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Pores and their number in bilayer lipid membranes

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Résumé. — Dans cet article on donne la définition d'un pore dans une bicouche lipidique. Le nombre des pores est déterminé par la surface S de la membrane et par l'énergie E nécessaire pour la formation d'un pore. Une comparaison est faite avec les méthodes existant jusqu'à présent pour l'explication de la formation des pores.

Abstract. — A proper definition of pores in lipid bilayers is given in this work. The pore number is determined by the area S of the membrane and by the energy E necessary for generation of one pore. A comparison is made with other existing approaches for the explanation of the pore formation.

1. Introduction.

The calculation of the pore number in lipid bilayers is considered in comparatively few papers [1-6]. Usually a clear and unambiguous definition of pore is not given in them. The intuitive concept used is that the pore is the place where the two-dimensional density of the lipid molecules is much less than the average density. Nevertheless, the calculations often use some kind of lattice models of the bilayer, and it is implied that the centre of the pore must coincide with some of the vertices of the lattice. In the present work an attempt is made to treat the pore formation starting from first principles, and to obtain the most general dependence of the number of these pores formed in the membrane on the area of the membrane and on the properties of the molecules constituting the lipid bilayer.

2. Definition of a pore with a radius R_0 in a lipid bilayer. Number of pores in the membrane.

In general, the lipid bilayer is formed by lipid molecules of different kinds. In our considerations we assume that all molecules of the bilayer are equal and indistinguishable. Each molecule can be represented as a three-dimensional body whose limits are determined by the van der Waals radii of the atoms constructing the molecule. We introduce a frame of reference XY in a plane, parallel to the membrane (we consider only flat membranes). Let us project the three-dimensional body representing the molecule on the plane XY . The limits of the projection of the molecule will depend on the coordinates of the atoms of the molecule. Later on when using the symbol « molecules » we will mean the figure restricted by the limits of the projection of the molecule on the plane XY .

Let R_0 be some distance greater than the average intermolecular distance, which is of the order of 10 Å in the lipid bilayer. Let us draw a circumference lying in the plane XY , and having a centre

(x_0, y_0) and a radius $R > R_0$. Let three or more molecules touch this circumference but no molecule is allowed to cross the circumference and or to lie within the disk restricted by it; let three of the points where molecules touch the circumference define an acute-angled triangle. When all these conditions are fulfilled, we will say that there is a pore in the membrane with a centre (x_0, y_0) and with a radius R . Of course the pore thus defined exists only for a fixed state of the membrane, i.e. for fixed coordinates of the atoms of these molecules constituting the bilayer. We choose a big enough value of R_0 so that the total area of the pores at any time is much less than the area of the membrane.

For a given state of the membrane we consider all the pores thus defined. For some states an overlap between two or more pores may appear. By proper choosing of large enough R_0 , it is possible to ensure that the possibility of this overlap is negligible (i.e. the case of an « ideal gas » of pores will be considered, see comments below). We do not exclude fluctuations of the pore from circular form. If, for example, an ellipsoidal defect arises in the membrane, the pore associated with it will have a radius equal to one half of the minor diameter of the ellipse. An integration over all the possible fluctuations of the molecules will be carried out later on (see formula (5)).

No restrictions are imposed upon the nature of the atoms touching the pore contour. If these are predominantly atoms of the hydrophilic head of the amphiphile, the pores will be hydrophilic, otherwise hydrophobic [8]. In the calculations we will not use any properties of the hydrophilic or hydrophobic pores. The pore will be considered as a statistical object, and in principle, for a given radius all the possible forms of the edge will be envisaged, the probability for the different edges (implicitly) depending on their energies. For a given radius, if the pore forms predominantly hydrophilic edges, the density of the projections of hydrophilic head atoms outside near the pore contour will be higher, while the corresponding density for atoms of hydrophobic parts will be lower in comparison with their mean values in a membrane without pores. In the case of hydrophobic pores the ratio of these quantities will not be changed even near the pores.

The pores are a kind of defects existing in the membrane. They increase the permeability of the membrane to a non-specific transport of particles for which the bilayer without the pores acts as a barrier.

