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A theoretical study of micro-domain formation in mixed lipid membranes

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The thermodynamic stability of micro-clusters in a membrane built-up by charged and uncharged lipid molecules is discussed. A simple variational function is proposed in order to describe the essential structure of such lipid domains. Solvent-screened electrostatic repulsion between the lipid ionic head groups, short-range forces between the lipid hydrophobic taisl and entropic effects are taken into account. The stability conditions as well as the composition and the size of the lipid micro-domains are calculated and expressed as a function of molecular parameters for the membrane and its environment (for example, short-range forces, surface charge density of the lipid bilayer, ion concentration of the electrolyte solution in contact with the lipid membrane and temperature). As an application, the effect of micro-domain formation on the number of adsorbed ions on a charged lipid membrane has been calculated.

1. Introduction

The clustering of lipid molecules into micro-domains richer in one component is an interesting phenomenon taking place both in natural and model membranes. This has been postulated as a rationale of facts concerning the mobility and function of proteins imbedded in the lipid bilayer [1, 2], the modulation of the trans-membrane potential [3, 4], neurotransmitter release [5] and fusion between lipid vesicles [6–10]. Different experimental techniques have been proposed to follow such a phenomenon. Among these are spin [11–14] and fluorescence [15–17] probes, differential scanning calorimetry [18–19], Raman spectroscopy [20] and freeze-fracture electron microscopy [21–23]. In addition the dramatic effects of some substances in inducing domain formation has been investigated (chiefly divalent cations (see e.g. [24])), H^+ [11] and proteins [16, 25–28].

Unfortunately, little attention has been paid in developing theoretical models of such a phenomenon, except some thermodynamic approaches based on the theory of binary mixtures [29–31]. An interesting computer simulation, performed by a Monte Carlo technique, suggests a large fluctuation of composition within a composite lipid membrane, the extent being strongly affected by the molecular properties of the lipid components [32, 33]. In this paper I propose a simple analytical model linking the structure of lipid micro-domains to the physical properties of individual components of the membrane. However, instead of following the usual methods employed to study order–disorder transitions (e.g. the calculation of short and long range order parameters [34]), I adopt a different standpoint, minimizing the energy of the system with respect to geometrical parameters of the micro-structures (mainly the size and the ratio between A and B lipid molecules within the clusters). This approach allows

the study of some interesting phenomena which depend strongly on the distribution of charged and neutral lipids over the membrane plane: for example the adsorption of charged molecules on the cell surface. This kind of approach requires some drastic assumptions in order to limit the number of variational parameters needing to describe the local microstructures. Its advantage is a relatively simple mathematical formalism, a physical picture of the main driving forces and the gain of information concerning the magnitude of compositional fluctuations within the lipid bilayers.

2. Theory

For structural considerations membrane lipids can be treated as bimodal rod-like molecules, one end of the rod is hydrophilic, the remainder hydrophobic. The hydrophobic tails tend to align themselves in such a way to minimize contact with water, while the hydrophilic polar heads interact favourably with water. The formation of micro-domains richer in one component will change the whole energy of the membrane. In particular, the energy contributions affected by the clustering are:

(*a*) The solvent-screened electrostatic repulsion between the ionic head groups of charged lipids. We expect increased repulsion as a consequence of cluster formation.

(*b*) The short range interactions (mainly van der Waals forces between the hydrocarbon tails of lipid molecules and/or salt bridges formed by divalent cations and ionic lipid ends [24]). We expect that strong interactions between like molecules favour the formation of local microstructures.

(*c*) Entropy effects. The mixing entropy decreases as a consequence of the clustering process.

Other energy contributions are little affected by variations in the lipid distribution within the membrane and are not considered here.

The next step is the choice of the more significant parameters defining the clusters structure. This choice is not unequivocal. In the simplest model only two parameters are needed to define the domain structure: the size D and the composition H. In more detail, H is defined as the maximum deviation of lipid B concentration from its mean value $\bar{X}_B$, while D is the mean distance between the centres of the clusters (see figure 1). Then, the local lipid B concentration $\varrho_B(X, Y)$ calculated at a generic point X, Y over the membrane plane can be expressed by means of the trial function

$$\varrho_B(X, Y) = \bar{X}_B + \frac{H}{2}(\cos \lambda X + \cos \lambda Y), \tag{1}$$

where λ is related to D through $\lambda = 2\pi/D$. The parameters H and λ are to be determined by a variational procedure. An implicit approximation in equation (1) is the assumption that the clusters size distribution is infinitely narrow, i.e. all the micro-domains have the same size. By inserting more terms in the Fourier series expansion of $\varrho_B(X, Y)$ we can take into account a broad distribution of domain size. On the other hand, it has been proved that simple variational functions often contain the essential features of a phenomenon. As an example, we recall the linear combination of atomic orbitals approximation widely used in quantum chemistry, or a recent variational procedure applied in a study of micelles [35].

Since we have defined the parameters to be optimized, in a further step we have to express each energy contribution as a function of such parameters. This can be done in the following way.

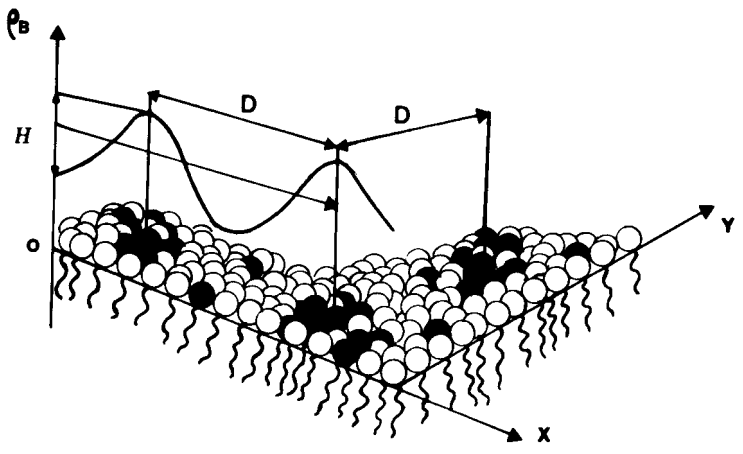

Figure 1. A schematic picture of a lipid membrane. The black and white dots represent the charged and neutral lipid head groups, respectively. On the Z axis perpendicular to the membrane plane is shown the charge density as a function of the spatial coordinates X and Y parallel to the lipid phase. D is the lattice constant of the lipid micro-domains.

