Magnetic field induced pattern formation in reactive membranes

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The effect of magnetic field on lipid bilayers has been a subject of interest to experimentalists in recent years. Here we examine a fluctuation model of reactive bilayer membranes, taking into view the compositioncurvature correlation in the presence of a homogeneous magnetic field. We show how the strength of the magnetic field in combination with the intensity of reaction induces instability into the system, at times leading to stationary pattern formation.

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I. INTRODUCTION

Lipid bilayers are the basic structural units of all cell membranes. Amphiphiles such as surfactants and phospholipids aggregate themselves in water to form bilayer sheets, with their polar head groups pointing toward water, and their hydrophobic tails facing each other $\lceil 1,2 \rceil$ $\lceil 1,2 \rceil$ $\lceil 1,2 \rceil$ $\lceil 1,2 \rceil$. These membranes have a fluidlike property and are highly elastic in nature. The study of the cell membrane and other intracellular activities demands a complete understanding of the bilayer. Also, lipid bilayers find widespread utility in medicinal research as a means of nanotechnology based artificial drug delivery $\lceil 3 \rceil$ $\lceil 3 \rceil$ $\lceil 3 \rceil$.

Starting from the seminal work by Helfrich on membrane shape deformation $\lceil 1 \rceil$ $\lceil 1 \rceil$ $\lceil 1 \rceil$, numerous theoretical studies have so far been carried out in this field $\left[4,5\right]$ $\left[4,5\right]$ $\left[4,5\right]$ $\left[4,5\right]$. Most of these deal with the fluctuations, phase separations, and transitions of bilayer structures [[6,](#page-6-5)[7](#page-6-6)]. For example, Taniguchi *et al.* have carried out extensive work in this direction $\left[8-10\right]$ $\left[8-10\right]$ $\left[8-10\right]$, giving us a concise mathematical expression of the effect of the inplane degrees of freedom toward shape deformations. Later, several other groups have taken up their approach of the composition-curvature relationship to study multicomponent membranes $\lceil 11 \rceil$ $\lceil 11 \rceil$ $\lceil 11 \rceil$ and membranes with active components [[12](#page-6-10)]. More recently, Lindenberg *et al.* [[2](#page-6-1)] have undertaken the study of shape fluctuations in reactive membranes, where they have shown how the competitive effect of the phase segregation and mixing due to a nonequilibrium interconvertion reaction between the two species of a membrane, induces a change in the curvature sequence of the bilayer, thus giving rise to stationary patterns. Many of these different properties (behavior) of the lipid bilayer have also been experimentally verified $[12,13]$ $[12,13]$ $[12,13]$ $[12,13]$.

The interaction between diffusion and nonlinearity in many natural processes often bring about spatiotemporal and stationary pattern formation in a spatially extended system, when the later is driven far away from equilibrium $[14]$ $[14]$ $[14]$. Numerous works have been done in this direction in order to study the effect of additive and multiplicative noise $[15]$ $[15]$ $[15]$, electric $[16,17]$ $[16,17]$ $[16,17]$ $[16,17]$ and magnetic fields $[18]$ $[18]$ $[18]$, photochemical induction $[19]$ $[19]$ $[19]$, etc. in such systems. Owing to the existing anisotropy of magnetic susceptibility of the lipid bilayer, magnetic field has a substantial effect on the orientation of magnetic fields bring about changes in the surface pressure and thus the structures of phospholipid membranes $[24-27]$ $[24-27]$ $[24-27]$. These phospholipid and glycolipid membranes being a major constitutent of most biological systems, the study of these membranes acts as a major step to the better understanding of the effects of magnetic fields on biomolecules $[28,29]$ $[28,29]$ $[28,29]$ $[28,29]$. From all these studies it has been concluded that magnetic field may play a decisive role in determining the structure and curvature of membranes. The study of magnetic field effects in membranes can be

the diamagnetic molecular domains within a membrane [[20–](#page-6-18)[23](#page-6-19)]. Several experimental studies have shown how static

dated back to the early works of Helfrich $[30]$ $[30]$ $[30]$, where he for the first time gave a mathematical expression for the orientational energy due to the application of a magnetic field on spherical membranes. In most of the theoretical studies following this, only spherical vescicles have been considered, there being an inherent simplicity in assuming the surface normal to coincide with the *z* axis, in this case. Moreover, to the best of our knowledge, studies of magnetic field effects on reactive membranes have not yet been carried out. In nature there are instances of many such reactive membranes that are locally flat in nature. It is therefore worthwhile to look for the imprints of magnetic field on the instability and spatiotemporal structure of nearly flat reactive membranes.

