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Phase-field modelling of the dynamics of Z-ring formation in liposomes: Onset of constriction and coarsening

C.B. Picallo^{1,2}, R.A. Barrio³, C. Varea³, T. Alarcón^{4,5}, and A. Hernandez-Machado^{1,4,a}

¹ Departament ECM, Facultat de Física, Universitat de Barcelona, Diagonal 647, E-08028 Barcelona, Spain

² Institut Lumière Matière, UMR5306 Université Lyon 1-CNRS, Université de Lyon 69622 Villeurbanne cedex, France

³ Instituto de Física, U.N.A.M., Apartado Postal 20-364, 01000 Mexico D.F., Mexico

⁴ Centre de Recerca Matemàtica, Campus de Bellaterra, Barcelona, Spain

⁵ Departament de Matemàtiques, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

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Abstract. We propose a model for the dynamics of the formation of rings of FtsZ on tubular liposomes which produce constriction on the corresponding membrane. Our phase-field model is based on a simple bending energy that captures the dynamics of the interplay between the protein and the membrane. The short-time regime is analyzed by a linear dispersion relation, with which we are able to predict the number of rings per unit length on a tubular liposome. We study numerically the long-time dynamics of the system in the non-linear regime where we observe coarsening of Z-rings on tubular liposomes. In particular, our numerical results show that, during the coarsening process, the number of Z-rings decreases as the radius of tubular liposome increases. This is consistent with the experimental observation that the separation between rings is proportional to the radius of the liposome. Our model predicts that the mechanism for the increased rate of coarsening in liposomes of larger radius is a consequence of the increased interface energy.

1 Introduction

Recent experimental results related to the origin of constrictive forces during cell division of rod-like bacteria have revealed that such forces are induced by the emergence of the so-called Z-rings. The major component of this structure is a filament formed by the FtsZ protein [1, 2].

A popular experimental setup to study this phenomenon *in vitro* consists of using a mixture of lipids and FtsZ protein. The lipids organise themselves into rod-like liposomes which resemble the structure of the bacterium membrane. The FtsZ protein, in turn, accumulates on this rod-shaped structure forming rings scattered over the length of the liposome. An important observation that results from these experiments is that the initially unstructured distribution of Z-rings evolves to a configuration in which the separation between two consecutive Z-rings is roughly equal to twice the radius of the liposome [1]. The results of these experiments demonstrate that FtsZ is enough for assembling Z-rings and therefore to generate constriction forces on the liposome.

There is a discussion in the literature as to what are the mechanisms of force generation. One hypothesis pos-

tulates that there are lateral bonds which induce lateral attraction between protein filaments. If, in addition, the overlapping ends are capable of sliding over each other and reducing the radius of the liposome, constriction would emerge as the increased number of lateral bonds makes this configuration more stable. A very different hypothesis consists of assuming that FtsZ protofilaments are curved and, when tethered to the membrane or liposome, produce a bending force [2].

These experimental results have motivated a number of modelling and theoretical studies examining both scenarios. The mechanism based on lateral bonding has been modelled and analysed by Hörger *et al.* using Langevin computer simulations [3, 4].

Regarding the second scenario where bending energy is the force-generating mechanism, Hörger *et al.* [3, 5] have proposed a model based on the Helfrich energy formalism with the addition of a constant spontaneous curvature term. This equilibrium model leads to the result that the constriction force is 50-100 pN in agreement with both *in vivo* and *in vitro* experimental results.

Cytrynbaum *et al.* [6], based on previous models of constriction generation via hydrolysis of FtsZ-GTP [7–9], have proposed a method for calculating the value of the bending modulus using a Langevin equation for the

^a e-mail: aurora@ecm.ub.es

movement of the rings. Their results are consistent with experimental data for the separation between Z-rings. The vast number of experimental studies on FtsZ, and in particular on its role in bacterial cell division in *E. coli*, provides access to reliable data [2, 10, 11].

Experiments have shown a dynamical evolution where a homogeneous distribution of FtsZ protein induces a constriction of the membrane of liposomes by the formation of Z-rings at regular spacings [1, 2, 11]. Several models have explored this topic [7, 8, 12–15]. Shlomovitz and Gov [14] have studied the role of the interaction between the membrane and FtsZ rings on their condensation and coalescence. Liu and Guo [15], by means of a combined phase-field model for the membrane and a kinetic description of the FtsZ ring, have analysed morphodynamics in bacterial cell division.

