Mitochondrial cristae modeled as an out-of-equilibrium membrane driven by a proton field

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As the place where the fuel of the cell, namely ATP, is synthesized, mitochondria are crucial organelles in eukaryotic cells. The shape of the invaginations of the mitochondria inner membrane, known as cristae, has been identified as a signature of the energetic state of the organelle. However, the interplay between the rate of ATP synthesis and the cristae shape remains unclear. In this work, we describe the cristae membrane using a pH-dependent Helfrich model, maintained out-of-equilibrium by a diffusive flux of protons. We show that such a model captures the shape change of the invagination, in particular the formation of necks between wider zones under variable, and especially oscillating, proton flux.

I. INTRODUCTION

Mitochondria are important organelles of eukaryotic cells often called the powerhouses of the cell, due to their role in the synthesis of Adenosine Tri-Phosphate (ATP) from Adenosine Di-Phosphate (ADP). These organelles of about one micrometer size comprise an inner membrane (IM), which delimits a region called the matrix, and an outer membrane (OM). The volume between the IM and the OM is called the intermembrane space (IMS). The inner membrane presents numerous tubular invaginations of nanometric size, called cristae, where ATP synthesis takes place. The liquid inside the cristae is isolated from the IMS by the cristae junction, an aggregate of proteins that limit the diffusion. Recently, it has been shown that cristae have a higher membrane potential than the intervening boundary membranes, involving confined proton loops and individual functioning of each cristae within the same mitochondria. It has been observed experimentally that the cristae assume wildly different shapes depending on the state of ATP production. A high rate of ATP production is associated with bumpy and wide cristae tubules (State III), while a low rate of ATP production is associated to a more regular cylindric shape (State IV) as illustrated by Fig. 1.

The endothermic reaction ADP → ATP, is catalyzed by the ATP-synthase which is located in the curved zone of the cristae membrane. This enzyme uses the pH difference present between the cristae and the matrix as energy supply. This pH difference is established and maintained by the respiratory chain which injects protons from the matrix inside the IMS. The proteins of the respiratory chain are located in the weakly curved zones of the invagination and thus are spatially separated from the ATP-synthases. Historically, the matrix and the IMS were described as pH buffers but a recent in vivo pH measurement gives access to a more precise view: the pH decreases along the cristae membrane between the proton source (the respiratory chain proteins) and the proton sink (the ATP synthase).

Membrane deformation driven by out-of-equilibrium chemical dynamics is an ubiquitous phenomenon in living cells. A mechanism of hydro-osmotic instabilities generated by ion pumps has been recently suggested to describe the dynamics of the contractile vacuole complex. Surface deformation driven by diffusion of an ‘active’ species is commonly observed in vivo such as the division of eukaryotic cell by accumulations of myosin motors at the cell ring. A phenomenological model has been proposed to study the coupling between diffusion of active agents and surface shape by introducing a modified Helfrich model associated with an active tension coupled to the 2-dimension diffusion of the chemical regulator on the deforming surface. Note that the coupling between a diffusive active agent and the bending of a membrane has not been included in the model.

The dynamical coupling between cristae shape and ATP production rate is a recent discovery and the physico-chemical mechanism at the origin of this coupling still needs to be characterized. The lipid composition of the IM has been pointed out as a key point for the ATP-synthesis machinery. Indeed, the cristae membrane is enriched in cardiolipin, and loss of mature cardiolipins affects the shape of the cristae and perturbs its function. These lipids possess a protic hydrophilic head and in vitro experiments have shown that tubular invaginations can be created by an externally controlled pH gradient in giant vesicles comprising cardiolipin.

FIG. 1. Cross section of a mitochondrion in different states of ATP production. (a) State III, for which sugar in excess is available leading to a high rate of ATP production. (b) State IV, for which no sugar is available leading to a vanishing ATP production rate.
A theoretical description modeling these giant vesicles as locally planar bilayer membranes with lipid density and composition heterogeneities in each monolayer has successfully reproduced the dynamics of the membrane in the regime of small deformation. In this model, composition can represent e.g. the acid and the basic form of cardiolipins, which is controlled by the local pH field. However, because the cristae membrane is enriched in proteins, representing up to 50 per cent of its mass, a detailed model describing a pure lipid bilayer and including the slippage between the two monolayers may not be necessary to describe this system. Therefore, here we consider a simpler and more phenomenological Helfrich model with pH-dependent parameters.

This work investigates the change of the cristae morphology between state IV and state III. We start with a reaction-diffusion system describing the proton flux on a cylinder (representing the cristae membrane) which contains a proton source, a proton sink and a reflecting barrier. The resulting proton concentration field will be considered as the driving force inducing the membrane deformation. We then propose a pH-dependent Helfrich model, in which the bending modulus, spontaneous curvature and tension depend on the local proton concentration, assuming small variations of this concentration. We derive the Green function of the system and study the phase diagram of the cristae shape in this model. Finally we solve the hydrodynamic equations of the system for a proton field oscillating between the state III and the state IV and show that such a model reproduces qualitatively the dynamical deformations observed in real cristae. The last part is devoted to the conclusion.