Let us consider all the states of the membrane with a fixed number N_1 of pores (as defined above). For given external conditions let the statistical sum be $Z(N_1)$ and the free energy $F(N_1) = -kT \ln Z(N_1)$, where T is the temperature of the membrane and k the Boltzmann constant. In order to proceed with the calculations, we consider membranes under the following external conditions: the area of the membrane S , the number of molecules constituting the bilayer N_0 and the temperature T . The general statistical sum $Z(N_0, S, T)$ is:

$$Z(N_0, S, T) = \sum_{N_1=0}^{\infty} Z(N_1, N_0, S, T) \quad (1)$$

The free energy $F(N_0, S, T)$ of the membrane, corresponding to this statistical sum is:

$$F(N_0, S, T) = -kT \ln [Z(N_0, S, T)]. \quad (2)$$

The mean number of pores in the membrane $\bar{N}_1(N_0, S, T)$ for given N_0 , S , and T must be equal to the value N'_1 at which the function $Z(N_1, N_0, S, T)$ reaches its maximum value with respect to N_1 . The reason for this is based on the fact that around its maximum the function $Z(N_1, N_0, S, T)$ is symmetrical according to the variable N_1 , and $N_1(N_0, S, T)$ depends mainly on the highest values of $Z(N_1, N_0, S, T)$. It can be written that:

$$Z(N_1, N_0, S, T) = \frac{1}{N_0! \lambda^{3nN_0}} \int_{\theta_{N_1}} \exp \left[-\frac{U(\mathbf{r}_1, \mathbf{r}_2, \dots, \mathbf{r}_{N_0})}{kT} \right] d\mathbf{r}_1 d\mathbf{r}_2 \dots d\mathbf{r}_{N_0} \quad (3)$$

where :

$$\lambda^2 = \frac{h^2}{2 \pi m k T}, \quad (4)$$

$U(\mathbf{r}_1, \mathbf{r}_2, \dots, \mathbf{r}_{N_0})$ is the Hamiltonian of the system, $\mathbf{r}_i$ is the set of the three dimensional radius-vectors of the atom of the i -th molecule, n is the number of these atoms per molecule, $m = \sqrt[n]{m_1 m_2 \dots m_n}$ where m_j , $j = 1, 2, \dots, n$, is the mass of the j -th atom of the molecule, θ_{N_1} is the area in the $3 n N_0$ dimensional space of the coordinates of the atoms of the molecules, each point of which corresponds to a state of the membrane with N_1 pores, and h is the Planck's constant.

Let us denote the indices of the molecules which determine the pores (three molecules per each pore) with $1, 2, \dots, 3 N_1$, and the indices of the other molecules with $3 N_1 + 1, 3 N_1 + 2, \dots, N_0$. The i -th pore is characterized by its radius R_i , by the radius vector $\mathbf{t}_i = (t_i^1, t_i^2)$ of the centre of the pore and by the three polar angles $\psi_i^1, \psi_i^2, \psi_i^3$ ($0 \leq \psi_i^1 < \psi_i^2 < \psi_i^3 < 2 \pi$) of the three points, fixing the pore; these angles are measured with reference to the X axis, say. Then the statistical sum $Z(N_1, N_0, S, T)$ can be presented as follows :

$$Z(N_1, N_0, S, T) = \frac{1}{N_0! \lambda^{3nN_0}} \cdot \left(\frac{N_0!}{N_1! (N_0 - 3 N_1)!} \right) \times \\ \times \int_{\theta_{N_1}} \exp \left[- \frac{U(R_1, \dots, R_{N_1}, \psi_1^1, \psi_1^2, \psi_1^3, \dots, \psi_{N_1}^1, \psi_{N_1}^2, \psi_{N_1}^3, \mathbf{t}_1, \mathbf{t}_2, \dots, \mathbf{t}_{N_1}, \mathbf{r}'_1, \dots, \mathbf{r}'_{3N_1}, \mathbf{r}_{3N_1+1}, \dots, \mathbf{r}_{N_0})}{kT} \right] \times \\ \times \prod_{i=1}^{N_1} [R_i^3 D(\psi_i^1, \psi_i^2, \psi_i^3) dR_i d\psi_i^1 d\psi_i^2 d\psi_i^3 dt_i] d\mathbf{r}'_1 \dots d\mathbf{r}'_{3N_1} d\mathbf{r}_{3N_1+1} \dots d\mathbf{r}_{N_0}. \quad (5)$$

