3. Case 3: $\tau_{-}(\eta) < \tau < \tau_{+}(\eta)$}

In this interval, $\Delta < 0$ and $M$ has two distinct complex conjugated eigenvalues, $\lambda$ and $\bar{\lambda}$, functions of $\eta$ and $\tau$. Let $\alpha, \beta \in \mathbb{R}$ be the real and imaginary parts of $\lambda$:

$$\alpha := \frac{2 + (\eta - 1)\tau}{4} > 0$$ $$\beta := \frac{\sqrt{-\Delta}}{2} > 0$$ $$\lambda(\eta, \tau) = \alpha + i\beta \quad (i^2 = -1).$$  \hfill (114)

Let $V$ (resp. $\bar{V}$) be a complex eigenvector of $N$, of eigenvalue $\lambda$ (resp. $\bar{\lambda}$), for instance:

$$V := B + \frac{\lambda}{\eta^2} A_{1} \quad \text{and} \quad \bar{V} := B + \frac{\bar{\lambda}}{\eta^2} A_{1}.$$  \hfill (115)
then the real and imaginary parts of \( V \), defined by \( V' := \frac{1}{2} (V + \overline{V}) \) and \( V'' := \frac{1}{2} (V - \overline{V}) \), form a basis of \( \mathbb{R}^2 \) on which \( N \) acts as an orthogonal matrix [19]:

\[
\frac{1}{2} \begin{pmatrix} \eta \tau & \eta \tau \\ -\tau & 2 - \tau \end{pmatrix} = U \begin{pmatrix} \alpha & \beta \\ -\beta & \alpha \end{pmatrix} U^{-1}
\]

(117)

\[
V' = U \begin{pmatrix} 1 \\ 0 \end{pmatrix} = \left( \frac{1}{2} \eta \tau - \frac{1}{2} \right) V'' = U \begin{pmatrix} 0 \\ 1 \end{pmatrix} = \left( \frac{0}{\eta \tau} \right)
\]

\( i.e. \) the matrix \( U \) has \( V' \) and \( V'' \) as columns:

\[
U = \begin{pmatrix} \frac{1}{2} & 0 \\ \frac{\eta \tau}{2 - (\eta \tau + 1) \tau} & \sqrt{\frac{\alpha}{2 \eta \tau}} \end{pmatrix}.
\]

(118)

Let \( s = \frac{\ell}{t_1} \). Since our evolution operator, the exponential of \( sN \), is:

\[
e^{sN} = e^{sN} U \begin{pmatrix} \cos(\beta s) & \sin(\beta s) \\ -\sin(\beta s) & \cos(\beta s) \end{pmatrix} U^{-1}
\]

we have:

\[
V'(s) = e^{sN} V'(0) = e^{sN} U \begin{pmatrix} \cos(\beta s) & \sin(\beta s) \\ -\sin(\beta s) & \cos(\beta s) \end{pmatrix} \left( \frac{1}{2} \eta \tau - \frac{1}{2} \right)
\]

(120)

\[
= e^{sN} \frac{1}{2 \eta \tau} \left( \frac{1}{2} \eta \tau - 0 \right) \begin{pmatrix} \eta \tau \cos(\beta s) + (2\alpha - \eta \tau) \sin(\beta s) \\ -\eta \tau \sin(\beta s) + (2\alpha - \eta \tau) \cos(\beta s) \end{pmatrix}
\]

\[
= e^{sN} \frac{1}{2 \eta \tau} \left( \frac{2\alpha - \eta \tau}{2 \eta \tau} \right) \cos(\beta s) + \left( \frac{2\alpha - \eta \tau}{2 \eta \tau} \right) \sin(\beta s)
\]

Similarly, we have the expression of \( V''(s) \):

\[
V''(s) = e^{sN} V''(0)
\]

(121)

\[
= \beta e^{sN} \frac{1}{\eta \tau} \begin{pmatrix} \frac{1}{2} \sin(\beta s) \\ \frac{1}{2 \eta \tau} \sin(\beta s) + \frac{\beta}{\eta \tau} \cos(\beta s) \end{pmatrix}.
\]