The aim of this paper is to formulate a novel phase-field model based on a simple bending energy that captures the dynamics of the interplay between the protein and the membrane, which allows to study the dynamic processes of ring formation and constriction. In particular, we propose a dynamic model where the spontaneous curvature depends on the local concentration of FtsZ protein [14]. We study numerically the long-time behaviour of the system where non-linear effects take over. The numerical results indicate the presence of coarsening of Z-rings in long tubular liposomes. Our results show that, during the coarsening process, the number of Z-rings decreases as the radius of tubular liposome increases, which is consistent with the experiments where the separation between rings has been observed to be proportional to the radius of the liposome [1]. The fact that our model is capable of reproducing this experimental results shows that our phase-field model could be extended, by removing certain constraints (*e.g.* membrane area conservation) and applied to account for more complex situations.

This paper is organised as follows. Section 2 is devoted to present the model and discuss its physical foundations. In sect. 3 we discuss, by means of a linear stability analysis, the onset of constriction. Section 4 is devoted to a numerical exploration of the non-linear behaviour that follows the initial instability. Last, in sect. 5, we discuss our results and present our conclusions.

2 The model

We model the constriction of the vesicle membrane in the presence of an external agent, the protein. We suppose that the main contribution to the membrane energy is bending, following the well-known approach of Canham-Helfrich [16]. We implement a Canham-Helfrich minimization scheme by means of a phase field that takes the value $\phi = 1$ inside the vesicle and $\phi = -1$ outside. Hence, the level set $\phi = 0$ gives us the membrane location. The bending free energy is given by [17]

$$F_b = A_b \int_V \Phi_b^2 dV \quad (1)$$

and

$$\Phi_b = -\phi + \phi^3 - \epsilon^2 \nabla^2 \phi + C_0 \epsilon (1 - \phi^2), \quad (2)$$

where A_b is the bending modulus which is the characteristic energy associated to bending, ϵ is the width of the interface, and C_0 is the spontaneous curvature term.

In our case, in the absence of FtsZ, the vesicle is a straight cylinder and hence the spontaneous curvature term in eq. (2) is zero. However, as the protein concentrates on the membrane, the cylindrical vesicle acquires curvature in the direction perpendicular to its axes, giving rise to the known constricted shapes shown in figs. 2 and 3 of refs. [1] and [2], respectively. Therefore, in our case, this spontaneous curvature term is proportional to a field u which gives the concentration of protein FtsZ in the domain. A non-linear dependence on the FtsZ concentration is imposed in the form $C_0 = C_0(u) = \beta u^2$, to enforce the positivity of u , where β measures the strength of the interaction, that is the ability of the protein to modify the spontaneous curvature. The rationale for choosing $C_0(u)$ to be a quadratic function of u can be given in terms of the model formulated in [3, 4]. This model proposes that constriction is produced by lateral bonding of two FtsZ fibers. Therefore, the constriction produced by the protein on the membrane, which, in our model, is accounted for through the spontaneous curvature, should be proportional to the protein concentration squared.

The protein-membrane adhesion free energy, V_s , has two wells, at the fixed points $u = u_{\max}$ (maximum concentration of protein per site) and $u = u_{\min}$ (minimum concentration of protein, usually taken to be zero). The parameter u_{\max} measures the affinity of the protein for the membrane. Since we know that FtsZ acts only on the surroundings of the vesicle membrane where the Z-rings locate to produce the constriction, further coupling of the protein and the vesicle reads

$$F_{sf} = \int_V (A_s V_s + A_f V_f) dV, \quad (3)$$

$$V_s = (\phi^2 - 1)^2 (u - u_{\max})^2 (u - u_{\min})^2 + \lambda |\nabla u|^2, \quad (4)$$

$$V_f = \phi^2 (u - u_{\text{far}})^2, \quad (5)$$

where λ is the surface tension of the protein field and u_{far} represents the average value of protein concentration in the environment of the system and can also be taken to be null. Similarly to A_b , A_s and A_f are characteristic energy scales. A_s is the energy associated to protein binding to the membrane, whereas A_f is the characteristic scale of the energy cost associated to the presence of protein away from the membrane (recall that $u_{\text{far}} = 0$). Therefore, the total free energy of our vesicle-protein system is given by

$$F = \int_V (A_b \Phi_b^2 + A_s V_s + A_f V_f + \sigma |\nabla \phi|^2) dV. \quad (6)$$