We propose to study a general model of bilayer membranes based on the fluctuation approach and the simultaneous kinetics of the composition and curvature in the presence of a magnetic field. This takes into account the out-ofequilibrium behavior of membranes in the presence of an externally applied constant magnetic field. We identify the onset of instability as a function of magnetic field strength to demonstrate the transition from homogeneity and the formation of stationary patterns. Our analytical results are corroborated with numerical simulations on the kinetic system in two dimensions.

II. THE MODEL

We consider a nearly flat membrane with a gradual height fluctuation $h(x, y)$ (Fig. [1](#page-1-0)). Moreover we assume that the membrane is composed of two kinds of amphiphiles, α and β , both of which are interconvertible by some procedure. We consider the interconversion to follow a simple first order

^{*}E-mail address: pcdsr@mahendra.iacs.res.in kinetics of the form,

FIG. 1. Schematic diagram of a lipid bilayer, where the amphiphiles are shown as components with a dark-colored polar head group and a chainlike lipid tail group.

$$
\begin{array}{rcl}\nk_+ \\
\alpha & \rightleftharpoons & \beta \\
k_- \n\end{array} \n\tag{2.1}
$$

Here α and β can be considered to be conformers of the same amphiphile or otherwise interconvertible by some simple reaction as seen for some of our visionary proteins and membranes involved in the nervous-synaptic process. Such examples are also seen in different mixtures of ionic or nonionic surfactants, mixtures of phospholipids (like phos-phatidyl choline and phosphatidyl glycerol), etc. [[13](#page-6-11),31-[33](#page-6-26)]. In our model we have assumed that the amphiphiles do not shuffle between the inner and outer layers.

Considering an almost planar unilamellar (one bilayer) membrane, we go about studying their undulations by a close look at the free energies. Since the bilayer is almost flat, and we consider the height fluctuations to be of very small magnitude, the Monge parametrization can be used to express the equation of the surface as $z=h(x, y)$. Following this, the free energy of bending and other deformations is given by $[8]$ $[8]$ $[8]$,

$$
F_1 = \frac{1}{2}\sigma \int (\nabla h)^2 dxdy + \frac{1}{2}k \int (\nabla^2 h)^2 dxdy, \qquad (2.2)
$$

where σ is the surface tension and *k* the bending modulus.

Now we define an order parameter, $\phi(x, y)$, as the relative composition of the two amphiphiles. The free energy of mixing can thus be written,

$$
F_2 = \int \left[-\frac{A}{2} \phi^2 + \frac{B}{4} \phi^4 \right] dx dy + \frac{1}{2} \gamma \int (\nabla \phi)^2 dx dy,
$$
\n(2.3)

where, the first two terms stand for the lateral free energy of mixing. These terms are homogeneous in nature. In what follows we show that their contribution is limited to controlling the dynamics of composition rather than height fluctuations. γ is the stiffness coefficient, and represents the resistance to local composition fluctuations of ϕ , with A, B, and γ being the coefficients of the Gingburg-Landau terms for phase separation.

The coupling between the composition and curvature is expressed as an interaction between the order parameter ϕ , and the even powers of the gradient of *h*. Thus the free energy arising out of this coupling is given by,

FIG. 2. Side view of a membrane depicting its curvature in a three-dimensional coordinate system. The angle (θ) made by the layer normal (**n**) with the magnetic field along *z* axis (B_0) , is also shown.

$$
F_3 = \Lambda \int \phi \nabla^2 h dx dy
$$
 (2.4)

where, Λ is the composition-shape coupling constant.

An external magnetic field exerts a torque on a magnetically anisotropic film. Similarly, if a uniform magnetic field, $B₀$, acts on our lipid bilayer along a direction parallel to the *z* axis, its effect can be taken into account by the orientational energy per unit area, that is given by $[1]$ $[1]$ $[1]$ (Fig. [2](#page-1-1)),

$$
F_4 = -\frac{1}{2}(\chi_n - \chi_t)d\cos^2\theta B_0^2.
$$
 (2.5)

Here, $\chi_n d$ and $\chi_i d$ are the susceptibilities per unit area for fields normal and tangential to the bilayer. *d* is the thickness of the bilayer and B_0 is the magnetic field strength.