Finally, \( A_1 \) and \( B \) are obtained from \( V' \) and \( V'' \) by the linear relations:

\[
B(s) = \frac{\beta V'(s) - \alpha V''(s)}{\beta - \alpha}
\]

(122)

\[
A_1(s) = \frac{\eta \tau}{\alpha - \beta} \left( V'(s) - V''(s) \right).
\]

**Appendix C. Smooth perturbation of cylindrical growth**

In this appendix, we compute the variation of the area and of the total mean curvature of a surface of revolution under a small variation of its generating curve. We will work in an orthonormal system of coordinates \((x, y, z)\). Let us suppose now that \( \Sigma \) is a revolution surface whose generating curve, rotated around the axis \{\(y = 0 = z\)\}, is given by:

\[
\sqrt{y^2 + z^2} = R(x) = R_0 + \delta R(x)
\]

(123)

with \(|\delta R(x)| \ll R_0\). The function \(\delta R\) represents an infinitesimal normal perturbation around the cylindrical shape. The variable \(x\) satisfies \(0 \leq x \leq \ell\) and the deformed cylinder is glued smoothly with two hemispherical caps of radius \(R_0\). In other words, we suppose that

\[
\delta R(0) = \delta R(\ell) = 0
\]

(124)

\[
\delta R'(0) = \delta R'(\ell) = 0.
\]

Let us compute the variations of area, \(\delta A\), of length, \(\delta \ell\), and of total mean curvature, \(\delta \mathcal{K}\), for a fixed volume.

**C.1. Isovolumic variation of the area.** \(A\) is a functional of the length, \(\ell\), the radius, \(R\), and its derivative, \(R'\):

\[
A(\ell, R, R') = \int_0^\ell dx 2\pi R \sqrt{1 + R'^2}.
\]

(125)

Its variation under infinitesimal changes of \(\ell\) and \(R\) is:

\[
\delta A = 2\pi R_0 \delta \ell
\]

(126)

\[
+ 2\pi \int_0^\ell dx \delta R \left( \sqrt{1 + R'^2} - \frac{d}{dx} \left( \frac{R'}{\sqrt{1 + R'^2}} \right) \right).
\]
Since
\[
\frac{d}{dx} \left( \frac{RR'}{\sqrt{1 + R'^2}} \right) = \frac{RR'' + R'^2}{\sqrt{1 + R'^2}} - \frac{R'R''}{(1 + R'^2)^{3/2}}
\]
we obtain
\[
\delta A = 2\pi R_0 \delta \ell + 2\pi \int_0^\ell dx \delta R \left( 1 + R'^2 \right)^{-3/2} (1 - RR'' - R^4).
\]
Similarly, the volume, \( V \), is a functional of \( \ell \) and \( R \):
\[
V(\ell, R) = \frac{4\pi R_0^3}{3} \int_0^\ell dx \pi R^2
\]
and its variation under infinitesimal changes of \( \ell \) and \( R \) is :
\[
\delta V = \pi R_0^2 \delta \ell + 2\pi \int_0^\ell dx R \delta R.
\]
If \( V \) is held constant, then \( \delta V = 0 \) and :
\[
(\delta \ell)_{V=\text{const.}} = -\frac{2}{R_0^2} \int_0^\ell dx R \delta R.
\]
Inserting this expression of \( \delta \ell \) into that of \( \delta A \), we obtain the isovolumic variation of area :
\[
(\delta A)_{V=\text{const.}} = 2\pi \int_0^\ell dx \delta R \left( 1 + R'^2 \right)^{-3/2} (1 - RR'' - R^4).
\]
**Theorem C.1.** The isovolumic variational derivatives of the length and of the area of a (nearly cylindrical) closed revolution surface are negative :
\[
\left( \frac{\delta \ell}{\delta R} \right)_{V=\text{const.}} < 0 \quad \text{and} \quad \left( \frac{\delta A}{\delta R} \right)_{V=\text{const.}} < 0.
\]
In other words, since the stuffing is incompressible whereas the gut is elastic, the length and the area of a squeezed sausage increase. We call this simple statement the **Squeezed Sausage Theorem (SST)**.