The numerical factor before the integral in equation (5) can be obtained in the following way. Let us consider at first that all molecules of the membrane are distinguishable. The indistinguishability can be introduced later on by adding the multiplier $\frac{1}{N_0!}$ in the statistical sum of the distinguishable molecules. The number of ways to choose the $3 N_1$ molecules from all the N_0 molecules is :

$$N_0(N_0 - 1) \dots (N_0 - 3 N_1 + 1) = \frac{N_0!}{(N_0 - 3 N_1)!}.$$

After carrying out the integration in [5] with this numerical factor, each state will be counted $N_1!$ times. Indeed if the i -th and j -th pores change their centres, radii and angles $\psi_i^1, \psi_i^2, \psi_i^3$ (this change appears in the course of the integration), the new state of the membrane will be identical to that prior this change, i.e. the pores in the membrane constituted of distinguishable molecules behave like indistinguishable objects. To avoid this unwanted factor, we multiply the integral by $\frac{1}{N_1!}$.

Adding the multiplier $\frac{1}{N_0!}$ leads to formula (5).

In (5) the quantities $\mathbf{r}'_l (i = 3(m - 1) + l, 1 \leq m \leq N_1, 1 \leq l \leq 3)$ represent the set of coordinates of the atoms of the i -th molecule which touches the m -th pore at the l -th point of the three points determining this pore ($\mathbf{r}'$ is a vector whose dimension is less by 2 than that of the vector $\mathbf{r}_i$); $R_i^3 D(\psi_i^1, \psi_i^2, \psi_i^3)$ is the determinant of transformation from the Cartesian coordinates of the atoms of the molecules, determining the i -th pore to the coordinates $R_i, \mathbf{t}_i, \psi_i^1, \psi_i^2, \psi_i^3, \mathbf{r}'_{3i-2}, \mathbf{r}'_{3i-1}, \mathbf{r}'_{3i}$. The area of integration of $\psi_i^1, \psi_i^2, \psi_i^3$ is determined by the requirement that the three points must be vertices of an acute angled triangle, and that $0 \leq \psi_i^1 < \psi_i^2 < \psi_i^3 < 2 \pi$.

If the coordinates of the $3 N_1$ points, determining the N_1 pores, are fixed, the $N_0 - 3 N_1$ molecules which do not touch any pore will dispose of an area $S - \sum_{i=1}^{N_1} \pi R_i^2$; other pores are not supposed to be present in this area. Consequently its free energy depends on the mean area per molecule [7], and on the total length of the edges as well as the radii of the pores. Because of the low number of pores, we assume that there are no common molecules touching different pores (this is equivalent to the assumption that the pores have a behaviour of an ideal gas of non-interacting pores. This assumption is more valid as the radius R_0 becomes larger, because an increase of R_0 results in a large decrease of the total area taken by the pores). We proceed by noting that the integration over the area without pores can be presented as :

$$\begin{aligned} & \frac{1}{\lambda^{3n(N_0-3N_1)}(N_0-3N_1)!} \times \\ & \times \int_{\theta_{N_1}} \exp \left[- \frac{U(R_1, \dots, R_{N_1}, \psi_1^1, \psi_1^2, \psi_1^3, \dots, \psi_{N_1}^1, \psi_{N_1}^2, \psi_{N_1}^3, \mathbf{t}_1, \mathbf{t}_2, \dots, \mathbf{t}_{N_1}, \mathbf{r}'_1, \dots, \mathbf{r}'_{3N_1}, \mathbf{r}_{3N_1+1}, \dots, \mathbf{r}_{N_0})}{kT} \right] \times \\ & \times d\mathbf{r}_{3N_1+1} \dots d\mathbf{r}_{N_0} = \\ & = \exp - \frac{1}{kT} \left\{ (N_0 - 3 N_1) \cdot f \left[\frac{S - \sum_{i=1}^{N_1} \pi R_i^2}{N_0 - 3 N_1} \right] + \tilde{F}'(R_1, \psi_1^1, \psi_1^2, \psi_1^3, \mathbf{r}'_1, \mathbf{r}'_2, \mathbf{r}'_3, a) + \right. \\ & \quad \left. + \dots + \tilde{F}'(R_{N_1}, \psi_{N_1}^1, \psi_{N_1}^2, \psi_{N_1}^3, \mathbf{r}'_{3N_1-2}, \mathbf{r}'_{3N_1-1}, \mathbf{r}'_{3N_1}, a) \right\} \quad (6) \end{aligned}$$

where « a » is the mean area per molecule, $a \approx \frac{S}{N_0}$, $f(a)$ is the free energy per molecule with a mean area « a » in a membrane without pores, and $\tilde{F}'(R_i, \psi_i^1, \psi_i^2, \psi_i^3, a)$ is the correction to the total free energy of the system caused by the presence of the i -th pore, when the three points defining it are fixed.