II. MODEL OF A MITOCHONDRIAL CRISTA

A. Proton field along the crista

We model the cristae as an axisymmetric cylinder of membrane of finite length \( L \) (see Fig. 2). The protons diffuse on the crista surface at the concentration

\[
[H^+]_S(s) = [H^+]_IV + h(s),
\]

where \( s \) is a coordinate parameterizing the position along the tube, while \([H^+]_IV\) represents the constant surface concentration in state IV taken as a reference, and \( h(s) \) is the variation in the proton concentration induced by the functioning of the respiratory chain and the ATP synthase. At one end of the cylinder, \( s = 0 \), one finds a reflecting barrier for the protons modeling the cristae junction, while at the other end, \( s = L \), a ring-shaped proton sink models the ATP synthase. Between the two, at \( s = L_s \), a ring-shaped proton source models the respiratory chain.

We assume that \( h(s) \) is small, i.e. \( h(s)/[H^+]_IV \ll 1 \), and that the tube shape does not deviate not much from a regular cylinder. In this framework, the equation governing the proton concentration on the surface can be written as follows,

\[
\frac{\partial h(s,t)}{\partial t} = D \frac{\partial^2 h(s,t)}{\partial s^2} + S_{in}(s,t) - S_{out}(s,t)
\]

\[
\frac{\partial h(s,t)}{\partial s} \bigg|_{s=0} = 0
\]

where Eq. (3) illustrates the reflecting barrier, \( D \) is the diffusion coefficient and \( S_{in}(s,t) \) and \( S_{out}(s,t) \) are the source and the sink of proton respectively. We consider a system oscillating with a period \( T = 2\pi/\omega \) between a state IV of homogeneous proton concentration and a state III associated with a maximal proton flux and write

\[
S_{in}(s,t) = k_{sc} (1 - \cos(\omega t)) \exp \left( \frac{-(s - L_s)^2}{2\Delta_1^2} \right)
\]

\[
S_{out}(s,t) = k_{sk} (1 - \cos(\omega t)) \left( [H^+]_IV + h(s,t) \right) \times \exp \left( \frac{-(s - L_s)^2}{2\Delta_2^2} \right)
\]

with \( k_{sc} \) the maximal rate of injection of the proton source, \( k_{sk}( [H^+]_IV + h(s,t) ) \) the maximal rate of the proton sink. The spatial extensions of the source and of the sink are modeled by two Gaussian functions of widths \( \Delta_1 \) and \( \Delta_2 \) respectively. The system oscillates between state IV \( (S_{in}(s,t)=0, S_{out}(s,t)=0) \) for \( t_{IV} = 0 \) modulo \( T \), noted \( [T] \), and state III (where the source and the sink function at their top rates) for \( t_{III} = T/2 \) \([T] \) with a frequency \( \omega \). We assume that there is no proton accumulation in the cristae during a period, i.e.

\[
\int_0^L ds \int_0^{2\pi/\omega} dt S_{in}(s,t) = \int_0^L ds \int_0^{2\pi/\omega} dt S_{out}(s,t)
\]

which sets the value of the ratio \( k_{sc}/k_{sk} \).
FIG. 3. Dynamics of the proton field along the cristae. The plots represent the field $h(s,t)$ solution of Eq. (3) for $h_0 = 7$, $D=3.5$, $\omega = \Pi/4$, $k_{sc} = 2.135$, $k_{sk} = 0.0045$, $L = 5$, $L_s = 2$, $\Delta_1 = 0.4$, $\Delta_2 = 0.4$ in dimensionless units and for $t_{IV} = 0$ [T], $t_{IV} \rightarrow III = T/4$ [T], $t_{III} = T/2$ [T], $t_{III} \rightarrow IV = 3T/4$ [T].

The proton concentration $h(s,t)$ is obtained by solving numerically Eq. (3) with an initial vanishing concentration field $h(s,0) = 0$. The profiles of the proton concentration along the tube at different times are shown in Fig. 3. The system oscillates between state IV, in which the proton concentration is approximately homogeneous between the junction ($L = 0$) and the source ($L = L_s$) and decreases between the source and the sink. These plots are obtained in the case of an oscillating period of the same order of magnitude as the typical diffusion time along the tube, $2\pi/\omega \approx L^2/D$.

B. Model of the membrane

To model the membrane of a mitochondrial crista, we start from the Helfrich model for elastic membranes, and include pH dependent parameters,

$$H = \int_{\Omega} \left[ \frac{1}{2} \kappa(s)(C - C_0(s))^2 + \sigma(s) \right] dA,$$

where $C$ is the local curvature of the surface and $dA$ the area of a surface element. The surface tension $\sigma(s)$, the bending modulus $\kappa(s)$, and the spontaneous curvature $C_0(s)$ are assumed to depend linearly on the proton concentration as follows,

$$\kappa(s) = \kappa_0 + h(s)\delta \kappa$$

$$\sigma(s) = \sigma_0 + h(s)\delta \sigma$$

$$C_0(s) = C_{00} + h(s)\delta C_0,$$

with $h(s)$ defined in Eq. (1). For simplicity the Gaussian curvature is not considered here. In this chemico-mechanical model, the chemical reaction of ATP synthesis generates a dynamical field $h(s,t)$ on the membrane and will drive the deformation of the cylinder.