C.2. **Isovolumic variation of the total mean curvature.** The circles \( \{ x = \text{cst.} \} \) and the meridians, obtained by rotating the generating curve of equation \( z^2 = R^2(x) \), form an orthogonal system of geodesics [7], and the mean curvature of \( \Sigma \) is given by :
\[
H = \frac{1}{2} \left( \frac{1}{R\sqrt{1 + R'^2}} + \frac{R''}{(1 + R'^2)^{3/2}} \right).
\]
The lateral area of a slice of width \( dx \), perpendicular to the axis of the surface, is :
\[
dA = 2\pi R \sqrt{1 + R'^2} \ dx
\]
and the total mean curvature is :
\[
\mathcal{H} := \int_\Sigma H \ dA
\]
\[
= \int_{\text{caps}} H \ dA + \int_0^\ell 2\pi R \sqrt{1 + R'^2} \ dx
\]
\[
= 4\pi R_0^2 \cdot \frac{1}{R_0} + 2\pi \int_0^\ell \frac{1}{2} \left( 1 + \frac{R R''}{1 + R'^2} \right) \ dx
\]
\[
= 4\pi R_0 + \pi \ell + \pi \int_0^\ell \frac{R R''}{1 + R'^2} \ dx.
\]
Since $\mathcal{H}$ is a functional of $\ell$, $R$, $R'$ and $R''$, its variation under a change $\delta R$ of the radius of gyration and a change of length $\delta \ell$, is obtained after a double integration by parts [10] :

$$
\delta \mathcal{H} = \pi \delta \ell + \pi \int_0^\ell dx \frac{R''}{1 + R'^2} + \frac{d}{dx} \left( \frac{2RR'R''}{(1 + R'^2)^2} \right)
+ \frac{d^2}{dx^2} \left( \frac{R}{1 + R'^2} \right) \delta R.
$$

Instead of computing each term of the integrand, let us make the approximation $R'^2 \ll 1$, valid when the initial cylinder is only slightly deformed. The expression of $\delta \mathcal{H}$ then simplifies to

$$
\delta \mathcal{H} \simeq \pi \delta \ell + \pi \int_0^\ell dx R R'' \\simeq \pi \delta \ell + 2\pi \int_0^\ell dx R'' \delta R.
$$

Using the expression of $\delta \ell = -\frac{2}{R_0^2} \int_0^\ell dx R \delta R$ when $V$ is held constant, we obtain :

$$
(\delta \mathcal{H})_{V=\text{cst.}} \simeq 2\pi \int_0^\ell dx \left( \frac{R'}{R_0^2} - \frac{R}{R_0^2} \right) \delta R.
$$

As long as $R_0^2|R''| \ll R$, the isovolumic variational derivative of $\mathcal{H}$ with respect to $R$ is negative :

$$
\left( \frac{\delta \mathcal{H}}{\delta R} \right)_{V=\text{cst.}} < 0 \quad \text{if} \quad R'^2 \ll 1 \quad \text{and} \quad R_0^2|R''| \ll R.
$$

When $\delta R$ approaches $-R_0$ and the protocell is ready to split, the two radii of curvature are small compared to $R_0$ but have opposite sign, hence the Gaussian curvature around the septum is large and negative. After the cut, when the two caps are formed, the mean curvature and the Gaussian curvature are positive again.

**Appendix D. Asymptotic expansion of $F(a)$**

The change of variable $t = \sqrt{a(s-1)}$ in the integral defining $F$ gives us :

$$
F(a) = \frac{e^{-a}}{a} \int_0^{+\infty} dt \ f(a,t)
$$

$$
f(a,t) := 2t^2 e^{-t^2} \left( 2\sqrt{t^2 + a} - \frac{1}{\sqrt{t^2 + a}} \right).
$$

