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A dynamical model for phospholipid–calcium binding

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A model for an isothermal gel–liquid crystalline transition induced by ionic binding is proposed. A Ginsburg–Landau functional was used to describe the long-range order that spontaneously arises during the transition. By calculation of the corresponding chemical potential we obtain the mass current of phospholipids in gel-phase described by an order parameter. In the conservation of mass equation the kinetics of the phospholipids–calcium interaction is introduced, together with the flux divergency. A circular membrane is considered for the analysis, so that the model can be studied in polar coordinates. A solution approximated to first order shows an heterogeneous distribution of domains of phospholipids in gel and liquid crystalline phases. These spatial domains have been detected experimentally by diverse methods in vesicles and cellular membranes. Spatial heterogeneities may cause destabilization of the membrane in the boundaries between domains. This may explain the enhanced vesicle fusion observed in the presence of Ca^{2+}.

1. Introduction

The gel-to-liquid crystal phase transition has been widely studied by both experimental and theoretical methods. There are a great number of studies and detailed models of thermotropic phase transitions involving the effects of different cellular components such as proteins and cholesterol [1–5].

The biological relevance of the thermotropic transitions in bacterial membranes seems to have been clearly recognized; however, in membranes of mammalian cells the isothermal phase transitions probably play a major regulatory role [6–8].

Studies of isothermal phase transitions have been performed mainly by using liposomes as relatively simple models of membranes. Among the factors that can trigger these transitions are ionic binding, pH variations [9], high concentrations of organic compounds such as alcohols [10], and cationic amphiphilic drugs [11], etc. These phase transitions may lead to spatial domains of high melting complexes [12–15].

The gel-to-liquid crystal phase transition in phospholipid bilayers has been described as a highly cooperative order–disorder transition [4,16]. Landau's theory has been particularly useful in describing this process. The results obtained show that, at least qualitatively, this theory helps to understand how different elements of the system influence or modify the transition process [5,17].

While in the aforementioned models only equilibrium phase transitions are described, in previous papers [8a and b] we proposed a model which predicts that, as a consequence of the phospholipid–ion binding, a non-equilibrium phase transition may occur. This type of mechanism seems to be more congruent with the dynamical conditions prevailing during the cellular regulation *in vivo*. As result of this isothermal non-equilibrium phase transition, domains of phospholipids in different phases are formed. This result seems to match well

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with the experimentally observed domains of high-melting point complexes [12–15], which could be responsible for different membrane phenomena [12,13]. In particular, we discussed the possible relevance of these packing defects in facilitating the vesicular fusion processes.

In the present work we study the properties of the model by using polar coordinates, which are more appropriate to the symmetry of the membrane. For the analysis a two time scale perturbation method, which permits to obtain and approximate solution in the limit $t \to \infty$, was used. We describe in the next section the justification of the model.

2. Model

The mean-field theory, in the Landau approximation, has proved to describe in a satisfactory manner the thermotropic phase transitions of phospholipids [1,5,18]. Thus, this approximation was used here to account for the long-range order, that spontaneously arises between phospholipids during an isothermal transition. In particular, the model will refer to phospholipid–Ca^{2+} binding, as this interaction has been more widely studied and more details are known.

To describe macroscopically the evolution of the transition induced by calcium binding we first define an order parameter [8]. To do this, let us assume that at a certain time of the process the phospholipid population in the membrane ν_t is constituted by unbounded lipids ν^{free} and by lipids bound to calcium ν^{Ca}. Moreover, we assume that there are two sub-populations of lipids bounded to calcium, that is, lipids in the fluid phase, $\eta^{\text{Ca}}_{\text{fluid}}$, and lipids in the gel phase, $\eta^{\text{Ca}}_{\text{solid}}$. With these considerations the order parameter may be defined as:

$$\xi = \frac{\nu^{\text{Ca}} - \eta^{\text{Ca}}_{\text{free}}}{\nu_t} = \frac{\eta^{\text{Ca}}_{\text{solid}}}{\nu_t} \tag{1}$$

This order parameter describes a positional order and may be related to the orientational order [5]. For values of $\eta^{\text{Ca}}_{\text{solid}}$ smaller than the critical point, the value of ξ equals 0. For values beyond the critical point, the maximum value that can be reached by ξ, which represents the fraction of lipids in the gel phase, is 1.