III. DYNAMICAL EQUATIONS OF THE SURFACE DEFORMATION

We consider an initial equilibrium state defined by a vanishing field $h(s,t) = 0$ and a finite cylinder of length $L$ and of radius $R$. In this case, the Hamiltonian given in Eq. (6) restricts to the Helfrich model in which the spontaneous curvature $C_{00}$ is a phenomenological parameter, illustrating an asymmetry in the membrane. A non-vanishing, non homogeneous proton field $h(s)$ will lead to a deformed cylinder. The initial surface and the deformed surface, can be respectively parametrized by the three-dimensional vectors $X_0$ and $X_t$, defined as follows:

$$X_t = X_0 + \delta X_t,$$

with

$$X_0 = \begin{pmatrix} R \cos(\theta) \\ R \sin(\theta) \\ z \end{pmatrix}, \quad \delta X_t = \begin{pmatrix} u_n(z,t) \cos(\theta) \\ u_n(z,t) \sin(\theta) \\ u_s(z,t) \end{pmatrix}$$

with $z \in [0,L]$, $\theta \in [0,2\pi]$. (11)

The surface is thus parametrized by the variables $z, \theta$ such that any point on this surface can be uniquely represented by a value of each of these parameters: $X_t(s,\theta)$. The deformed state $X_t$ is characterized by two fields $u_n(z,t)$ and $u_s(z,t)$ defined in Fig. 2B. In this work, we consider only small deformations, i.e. $u_s(z,t)/R$, $u_n(z,t)/R$ much smaller than 1. In the following, we will work in the intrinsic basis of the deformed surface represented in Fig. 2B, and use the curvilinear abscissa $s$. To first order, the derivatives with respect to $z$ and $s$ of the deformation fields are equal: $\partial_s u_i(z,t) = \partial_z u_i(z,t)$, with $i = s, n$, and we will use the second ones.

A. Some elements of differential geometry for an axisymmetric membrane

We wish to describe the dynamics of the axisymmetric membrane $X_t$ driven by the concentration field $h(t)$. Let us first introduce some terminology and results from differential geometry and their expressions to the first order in the deformation field.

The tangent vectors on the surface are defined as

$$e_s = \partial_s X = \begin{pmatrix} u_n'(s) \cos(\theta) \\ u_n'(s) \sin(\theta) \\ 1 + u_s'(s) \end{pmatrix},$$

$$e_\theta = \partial_\theta X = \begin{pmatrix} -(R + u_n(s)) \sin(\theta) \\ (R + u_n(s)) \cos(\theta) \\ 0 \end{pmatrix}$$

with $u' = \partial_s u$. The normal vector can be expressed as

$$n = \frac{e_s \wedge e_\theta}{|e_s \wedge e_\theta|} = \begin{pmatrix} -u_n'(\cos(\theta) \\ -u_n'(\sin(\theta) \\ 1 \end{pmatrix},$$

(13)
and the metric of the surface is defined as $g_{ab} = e_a \cdot e_b$, with $(a = (s, \theta), b = (s, \theta))$ and is equal to

$$g_{ab} \approx \left(\begin{array}{cc} R^2 + 2Ru_n & 0 \\ 0 & 1 + 2u'_n \end{array}\right).$$

The curvature tensor, also known as the second fundamental form, is defined as $K_{ab} = e_a \cdot \partial_b n$ using the convention that for a pointing outward normal vector, the curvature is positive. It gives:

$$K_{ab} = \left(\begin{array}{cc} R + u_n & 0 \\ 0 & -u''_n \end{array}\right).$$

Finally, the sum $C = K^s + K^\theta$ of the principal curvatures can be written as,

$$C = \frac{1}{R} - \left(\begin{array}{c} u_n \\ R \end{array}\right).$$

using $K^b = K^s g^{bb}$, with $g^{ab} = (g_{ab})^{-1}$.

Finally, we recall the expression of the covariant derivative of a tangential vector $x^a e_a$,

$$\nabla_a x^b = \partial_a x^b + \Gamma^b_{ac} x^c$$

and of a tensor $t^{ab} e_a \otimes e_b$,

$$\nabla_a t^{bc} = \partial_a t^{bc} + \Gamma^d_{ac} t^{bd} + \Gamma^c_{ad} t^{bd},$$

where the Christoffel symbols can be written as

$$\Gamma^a_{ab} = \left(\begin{array}{cc} u''_n(s) \\ 0 \\ -Ru'_n(s) \end{array}\right), \quad \Gamma^b_{ab} = \left(\begin{array}{c} 0 \\ -u'_n(s) \\ R \end{array}\right).$$

To characterize the dynamical deformation of the tube, we introduce the flow velocity of the surface elements of the membrane

$$\mathbf{v}(s, t) = v_s(s, t)e_s + v_n(s, t)n$$

with $v_s = \partial_t u_s$ and $v_n = \partial_t u_n$. It is composed of an in-plane flow $\mathbf{v}_s = v_s e_s$ and a term describing the deformation of the surface $\mathbf{v}_n = v_n n$.

### B. Stress tensor acting on the deforming surface

Next, we determine the stress tensor $f$ acting on the surface $X_t$. It is the sum of two contributions: a mechanical stress tensor $f_H$ deriving from the Helfrich energy given in Eq. (6) and a viscous stress tensor $f_n$.

The mechanical stress tensor $f_H$ can be written as a 3x2 tensor $^{18,19}$ that can be decomposed into a surface stress tensor $f_H^{ab}$ generating forces tangent to the surface and a 2x1 tensor $f_H^{an} = f_H^{an} n \times e_a$ generating forces normal to the surface. To derive the expression of $f$, we follow the approach developed by Guven and coworkers, presented in Appendix A, which involves a minimization under constrain of the Hamiltonian yielding the surface energy. It gives $^{20}$

$$f_H^{ab} = T^{ab} - \mathcal{H}^{bc} K^b$$

$$f_H^{an} = -\left(\nabla_n f_{ab}\right)$$

with $\mathcal{H}$ defined in Eq. (6).