Let

$$
G(a) := \int_0^{+\infty} dt \ f(a,t) = ae^a F(a).
$$

The function $f(0, \cdot)$ is integrable over the half line $[0, +\infty[$ and

$$
G(0) = \int_0^{+\infty} dt \ f(0,t)
= \int_0^{+\infty} dt \ 2t e^{-t^2}(2t^2 - 1)
= \int_0^{+\infty} du \ e^{-u}(2u - 1) = 1.
$$
Let us compute the asymptotic expansion of \( G(a) \) when \( a \to 0^+ \):
\[
G(a) - G(0) = \int_0^{+\infty} dt \left( f(a, t) - f(0, t) \right)
\]
\[
= 2 \int_0^{+\infty} dt e^{-t^2} \left( 2 \left( \sqrt{t^2 + a} - t \right) - \left( \frac{1}{\sqrt{t^2 + a}} - \frac{1}{t} \right) \right)
\]
\[
= \int_0^{+\infty} 2t dt e^{-t^2} \left( \sqrt{t^2 + a} - t \right) \left( 2t + \frac{1}{\sqrt{t^2 + a}} \right)
\]
\[
= 2 \int_0^{+\infty} du e^{-u} \left( \sqrt{u + a} - \sqrt{u} \right) \left( 2\sqrt{u} + \frac{1}{\sqrt{u + a}} \right)
\]
\[
= 2 \int_0^{+\infty} du e^{-u} \sqrt{u(u + a)} + \int_0^{+\infty} du e^{-u}(1 - 2u) - \int_0^{+\infty} du e^{-u} \sqrt{\frac{u}{u + a}}
\]
\[
= 2 \int_0^{+\infty} du e^{-u} \sqrt{u(u + a)} - 1 - \int_0^{+\infty} du e^{-u} \sqrt{\frac{u}{u + a}}.
\]
Hence:
\[
G(a) = \varphi(a) - \varphi'(a)
\]
where
\[
\varphi(a) := 2 \int_0^{+\infty} du e^{-u} \sqrt{u(u + a)}
\]
\[
= 2a^2 \int_0^{+\infty} dx e^{-ax} \sqrt{x(x + 1)}.
\]
\( \varphi(a) \) being the Laplace transform of the function \( x \mapsto 2a^2 \sqrt{x(x + 1)} \), its expansion as \( 0^+ \) is given by integrating the expansion of \( \sqrt{x(x + 1)} \) at \( +\infty \) term by term:
\[
\sqrt{x(x + 1)} = x + \frac{1}{2} - \frac{1}{8x} + \mathcal{O}(x^{-2})
\]
\[
\varphi(a) = 2a^2 \left( \frac{1}{a^2} + \frac{1}{2a} - \frac{1}{8} \int_1^{+\infty} dx \frac{e^{-ax}}{x} + \mathcal{O}(1) \right)
\]
\[
= 2 + a - \frac{a^2}{4} \ln(a) + \mathcal{O}(a^2).
\]
Similarly, for \( \varphi'(a) \) we have:
\[
\sqrt{x(x + 1)} = 1 - \frac{1}{2x} + \frac{3}{8x^3} + \mathcal{O}(x^{-3}) \quad (x \to +\infty)
\]
\[
\varphi'(a) = a \int_0^1 dx e^{-ax} \sqrt{\frac{x}{x + 1}}
\]
\[
+ a \int_1^{+\infty} dx e^{-ax} \sqrt{\frac{x}{x + 1}}
\]
\[
= a \int_0^1 dx \left( e^{-ax} - \frac{1}{2} \int_1^{+\infty} dx \frac{e^{-ax}}{x} + \mathcal{O}(1) \right)
\]
\[
= 1 - \frac{a \ln(a)}{2} + \mathcal{O}(a).
\]
Consequently:
\[
G(a) = 1 + \frac{a \ln(a)}{2} + \mathcal{O}(a)
\]
and
\[
F(a) = e^{-a} + \frac{e^{-a} \ln(a)}{2} + \mathcal{O}(a)
\]
\[
= \frac{1}{a} + \frac{1}{2} \ln(a) + o(1).
\]
The asymptotic expansion of \( \frac{\partial \ln F}{\partial \ln a} \) is therefore:
\[
\frac{\partial \ln F}{\partial \ln a} = -1 + \frac{a \ln(a)}{2} + \mathcal{O}(a).
\]
In particular, since $a \ln(a) < 0$ for $0 < a < 1$, we have
\begin{equation}
\frac{\partial \ln F}{\partial \ln a} < -1 \quad (a \rightarrow 0^+).
\end{equation}

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