(*a*) *Electrostatic energy*

The solvent screened electrostatic repulsion between the charged head groups of lipid molecules can be written as

$$E_e = \frac{1}{2}\sum_i \sum_{j\neq i} q_i^B q_j^B \Phi(r_{ij}), \tag{2}$$

where q_i^B is the net charge of the head groups, $\Phi(r_{ij})$ is the electrostatic potential (dimensionally corrected) between these charged ends and the sum is over the charged species B. A simple but reliable analytical function for $\Phi(r_{ij})$ is [36] $\exp(-\kappa r_{ij})/\varepsilon r_{ij}$, κ being the Debye kappa ($\kappa^2 = 8\pi q^2 C_0/\varepsilon kT$), C_0 and ε are the ion concentration and dielectric permittivity of the electrolyte solution in contact with the lipid ends. The evaluation of equation (2) can be easily performed by using cylindrical coordinates, r and ϕ. This allows us to write the interaction of a generic i-th charged end with its neighbours as

$$\sum_{j\neq i} q_j^B \Phi(r_{ij}) \to \frac{q}{\varepsilon a^2}\int_0^{2\pi}\int_a^{Na} \varrho_B(X_j, Y_j)\frac{\exp(-\kappa r)}{r}\, r\,dr\,d\phi, \tag{3}$$

where a is the mean distance between the lipid ends and N is the number of lipids forming the membrane ($N \to \infty$). The concentration of the charged ends $\varrho_B(X_j, Y_j)$ is given by equation (1). In order to evaluate the double integral appearing in equation (3) we must express the cartesian coordinates X_j and Y_j as a function of r and ϕ. This can be done noticing that the origin of the coordinate system has been put on the i-th atom (see figure 2); then

$$\left.\begin{aligned} X_j &= X_i + r\cos\phi, \\ Y_j &= Y_i + r\sin\phi. \end{aligned}\right\} \tag{4}$$

Combining equations (1), (3) and (4) and rearranging we obtain

$$\sum_{j\neq i} q_j \Phi(r_{ij}) \to \frac{q}{\varepsilon a^2}\int_0^{2\pi}\int_a^{Na}\left(\bar{X}_B + \frac{H}{2}(f_i\cos\lambda X_i - f_2\sin\lambda X_i + f_3\cos\lambda Y_i - f_4\sin\lambda Y_i)\right) \times \exp(-\kappa r)\, dr\, d\phi, \tag{5}$$

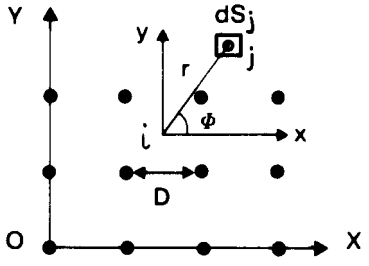

Figure 2. The coordinate system used in evaluating the electrostatic potential of a planar periodic charge distribution (see equations (2)–(5)). X and Y lie on the membrane plane, D is the lattice constant of the lipid micro-domains (see figure 1). The remaining parameters are defined in the text.

where

$$f_v \equiv \begin{Bmatrix} \cos \\ \sin \end{Bmatrix} \lambda r \begin{Bmatrix} \cos\phi \\ \sin\phi \end{Bmatrix}.$$

Re-inserting equation (5) into equation (2) and using equation (1) we find

$$E_e = \frac{q^2}{\varepsilon a^2} \sum_{X_i} \sum_{Y_i} \left(\bar{X}_B + \frac{H}{2} (\cos \lambda X_i + \cos \lambda Y_i) \right) \eta(X_i Y_i), \tag{6}$$

where

$$\eta(X_i, Y_i) \equiv \left(\bar{X}_B F_0 + \frac{H}{2} (F_1 \cos \lambda X_i - F_2 \sin \lambda X_i + F_3 \cos \lambda Y_i - F_4 \sin \lambda Y_i) \right);$$

F_0 and F_v are the double integrals over r and ϕ

$$F_0 \equiv \int_0^{2\pi} \int_a^{Na} \exp(-\kappa r)\, dr\, d\phi; \quad F_v \equiv \int_0^{2\pi} \int_a^{Na} f_v(r, \phi) \exp(-\kappa r)\, dr\, d\phi. \tag{6a}$$

The sums over X_i and Y_i are very easy, the final result is

$$E_e = \tfrac{1}{2} N \frac{q^2}{\varepsilon a^2} \left[\bar{X}_B^2 F_0 + \frac{H^2}{8} (F_1 + F_3) \right], \tag{7}$$

where

$$F_0 \equiv \int_0^{2\pi} \int_a^{Na} \exp(-\kappa r)\, dr\, d\phi$$

$$= \frac{2\pi}{\kappa} \exp(-\kappa a)$$

The double integrals F_1 and F_3 can be factorized into the sum of products of simple integrals with the aid of the formulae [37]

$$\cos(\lambda r \cos\phi) = J_0(\lambda r) + 2 \sum_{k=1}^{\infty} (-1)^k J_{2k}(\lambda r) \cos 2k\phi, \tag{8a}$$

$$\cos(\lambda r \sin\phi) = J_0(\kappa r) + 2 \sum_{k=1}^{\infty} J_{2k}(\lambda r) \cos 2k\phi, \tag{8b}$$

where $J_n(X)$ are Bessel functions of the first kind. The integration over ϕ is trivial and leads to: $F_1 = F_3$. More difficult is the integration over rf. It can be performed analytically and is described in Appendix 1. Combining these results with equation (7), we obtain, eventually

$$E_e = E_e^{(0)} + \pi N \frac{q^2}{\varepsilon a^2} \cdot \frac{H^2}{4} \exp(-\kappa a) \left(\frac{J_0(\lambda a)}{(\kappa^2 + \lambda^2)^{1/2}} + 2 \frac{J_1(\lambda a)}{\lambda} \left(\frac{\kappa}{(\kappa^2 + \lambda^2)^{1/2}} - 1 \right) \right)$$

$$\simeq E_e^{(0)} + \pi N \frac{q^2}{\varepsilon a^2} \frac{H^2}{4} \frac{\exp(-\kappa a)}{\kappa} \left(1 - \frac{\lambda}{2\kappa^2} (1 + \kappa a + \tfrac{1}{2}\kappa^2 a^2) \right)$$

$$+ O(\lambda^4 a^4, \lambda^4/\kappa^4) \tag{9}$$

where

$$E_e^{(0)} \equiv \pi N \frac{q^2}{\varepsilon a^2} \cdot \frac{\exp(-\kappa a)}{\kappa} \bar{X}_B^2.$$

Equation (9) is exact; it is an expansion in a power series of λa and it is a good approximation because both the interlipid distance a (≈ 10 Å [38]) and $1/\kappa$ (≈ 10 Å at physiological salt concentration) are much smaller than D ($\lambda = 2\pi/D$, see figure 1). Equation (9) is the required formula connecting the electrostatic repulsion between the charged lipid ends to H and λ, the variational parameters describing the micro-domains structure. As expected, when $H \to 0$ we obtain the formula for a uniformly charged plate imbedded in an electrolyte solution. Moreover, when $\lambda a \to 0$ (complete lateral phase separation) the electrostatic repulsion shows a maximum.