In our case, the mechanical stress associated to the surface $X_t(s)$ depends both on the deformation fields $(u_s(s, t), u_n(s, t))$ and on the concentration field $h(s, t)$. The surface stress tensor $f_H^{ab}$ and the normal stress tensor $f_H^{an}$ expanded to first order in the fields can be expressed as

$$f_H^{ab} = f_H^{ab} + f_H^{ab}$$

and

$$f_H^{an} = f_H^{an} + f_H^{an}$$

with

$$f_H^{ab} = \left(\begin{array}{c} \frac{X^0}{X^0} - \sigma_0 \\ 0 \end{array}\right)$$

and $f_H^{an} = 0$, and where the first order part,

$$f_H^{ab} = f_H^{ab} + f_H^{ab}$$

and $f_H^{an} = 0$, and where the first order part,

$$f_H^{ab} = f_H^{ab} + f_H^{ab}$$

is the sum of a term $f_H^{ab}$ depending on the concentration field $h(s, t)$ and of a term $f_H^{ab}$ depending on the deformation fields $u_n(s, t), u_s(s, t)$.

Using the expression of the Hamiltonian given in Eq. (6), the dependences of $\kappa, C_{00}$ and $\sigma$ on $h(s, t)$ given in Eqs. (7) and the expression of the stress tensor given in Eq. (30, 31), we derive the expression of the stress tensor to first order in the fields and find,
The stress tensor depending on the proton field involves both the field \( h(s, t) \) in its tangential component \( f_{1h}^{ab} \), and its spatial derivative \( h'(s, t) \) in its normal component \( f_{1n}^{ab} \). The latter contribution will vanish for a constant field \( h \). In fact, a constant field \( h \) simply induces a renormalisation of the parameters of the Helfrich model. By contrast, a spatially non homogeneous field \( h(s, t) \) will generate forces on the normal direction that tend to pinch or expand the tube.

The viscous stress tensor for a deforming membrane is a surface tensor that can be obtained from the two-dimensional strain rate,

\[
v_{ab} = \frac{1}{2} \left( \nabla_a v_b + \nabla_b v_a \right) + K_{ab} v_n
\]

where \( \mathbf{v} \) is given in Eq. (20), that is derived from the strain tensor of a three dimensional fluid shell taken in the limit of a small thickness.\(^2\) The viscous stress in compressible thin films can be expressed as

\[
f_{ab, \eta} = 2\eta_s (v_{ab} - \frac{1}{2} \mathbf{v} \times \mathbf{g} ab) + \eta_b v_c g_{ab},
\]

which involves the two-dimensional strain rate \( v_{ab} \) and phenomenological coefficients \( \eta_s \) and \( \eta_b \) that are the shear and bulk viscosity of the film. The viscous stress \( f_{ab, \eta} = f_{ab, \eta} \mathbf{g} a \times \mathbf{g} b \) can be written as 2x2 matrix, whose components are given in covariant coordinates in Eq. (35).\(^2\) It yields the force generated by the flow within the membrane surface. Note that we do not account for the bulk viscosity of the fluid surrounding the membrane in our force balance. Indeed, its contribution can be neglected for strongly curved membrane buds\(^2\) and tubes\(^2\) with characteristic sizes smaller than a few micrometers, which is the appropriate regime for cristae.

In the absence of deformation, i.e. when the fields \( v_n, v_s, u_n, u_s, h \) vanish, the viscous stress also vanishes and the equilibrium shape of the surface can be obtained by setting to zero the divergence of the stress tensor \( f_{H0,0} \) given in Eq. (27).

\[ \nabla_a f_{ab} + K_{ab} f_n = 0 \]

\[ \nabla_a f_{an} - K_{ab} f_{ab} = 0 \]

C. Hydrodynamic equations for the tubular membrane

We now consider the response of the membrane to a time varying field, \( h(s, t) \). In the absence of inertia, corresponding to the low-Reynolds number regime which is appropriate at the length scales considered, the dynamics of the system derives from the force balance, which can be written in the tangential basis of the surface as,

\[
\nabla_a f_{ab} + K_{ab} f_n = 0, \quad (36)
\]

\[
\nabla_a f_{an} - K_{ab} f_{ab} = 0. \quad (37)
\]

The force balance along \( \mathbf{g}_a \) vanishes for symmetry reasons. Replacing the mechanical tensors \( f_{ab}^{aH}, f_{1H}, f_{1H}^{an} \) by their expressions given in Eqs. (27,28,29), we finally obtain the dynamical equations of the system as a function of the velocity, displacement and concentration fields:

\[
a_1 v'_n + a_2 v''_n + a_3 h' = 0 \quad (40)
\]

\[
b_1 v_n + b_2 v'_s + b_3 u_n + b_4 u'_n + b_5 u''_n + b_6 h + b_7 h'' = 0 \quad (41)
\]