In our case, it takes the following form (Appendix A):

$$
F_4 = -\frac{1}{2}(\chi_n - \chi_t)d\left[\frac{1}{1 + (\nabla_x h)^2 + (\nabla_y h)^2}\right]B_0^2.
$$
 (2.6)

Considering only the linear terms arising from a binomial expansion of the later fraction of the above equation, the free energy of orientation becomes,

$$
F_4 = -\frac{1}{2} \int d(\chi_n - \chi_t) B_0^2 [1 - (\nabla_x h)^2 - (\nabla_y h)^2] dx dy.
$$
\n(2.7)

Thus, the total free energy of the system over the entire surface, being the accumulative total of the four kinds of free energy, viz. bending, mixing, composition-curvature coupling, and magnetic orientation, is given by,

$$
F = \int \left[\frac{\sigma}{2} (\nabla h)^2 + \frac{k}{2} (\nabla^2 h)^2 - \frac{A}{2} \phi^2 + \frac{B}{4} \phi^4 + \frac{\gamma}{2} (\nabla \phi)^2 + \Lambda \phi \nabla^2 h - \frac{d}{2} (\chi_n - \chi_t) B_0^2 (1 - (\nabla_x h)^2 - (\nabla_y h)^2) \right] dx dy.
$$
\n(2.8)

We nondimensionalize the energy by dividing both sides by *k* (with a dimension of energy, units of $k_B T$). We also express the lengths in units of *d*.

$$
F_a = \int \left[\frac{a_1}{2} (\nabla_a h_a)^2 + \frac{1}{2} (\nabla_a^2 h_a)^2 - \frac{a_2}{2} \phi^2 + \frac{a_3}{4} \phi^4 + \frac{a_4}{2} (\nabla_a \phi)^2 + a_5 \phi \nabla_a^2 h_a - \frac{a_6}{2} (\chi_n - \chi_t) (1 - (\nabla_{x_a} h_a)^2 - (\nabla_{y_a} h_a)^2) \right] dx_a dy_a, \tag{2.9}
$$

where, $F_a = \frac{F}{k}$, the dimensionless form of free energy, $\nabla_{a} = d\nabla, h_a = \frac{\hbar}{d}, a_1 = \frac{\sigma d^2}{k}, a_2 = \frac{A}{k}, a_3 = \frac{B}{k}, a_4 = \frac{\gamma}{k}, a_5 = \frac{\Delta d}{k}$, and a_6 $=\frac{B_0^2 d^3}{k}$ $\frac{m}{k}$.

The kinetic equations governing ϕ and the time evolution of the height field is given by $[2]$ $[2]$ $[2]$,

$$
\frac{\delta \phi}{\delta t} = D \nabla^2 \left[\frac{\delta F_a}{\delta \phi} \right] - \Gamma (\phi - \phi_0), \tag{2.10}
$$

where, $\Gamma = k_+ + k_-$ and $\phi_0 = \frac{k_- - k_+}{k_- + k_+}$; and

$$
\frac{\delta h_a}{\delta t} = -M \frac{\delta F_a}{\delta h_a},\tag{2.11}
$$

where *M* is the mobility parameter so that $M \propto \tau_h^{-1}$; τ_h being the relaxational time.

We nondimensionalize the equations by multiplying Eqns. (2.10) (2.10) (2.10) and (2.11) (2.11) (2.11) with τ_h , and expressing all lengths in units of *d*.

This gives,

$$
\frac{\delta \phi}{\delta t_a} = b_1 \nabla_a^2 \left[\frac{\delta F_a}{\delta \phi} \right] - \Gamma_a (\phi - \phi_0)
$$
 (2.12)

and

$$
\frac{\delta h_a}{\delta t_a} = -b_2 \frac{\delta F_a}{\delta h_a},\tag{2.13}
$$

where $t_a = \frac{t}{\tau_h}$; $b_1 = \frac{D\tau_h}{d^2}$; $\Gamma_a = \Gamma \tau_h$; $b_2 = M\tau_h \approx 1$.

From Eqns. (2.12) (2.12) (2.12) , (2.13) (2.13) (2.13) , and (2.9) (2.9) (2.9) , we have the final forms of the kinetic equations as follows:

$$
\frac{\delta\phi}{\delta t_a} = -b_1 a_2 \nabla_a^2 \phi + 3b_1 a_3 \phi^2 \nabla_a^2 \phi + b_1 a_4 \nabla_a^4 \phi + b_1 a_5 \nabla_a^4 h_a
$$

$$
- \Gamma_a (\phi - \phi_0)
$$
(2.14)

and

$$
\frac{\delta h_a}{\delta t_a} = -b_2 \left[a_1 \nabla_a^2 h_a + \nabla_a^4 h_a + a_5 \nabla_a^2 \phi + a_6 (\chi_n - \chi_t) \nabla_a^2 h_a\right].
$$
\n(2.15)

In the succeeding equations, we denote h_a by h , ∇_a by ∇ , and *ta* by *t* for simplicity.