(*b*) *Short range energy*

The variation of the short range energy (mainly van der Waals interactions between the hydrocarbon tails of lipid molecules) as a function of the micro-domain structure can be calculated following the same procedure which was used in the previous sub-section. The short range energy can be written as

$$E_{SR} = \frac{1}{2} \sum_{S,S'} \sum_i \sum_{j \neq i} m_i^S m_j^{S'} G^{SS'}(r_{ij}), \tag{10}$$

where $G^{SS'}(r_{ij})$ is the short range energy per unity of mass between S and S' lipid molecules located at the generic sites i and j (S and $S' = A$ or B). m_i^S is the probability of finding a molecule of type S at site i; an analogous definition is valid for $m_j^{S'}$. In contrast to the electrostatic energy, the sum is now over A and B species. Since the short range energy decreases rapidly with the intermolecular distance, we can approximate $G^{SS'}(r_{ij})$ by

$$G^{SS'}(r_{ij}) = \begin{cases} +\infty, & r_{ij} < a, \\ -W_{SS'}, & r_{ij} = a, \\ 0, & r_{ij} > a, \end{cases} \tag{11}$$

where a is the interlipid distance. Assuming the distribution given by equation (1), we have

$$m_i^B = \varrho_B(X_i, Y_i)$$

and

$$m_i^A = 1 - \varrho_B(X_i, Y_i).$$

Combining the previous equations and using the same procedure adopted in the previous sub-section, we obtain, eventually

$$E_{SR} = E_{SR}^{(0)} - \pi N \frac{H}{4} W J_0(\lambda a)$$

$$\approx E_{SR}^{(0)} - \pi N \frac{H^2}{4} W \left(1 - \frac{\lambda^2 a^2}{4}\right), \tag{12}$$

where

$$E_{SR}^{(0)} = -\pi N(\bar{X}_A W_{AA} + \bar{X}_B^2 W_{BB} + 2\bar{X}_A \bar{X}_B W_{AB})$$

and the parameter W is defined as

$$W \equiv \tfrac{1}{2}(W_{AA} + W_{BB} - 2W_{AB}).$$

Equation (12) gives the variation of the short range energy as a function of the variational parameters H and λ. $E_{SR}^{(0)}$ is the contribution corresponding to a random distribution of A and B lipid molecules, while the remaining term in the right hand size of equation (12) represents the correction caused by lateral phase separation. Since $\lambda a \ll 1$, it follows that $J_0(\lambda a) > 0$, so that the sign of the correction depends upon the sign of W. In consequence strong interactions between like lipid molecules favour the thermodynamic stability of micro-domains.

(c) *Entropy*

Insofar as we have considered only the energy contributions to the total free energy, now we want to calculate the mixing entropy term given by:

$$F_{\text{mix}} = -T\Delta S_{\text{mix}}$$

$$= -kT \log g(H, \lambda). \tag{13}$$

The combinatorial factor $g(H, \lambda)$ is the number of ways to arrange N_A and N_B molecules in such a way that the local density of A and B follows a prescribed function. When all the lattice sites are equivalent, g is given by the formula [39]: $[(N_A + N_B)!]/(N_A!\, N_B!)$. On the other hand, when all the sites are different we have $g = 1$. In the present case we expect an intermediate situation between these two limiting cases.

Let M be the number of different sub-lattices each having the same composition (i.e. the same A/B ratio), then, $g(H, \lambda)$ can be written as [40]

$$g(H, \lambda) = \prod_{n=1}^{M} g_n, \tag{14}$$

where g_n is the combinatorial factor for each sub-lattice. Instead of the discrete variable n, it is useful to introduce a continuous one, say ξ, defined as the deviation of the local density from its mean value

$$\xi \equiv \varrho_B(X, Y) - \langle \varrho_B \rangle$$

$$= \varrho_B(X, Y) - \bar{X}_B.$$

The limiting values of ξ are: $-H \leqslant \xi \leqslant +H$ (see figure 3). If we let $N_A(\xi)$ and $N_B(\xi)$ be the number of A and B molecules in each sub-lattice ξ, we have

$$g_n \to g(\xi) = \frac{(N_A(\xi) + N_B(\xi))!}{N_A(\xi)!\, N_B(\xi)!}; \tag{15}$$

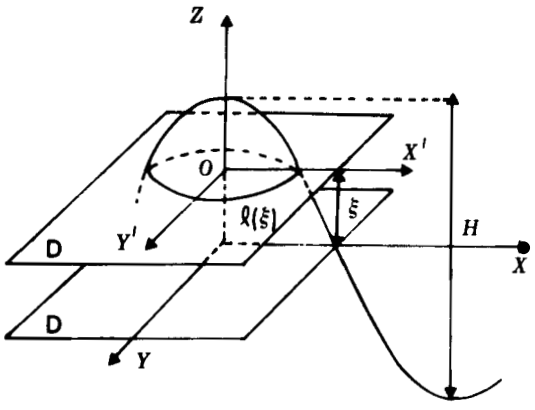

Figure 3. Graphical calculation of the number of sites having the same charge density. ξ is the deviation of the density from its mean value, while the spatial coordinates X and Y are parallel to the membrane surface and D is the lattice constant of the lipid micro-domains (see figure 1). The number of sites is proportional to $l(\xi)$ (see text).