The integration with respect to the quantities $\mathbf{r}'_j$ will give as a result a change of the quantities $\tilde{F}'_i$:

$$\begin{aligned} & \frac{1}{\lambda^{3(n-2)N_1}} \int \exp - \frac{1}{kT} \left\{ (N_0 - 3 N_1) \cdot f \left[\frac{S - \sum_{i=1}^{N_1} \pi R_i^2}{N_0 - 3 N_1} \right] + \tilde{F}'(R_1, \psi_1^1, \psi_1^2, \psi_1^3, \mathbf{r}'_1, \mathbf{r}'_2, \mathbf{r}'_3, a) + \right. \\ & \quad \left. + \dots + \tilde{F}'(R_{N_1}, \psi_{N_1}^1, \psi_{N_1}^2, \psi_{N_1}^3, \mathbf{r}'_{3N_1-2}, \mathbf{r}'_{3N_1-1}, \mathbf{r}'_{3N_1}, a) \right\} d\mathbf{r}_1 \cdot d\mathbf{r}_2 \dots d\mathbf{r}_{3N_1} = \\ & = \exp - \frac{1}{kT} \left\{ (N_0 - 3 N_1) \cdot f \left[\frac{S - \sum_{i=1}^{N_1} \pi R_i^2}{N_0 - 3 N_1} \right] + \tilde{F}(R_1, \psi_1^1, \psi_1^2, \psi_1^3, a) + \right. \\ & \quad \left. + \dots + \tilde{F}(R_{N_1}, \psi_{N_1}^1, \psi_{N_1}^2, \psi_{N_1}^3, a) \right\} \quad (6') \end{aligned}$$

$$f \left[\frac{S - \sum_{i=1}^{N_1} \pi R_i^2}{N_0 - 3 N_1} \right] = f \left(\frac{S}{N_0} \right) - \left. \frac{\partial f}{\partial a} \right|_{a=\frac{S}{N_0}} \times \left[\sum_{i=1}^{N_1} \left(\frac{\pi R_i^2}{N_0} - 3 \frac{S}{N_0^2} \right) \right]. \quad (7)$$

But :

$$\left. \frac{\partial f}{\partial a} \right|_{a = \frac{s}{N_0}} = \sigma \quad (8)$$

where σ is the stress applied to the membrane if there are no pores in it. For a given area, the membrane will have different stresses depending on the presence or absence of pores [6]. But because of their low number we disregard this difference and consider σ as the real tension of the membrane. Then the following equality is fulfilled :

$$(N_0 - 3 N_1) f \left[\frac{S - \sum_{i=1}^{N_1} \pi R_i^2}{N_0 - 3 N_1} \right] = N_0 f \left(\frac{S}{N_0} \right) + \sum_{i=1}^{N_1} \left[-3 f \left(\frac{S}{N_0} \right) - \sigma \left(\pi R_i^2 - 3 \frac{S}{N_0} \right) \right] \quad (9)$$

We denote :

$$E(\sigma, R_0) = -kT \ln \left\{ \frac{1}{\lambda^4} \int_{R=R_0}^{R_{\max}} dR G(\sigma, R) \right\} \quad (10)$$

where :

$$G(\sigma, R) = \int d\psi^1 d\psi^2 d\psi^3 R^3 D(\psi^1, \psi^2, \psi^3) \times \\ \times \exp \left[- \frac{\tilde{F}(R, \psi^1, \psi^2, \psi^3, a) - \sigma \left(\pi R^2 - 3 \frac{S}{N_0} \right) - 3 f \left(\frac{S}{N_0} \right)}{kT} \right] \quad (10')$$

In (10) the integration with respect to R is performed up to $R_{\max}$ and not to ∞ . $R_{\max}$ is the value of R , at which the function $G(\sigma, R)$ reaches its maximum value as a function of R at a given σ . If a pore appears with a radius $R > R_{\max}$, then the subsequent increase of R will decrease the energy of the membrane, and because this process cannot be stopped, the membrane will rupture [1-3, 8]. $R_{\max} < \infty$ exists only if $\sigma > 0$. Consequently if $\sigma > 0$, we consider only pores whose radius is less than $R_{\max}$. Strictly speaking, for $\sigma > 0$ the membrane has finite lifetime, because a greater than zero possibility exists for formation of a pore with a radius $R > R_{\max}$ [2, 3].