III. LINEAR STABILITY ANALYSIS

A look at the final equations reveals the existence of a spatially uniform steady state $(\phi = \phi_0, h = h_0)$, of the dynamical system. Expressing the variables as follows:

$$
\phi = \phi_0 + \overline{\delta\phi(x, y, t)} e^{i(q_x x + q_y y + \omega t)}, \tag{3.1}
$$

$$
h = h_0 + \overline{\delta h(x, y, t)} e^{i(q_x x + q_y y + \omega t)}, \tag{3.2}
$$

and substituting the above into Eqns. (2.14) (2.14) (2.14) and (2.15) (2.15) (2.15) , we have,

$$
i\omega\overline{\delta\phi} = b_1 a_2 q^2 \overline{\delta\phi} - 3 \phi_0^2 b_1 a_3 q^2 \overline{\delta\phi} + b_1 a_4 q^4 \overline{\delta\phi} + b_1 a_5 q^4 \overline{\delta h}
$$

$$
- \Gamma_a \overline{\delta\phi}, \tag{3.3}
$$

$$
i\omega\overline{\delta h} = b_2[a_1q^2\overline{\delta h} - q^4\overline{\delta h} + a_5q^2\overline{\delta\phi} + a_6(\chi_n - \chi_t)q^2\overline{\delta h}]
$$
\n(3.4)

where, $q = q_x + q_y$.

The system of equations (3.3) (3.3) (3.3) and (3.4) (3.4) (3.4) can be put in the form of a matrix equation as follows:

$$
L\left(\frac{\delta\phi}{\delta h}\right) = 0,\t(3.5)
$$

where

$$
L = \begin{pmatrix} l_{11} - i\omega & l_{12} \\ l_{21} & l_{22} - i\omega \end{pmatrix}.
$$

The detailed expressions of the elements of the above matrix are as follows:

$$
l_{11} = (b_1 a_2 - 3b_1 a_3 \phi_0^2) q^2 + b_1 a_4 q^4 - \Gamma_a,
$$
 (3.6)

$$
l_{12} = b_1 a_5 q^4, \tag{3.7}
$$

$$
l_{21} = b_2 a_5 q^2, \tag{3.8}
$$

$$
l_{22} = b_2 [a_1 q^2 + (\chi_n - \chi_t) a_6 q^2 - q^4]. \tag{3.9}
$$

To examine the stability, we now write the following determinantal equation for the eigenvalue problem,

$$
|L| = 0.\tag{3.10}
$$

Supposing ω with complex values, the eigenvalues of the Jacobian can be written

$$
\omega(q^2) = \frac{\operatorname{Tr}[L] \pm \sqrt{\Delta[L]}}{2},\tag{3.11}
$$

where, $Tr[L] = l_{11} + l_{22}$ and, $Δ[L] = Tr[L]^2 - 4 Det[L]$; $Det[L]$ $=$ *l*₁₁*l*₂₂−*l*₁₂*l*₂₁.

The instability sets in as $\text{Re}(\omega(q^2))$ attains a positive value. We now look for the range of q^2 values for which the real part of the eigenvalues, $\omega(q^2)$ attain a positive value for the given strength of the applied magnetic field, B_0 , and a particular value of the kinetic parameter, Γ .

In our studies, we consider the value of the magnetic field strength over the range of 0 to 1×10^5 oersted, that in the case of biological membranes can be held equivalent to a magnetic field induction of 0 to 10 Tesla. The other experimentally admissible values of the constants $\lceil 1,12,24 \rceil$ for our system will be of the order of, $\sigma \approx 1-5$ *dyne* cm⁻¹, $\kappa \approx 5 \times 10^{-13}$ *erg*, $d \approx 5 \times 10^{-7}$ cm, $\Gamma \sim 10^{-9}$ *dyne*, and $\chi_n - \chi_t \approx 1.0 \times 10^{-7}$ *erg* cm⁻³ *oersted*⁻². The corresponding nondimensional parameters will thus take the values, $a_1 = 0.1$; $a_2 = 0.01$; $a_3 = 0.01$ $a_4 = 0.01$; $a_5 = 0.001$; $b_1 = 1.0$ and

FIG. 3. Dispersion relation (plot of ω versus q^2), for varying B_0 . Γ_a =0.5 and other parameters are as mentioned in the text.

 b_2 = 1.0. And the initial conditions used are ϕ_0 = 0.1 and h_0 =0.0. The Γ_a values range from 0.1 to 2.0.