$N_A(\xi)$ and $N_B(\xi)$ can be calculated as follows. First we need to calculate the number of sites $N(\xi)$ having the same ξ value. This can be done in an easier way by considering an elementary cell whose dimensions are $D \times D$ (see figure 1). Let $n(\xi)$ be the number of sub-lattices within this cell, it is proportional to $l(\xi)$ which is defined as the length of the cut between the surface defined by

$$Z = \varrho_B(X, Y) - \langle \varrho_B \rangle$$

and the plane $Z = \xi$ (see figure 3). Clearly $n(\xi) = l(\xi)/a$, where a is the diameter of a lipid molecule. $N(\xi)$ can be obtained by multiplying $n(\xi)$ by the number $\mathcal{N}$ of elementary cells lying on the membrane plane. Let N be the total number of lipid molecules, we must have then $\mathcal{N}D^2 = Na^2$ or $\mathcal{N} = N(a/D)^2$. Combining the previous equations, we find

$$\begin{aligned} N(\xi) &= \mathcal{N} n(\xi) \\ &= Nl(\xi) \frac{a}{D^2}; \end{aligned} \tag{16}$$

the calculation of $l(\xi)$ can be performed following standard methods of differential geometry to give

$$l(\xi) = 2 \int_{-D/2}^{+D/2} \left[1 + \left(\frac{\partial Y}{\partial X} \right)^2 \right]^{1/2} dx, \tag{17}$$

where $Y = Y(X)$ is defined implicitly by

$$\xi = \varrho_B(X, Y) - \bar{X}_B.$$

Inserting equation (1) into the previous expression and rearranging we have

$$Y(\xi, X) = \frac{1}{\lambda} \arccos \left(\frac{2\xi}{H} - \cos \lambda X \right).$$

Unfortunately, the integral (17) cannot be expressed in closed form. However, since $\xi < 1$, we may expand $l(\xi)$ in a power series as

$$l(\xi) = \alpha_0 + \alpha_1 \xi + \alpha_2 \xi^2 + \alpha_3 \xi^3 + \ldots, \tag{8}$$

where the coefficients α_i are to be determined. Since $l(\xi)$ must remain unchanged when we replace ξ by $-\xi$, it follows that $\alpha_1 = \alpha_3 = 0$. Moreover, when $\xi \to |H|$, $l(\xi)$ must tend to zero (see figure 3). On the other hand, when $\xi \to 0$, the integral (17) can be calculated analytically yielding

$$l(0) = 2^{3/2}D. \tag{19}$$

Making use of these results we can calculate α_0 and α_2, allowing us to rewrite equation (18) as

$$l(\xi) \approx 2^{3/2}D\left(1 - \frac{\xi^2}{H^2}\right) \tag{20}$$

and combining equations (16) and (20) we obtain the required formula

$$N(\xi) \approx N \cdot 2^{3/2}\frac{a}{D}\left(1 - \frac{\xi^2}{H^2}\right). \tag{21}$$

Obviously, the upper limit of ξ, say $\xi_{\max}$, must be selected in such a way that the normalization condition $\Sigma^{\xi_{\max}} N(\xi) = N$ is fulfilled

Knowledge of $N(\xi)$ allows us to calculate $N_A(\xi)$ and $N_B(\xi)$ in a straightforward way, in fact they are given by

$$\begin{cases} N_A(\xi) = N(\xi) \cdot \varrho_A(X, Y) \\ \qquad\quad = N(\xi)(\bar{X}_A - \xi) & (22a) \\ N_B(\xi) = N(\xi) \cdot \varrho_B(X, Y) \\ \qquad\quad = N(\xi)(\bar{X}_B + \xi). & (22b) \end{cases}$$

Now, inserting equation (22) into equations (14) and (15), applying Stirling's approximation

$$\log P! = P\log P - P, \quad (P \gg 1)$$

and remembering that $\bar{X}_A + \bar{X}_B = 1$, we obtain

$$\log g \approx \int_\xi N(\xi)[-(\bar{X}_A - \xi)\log(\bar{X}_A - \xi) - (\bar{X}_B + \xi)\log(\bar{X}_B + \xi)]\, d\xi$$

$$\equiv -\int_\xi N(\xi)\tau(\xi)\, d\xi, \tag{23}$$

where $N(\xi)$ is given by equation (21).

The last step is the integration of equation (23). This can be done in a simpler manner by means of the saddle point method [41]. In fact, when we confine our analysis to the region of equimolecular composition ($\bar{X}_A = \bar{X}_B = \frac{1}{2}$), the greatest contribution to the integral arises in the neighbourhood of $\xi = 0$, where both $N(\xi)$ and $\tau(\xi)$ have a maximum. Then, making use of the identity

$$\theta(\xi) = \exp\log\theta(\xi)$$

and expanding $\theta(\xi) \equiv N(\xi)\tau(\xi)$ in a power series in ξ

$$\log\theta(\xi) = \log\theta(0) + \frac{1}{2}\left|\frac{\partial^2}{\partial\xi^2}\log\theta(\xi)\right|_{\xi=0} \cdot \xi^2 + \ldots \tag{24}$$

we can rewrite integral (23) as

$$\log g \approx \theta(0) \int_0^\infty \exp\left[-\frac{1}{2} \left| \frac{\partial^2}{\partial \xi^2} \log \theta(\xi) \right|_{\xi=0} \xi^2 \right] d\xi. \tag{25}$$

Since the main contribution to the integral occurs in the neighbourhood of $\xi = 0$, we have replaced ξ_{max} with ∞; then

$$\log g \approx \left[-\frac{2\pi\theta^3(0)}{[\partial^2\theta(\xi)/\partial\xi^2]|_{\xi=0}} \right]^{1/2}. \tag{26}$$

Remembering that

$$\theta(\xi) \equiv N(\xi) \cdot \tau(\xi),$$

$N(\xi)$ and $\tau(\xi)$ being defined by equations (21) and (23), and that

$$\left. \frac{\partial N(\xi)}{\partial \xi} \right|_{\xi=0} = \left. \frac{\partial \tau(\xi)}{\partial \xi} \right|_{\xi=0} = 0,$$

we obtain

$$\log g \approx N 2^{3/2} \frac{a}{D} \cdot \frac{1}{H} \left(\frac{\pi(\log 2)^3}{2 + \log 2} \right)^{1/2}, \tag{27}$$

which, combined with equation (13) gives the required formula for the entropy contribution o the free energy expressed as a function of the variational parameters H and λ

$$F_{MIX} = -N \frac{\lambda a}{H} \left(\frac{2(\log 2)^3}{\pi(2 + \log 2)} \right)^{1/2} kT; \quad \bar{X}_A = \bar{X}_B = \tfrac{1}{2}. \tag{28}$$