In general, it is considered that the phospholipid phase transition is of first order. Under this consideration, the expression for the chemical potential is obtained by minimizing the Landau free energy functional [8]. Then, we have

$$\mu = b + c\xi + d\xi^2 + e\xi^3 \cdots - \mathrm{k}\ \nabla^2\xi \tag{2}$$

This chemical potential induces a mass current equal to

$$\begin{aligned} J &= -D\ \text{grad}\ \mu \\ &= -D\ \text{grad}\left(b + c\xi + d\xi^2 + e\xi^3 \cdots - k\ \nabla^2\xi\right) \end{aligned} \tag{3}$$

where grad stands for the gradient of the chemical potential, the coefficients b, c, d,... are analytical functions of T and T_c, the temperature and critical temperature, respectively; D represents the lateral diffusion constant of the lipids in the membrane, and k represents the product of the interaction energy between lipids and the square of the interaction energy. We assume that k is always positive, which implies that forces between lipids are attractive (see Refs. [5] and [8] for a wider discussion and experimental values of these parameters). The respective mass balance is then:

$$\frac{\partial \xi}{\partial t} = -\,\text{div}\, J + R(\xi) \tag{4}$$

where the first term on the right represents the divergence of the flux and $R(\xi)$ the kinetics of the lipid–Ca^{2+} interaction.

In Appendix A the reported stoichiometry for the lipids–Ca^{2+} interaction was taken as a basis for deducing the kinetic equation $R(\xi)$. By substituting (3) in (4), and taking the kinetic equation (A.10) of Appendix A for $R(\xi)$, the equation which governs the dynamics for the lipids phase transition is obtained. The final form of the non-dimensional equation, is:

$$\begin{aligned} \frac{\partial \xi}{\partial t^*} = &-\alpha\ \nabla^{*4}\xi + (A + 2B\xi)\ \nabla^{*2}\xi + 2B(\nabla^*\xi)^2 \\ &+ k_f^*(1-\xi)^2 - k_b^*\xi \end{aligned} \tag{5}$$

where the following definitions are used:

$$t^* = Dt/q^2;\ \nabla^* = \nabla q^2;\ \alpha = K/q^2$$

$$k_f^* = k_f q^2/D;\ k_b^* = k_{-2} q^2/D;\ A = c;\ B = 2d$$

q being a scale factor, and only considering up to second order terms for the expansion of μ. For simplicity, the asterisks of the above definitions in eq. (5) are dropped.