We also impose a restriction on the vesicle area (see [17]) that mimics the limited number of lipids that form the membrane. This is done by means of the Lagrange multiplier σ that is calculated by imposing surface area conservation of the membrane [18]. Both the protein

concentration u and the order parameter for the vesicle ϕ are conserved quantities and hence their evolution is given by Cahn-Hilliard (or model B) equations [19, 20]

$$\frac{\partial \phi}{\partial t} = D_\phi \nabla^2 \left(\frac{\delta F}{\delta \phi} \right), \quad (7)$$

$$\frac{\partial u}{\partial t} = D_u \nabla^2 \left(\frac{\delta F}{\delta u} \right), \quad (8)$$

where D_ϕ and D_u are the corresponding diffusion coefficients, providing the time scales for the system.

Our model is fundamentally different from previous ones such as [14] in a significant aspect, namely, they consider that the radius of the liposome, $r(z)$, where z is the coordinate along the axis of the cylinder, is given by $r(z) = R + h(z)$ with $h(z)$ is considered to be a small deviation. By contrast, our model does not make such an assumption regarding small perturbations. On the contrary, our model is formulated in terms of a phase-field theory which is intrinsically non-linear and, therefore, accounts for the dynamics of the membrane in an accurate way in all the regimes of its evolution. In the next section, we carry out a linear stability analysis in the standard form, *i.e.* we linearise the phase-field dynamical equations and consider a small, plain wave perturbation of a uniform state.

3 Onset of constriction

In order to analyse the stability of a membrane with homogeneous distribution of protein, one can study the effect of small perturbations of a flat interface. A more detailed description of the derivation of the dispersion relation is given in appendix A. In particular, we consider that perturbations take the form of plane waves: $\phi = \phi_0 e^{ikz + \omega t}$ and $u = u_h + u_0 e^{ikz + \omega t}$ around the membrane ($\phi = 0$) and u_h which is the concentration of protein, initially homogeneously distributed on the membrane. We further assume that the amplitudes $\phi_0 \ll 1$ and $u_0 \ll 1$. Substituting these expression in the linearized version of eqs. (7), (8), we obtain two different roots, $\omega_1(k)$ and $\omega_2(k)$, of the dispersion relation $\omega(k)$. One of them is shown to exhibit a region of values of k such that $\omega_1(k) > 0$, which indicates instability. The wavelength where $w(k)$ changes sign is associated to the inverse of the characteristic length scale, $l_c = \pi/k$, of the separation between protein rings in our system. In fig. 1 the unstable branch of the dispersion relation is shown for typical values of the parameters. We observe that the corresponding value of l_c is $l \simeq 3.0$.

4 Non-linear behaviour

We integrate numerically equations (7) and (8) using second-order finite differences for the spatial dependence and an Euler scheme for the time dependence. Since the standard second-order finite differences is a consistent finite difference method, the time step was chosen following the Courant-Friedrichs-Lewy stability criterion: $\Delta t \leq c \Delta x$, where Δx is the mesh size and c is a positive constant [21]. We choose our units so that $\Delta x = 1$. We impose