We call the quantity $E(\sigma, R_0)$ the energy of a pore with a radius R_0 .

$$Z(N_1, N_0, S, T) = \frac{1}{N_1! \lambda^{2N_1}} \int dt_1 \dots dt_{N_1} \exp \left[- \frac{N_0 f \left(\frac{S}{N_0} \right) + N_1 E(\sigma, R_0)}{kT} \right].$$

By means of the Stirling formula $N! \approx \left(\frac{N}{e} \right)^N$, where e is the base of the natural logarithms :

$$Z(N_1, N_0, S, T) = \left(\frac{S}{\lambda^2} \right)^{N_1} \frac{1}{\left(\frac{N_1}{e} \right)^{N_1}} \exp \left[- \frac{N_0 f \left(\frac{S}{N_0} \right) + N_1 E(\sigma, R_0)}{kT} \right]. \quad (11)$$

The free energy $F(N_1, N_0, S, T)$ is :

$$F(N_1, N_0, S, T) = -kT \ln Z(N_1, N_0, S, T) \\ = N_0 f(a) + N_1 E(\sigma, R_0) - kTN_1 \ln \left(\frac{S}{\lambda^2} \right) + kTN_1 \ln \left(\frac{N_1}{e} \right) \quad (12)$$

where « a » is the mean area per molecule in the membrane, $a = S/N_0$.

The function $F(N_1, N_0, S, T)$ reaches its minimum with respect to N_1 when $\partial F / \partial N_1 = 0$, which gives as a result :

$$\frac{E(\sigma, R_0)}{kT} - \ln \left(\frac{S}{\lambda^2} \right) + \ln \bar{N}_1 = 0. \quad (13)$$

After some calculations :

$$\bar{N}_1(R_0, N_0, S, T) = N_0 \frac{a}{\lambda^2} \exp \left[- \frac{E(\sigma(a), R_0)}{kT} \right] \quad (14)$$

where $a = S/N_0$.

Formula (10) shows that the quantity $E(\sigma, R_0)$ depends on T as well as on σ and R_0 .

In principle σ depends not only on a , but on N_1 as well. But this dependence is important when the area, taken by the pores, is a large enough part of the membrane. This case is not considered by us. Its treatment requires knowledge of the energy of interaction between two pores the distance between them being of the order of their radii.

3. Discussion.

Formula (14) presents the basic result of our work. It shows that the mean number of the pores in the membranes is proportional, at fixed external conditions, to N_0 .

Naturally the question arises : what is the physical meaning of the quantity $E(\sigma, R_0)$? To clarify it, we make the following considerations.

Up to now we considered the pores as objects which can change their positions. Let us consider now a static defect with a fixed centre in the membrane. Let the defect be of such a kind, that in a circle with a radius R_c around a centre (x_c, y_c) there are no lipid molecules. We can then assume that the change ΔF of the free energy of the whole membrane due to the presence of this defect, according to [1] is :

$$\Delta F = 2 \pi \gamma(R_c, \sigma) \cdot R_c - \pi \sigma R_c^2 \quad (15)$$

where $\gamma(R_c, \sigma)$ is the edge energy of a curved edge with a radius R_c at a tension σ of the membrane [8]. The quantity ΔF can be expressed in terms of $E(\sigma, R_c)$. To do this, we take into account the fact that the static defect can exist only if a pore, as described in the beginning of the present work, always exists and this pore includes the circle with radius R_c and centre (x_c, y_c) (it is not necessary that the centre of the pore coincides with (x_c, y_c)). The statistical sum Z_{def} of the system with such a defect is :

$$Z_{\text{def}} = \frac{1}{N_0 !} \frac{1}{\lambda^{3N_0 n}} \int \exp \left[- \frac{U(\mathbf{r}_1, \mathbf{r}_2, \mathbf{r}_3, \mathbf{r}_4, \dots, \mathbf{r}_{N_0})}{kT} \right] d\mathbf{r}_1 \dots d\mathbf{r}_{N_0}. \quad (16)$$