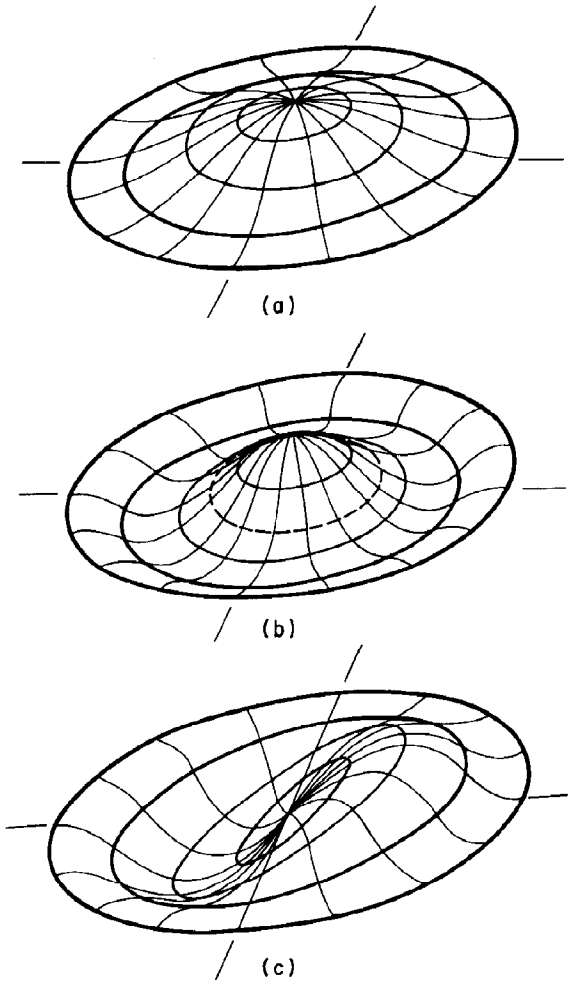

Fig. 1. Normal modes for three different values of the bifurcation parameter k_f: (a) $k_f = 2.67$, (b) $k_f = 624.5$, and (c) $k_f = 51.174$. Values greater than the homogeneous steady state correspond with a higher fraction of phospholipids in gel phase.

Equation (5) was solved using a perturbation method, with two time scales. We consider a circular membrane extending over a domain D given by $0 < r < 1$, where the boundary of the domain is a unit circle. Because of the shape of the boundary, polar coordinates are most appropriate for this analysis. The bifurcation parameter was k_f, which is defined in eq. (A.11) in terms of the specific rate constants of the different steps of the kinetics and the calcium concentration.

The linear analysis for stability of the steady states of eq. (5) is shown in Appendix B. The perturbative method used to calculate an approximated solution of (5) is shown in Appendix C. The first-order solution obtained, with is valid only in the vicinity of the critical point, is of the form

$$\begin{aligned}\xi(r,\theta) = {} & A_{mnc}(\tau)\left[I_m(k_{mn})J_m(k_{mn}r)\right. \\ & \left.-J_m(k_{mn})I_m(k_{mn}r)\right]\cos m\theta \\ & + A_{mns}(\tau)\left[I_m(k_{mn})J_m(k_{mn}r)\right. \\ & \left.-J_m(k_{mn})I_m(k_{mn}r)\right]\sin m\theta \end{aligned} \qquad (6)$$

where the coefficients A_{mn} are only dependent on the slow time τ. The asymptotic solution (6) represents the predominating normal mode, which will make the most important contribution to the form of the solution [19]. The values of k_{mn} correspond to values of k_f beyond the bifurcation point, where the homogeneous solution is unstable (see linear analysis in Appendix B). For a different value of the bifurcation parameter k_f, a different normal mode predominates; thus, for diverse calcium concentrations, different spatial patterns may be generated (see A.11) in Appendix A for the definition of k_f). Three of the normal modes for different values of the bifurcation parameter k_f are shown in Fig. 1.

3. Discussion

The results obtained show that by binding to calcium a spatial pattern of crystalline and fluid phospholipids is formed, which is a consequence of a non-equilibrium phase transition. For different values of the bifurcation parameter k_f, which

is defined in terms of the calcium concentrations in the medium, a different spatial distribution of lipids in the liquid-crystalline and gel phases may be obtained (Fig. 1). Each stable distribution corresponds to a predominating normal mode, as indicated by eq. (6), and suggests that by exposing the phospholipids to various concentrations of calcium various spatial patterns may be generated.

The solution obtained is only qualitative, and very much approximated. However, it clearly describes a heterogeneous spatial distribution of domains of phospholipids in gel and liquid-crystalline phases. By using a less approximated method, including higher order non-linearities, we expect to obtain less symmetrical solutions, which should correspond to a less regular distribution of phospholipids in several phases.

Experimentally, the formation of domains of lipids in gel and fluid phases has been determined by different procedures [14,20–22]. For instance, with spin-labeled lipids spin–spin interactions have been detected, arising from clustering of spin labels when Ca^{2+} was added. This was interpreted as due to the exclusion of the spin-labeled lipids from the solid phase of Ca^{2+} complexed to the phospholipids [23–25]. Klausner and Kleinfeld [15] extensively discussed on the existing evidence for domain structures in biological membranes.