IV. STATIC GREEN FUNCTION OF AN INFINITE MEMBRANE CYLINDER

To gain physical insight on the model and on the static solutions of Eqs. (38,39), we derive the static Green
function of the system. We consider as a reference equilibrium state an infinite cylinder of radius $R$ and a vanishing field $h(s)=0$. The shape equation for this system, given in Eq. (37), can be written as

$$\sigma_0 - \frac{(1 - X^2)\kappa_0}{2R^2} = 0,$$  \hspace{1cm} (42)

where we have employed the expression of $f_{H0}^{ab}$ given in Eq. (27). Given that $R$, $\sigma$, and $\kappa$ are necessarily positive, this equation admits a solution for $X = C_{00} R \in ]-1,1[. \hspace{1cm} (43)$

$$\frac{4R\sigma_0\delta C_0}{1 + X} - 2\delta \sigma - \frac{\delta \kappa (1 - X^2)}{R^2} = 0 \hspace{1cm} (47)$$

between the perturbation parameters, $(\delta \kappa, \delta \sigma, \delta C_0)$, is satisfied. Assuming that this condition holds, we express $\delta \kappa$ as a function of $\delta \sigma$ and $\delta X$,

$$\delta \kappa = \frac{4R\sigma_0}{(1 - X)^2(1 + X)} \delta C_0 - \frac{2R^2}{(1 - X)^2} \delta \sigma. \hspace{1cm} (48)$$

Let us now consider a localized perturbation in the proton concentration: $h(s) = h_0 \delta(s)$ with $h_0 = 1$ introduced to dimension $h(s)$, and derive the Green function $G_n(s)$ yielding the deformation field $u_n(s)$ in response to this perturbation. Performing a Fourier transform of the shape equation given in Eq. (40) and introducing $\tilde{u}_n(q) = 1/2\pi \int dse^{iqs}u_n(s)$, the normal deformation field in the Fourier space, we find

$$\tilde{u}_n(q) = \tilde{G}_n(q) = \frac{R(q^2 R^2 - 1)(1 + X)\delta \sigma - R\sigma_0 \delta C_0}{\sigma_0 \sqrt{2\pi} (1 - 2X q^2 R^2 + q^4 R^4)}. \hspace{1cm} (49)$$

Performing the inverse Fourier transform of $\tilde{G}_n$ given in Eq. (49), we obtain the expression of the Green function in real space

$$G_n(s) = G_n R \sin \left( \frac{|s|}{R} \sqrt{\frac{1 + X}{2}} \right) \exp \left( - \frac{|s|}{R} \sqrt{\frac{1 - X}{2}} \right). \hspace{1cm} (50)$$

In this range (see Fig. 4A), the radius of the equilibrium cylinder $R$ can be expressed as a function of the parameters of the non-perturbed Helfrich model,

$$R = \frac{1}{\sqrt{C_{00}^2 + \frac{2\sigma_0}{\kappa_0}}}. \hspace{1cm} (44)$$

The stationary shape of a deformed cylinder associated with a perturbation field $h(s) \neq 0$ is given by the displacement fields $(u_n(s), u_s(s))$ that are solutions of the Eqs. (40,41) where $v_n$ and $v_s$ are set to zero. Replacing the coefficients $(a_i, b_j)$ by their expressions given in Appendix 2, we obtain,

$$G_n = G_{n\sigma}(X) \frac{\delta \sigma}{\sigma} + G_{nC}(X) R \delta C_0 \hspace{1cm} (51)$$

with

$$G_{n\sigma}(X) = - \frac{(1 + X)}{\sqrt{2\pi} \sqrt{1 + X}}, \hspace{1cm} G_{nC}(X) = \frac{1}{\sqrt{2\pi} \sqrt{1 + X}} \hspace{1cm} (52)$$

The deformation induced by a localized perturbation is thus an oscillating and exponentially decaying function, as shown in Fig. 4B1. The characteristic lengths of oscillation $\lambda_\sigma$ and decay $\lambda_d$ are given respectively by

$$\lambda_\sigma = R \sqrt{\frac{2}{1 + X}}, \hspace{1cm} \lambda_d = R \sqrt{\frac{2}{1 - X}}. \hspace{1cm} (53)$$

Fig. 4B2 represents $\lambda_\sigma$ and $\lambda_d$ as functions of $X$ for the range of possible equilibrium cylinders. The decay length $\lambda_d$ is an increasing function of $X$ that diverges for $X = 1$. Thus, cylinders with larger spontaneous curvatures are deformed on a longer range by a heterogeneous $h$. The oscillation length $\lambda_\sigma$, on the contrary, is a decreasing function of $X$.

The respective amplitudes $A_{n\sigma}$, $A_{nC}$ of the deformations induced by perturbations in $\sigma$ or in $C_0$, and defined as

$$A_{n\sigma}(X) = G_{n\sigma}(X) \frac{G_n(s_m)}{G_n}, \hspace{1cm} (54)$$

$$A_{nC}(X) = G_{nC}(X) \frac{G_n(s_m)}{G_n}, \hspace{1cm} (55)$$

are plotted with respect to $X$ in Fig. 4B3. Here, we have introduced $s_m = \sqrt{2/\sqrt{X + 1}} \sin^{-1}(\sqrt{X + 1}/\sqrt{2})$, which
is the coordinate of the maximum of $G_n(s)/G_n$. The absolute values of the amplitudes $A_{nσ}, A_{nC}$ are increasing functions of X but possess opposite signs. An increase of the tension ($δσ > 0, h(s) > 0$) leads to a constriction of the cylinder (since $A_{nσ} < 0$). On the contrary, an increase of the spontaneous curvature ($δC_0 > 0, h(s) > 0$) leads to a dilatation of the cylinder (since $A_{nC} > 0$). A perturbation in the spontaneous curvature $δC_0 R = 0.1$ (respectively in the surface tension $δσ/σ = 0.1$) will induce a variation of the radius of 5 percent (respectively 10 percent), for $X → 1$. For $X$ negative or $0 < X << 1$, the deformation induced by a variation of $h$ is negligible.