In (16) $\mathbf{r}_1, \mathbf{r}_2, \mathbf{r}_3$ are the sets of the coordinates of the atoms of the molecules, determining the pore with the above mentioned properties. The calculation of Z_{def} gives :

$$Z_{\text{def}} = \frac{2\pi}{\lambda^2} \int_0^{(R_{\text{max}} - R_c)} \exp\left[-\frac{N_0 f(a) + E(\sigma, \rho + R_c)}{kT}\right] (\rho + R_c) d\rho \quad (17)$$

$$\Delta F = F(\text{membrane with defect}) - F(\text{membrane without defect}) =$$

$$= -kT \ln \left[\frac{2\pi}{\lambda^2} \int_0^{(R_{\text{max}} - R_c)} \exp\left[-\frac{E(\sigma, \rho + R_c)}{kT}\right] (\rho + R_c) d\rho \right]. \quad (18)$$

From equations (14), (15) and (18) :

$$2\pi\gamma(R_c, \sigma) \cdot R_c - \pi\sigma R_c^2 = -kT \ln \left[\frac{2\pi}{a} \int_0^{(R_{\text{max}} - R_c)} \frac{\bar{N}_1[(\rho + R_c), N_0, S, T]}{N_0} d\rho \right] (\rho + R_c) d\rho. \quad (19)$$

After changing the variables $\rho' = \rho + R_c$, differentiating with respect to R_c and some algebra, the following result is obtained ($R_c < R_{\text{max}}$) :

$$\frac{\bar{N}_1(R_c, N_0, S, T)}{N_0} = \frac{a}{kT} \left\{ \exp\left[-\frac{2\pi\gamma(R_c, \sigma) \cdot R_c - \pi\sigma R_c^2}{kT}\right] \right\} \cdot \left[\frac{\gamma(R_c, \sigma)}{R_c} + \frac{\partial\gamma(R_c, \sigma)}{\partial R_c} - \sigma \right]. \quad (20)$$

If R_c is large enough, with however $R_c \ll R_{\text{max}}$, the derivative $\frac{\partial\gamma(R_c, \sigma)}{\partial R_c} \approx 0$, and $\gamma(R_c, \sigma) \approx \gamma$, where γ is the edge energy of the straight edge of the membrane. The value of R_{max} is of the order of γ/σ [1, 8], consequently, if $R_c \ll R_{\text{max}}$, γ/R_c is greater than σ . Under these conditions, equation (20) can be presented in the simpler form :

$$\bar{N}_1(R_c, N_0, S, T) = N_0 \frac{a\gamma}{kT R_c} \exp\left[-\frac{2\pi\gamma(R_c, \sigma) - \pi\sigma R_c^2}{kT}\right]. \quad (21)$$

Comparison with equation (14) shows that in this case the following equality is fulfilled :

$$E(\sigma, R_c) \approx 2\pi\gamma R_c - \pi\sigma R_c^2. \quad (22)$$

This result has been used elsewhere [1, 8].

The mean number of pores having a radius in the interval $(R_c, R_c + dR_c)$ is :

$$\begin{aligned} \Delta N(R_c, R_c + dR_c, N_0, S, T) &= \bar{N}_1(R_c, N_0, S, T) - \bar{N}_1(R_c + dR_c, N_0, S, T) \\ &= N_w(R_c, N_0, S, T) \cdot dR_c. \end{aligned} \quad (23)$$

The last equation can be considered as a definition for the density $N_w(R_c, N_0, S, T)$ of the number of pores, having a radius R_c . The result for $N_w(R_c, N_0, S, T)$ is :

$$\begin{aligned} N_w(R_c, N_0, S, T) &= \frac{N_0 a}{kT} \left\{ \exp\left[-\frac{2\pi\gamma(R_c, \sigma) - \pi\sigma R_c^2}{kT}\right] \right\} \times \\ &\times \left\{ \frac{2\pi R_c}{kT} \left[\frac{\gamma(R_c, \sigma)}{R_c} + \frac{\partial\gamma(R_c, \sigma)}{\partial R_c} - \sigma \right]^2 - \frac{1}{R_c} \frac{\partial\gamma(R_c, \sigma)}{\partial R_c} + \frac{\gamma(R_c, \sigma)}{R_c^2} - \frac{\partial^2\gamma(R_c, \sigma)}{\partial R_c^2} \right\}. \end{aligned} \quad (24)$$