The spatially distributed domains of phospholipids in several phases originate from zones of bilayer destabilization in the boundaries between fluid and gel phases. Packing defects, formed by a dynamic mechanism which is far from being in equilibrium, may be the cause of different membrane phenomena observed in the presence of calcium [12–14].

Papahadjopoulos et al. [12,13] suggested that the formation of rigid crystalline domains may induce the fusion of phospholipid vesicles. Specifically, they assume [26] that a small domain of a more condensed or crystalline lipid bilayer may act as a local perturbation of the lipid bilayer structure. Other authors also agree with the necessity of a destabilization of the bilayer as a prerequisite for vesicle and cellular fusion, although they attribute this destabilization to different factors [17,27].

It has been reported that, if Ca^{2+} ions are introduced asymmetrically into only one side of a negatively charged free bilayer, the membrane fuses [28]. But, if this ion is introduced symmetrically into both sides, the bilayers adhere to each other and do not fuse. The authors conclude that fusion resulting from such ionic interactions must operate through bilayer stresses that first destabilize the bilayer.

Some authors proposed that destabilization previous to vesicle fusion can be transient. Wilschut and Hoekstra [29] assume that such types of destabilization may be induced by contact between vesicles. However, they mention that for this to happen, a concomitant dehydration of the vesicles in the site of interaction, provoked by the Ca^{2+} binding, would be necessary. In this sense, Leikin et al. [30] indicate that ions such as La^{3+}, Mn^{2+}, and Ba^{2+}, which have stronger 'fusogenic' activity than Ca^{2+}, do not exhibit formation of dehydrated contacts. These authors suggest that out-of-plane thermal fluctuations may lead to local overcoming of hydration repulsion, and to local close approach of the membranes. This process is enough to induce the rupture of interacting monolayers and the formation of a monolayer stalk (however, for Ba^{2+}, see a different proposal in [31]).

More recently, it was reported that, indeed, bilayers do not need to overcome a repulsive force barrier of hydration to fuse [28], and that once bilayers are near enough to each other, local deformations and molecular rearrangements may surmount these forces (for another point of view see [32]). Nevertheless, it was proposed [28] that fusion resulting from ionic binding must operate by a completely different mechanism, such as bilayer stresses that first destabilize the membrane.

It then seems that an extended proposition would be that fusion should happen via molecular packing defects. This instability in agreement with our results, may be produced by an out-of-equilibrium phase transition process. This dynamical mechanism, which differs from a classical phase transition, induces the formation of microdomains which represent points of bilayer destabilization. These heterogeneities have been detected experimentally, which proves the predictions of the model are correct, independently from the fact

that the origin of the experimental ion-facilitated vesicle fusion may still be in controversy.

It must also be noted that the results predicted by the model may be generalized to any phospholipid–ion binding, with stoichiometries and kinetics similar to those considered in the model. We then have that Mn^{2+} [33], La^{3+} [34], and Ba^{2+} [6] bind to phospholipids with stoichiometries and association constants similar to that of Ca^{2+} [6,34]. Thus, the similar fusogenic activity of these ions, compared to Ca^{2+} [35], seems to match well with their capability for bilayer destabilization, as predicted by the model. The differences in their degree of fusogenic activity may be explained in terms of size, which may modify the structural characteristics of the complexes formed [29]. Other factors which depending on the conditions of the experiment may facilitate the fusion processes to a lesser or greater extent are variations in the radii of the vesicles, changes in the ionic concentration of monovalent ions in the reaction medium (which favor vesicle aggregation) etc.