Fig. 4C shows three-dimensional representations of the non-perturbed and of the perturbed cylinder for $X = 0.9$, and with $δσ > 0$ and $δσ < 0$, respectively. This illustrates that the model proposed in Eq. (6) can generate tubular membrane shapes of various curvature that can resemble mitochondrial cristae.

Using the expression of the Green function given in Eq. (56), we can find the stationary shapes generated by any proton concentration field $h(s)$ through

$$u_n(s) = ∫_{−∞}^{∞} h(x)G_n(s − x)dx.$$  \hspace{1cm} (56)

As a practical illustration, in Fig. 5, we consider a step function for $h(s)$,

$$h(s) = −1 \hspace{0.5cm} s < 0, \hspace{0.5cm} 1 \hspace{0.5cm} s > 0$$ \hspace{1cm} (57)

(see Fig. 5A). The corresponding deformation field $u_n(s)$ obtained using Eq. (56) is continuous, unlike the input step function, and is an odd function of $s$ that oscillates before reaching a plateau of constant value, as shown in Fig. 5B. The resulting stationary shape of the tube, represented in Fig 5C, corresponds to two cylinders of different radii welded together through an oscillating neck.

**V. APPLICATION TO MITOCHONDRIA CRISTAE**

In this section, we describe the dynamical deformation of a finite tube of membrane of length $L$ submitted to the proton field $h(s, t)$ solution of Eq. (6) and represented in Fig. 3, which models a crista oscillating between state IV and state III.

To do so, we first specify the external mechanical forces exerted on the axisymmetric membrane. Here, we do not take into account extra external pressures or viscous forces that could be applied to the membrane and we consider that a constant tension $f_{ext}$ is exerted by the rest of the mitochondria on the tubule boundary rings in $s = 0$ and in $s = L$. This force,

$$f_{ext}(0) = −\left(σ + (1 − X^2)\frac{κ_0}{2R^2}\right)e_s,$$

$$f_{ext}(L) = \left(σ + (1 − X^2)\frac{κ_0}{2R^2}\right)e_s$$ \hspace{1cm} (58)

balances the effective tension of the undeformed Helfrich cylinder, defined by $X_0$ in Eq. (11), and derives from the zeroth order stress tensor $f_{H0}$ given in Eq. (27). Consequently, the first order forces deriving from the cylinder deformation vanish at the boundary of the tubule, and
the first order stress tensors satisfy:
\[ f_{1,s}^r (0, t) = 0, \quad f_{1,s}^r (L, t) = 0 \]  \hspace{1cm} (59)
\[ f_{H1}^n (0, t) = 0, \quad f_{H1}^n (L, t) = 0. \]  \hspace{1cm} (60)
where \( f_{1,s}^r = f_{H1,s}^r + f_{H,s,n}^r \), and where the expression of the stress is given in Eqs. \[25,29,35\]. Moreover, the edges of the cylinder are assumed to be pinned in \( s = 0 \) and \( s = L \), which leads to a vanishing tangential velocity \( v_s \) in \( s = 0 \) and \( s = L \),
\[ v_s(0, t) = 0, \quad v_s(L, t) = 0. \]  \hspace{1cm} (61)

In order to facilitate the numerical resolution of the hydrodynamical equations given in Eqs. \[40,41\], the deformation fields \( u_n(s, t), u_n(s, t) \) are expressed as function of the velocity fields \( v_n(s, t) \) and \( v_s(s, t) \) and of the deformation field at the time \( t - dt \), using the backward Euler method and a discretisation of the time with a time step \( dt \),
\[ u_s^t = u_s^t - dt + dt \times v_s^t \]  \hspace{1cm} (62)
\[ u_n^t = u_n^t - dt + dt \times v_n^t, \]  \hspace{1cm} (63)
where we have introduced the notation \( (u_s^t, v_s^t) \) for \( (u_n(s, t), u_s(s, t)) \). Inserting the expressions in Eqs. \[62,63\], the deformation fields into Eqs. \[40,41\] leads to the following coupled system of equations for \( v_s^t \) and \( v_n^t \)
\[ \begin{align*}
& a_1 \partial_t v_s^t + a_2 \partial_s^2 v_s^t + a_3 \partial_t h^t = 0 \quad (64) \\
& (b_1 + b_2 \times dt) v_n^t + b_3 \times dt \partial_s v_n^t + b_4 \times dt \partial_t v_n^t \\
& + b_5 \partial_t v_n^t = 0 \quad (65)
\end{align*} \]

Assuming that the geometry of the system at the time \( t - dt \) is known, the system to solve is a couple of ordinary differential equations in space for \( v_s^t \) and \( v_n^t \) for which the 6 necessary boundary conditions are given in Eqs. \[59,61\]. The complete dynamic of the system is obtained by solving Eqs. \[64,65\] starting at \( t = 0 \), when all the fields, \( h^0, u_s^0, v_s^0 \), vanish. Then, knowing the geometry of the system at the time \( t - dt \), i.e., the deformation fields \( u_s^{t - dt} \), and the concentration field at the time \( t, h^t \), the velocities \( (v_s^t(s), v_n^t(s)) \) are determined as solutions of Eqs. \[64,65\]. Next, the deformation fields \( u_n^t, u_s^t \) at time \( t \) are calculated using Eqs. \[62,63\]. The procedure can then be iterated to obtain the state of the system at time \( t + dt \), and so forth. Further details on the numerical resolution are given in Appendix 3.