Equation (28) shows the correct trend; in fact, large values of H or small values of λ (large domains) lead to small mixing entropy contribution. Unfortunately, equation (28) does not give the correct limit as H tends to zero. This is due to the failure of the saddle-point integration technique which can be applied only when the integrand shows a sharp peak for some values of the variable of integration. When we consider small inhomogeneities in the lipid distribution (small H values) the integrand shows a broader peak leading to the break-down of the saddle-point approximation. However, in the limit $H \to 0$, the calculation of the entropic contribution can be performed starting from equation (14) and putting here $M = 1$. The final result is the well-known formula

$$F_{MIX} = -NkT \log 2; \quad \bar{X}_A = \bar{X}_B \bar{A}_B = \tfrac{1}{2}. \tag{29}$$

(*d*) *Minimization procedure*

The total free energy of the lipid membrane expressed as a function of the variational parameters can be obtained by summing the electrostatic (equation (9)), short range (equation (12)) and entropy (equation (28)) contributions. Rearranging we have

$$F_{TOT/N} = F^{(0)}_{TOT/N} - \frac{C}{H} \lambda a + H^2(A - B\lambda^2 a^2), \tag{30}$$

where $F^{(0)}_{TOT}$ is the total free energy of the membrane, calculated assuming a random distribution of A and B molecules, and the other symbols are

$$A \equiv \frac{\pi}{4}\left(\frac{q^2}{\varepsilon\kappa a^2}\exp(-\kappa a) - W\right), \tag{31 a}$$

$$B \equiv \frac{\pi}{16}\left(\frac{2q^2}{\varepsilon\kappa^3 a^4}(1 + \kappa a + \tfrac{1}{2}\kappa^2 a^2)\exp(-\kappa a) - W\right), \tag{31 b}$$

$$C \equiv \left(\frac{2(\log 2)^3}{\pi(2 + \log 2)}\right)^{1/2} kT. \tag{31 c}$$

All the other symbols have been defined previously. The parameters H and λ can be obtained by the standard procedure. Differentiating equation (30) with respect to λa we find

$$\frac{1}{N}\frac{\partial F_{TOT}}{\partial(\lambda a)} = -\frac{C}{H} - 2BH^2\lambda a = 0, \tag{32}$$

then

$$\lambda a = -\frac{C}{2BH^3}. \tag{33}$$

Since λ must be positive (λ is the reciprocal of a length) and both C (see equation (31 c)) and H are positive, it follows that the condition $B < O$ must be fulfilled. Within this condition, it is easy to see that the extremal point (33) is a minimum. Inserting now equation (33) into equation (30) we have

$$(F_{TOT/N})_{\lambda=\lambda_{MIN}} = F^{(0)}_{TOT/N} + AH^2 + \frac{C^2}{4BH^4}. \tag{34}$$

Since it can be proved that the condition $B < 0$ implies also $A < 0$ (see equation (31)), we conclude that the function defined by equation (34) must have a maximum in the range of the allowed values of H ($0 \leqslant H \leqslant \frac{1}{2}$ for $\bar{X}_A = \bar{X}_B = \frac{1}{2}$). These trends are shown qualitatively in figure 4 where on the z axis we give the total free energy per lipid molecule, while on the remaining axes we show the parameters H and λ. The

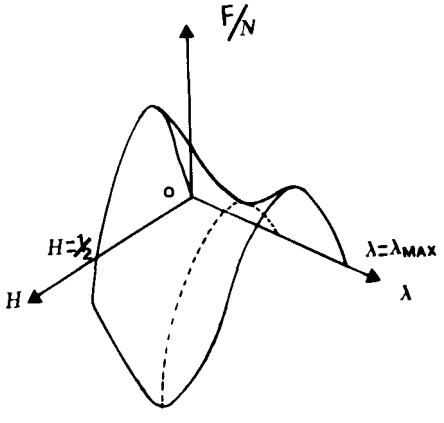

Figure 4. Free energy per lipid molecule versus the H and λ parameters (schematic). The more likely H values are 0 or 1/2.

real shape of the energy surface depends on physical parameters of the membrane and its environment (salt concentration, temperature, short-range forces etc.) but the general trend is that shown in the figure. As a consequence of this particular shape, we expect that the most likely values of H are 0 or $\frac{1}{2}$, depending on the relative energy of the system calculated at these points. Then, the thermodynamic condition for the stability of the micro-domains is that the difference between $(F_{\mathrm{TOT}/N})_{\substack{\lambda=\lambda_{\mathrm{MIN}} \\ H=1/2}}$ and $(F_{\mathrm{TOT}/N})_{\mathrm{RANDOM}}$ is less than zero. Making use of equation (34) we have

$$\frac{\Delta F_{\mathrm{TOT}}}{N} = \left(\frac{F_{\mathrm{TOT}}}{N}\right)_{\substack{\lambda=\lambda_{\mathrm{MIN}} \\ H=1/2}} - \left(\frac{F_{\mathrm{TOT}}}{N}\right)_{\mathrm{RANDOM}}$$

$$= \frac{4C^2}{B} + \frac{A}{4} + T\Delta S^{(0)} < 0, \tag{35a}$$

where $\Delta S^{(0)}$ is the mixing entropy contribution calculated for a random distribution of charged and neutral lipid molecules ($\Delta S^{(0)} = Nk\log 2$). This inequality must be fulfilled within the other condition (see equation (34))

$$B < 0. \tag{35b}$$

Equations (35), together with equation (33) are the main results of this paper. They give the thermodynamic conditions for micro-domain formation as a function of the molecular structure of the membrane as well as some information about the size of such aggregates; this topic will be discussed in the Results section. The validity range of the previous equations is limited by the constraints $\lambda a \ll 1$ and $\lambda/\kappa \ll 1$. These conditions allow us to study only large domains, far from the transition point. These limitations could be avoided by retaining more terms in the series expansions of equations (9) and (12). Unfortunately, this procedure leads to very complicated non-linear equations which could only be solved numerically.

(*e*) *Ion adsorption on a charged lipid membrane*

As an application of this model, let us calculate the effect of micro-domain formation on the number of adsorbed ions on a charged lipid membrane. Since the release or uptake of ions by biological membranes can be modulated by lateral phase separation of the membrane lipid components, this model may be used to investigate receptorial properties of charged lipid bilayers.