Appendix A

Equations for the phospholipid–calcium interaction

There are several reports [7,12,36] which suggest that calcium binds phospholipids obeying the following scheme of reaction:

$$\nu^{\text{free}} + Ca^{2+} \rightarrow \eta^{Ca}_{\text{fluid}} \tag{A.1}$$

$$\nu^{\text{free}} + \eta^{Ca}_{\text{fluid}} \rightarrow \eta^{Ca}_{\text{cryst}} \tag{A.2}$$

$$2\nu^{\text{free}} + Ca^{2+} \rightarrow \eta^{Ca}_{\text{cryst}} \tag{A.3}$$

where the meaning of each symbol was given previously in the text. The corresponding equations which govern the time evolution of the reaction are:

$$\frac{d\eta^{Ca}_{\text{fluid}}}{dt} = k_1[\nu^{\text{free}}][Ca^{2+}] - k_{-1}[\eta^{Ca}_{\text{fluid}}] \tag{A.4}$$

$$\frac{d\eta^{Ca}_{\text{cryst}}}{dt} = k_2[\nu^{\text{free}}][\eta^{Ca}_{\text{fluid}}] - k_{-2}[\eta^{Ca}_{\text{cryst}}] \tag{A.5}$$

Moreover, the following conservation equation must be satisfied:

$$\nu_t = \nu^{\text{free}} + \eta_{\text{fluid}} + \eta^{Ca}_{\text{cryst}} \tag{A.6}$$

We assume that eq. (A.5) represents the rate-determining step of the reaction, and that (A.4) is a fast reaction. It is then possible to consider that (A.4) reaches the steady state rapidly, so that it can be rewritten in the form:

$$\eta^{Ca}_{\text{fluid}} = \frac{\nu_t - \eta^{Ca}_{\text{cryst}}}{1 + K_m}, \tag{A.7}$$

here it is considered that calcium is always in excess with respect to lipid concentrations, and where K_m is defined by:

$$K_m = k_{-1}/k_1[Ca^{2+}]. \tag{A.8}$$

Thus K_m represents the dissociation constant of the phospholipid–calcium complex, multiplied by the inverse of the concentration of the ion in excess.

By substituting (A.7) in (A.5) and using the conservation equation (A.6), the equation for the kinetics of the reaction takes the form:

$$\frac{d\eta^{Ca}_{\text{cryst}}}{dt} = \frac{k_2 K_m\left(\nu_t - \left[\eta^{Ca}_{\text{cryst}}\right]\right)^2}{(1 + K_m)^2} - k_{-1}\eta_{\text{cryst}}. \tag{A.9}$$

By dividing both members of the eq. (A.9) by ν_t, and substituting the order parameter defined by equation (1), the final form of the phospholipid–calcium reaction rate equation becomes:

$$\frac{d\xi}{dt} = k_f(1 - \xi)^2 - k_{-2}\xi. \tag{A.10}$$

where k_f is defined as:

$$k_f = k_2 K_m \nu_t/(1 + K_m)^2. \tag{A.11}$$

This apparent specific constant of reaction depends on the excess concentration of calcium; as indicated in (A.8), in the definition of K_m. Thus, in order to study the different behaviors of the phospholipid–calcium interaction, generated by different concentrations of calcium, k_f was selected as bifurcation parameter.

Appendix B

Linear analysis of the model, given by eq. (5)

The steady states of the homogeneous system are given by:

$$\eta_0 = \frac{0.5}{k_f}\left\{(2k_f + k_b) \pm \left(4k_b k_f + k_b^2\right)^{0.5}\right\} \qquad (B.1)$$

Considering small perturbations around the homogeneous steady state and neglecting non-linear terms, the linearized form of system (5) is

$$\left\{-\delta_t - \alpha\,\nabla^4 + (A + 2B\eta_0)\,\nabla^2 + \left[2k_f(\eta_0 - 1) - k_b\right]\right\}\xi = L\xi = 0 \qquad (B.2)$$

Considering the symmetry of the problem, polar coordinates are more suitable. An approximated solution is given in Appendix C using these coordinates.