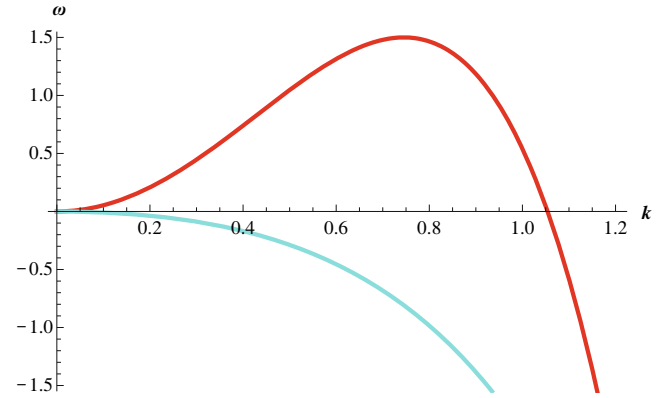


Fig. 1. Dispersion relation showing an unstable branch (red line) for a finite interval of the wave number k . Parameter values: $D_\phi = 1$, $D_u = 2.7$, $A_b = 0.2$, $A_s = 2$, $A_f = 2$, $\epsilon = 0.01$, $\beta = 0.1$, $u_{\min} = 0$, $u_{\max} = 1$, $\lambda = 0.45$, $u_{\text{far}} = 0$, $\sigma = 1$, and $u_h = u_{\max}/2$.

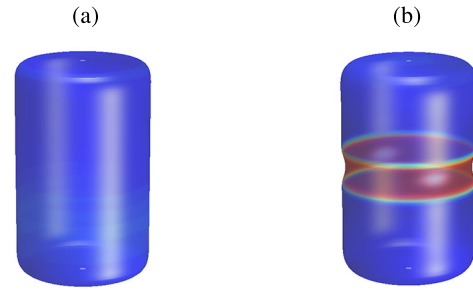


Fig. 2. FtsZ ring formation as a function of length of the short tubular liposome. Plot (a) corresponds to length $l = 3$ and plot (b) to $l = 4$. Parameter values are given in fig. 1.

zero-flux boundary conditions on the edges of the planar rectangular domain, within which the membrane is imbedded. The initial condition for the membrane is formed by a cylinder with two semispherical caps of constant radius at its ends, corresponding to the shape of the membrane of a short liposome. Regarding the variable u , *i.e.* the concentration of FtsZ protein, we consider it to be uniformly distributed over the simulation domain. No-flux boundary conditions are taken at the exterior boundaries of the domain. The protein is uniformly distributed over the integration domain with a small random noise on the uniform concentration u_h . As time progresses, the protein gets attached to the membrane forming rings. The short-time behaviour is controlled by the length scale given by the linear dispersion relation, whereas the long-time behaviour depends on the length of the cylindrical membrane.

In agreement with our analytical results, short tubular liposome ($l = 3$, close to the critical length scale provided by the dispersion relation, l_c) show no ring formation and, the protein distributes homogeneously over the surface of the membrane, and, therefore, there is no constriction (see fig. 2(a)). For lengths of the larger than l_c ($l = 4$), we observe that only one constriction is produced where, as time progresses, all the protein will eventually accumulate (see fig. 2(b)).

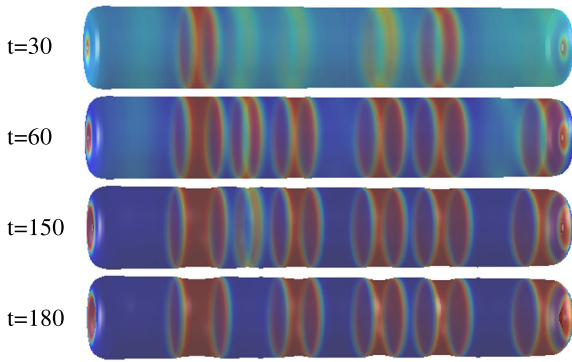


Fig. 3. FtsZ ring formation as a function of time in long tubular liposomes. This figure shows the emergence of long-time coarsening as the number of rings is observed to decrease in time. Parameter values as given in fig. 1.

Longer tubular liposomes whose length is several times l_c , show a richer behaviour. Initially, in agreement with the behaviour predicted by the linear analysis, a number of rings are formed, located at distances approximately given by l_c . At longer times, a non-linear regime ensues in which rings exhibit coarsening where rings move over the membrane and eventually fuse, as shown in fig. 3.

We could also investigate the number of Z-rings when varying the surface tension parameter of the protein, keeping the length of the liposome constant, since this term gives an important contribution to the dispersion relation. In fig. 4, we show that the number of rings that appear increases as λ decreases. This is to be expected, since the energy associated to create an interface is smaller.