It is apparent from the expression of $\omega(q^2)$ in Eq. ([3.11](#page-2-9)), that

$$
\Delta[L] = (l_{11} - l_{22})^2 + 4l_{12}l_{21} \Rightarrow \Delta[L] > 0 \tag{3.12}
$$

so that, Im($\omega(q^2)$)=0. This suggests an absence of wave bifurcation.

Figure [3](#page-3-0) depicts the range of the q^2 values for varying magnetic fields (B_0) and a given Γ_a value for which a small perturbation may bring about an instability, initiating a transition to a pattern stationary in time.

IV. NUMERICAL SIMULATIONS AND DISCUSSIONS

We carry out numerical simulations of the kinetic system [Eqs. (2.14) (2.14) (2.14) and (2.15) (2.15) (2.15)], using the explicit Euler method for integration of the equations, following the discretization of space and time. A finite system size of 128×128 grid points has been chosen. Zero flux boundary conditions have been considered along all the four walls. A time interval Δt = 0.0001, and a cell size, Δx = 1.0, have been found to be appropriate for the purpose.

We have carried out our numerical simulations for different values of Γ_a ranging from 0.1 to 2.0, and with magnetic field strength, B_0 , varying between 0.0 T and 10.0 T. The initial conditions are taken to be $\phi_0=0.1$, $h_0=0.0$, with a random and small perturbation over the whole concentration space, ϕ . We plot the contour values of the concentration order parameter, ϕ in the Figs. [4](#page-3-1)[–7.](#page-5-0) Dark colors represent a greater concentration of species α , and light colors depict more concentration of amphiphile β . Simultaneously is given a surface plot of the *h* values over a portion of the whole space, to give a real feel of the shape and curvature of the membrane surface.

In Fig. [4](#page-3-1) we show both the contour plot of ϕ and surface plot of *h* to depict the formation of patterns stationary in time. In Figs. $5-7$ $5-7$, we plot ϕ and *h* after a considerably long time period. While, in Fig. [5](#page-4-0) we show how with the increase in the value of Γ_a from 0.1 to 1.0, the spots become more and more prominent for a particular strength of the magnetic field $(B_0= 5.0 \text{ T})$; in the Figs. [6](#page-4-1) and [7](#page-5-0) is shown the change in pattern with increasing magnetic field for a particular value of $\Gamma_a(=2.0, 0.8)$.

As Γ_a varies, the nature of the pattern also changes (Fig. [5](#page-4-0)). For small values of Γ_a (=0.1), the perturbed system becomes almost homogeneous with time. With an increase in the value of Γ_a , patterns begin to appear. For $\Gamma_a = 0.5$, spots emerge out of stripes. As Γ_a moves to still higher values $(=1.0)$, it gives rise to prominent spots. These observations are in keeping with the studies of Lindenberg *et al.* [[2](#page-6-1)], where stripes indicate a dominance of the phase separation for low values of Γ_a whereas for higher values, the symmetric dropletlike patterns shown by the ϕ - and *h*-field profiles are due to the increased strength of the reaction. The presence of magnetic field in this case allows for the presence of a nearly flat homogeneous state for very low values of Γ_a , possibly due to a greater prominence of the magnetic field orientation over the other two factors.

FIG. 4. Time variation of the concentration order parameter (ϕ) and the height field (h) for $\Gamma_a = 0.5$ and $B_0 = 5.0$ T (other parameters are as mentioned in the text).

FIG. 5. Variation of the concentration order parameter (ϕ) and the height field (h) for $\Gamma_a = 0.1$, 0.5, 1.0, and $B_0 = 5.0$ T other parameters are as mentioned in the text).

On the other hand, changes in the magnetic field lead to considerable changes in the spatial patterns (Figs. 6 and 7). For zero or very low values of magnetic field, one observes the transition to a homogeneous phase. As B_0 increases, spotlike or dropletlike formations appear. With a further rise in the value of B_0 , there is an increase in the density of the spots, while their size decreases. The results of the numerical simulations show, in general, a good conformation to the theoretical analysis, as shown in Fig. [7.](#page-5-0) The orientational energy being a function of the square of the magnetic field $B₀$, there are similar effects for both positive and negative values of magnetic field, i.e., sign of the magnetic field is immaterial to its orientational effect.