Now we look for solutions of the type

$$\xi = \sum_m \sum_n \eta_{mn}(r, \theta)\, e^{\sigma t}; \qquad (B.3)$$

The usual stability theory yields the characteristic equation [37]

$$\sigma = -\alpha k_{mn}^4 - (A + 2B\eta_0)k_{mn}^2 + 2k_F(\eta_0 - 1) - k_b \qquad (B.4)$$

such that k_{mn} are the eigenvalues of the biharmonic operator in a uniform circular membrane, and σ represents the relation dispersion. To obtain the eigenvalues k_{mn}, for a given m and subject to the boundary conditions

$$\xi(1, \theta) = 0$$
$$\frac{\partial \xi(r, \theta)}{\partial r} = 0 \qquad (B.5)$$

one must solve numerically the following equation:

$$I_m(k_{mn})J_{m-1}(k_{mn}) - J_m(k_{mn})I_{m-1}(k_{mn}) = 0 \qquad (B.6)$$

In Fig. 2 the value of σ is plotted versus k_{mn}^2, for different values of the parameter k_f. These curves have a maximum in k_{mn}^2 different from

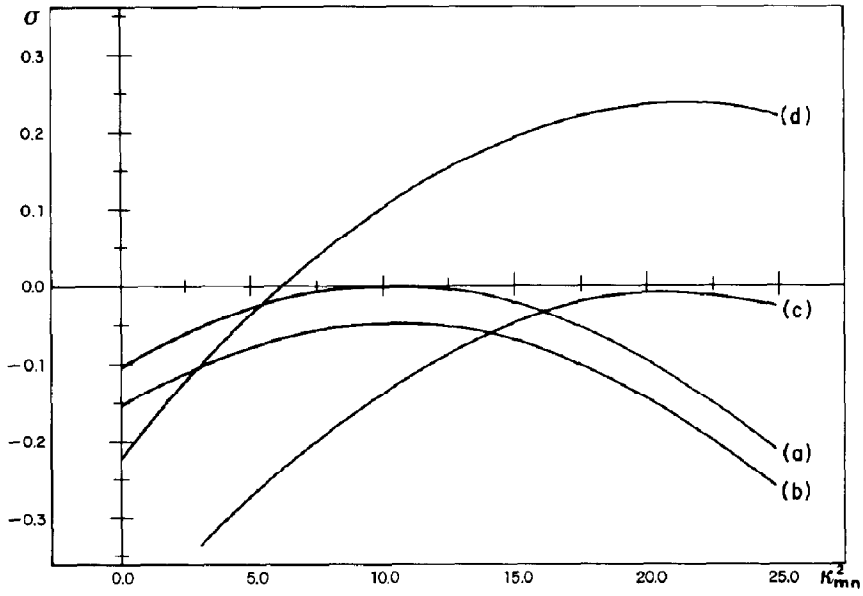

Fig. 2. Dispersion relation (B.4), plotted for different values of the apparent specific rate constant k_f: (a) $k_f = 2.67$ ($A = -0.854$, $\eta_0 = 0.98$), (b) $k_f = 4$, (c) $k_f = 51.174$ ($A = -0.885$, 0.9956), and (d) $k_f = 50$. The solution loses stability for values of k_f greater than a critical value given by eq. (B.7). Other parameter values are α-0.001, $B = 0.4249$, $k_b = 10^{-3}$ (values of A and B were estimated from Ref. [5]).

zero. Moreover, from a certain value of k_f the value of σ takes positive values, whence the solution loses stability. It is then possible that solutions beyond these points of instability show heterogeneous spatial distributions of phospholipids in different phases. To look into this possibility, an approximated solution, only valid in a neighborhood of the bifurcation point, is calculated in Appendix C. The critical value of k_f, determined in the point of marginal stability $\sigma = 0$, is determined from

$$k_{fc} = \frac{4\alpha k_b - (A + 2B\eta_o)^2}{8\alpha(\eta_0 - 1)} \tag{B.7}$$

In Appendix C the model system given by eq. (5) is solved, in the vicinity of the bifurcation points given by (B.7).

Appendix C

Non-linear analysis

It is interesting to study the dynamics of the model given by eq. (5), and how it is modified by different concentrations of calcium. Using a perturbative method around a bifurcation point, we look for an approximated solution which is induced by changes in the parameter k_f. This parameter implicitly reflects changes of calcium concentrations.