The non-linear regime is particularly relevant for longer systems (*e.g.* tubular liposomes several times l_c in length). In these systems, according to the results of the linear analysis, a periodic pattern of peaks in protein concentration is formed. As time progresses, this periodic structure goes through a process of coarsening which leads to the movement of rings over the membrane which eventually merge. The coarsening rate is, according to our numerical results, increased in tubular liposomes with larger radius as shown in fig. 5. In accordance with experimental results by [1], the long-time behaviour of the distance between rings is bigger in tubular liposomes with larger radius since the number of rings is observed to be inversely proportional to the radius. Our model predicts that the mechanism for the increased rate of coarsening in bigger liposomes is a consequence of the increased interface energy, as determined by the surface tension parameter λ . Z-rings on tubular liposomes with larger radius will have a larger interface than rings on smaller radius, thus yielding a larger contribution to the total energy, which in turn leads to an increase of the effective diffusion coefficient of the rings.

5 Discussion and conclusions

We have proposed a model to study the dynamics of the constriction and ring formation on tubular liposomes. This

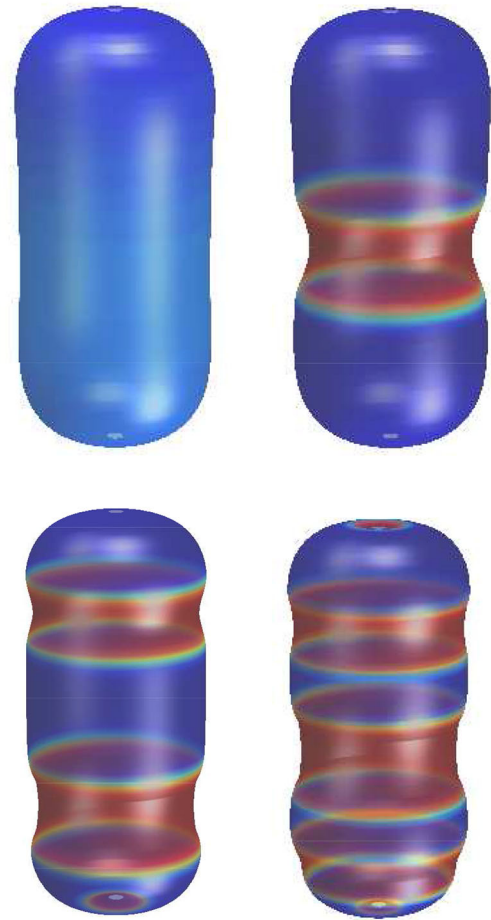


Fig. 4. The dependence of Z-ring formation as a function of the value of the surface tension of the protein field λ . From top left to bottom right: $\lambda = 1.89, 0.54, 0.36,$ and 0.225 . Parameter values are as in fig. 1.

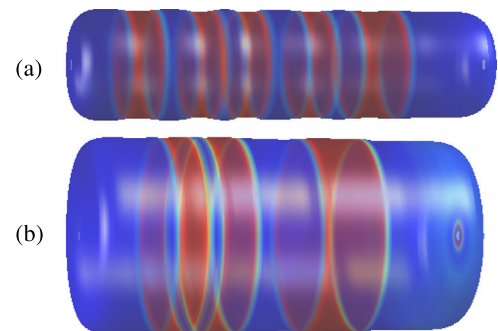


Fig. 5. This figure shows the increase of the coarsening rate with the radius of liposome. We show the state at a given time of two tubular liposomes with different radius: Plot (a) corresponds to radius R and plot (b) to radius $2R$. Parameter values as given in fig. 1.

model is based on a free energy functional accounting for the bending energy of the membrane including spontaneous curvature. The FtsZ protein is coupled to the membrane through the spontaneous curvature term. Additionally, there are two terms in the free energy, one describes

the adhesion of the protein to the membrane, and the other determines the concentration of protein in the bulk, far from the membrane. Furthermore, there is a term that takes into account the energy to create a surface of FtsZ ring on the membrane.

The dynamical model has been studied analytically by means of a linear stability analysis. The results show the occurrence of an instability which leads to a periodic pattern of the concentration of protein and to the onset of constriction, eventually leading to the formation of Z-rings.

By means of a dispersion relation found in the linear analysis we determined a minimum length, l_c , which depends on the model parameters, below which the membrane would not be able to constrict.