We have repeated both stability analysis and numerical simulation by excluding the first two terms of F_2 , that stands for the lateral free energy of mixing. It was observed that there is only a very slight quantitative change in the $\omega(q^2)$ values, with the dispersion curves remaining almost identical. More so in the case of numerical simulation, there seems to be no visible effect on the reaching of instability and the consequent formation of patterns. This may be due to the homogeneity of these terms that makes them less effective in inducing instability as compared to the rest of the terms, that were inhomogeneous in nature in this form of the free energy functional.

Keeping in view of the recent developments, it is apparent that theoretical studies on such pattern-forming effects of magnetic field on membranes have not been looked into until now. The above work also gains significance from the experimental investigations carried out in related context in recent years $\lceil 21,25 \rceil$ $\lceil 21,25 \rceil$ $\lceil 21,25 \rceil$ $\lceil 21,25 \rceil$ where it has been shown that homogenous magnetic fields orient membranes and thus bring about substantial changes in biological systems. Phospholipid molecules have an intrinsic anisotropy in their magnetic behavior, and this leads to nonzero $|\Delta \chi|$ values of the order of 10−7 *erg* cm−3 *oersted*−2 at room temperatures, for a bilayer of nearly 60 Å thickness $[24]$ $[24]$ $[24]$. This property of bilayers makes them susceptible to magnetic field effects. Small

FIG. 6. Variation of the concentration order parameter (ϕ) and the height field (h) for $\Gamma_a = 2.0$ and $B_0 = 0.0$ T, 5.0 T, and 8.0 T (other parameters are as mentioned in the text).

FIG. 7. Dispersion relation (plot of ω versus q^2) and the variation of the concentration order parameter (ϕ) and the height field (h) for $\Gamma_a = 0.8$ and $B_0 = 0.0$ T, 5.0 T, and 10.0 T other parameters are as mentioned in the text).

angle neutron scattering studies of dimyristoylphosphatidylcholine molecules have shown, that they orient themselves in spherical or elliptical shape in the presence of magnetic field of greater than 6 Tesla $(6 \times 10^4 \text{ oersted})$ strength [[34](#page-6-29)]. These structural changes can be monitored by the techniques like magnetic resonance, optical spectroscopy, x-ray scattering, and birefringence measurements $[35,36]$ $[35,36]$ $[35,36]$ $[35,36]$. In the spirit of liquid crystal studies, we believe that the effect we have discussed here can easily be probed through birefringencemeasurement in the presence of magnetic fields of the order of $1-10$ Tesla, using suitable polarizing microscope.

V. CONCLUSION

In this paper, we have studied the orientational effects of a homogeneous magnetic field on reactive membranes, both analytically and numerically. Beginning with a fluctuation approach to our problem, we have considered in detail the composition-curvature relationships and identified the role of magnetic field in initiating instability into the system, thus leading to spatial structure formation. It has been shown that, while a reactive membrane in the absence of magnetic field has two competing effects of phase segregation and mixing due to reaction, the presence of the field imposes a further restriction to the induction of instability. We hope this approach will be useful for exploring the effects of magnetic field in pattern formation and selection in biological systems and also as a supporting document to the utility of bilayer membranes as a potent tool for site specific drug delivery or magnetotherapy.

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APPENDIX A

The equation of a surface $S(x, y, z)$, such that $z=h(x, y)$, can be written

$$
z - h(x, y) = 0.
$$
 (A1)

 $\vec{\nabla}(z - h(x, y)) = 0$ (A2)

 $(A2)$

The normal to the surface at any point (x, y, z) is given by the gradient of *S* at that point $|37|$ $|37|$ $|37|$,

or,

$$
\left(\hat{z} - \frac{\delta h}{\delta x}\hat{x} - \frac{\delta h}{\delta y}\hat{y}\right) = 0\tag{A3}
$$

Thus, the angle (θ) made by the normal to the *z* axis is given by,

$$
\cos \theta = \frac{\hat{z} \cdot (\vec{\nabla}S)}{\sqrt{(\hat{z})^2} \sqrt{(\vec{\nabla}S)^2}}
$$
(A4)

or,

$$
\cos \theta = \frac{1}{\sqrt{\left(1 + \left(\frac{\delta h}{\delta x}\right)^2 + \left(\frac{\delta h}{\delta y}\right)^2\right)}}.
$$
 (A5)

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