When the uniform state is unstable, solutions to system (5), starting with initial conditions near one of the steady states given by (B.1), can be approximated by a uniformly valid asymptotic solution of the form [38–41]:

$$\xi = \sum_{i=0}^{\eta} \epsilon^i \eta_{i+1} \tag{C.1}$$

Moreover, we define a perturbation parameter ϵ ($\ll 1$) and a slow time τ with:

$$k_f = k_{fc} + \epsilon^2 \qquad \tau = \epsilon^2 t \tag{C.2}$$

By substituting (C.1) and (C.2) into eq. (5), and equating the same powers of ϵ we obtain, to $O(\epsilon^3)$, the terms:

$$\epsilon\colon\ L\eta_1 = 0 \qquad \epsilon^2\colon\ L\eta_2 = H_1(\eta_1, k_c)$$
$$\epsilon^3\colon\ L\eta_3 = H_2(\nu_1, \eta_2, k_c) \tag{C.3}$$

where L corresponds to the linearized operator defined by (B.2), and H_1 and H_2 are non-linear terms.

To solve system (C.3) the linear equation to $O(\epsilon)$ is solved and substituted in the non-linear part H_1 of the equation to $O(\epsilon^2)$. This new linear equation is solved and substituted in the non-linear part H_2 of the equation to $O(\epsilon^3)$ [38–41]. In each stage of the analysis one looks for possible secular terms [38–41].

Solutions for the first-order approximation of system (C.3), with the conditions that it is finite, single valued and subject to the following boundary conditions:

$$u(1, \theta) = 0 \tag{C.4}$$
$$\partial u(r, \theta)/\partial r = 0$$

is of the form:

$$u_1(r, \theta) = \sum_n \sum_m \{ A_{mnc}(\tau)[I_m(k_{mn})J_m(k_{mn}r) - J_m(k_{mn})I_m(k_{mn}r)] \cos m\theta + A_{mns}(\tau)[I_m(k_{mn})J_m(k_{mn}r) - J_m(k_{mn})I_m(k_{mn}r)] \sin m\theta \} e^{\sigma t} \tag{C.5}$$

where σ is given by the dispersion relation (B.4). We are concerned here with the ultimate steady state solution which exhibits spatial heterogeneity. The procedure can be simplified considerably if we perform an asymptotic approach for large time at every step. Hence, considering that we started from a steady state and we are near the bifurcation point, the influence of a perturbation on σ is of order ϵ^2. We can thus assume that in a vicinity of the critical point it is possible to consider only the influence of the slow time. Furthermore, considering that in the linear analysis was found that $\sigma(k_{mn}^2)$ has a maximum at k_{fc}, we can use the

standard method of Laplace [38] to get the asymptotic form of eq. (C.5). This yields

$$\xi(r,\theta) = A_{mnc}(\tau)\left[I_m(k_{mn})J_m(k_{mn}r) - J_m(k_{mn})I_m(k_{mn}r)\right]\cos m\theta + A_{mns}(\tau)\left[I_m(k_{mn})J_m(k_{mn}r) - J_m(k_{mn})I_m(k_{mn}r)\right]\sin m\theta \qquad (C.6)$$

The coefficients A_{mnc}, A_{mns} are undetermined at this stage. They are determined by suppressing secular terms at a later stage, which in our problem is at $O(\epsilon^3)$. Using polar coordinates this search proves to be very cumbersome, so that it was not persued beyond first-order approximation. Instead, we considered that a solution at this order of approximation may reflect, at least in an approximated way, and restricted to the neighborhood of the bifurcation point, part of the dynamical behavior shown when terms of higher order are used. Furthermore, given that in a previous work the solution of the secular equation was obtained using cartesian coordinates, and resulted to be stable [41], we assume here that the solution to eq. (5) in polar coordinates, is also stable (numerical calculations to prove this assumption are now being developed).

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