We further study the phenomenon of ring formation by means of extensive numerical analysis, which allowed us to examine the coarsening phenomena that occur as a result of the non-linear dynamics. As coarsening progresses, some rings coalesce so the number of rings decreases with time. The number of rings could be controlled by the surface tension parameter λ , which increases as λ decreases.

The radius of the tubular liposome is also important in the coarsening process. We found that the number of rings in a liposome of fixed length decreases as its radius increases, in such a way that for a liposome twice the radius, we get half the rings (see fig. 5), which has been reported in *in vitro* experiments in liposomes [1].

This model allows the study the dynamics of formation of FtsZ rings, which is relevant to explain and predict phenomena observed in experiments with liposomes *in vitro*. This is an indispensable step if one is interested in understanding pinching of the membrane in bacterial division.

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Appendix A. Linear stability analysis

In this appendix, we present the details of our analytical calculation of the dispersion relation by means of a linear stability analysis of our model. We present the model equations corresponding to the short-time regime and discuss the linear stability analysis for infinite systems.

The model equations in explicit form can be derived from eqs. (7) and (8), and taking into account the definition $\frac{\delta}{\delta\phi}F(\phi, \nabla\phi, \nabla^2\phi) = \frac{\partial F}{\partial\phi} - \nabla\frac{\partial F}{\partial\nabla\phi} + \nabla^2\frac{\partial F}{\partial\nabla^2\phi}$, we obtain

the expression of our dynamical equations:

$$\begin{aligned} \frac{\partial\phi}{\partial t} = & D_\phi\nabla^2(A_b(2\Phi_b(-1+3\phi^2-2\epsilon\beta u^2\phi) - 2\epsilon^2\nabla^2\Phi_b) \\ & + A_s4\phi(\phi^2-1)(u-u_{\min})^2(u-u_{\max})^2 \\ & + A_f2\phi(u-u_{\text{far}})^2 - 2\sigma\nabla^2\phi), \end{aligned} \quad (\text{A.1})$$

$$\begin{aligned} \frac{\partial u}{\partial t} = & D_u\nabla^2(A_b4\epsilon\beta u(1-\phi^2)\Phi_b + A_s2((u-u_{\min}) \\ & \times (u-u_{\max})(2u-(u_{\min}+u_{\max}))(\phi^2-1)^2 \\ & - \lambda\nabla^2u) + A_f2\phi^2(u-u_{\text{far}})). \end{aligned} \quad (\text{A.2})$$

By following the standard linearisation procedure (see sect. 3), we obtain a system of two linear equations. The linear stability of the flat interface state is analysed by diagonalising the associated 2×2 matrix, $A = (a_{ij})$.

By means of this linear analysis, we can determine that the spontaneous curvature, which couples perturbations on a homogeneous concentration of protein with the perturbation of the shape of the membrane, is responsible for the onset of the instability. Intuitively, the local increase in the concentration of protein increases the spontaneous curvature. Mathematically, this can be seen from the above equations, in particular the terms which explicitly depend on β in eqs. (A.1) and (A.2) are the ones which give positive contribution to the linearised equations, and, therefore responsible for unstable behaviour.

The variables used in our model are different from the ones considered in [14]. Whereas [14] use the variable $h(z)$, our model considers an order parameter within the phase-field framework, ϕ , which takes values between -1 and $+1$ with the membrane located in the geometrical locus of points that satisfy $\phi = 0$.

In order to make a comparison with the linear analysis carried out in [14], we consider the $\epsilon \rightarrow 0$ limit of our linearised dynamical equations, which corresponds to study the limit of small wave numbers. After performing this limit, we derive that the entries of the matrix $A = (a_{ij})$ have the following form:

$$a_{ij} = A_{ij}k^2 + B_{ij}k^4.$$

Note that the signs of the quantities A_{ij} and B_{ij} are determined by the values of the model parameters.

We observe, as a consequence of eqs. (7) and (8) our model variables are both conserved, which is reflected in the dependence on k of a_{ij} . This is in contrast with the linear result obtained in [14], where instability is generated by the growth of the perturbation $h(z)$ (which is proportional to k^0) due to the presence of the protein, and non-homogeneous diffusion of the protein on the surface of the liposome (which is proportional to k^2).

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