Fig. 6A represents the shape of the tube for the oscillating proton field given in Fig. 3. The tube alternates between a quasi-non-deformed cylinder for a quasi vanishing proton field (see Fig. 3) and a tubular invagination presenting a bump and a neck for the top proton flux. These oscillations are obtained in the regime of a fast mechanical relaxation time \( \tau_0 = 2 \eta_3 \eta_4 / \sigma_0 (\eta_s + \eta_b) \) with respect to the chemical diffusion time \( \tau_c = L^2 \sigma_0 / D \). It is thus the dynamics of the diffusive process that governs the shape change dynamics in this case. Fig. 6B represents the deformation fields associated with these shapes, and shows that the model developed in this work qualitatively captures the salient features of the shape of the cristae in state III and IV observed experimentally and represented in Fig. 1 (regular tubes in state IV, bumpy tubes in state III).

VI. CONCLUSION

Mitochondrial cristae are membrane protrusions that confine the ATP-synthesis machinery. Experimental observations have shown that the shape of these protrusions is coupled to the energetic state of mitochondria, as seen in Fig. 1. However, the underlying physical mechanisms controlling this coupling remain to elucidate.

We considered the hypothesis that the shape of the invagination is driven by the flux of protons diffusing on the membrane. Indeed, the absence of cardiolipins in cristae membrane induces anomalous cristae shapes, and local pH heterogeneities induce cristae-like deformations of GUVs comprising cardiolipins.\[43\] We described the mechanical properties of mitochondrial cristae membrane using a pH-dependent Helfrich model coupled to a diffusive proton concentration field on the surface. We first derived the stationary Green function of this system and showed that it can qualitatively reproduce the bumpy shapes of the mitochondrial invaginations observed experimentally. We then studied the dynamical shape change of the invagination for a membrane tubule subjected to an oscillating proton concentration field, modeling the oscillations between state IV (no ATP syn-
thesis) and state III (high rate of ATP synthesis). We showed that, for appropriate values of the parameters, it can reproduce the salient features of the experimentally-observed shapes of the mitochondrial cristae, oscillating between regular tubes in state IV and bumpy tubular protrusions in state III.

The phenomenological model introduced here is a coarse-grained description of a membrane containing different lipids, proteins, etc. It will be interesting to consider a more microscopic model of the membrane to gain insight on the values of the parameters \( \delta \kappa, \delta C_\text{0}, \delta \sigma \) of this model, and test whether realistic values yield membrane shapes resembling experimental observations.

Finally, a coupling between the shape and the concentration field could be introduced by considering the advection of the protons on the deformed membrane.

\[
H_c = H_\Omega + \int_{\Omega} \lambda^{ab}(g_{ab} - e_a \cdot e_b)dA + \int_{\Omega} \Lambda^{ab}(K_{ab} - e_a \cdot \nabla_b \mathbf{n})dA + \int_{\Omega} f^a \cdot (e_a - \nabla_a \mathbf{X})dA
\]

in which the matrices \( \lambda^{ab} \) and \( \Lambda^{ab} \) enforce the definition of metric and the curvature, \( f^a \) pins the basis vector to the tangent of the surface, \( \lambda_1^a \) enforces the normal vector to be perpendicular and \( \lambda_n \) its normalisation. The minimisation of the Hamiltonian Eq. (68) with respect to the, now, 12 independent functions give the following equations,

\[
\frac{\delta H_c}{\delta \mathbf{X}} = \nabla_a f^a \quad (69)
\]

\[
\frac{\delta H_c}{\delta e_a} = -f^a + (\Lambda^{ac} K_c + 2\lambda^{ab})e_b - \lambda_1^a \mathbf{n} \quad (70)
\]

\[
\frac{\delta H_c}{\delta \mathbf{n}} = (\nabla_b \Lambda^{ab} + \lambda_n^a) e_a + (2\lambda_n - \Lambda^{ab} K_{ab}) \mathbf{n} \quad (71)
\]

\[
\frac{\delta H_c}{\delta K_{ab}} = \Lambda_{ab} + \mathcal{H}^{ab} \quad (72)
\]

\[
\frac{\delta H_c}{\delta g_{ab}} = \lambda^{ab} + \lambda_\rho \rho \sqrt{g} \frac{\mathcal{g}^{ab}}{2} + \lambda_\phi \phi \sqrt{g} \frac{\mathcal{g}^{ab}}{2} - \frac{1}{2} T^{ab} \quad (73)
\]

where \( \mathcal{H}^{ab} \) and \( T^{ab} \) are given by

\[
\mathcal{H}^{ab} = \frac{\delta \mathcal{H}}{\delta K_{ab}} \quad (74)
\]

\[
T^{ab} = -\frac{2}{\sqrt{g}} \frac{\delta \sqrt{g} \mathcal{H}}{\delta g_{ab}} \quad (75)
\]

We notice that the Lagrange multiplier \( f^a \) obeys a conservation law (\( \nabla_a f^a = 0 \)). It can be identified as the stress tensor \( \mathcal{H}^{ab} \). Using that Eqs. (69,73) vanish, we eliminate the Lagrange multipliers present in Eq. (70) and find

\[
f^a = (T^{ab} - \mathcal{H}^{ac} K_c^b)e_b - (\nabla_a \mathcal{H}^{ab})\mathbf{n}. \quad (76)
\]

APPENDIX

1. Covariant stress tensor for a membrane

In this subsection we derive the stress tensor for a generic Hamiltonian associated with energy penalties for bending of an arbitrary inhomogeneous surface,

\[
H_\Omega[g_{ab}, K_{ab}] = \int_{\Omega} \mathcal{H}[g_{ab}, K_{ab}]dA. \quad (66)
\]

where \( g_{ab} \) and \( K_{ab} \) are the metric and the curvature tensors and \( dA \) is the area of a surface element. The local force density of a membrane in an equilibrium shape vanishes \(^{12}\), and this force density is the covariant divergence of the local surface stress.