The excess of charge at the point z from a charged surface is defined as

$$\delta c(z) = C_+(z) - C_-(z)$$

$$= C_0\left(\exp\left(\frac{q\Phi(z)}{kT}\right) - \exp\left(-\frac{q\Phi(z)}{kT}\right)\right), \tag{36}$$

where C_0 is the bulk concentration of the electrolyte solution, q is the ion charge and $\Phi(z)$ is the solvent-screened electrostatic potential calculated at a distance z from the charged surface. The potential $\Phi(z)$ can be partitioned in two contributions. The first, $\Phi^{(0)}$, is that produced by a uniform charge distribution on the membrane surface; it depends only on the z coordinate. The second, $\Phi^{(1)}$, takes into account the effects arising from inhomogeneities of charge distribution and depends also on the x and y coordinates (x and y are parallel to the membrane plane). Since $\Phi^{(0)} \gg \Phi^{(1)}$ we can

develop equation (36) in a power series of $\Phi^{(1)}$; simple albegra gives

$$\frac{\delta C(z)}{C_0} = 2\sinh\frac{q\Phi^{(0)}}{kT} + \frac{q\Phi^{(1)}}{kT}\,2\cosh\frac{q\Phi^{(0)}}{kT} + \left(\frac{q\Phi^{(1)}}{kT}\right)^2\sinh\frac{q\Phi^{(0)}}{kT}. \tag{37}$$

The mean excess of charge calculated at a distance z from the membrane plane can be calculated by averaging equation (37) with respect to x and y. This can be done if we have an explicit equation for $\Phi^{(1)}$ as a function of the x and y coordinates. The calculation can be performed following the same procedure used in deriving the mean electrostatic energy of a charged membrane, the only difference being that we must replace r by $(r^2 + z^2)^{1/2}$ in the equation for the electrostatic potential (equation (3)). Simple algebraic manipulations lead to

$$\Phi(z) = \sum_{j\neq i} q_i \frac{\exp(-\kappa r_{ij})}{r_{ij}} \to \frac{Q}{\varepsilon a^2}\int_0^{2\pi}\int_0^{Na} \varrho^B(X_j, Y_j)\frac{\exp[-\kappa(r^2 + z^2)^{1/2}]}{(r^2 + z^2)^{1/2}}\,r\,dr\,d\phi, \tag{38}$$

where the symbols have been defined in equation (3). Equation (38) can be put in a more useful form, viz

$$\Phi(z) = \Phi^{(0)} + \Phi^{(1)}$$

where

$$\Phi^{(0)} = \frac{Q}{\varepsilon a^2}\bar{X}_B G_0, \tag{39a}$$

$$\Phi^{(1)} = \frac{Q}{\varepsilon a^2}\cdot\frac{H}{2}\,(G_1\cos\lambda X_i - G_2\sin\lambda X_i + G_3\cos\lambda Y_i - G_4\sin\lambda Y_i) \tag{39b}$$

and

$$G_0 \equiv \int_0^{2\pi}\int_0^{Na}\frac{\exp[-\kappa(r^2 + z^2)^{1/2}]}{(r^2 + z^2)^{1/2}}\,r\,dr\,d\phi, \tag{40a}$$

$$G_v \equiv \int_0^{2\pi}\int_0^{Na} f_v(r, \phi)\frac{\exp[-\kappa(r^2 + z^2)^{1/2}]}{(r^2 + z^2)^{1/2}}\,r\,dr\,d\phi; \tag{40b}$$

the functions $f_v(r, \phi)$ are defined in equation (5). Averaging now equation (37) with respect to x and y and making use of equations (39), eventually we obtain

$$\langle\delta c(z)\rangle_{X,Y} \approx 2C_0\sinh\left(\frac{q\Phi^{(0)}}{kT}\right)\left[1 + \frac{1}{2}\left(\frac{q}{kT}\right)^2\langle\Phi^{(1)2}\rangle_{X,Y}\right], \tag{41}$$

where

$$\langle\Phi^{(1)2}\rangle = \frac{Q^2}{\varepsilon^2 a^4}\cdot\frac{H^2}{4}\cdot G_1^2$$

and $\Phi^{(0)}$ is defined by equation (39a). The last step is the evaluation of the integrals G_0 and G_1 appearing in equations ((39a) and (41)). The calculation of G_0 is simple and the result is

$$G_0 = 2\pi\frac{\exp(-\kappa z)}{\kappa}. \tag{42a}$$

The evaluation of G_1 is more difficult; no analytic expression can be obtained. However, if we are interested in the region near the membrane surface, a simple asymptotic equation can be obtained. Its proof is given in appendix 2 although the final result is

$$G_1 \approx 2\pi \frac{\exp(-\kappa z)}{\kappa}\left(1 - \frac{\lambda^2}{2\kappa^2}(1 + \kappa z + \kappa^2 z^2)\right) + O(\kappa^3 z^3) \qquad (42\,b)$$

Combining now equations (39) to (42), we obtain eventually

$$\langle \delta c(z)\rangle_{X,Y} \approx 2C_0 \sinh\left(\frac{q}{kT}\pi \frac{Q}{\varepsilon\kappa a^2}\exp(-\kappa z)\right)$$

$$\times \left\{1 + \frac{1}{2}\left(\frac{q}{kT}\right)^2 \cdot \left[\pi \frac{Q}{\varepsilon\kappa a^2} \cdot H \cdot \exp(-\kappa z)\left(1 - \frac{\lambda^2}{2\kappa^2}(1 + \kappa z)\right)\right]^2\right\}. \qquad (43)$$

In the framework of the present theory, H can assume only the values 0 or $\frac{1}{2}$. $H = 0$ corresponds to a random distribution of A and B molecules, while $H = \frac{1}{2}$ indicates the formation of lipid domains. The other parameter λ (linked to the domain size D through: $\lambda = 2\pi/D$) can be obtained from equation (33). It is not an independent parameter, being related to structural properties of the lipid membrane and its environment (surface charge density, ionic strength, temperature, etc.). Consequently, the dependence of $\langle \delta c(z)\rangle_{X,Y}$ on the electrolyte concentration is quite complex. On one hand, increasing the ion concentration screens the electrostatic potential inside the aqueous phase and so reducing the number of adsorbed ions. On the other hand, high ionic strength favours the formation of lipid micro-domains and, consequently, a stronger electrostatic potential is produced, leading to a larger number of adsorbed ions. A detailed analysis of such effects will be discussed in the following section.