We consider an infinitesimal deformation of the surface \( \mathbf{X} \rightarrow \mathbf{X} + \delta \mathbf{X} \). Instead of tracking the variation of the intrinsic basis and the fundamental forms of the surface induce by this deformation, we enforce these geometrical constraints by introducing Lagrange multipliers and we work with a generalised Hamiltonian \( H_c \),

\[
\mathcal{X} = \mathcal{X}(\mathbf{X}) + \delta \mathbf{X} \quad \rightarrow \quad X = X(\mathbf{X}) + \delta \mathbf{X}
\]

\[
\mathcal{H}^{ab} = \frac{\delta \mathcal{H}}{\delta K_{ab}} \quad (74)
\]

\[
T^{ab} = -\frac{2}{\sqrt{g}} \frac{\delta \sqrt{g} \mathcal{H}}{\delta g_{ab}} \quad (75)
\]

We notice that the Lagrange multiplier \( f^a \) obeys a conservation law (\( \nabla_a f^a = 0 \)). It can be identified as the stress tensor \( \mathcal{H}^{ab} \). Using that Eqs. (69,73) vanish, we eliminate the Lagrange multipliers present in Eq. (70) and find

\[
f^a = (T^{ab} - \mathcal{H}^{ac} K_c^b)e_b - (\nabla_a \mathcal{H}^{ab})\mathbf{n}. \quad (76)
\]

2. Coefficients for dynamical force balance equations

The full expression for the dynamical force balance equations Eqs. (40),(41) can be obtained by inserting
the coefficients which are given as follows:

\[ a_1 = \frac{\eta_0 - \eta_s}{R} \quad (77a) \]
\[ a_2 = \frac{\eta_s + \eta_0}{R^2} \quad (77b) \]
\[ a_3 = -\frac{(1 - X)^2}{2R^2}\delta \kappa - \delta \sigma + \frac{2R\sigma_0}{1 + X}\delta C_0 \quad (77c) \]
\[ b_1 = \frac{\eta_0 + \eta_s}{R^2} \quad (77d) \]
\[ b_2 = \frac{\eta_s - \eta_0}{R} \quad (77e) \]
\[ b_3 = \frac{2\sigma_0}{R^2(1 - X^2)} \quad (77f) \]
\[ b_4 = \frac{4X\sigma_0}{1 - X^2} \quad (77g) \]
\[ b_5 = \frac{2R^2\sigma_0}{1 - X^2} \quad (77h) \]
\[ b_6 = -\frac{(1 - X^2)}{2R^3}\delta \kappa + \frac{1}{R}\delta \sigma + \frac{2X\sigma_0}{1 - X^2}\delta C_0 \quad (77i) \]
\[ b_7 = \frac{1}{R(1 - X^2)}\left(2R^3\delta C_0\sigma_0 - (1 - X)^2(1 + X)\delta \kappa \right) \quad (77j) \]

Here \( \kappa_0 \) has already been substituted in terms of \( \sigma_0, R \) and \( X \) using the relation between the Helfrich parameters \( [44] \). If we set \( a_1, a_2, b_1, \) and \( b_2 \) to zero (i.e. no viscosities), we obtain the steady state force balance equations for the Helfrich cylinder model.

3. Details of the numerical resolution of hydrodynamics equations

The system of equations Eqs. \([44, 45]\) is solved numerically at time \( t \) using the command DSolve of the Mathematica software. The solutions obtained for \( v_i^t \) and \( v_s^t \) are discretized in space on a regular grid with a step equal to \( 10^{-2} \) for a cylinder with radius \( R = 1 \). The discrete spatial derivatives of the velocities \( (v_i^t)_{i,\text{vec}} \), \( (i = 1, ... , 4) \), \( (v_s^t)_{j,\text{vec}} \), \( (j = 1, 2) \) are derived on this grid.

The vectors are interpolated using polynomials of degree 18 to obtain analytical functions \((\partial_i v_{i}^{\text{pol}}, \partial_j v_{j}^{\text{pol}}), i = 1, ..., 4, j = 1, ..., 2\) that are used to derive the deformation fields and their derivatives at the time step \( t \),

\[
\partial_t u_i^{\text{pol}}(s) = \partial_i^{\text{pol}} u_i^{\text{vec}} + dt \times \partial_i^{\text{pol}} v_{i}^{\text{pol}}, \quad i = 1, ..., 2 \quad (78)
\]
\[
\partial_s u_i^{\text{pol}}(s) = \partial_s^{\text{pol}} u_i^{\text{vec}} + dt \times \partial_s^{\text{pol}} v_{j}^{\text{pol}}, \quad j = 1, ..., 4 \quad (79)
\]