3. Results and discussion

We now calculate the size and stability of lipid micro-domains by means of the formulae developed in the previous section. In particular, equation (33) gives the mean lattice constant of the lipid aggregates, while equations (35) determine their stability condition.

The parameters temperature and interlipid distance were kept constant and set equal to 298 K and 10 Å, respectively. This latter figure is a common value found for phospholipid bilayers [38]. The dielectric constant of the lipid-water interface region ε was set equal to 30 [42–44] and the ionic concentration of the univalent symmetric electrolyte was considered as a variable. In the limit of zero ionic strength the present theory is no longer valid, so the condition $\lambda/\kappa \ll 1$ has always been satisfied in our numerical calculations. The short range forces parameter W (see equation (12)) was taken as an adjustable coefficient as will be discussed later. The results are shown in figure 5 where we show the mean size of lipid clusters D versus the ion concentration of the electrolyte solution in contact with the membrane. A rapid growth of D followed by saturation can be observed. At physiological ion concentration ($\approx 0{\cdot}1$ mole l^{-1}) the salt effect is still relevant. Increase of the short range forces parameter W leads to larger D values, the dependence of D on W being almost linear. Finally, the stability of lipid domains was tested by the two inequalities given in equations (35); in all the cases examined the domain structure is more stable than the random one.

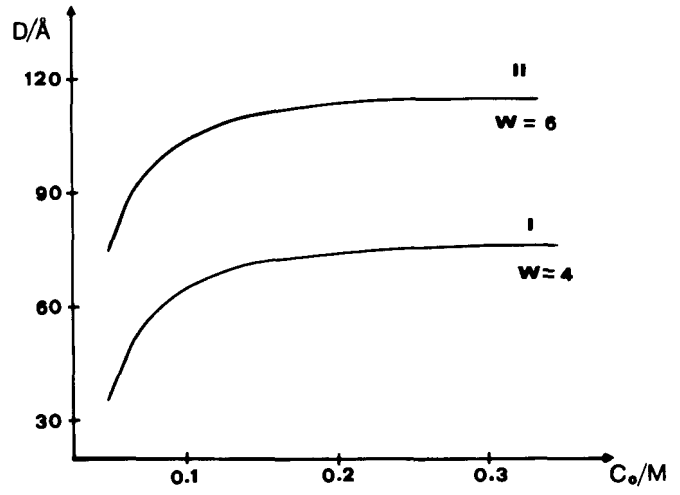

Figure 5. Lattice constant D versus ionic concentration C_0 of the electrolyte solution in contact with the lipid membrane. Curves I and II have been calculated putting the short-range forces parameter W equal to 17 and 25 kJ/mole, respectively.

The ionotropic formation of lipid aggregates is a well-known phenomenon. Unfortunately, most of the data refer to divalent cations (mainly Ca^{2+} [24]) generally mixed with buffers and/or non-specific monovalent ions. Moreover, only very indirect information about domain size can be drawn from the spectroscopic or calorimetric variations observed as a result of the lateral phase separation of lipid components. Consequently, the main aim of this work is to show that despite the unfavourable entropic and electrostatic interactions, the formation of stable micro-domains is a likely phenomenon which can be triggered by spending a little amount of energy. In fact, numerical calculations showed that an energy difference of about 17–25 kJ/mol between like and unlike lipid molecules it is sufficient to produce large micro-domains (~ 100 Å in diameter). This energy can be supplied by divalent cations which form salt bridges between the charged head groups of ionic lipids [45].

Another interesting, related aspect is the study of the effects of lateral phase separation on the number of adsorbed ions on the membrane plane. These effects have been calculated by means of equation (43) and the results are shown in figure 6. Here, we report the difference of concentration $\langle \delta c(z) \rangle_{X,Y}$ between positive and negative ions calculated at a distance z from the membrane plane versus the bulk ion concentration c_0 of the electrolyte solution in contact with the membrane. Curve a has been calculated assuming a random distribution of charged and neutral lipid molecules, curves b and c have been obtained taking into account domain formation at the interface and putting the short range forces parameter W equal to 17 and 25 kJ/mole, respectively. The parameter λ appearing in equation (43) has been calculated by equation (33) and H was set equal to $\frac{1}{2}$ (see the previous section). z was put equal to 5 Å and the values of the other parameters have been stated previously. As we can see, the effect is quite large; the formation of micro-domains always increases the number of adsorbed ions, the effect being dramatic for large values of W and at low ionic strength. However, the effect is still relevant at physiological electrolyte concentrations. Modulation of adsorbed ions concentration through lateral phase separation of lipid components could be a common mechanism in living

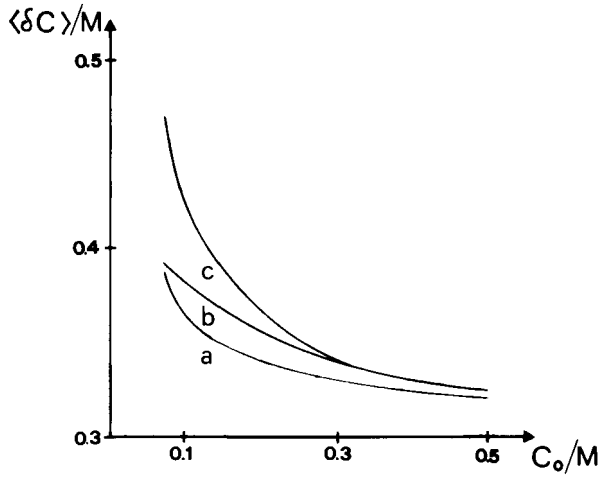

Figure 6. The excess charge $\langle \delta c(z) \rangle_{X,Y}$ versus ionic concentration C_0 of the electrolyte solution in contact with the lipid membrane. All of the calculations were performed by putting $z = 5$ Å. Curve *a* was calculated assuming a random distribution of charged and neutral lipid molecules. Curves *b* and *c* were obtained for a lipid membrane showing lateral phase separation putting the short range forces parameter W equal to 17 and 25 kJ/mole, respectively

cells, as suggested by some authors [3, 4]. Its reliability is confirmed by the present calculations; however a deeper analysis deserves more work and will appear in a forthcoming paper.

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Appendix 1

The calculation of the electrostatic energy of a periodic planar charge distribution (see equation (2)) requires the evaluation of the integral

$$I_n \equiv \int_z^\infty J_n(\lambda t) \exp(-\kappa t)\, dt, \tag{1A 1}$$

where $J_n(\lambda t)$ is a Bessel function. Making use of the integral representation of Bessel functions [46],

$$J_n(X) = \frac{1}{\pi} \int_0^\pi \cos(X \sin\theta - n\theta)\, d\theta, \tag{1A 2}$$

and interchanging the order of integration, we can rewrite equation (1A 1) as

$$I_n = \frac{1}{\pi\lambda} \int_0^\pi \cos n\theta \left(\int_p^\infty \exp(-vx) \cos bX\, dX \right) d\theta$$

$$+ \frac{1}{\pi\lambda} \int_0^\pi \sin n\theta \left(\int_p^\theta \exp(-vx) \sin bX\, dX \right) d\theta, \tag{1A 3}$$

where we have used the shortened notation $b \equiv \sin\theta$, $v \equiv \kappa/\lambda$ and $p \equiv \lambda z$. The integration over dX can be carried out analytically and the final result is

$$I_n = \frac{\exp(-\kappa z)}{\pi\lambda}\int_0^{\pi}\{\cos n\theta(v\cos(p\sin\theta) - \sin\theta\sin(p\sin\theta)) + \sin n\theta(v\sin(p\sin\theta) + \sin\theta\cos(p\sin\theta))\}\cdot\frac{d\theta}{v^2 + \sin^2\theta}. \quad (1A\,4)$$

Expanding $\cos(p\sin\theta)$ and $\sin(p\sin\theta)$ in a power series of Bessel functions [37] and inserting them into equation (1A 4), we can evaluate the integral I_n. It is easy to prove that most of the integrals appearing in the series expansion of I_n vanish. Collecting the surviving terms, we obtain, after some algebra,

$$\begin{aligned} I_n = \frac{\exp(-\kappa z)}{\pi\lambda}[&J_0(p)(v\delta_{n0} + \tfrac{1}{2}\delta_{n1})F_0 + 2J_n(p)(v\gamma_n F_n + (1 - \gamma_n - \delta_{n0})(F_0 - F_n)) \\ &+ J_{n-1}(p)((\gamma_n - \delta_{n0})(F_0 - F_{n-1}) + (1 - \gamma_n - \delta_{n0} - \delta_{n1})F_{n-1}) \\ &- J_{n+1}(p)((\gamma_n + \delta_{n0})(F_0 + F_{n+1}) + (1 - \gamma_n - \delta_{n0})F_{n+1})], \end{aligned} \quad (1A\,5)$$

where the function γ_n is defined as

$$\gamma_n = \begin{cases} 1, & n = 2, 4, 6, \ldots, \\ 0, & n = 0, 1, 3, \ldots, \end{cases}$$

δ_{ij} is the kronecker delta and F_n is the integral [47]

$$F_n \equiv \int_0^{\pi}\frac{\cos^2 n\theta}{v^2 + \sin^2\theta}\,d\theta = \pi\frac{b}{(1 - b^2)^{1/2}}\left[1 + (-1)^n\left(\frac{(1 - b^2)^{1/2} - 1}{b}\right)^n\right] \quad (1A\,6)$$

where

$$b \equiv (2v^2 + 1)^{-1}.$$

when $n = 0$, equation (1A 5) reduces to the result reported in equation (9). It is worth noting that in the limit $z \to 0$ we obtain: $I_0 = (\kappa^2 + \lambda^2)^{-1/2}$, a well-known result [46].

Appendix 2

The integration over $d\phi$ of the double integral G_1 defined in equation (40 *b*) can be performed following the same procedure employed to evaluate equation (6). Expanding $f_1(r, \phi) \equiv \cos(\lambda r\cos\phi)$ in a power series of Bessel functions [37] and integrating over $d\phi$, equation (40 *b*) reduces to

$$G_1 = 2\pi\int_0^{\infty} J_0(\lambda r)\frac{\exp(-\kappa(r^2 + z^2)^{1/2})}{(r^2 + z^2)^{1/2}}\,rdr, \quad (2A\,1)$$

where we have replaced Na by ∞. Making the change of variable $t \equiv (r^2 + z^2)^{1/2}$, equation (2A 1) becomes

$$G_1 = 2\pi\int_z^{\infty} J_0(\lambda(t^2 - z^2)^{1/2})\exp(-\kappa t)\,dt. \quad (2A\,2)$$

Using the series expansion [37]

$$J_v(ab) = a^v\sum_{\sigma=0}^{\infty}\frac{(-1)^{\sigma}}{\sigma!}(a^2 - 1)^{\sigma}\left(\frac{b}{2}\right)^{\sigma}J_{v+\sigma}(b), \quad (2A\,3)$$

we can rewrite equation (2A 2) as

$$G_1 = 2\pi \int_z^\infty J_0(\lambda t) \exp(-\kappa t)\, dt + 2\pi \sum_{\sigma=1}^{\infty} \frac{1}{\sigma!} \left(\frac{\lambda z^2}{2}\right)^\sigma \cdot \int_z^\infty \frac{J_\sigma(\lambda t)}{t^\sigma} \exp(-\kappa t)\, dt. \tag{2A 4}$$

The first integral has been calculated in Appendix 1. An exact evaluation of the remaining integrals is not necessary because their coefficients are proportional to λ^σ ($\sigma \geqslant 1$), which is a small number if z is not too large. Confining the calculation in the range of small z values, we note that $J_\sigma(\lambda t)/t^\sigma$ is a slowly varying function with respect to $\exp(-\kappa t)$ ($J_\sigma(\lambda t) \simeq (\lambda t/2)^\sigma/\Gamma(\sigma + 1)$ see [37], and then can be put outside the integral symbol. Performing the integrations, the final result is

$$G_1 \simeq 2\pi \exp(-\kappa z) \left[\frac{J_0(\lambda z)}{(\kappa^2 + \lambda^2)^{1/2}} + 2J_1(\lambda z) \frac{1}{\lambda} \left(\frac{\kappa}{(\kappa^2 + \lambda^2)^{1/2}} - 1\right)\right]$$
$$+ 2\pi \frac{\exp(-\kappa z)}{\kappa} \sum_{\sigma=1}^{\infty} \frac{1}{\sigma!} \left(\frac{\lambda z}{2}\right)^\sigma J_\sigma(\lambda z). \tag{2A 5}$$

Expanding equation (2A 5) in a power series of λZ and λ/κ and disregarding terms higher order than λ^2, we obtain the result reported in equation (42*b*).

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