In the limit of R_c large enough, with $R_c \ll R_{\max}$, the expression (24) becomes :

$$N_w(R_c, N_0, S, T) = \frac{N_0 a}{(kT)^2} \frac{2\pi}{R_c} \gamma^2 \exp\left[-\frac{2\pi\gamma R_c - \pi\sigma R_c^2}{kT}\right]. \quad (25)$$

Equations (24) and (25) allow calculation of the pore size distribution once knowing the pore radius dependence of the edge energy γ . This dependence can be obtained on the basis of molecular models [8].

The model of a pore, used in [8], is exactly similar to the model of the static defect described here ; there the pore is represented as the inner half of a torus, which connects the surfaces where the hydrophilic heads of the molecules of the two monolayers comprising the bilayer are situated ; there are no other restrictions on the positions of the heads and the nearest molecules can, in principle, be arbitrarily far from the pore. Equations (15) and (18) give a relation between σ , $\gamma(R_c, \sigma)$ and $E(\sigma, R_c)$.

Up to now, the pores have been considered circular. The presentation of the simultaneous pore with three molecules allows to keep the total number of degrees of freedom of all the molecules. Each other presentation of these pores with more than three molecules touching the same circumference (which is a widely spread concept for the pores) will restrict the membrane to a « surface » with a lower dimension in the configurational space of coordinates of molecules forming the membrane. Consequently, the probability of appearance for such an object will be equal to zero.

In principle, the approach presented above can be generalized for pores with arbitrary shapes. Any other shape, different from the circumference, has not a circular symmetry. The pore with such a shape must be characterized with its position (this can be the mass centre of the figure, restricted by the contour), with its size (this can be the half of the maximal distance between two points lying on the contour), and with its a direction (this can be the angle between the vector, connecting two peculiar points of the contour, and the axis X). In general, if a contour different from a circumference is given, this contour must be specified with 4 points (for example, through 4 points one can draw one ellipse only with a given eccentricity ; it is possible to choose four other points, for which this is not true, but the probability to find them is equal to zero). These 4 points must be chosen so that each infinitesimal rotation and translation of the pore will give as a result, that at least one of these points will be found in the interior of the pore (this is the analog of the requirement that the three points defining the circular pore, form an acute angled triangle). The degree of freedom of the molecules will not be reduced ; the degrees of freedom lost because of the existence of a pore are compensated by the two degrees of freedom of the centre of the pore, and by the possibility for the pore to change its dimensionality and orientation. All considerations for obtaining the formula (14) can therefore be repeated, using 4 points per pore ; in the integrals, analogous to (6) and (6'), an additional integration towards the direction of the pore will appear. If we denote the contour with L , the formula, analogous to (14) will be :

$$\overline{N}_1^L(R_0, N_0, S, T) = N_0 \frac{a}{\lambda^2} \exp\left[-\frac{E^L(\sigma(a), R_0)}{kT}\right] \quad (26)$$

where R_0 is the generalized size of the pore, $\overline{N}_1^L(R_0, N_0, S, T)$ and $E^L(\sigma(a), R_0)$ are the analogues of $\overline{N}_1(R_0, N_0, S, T)$ and $E(\sigma(a), R_0)$ in (14) for a pore with the shape of an L ; all other notations as in (14). Formula (15) must also be generalized because the edge energy of the static defect with the shape of an L and with size R_c depends on the local radius of curvature and on all its derivatives along the contour ; πR_c^2 from (15) must be replaced by $b R_c^2$, where b is a coefficient depend-

ing on the form of the contour L. Hence, the analog of (15) :

$$\Delta F = \oint_L dl \left\{ \gamma \left[\sigma, R(l), \frac{\partial R(l)}{\partial l}, \frac{\partial^2 R(l)}{\partial l^2} \dots \right] \right\} - \sigma b R_c^2$$

where $R(l)$ is the radius of curvature of the contour in the point of coordinate l .

For the determination of a Z_{def} similar to that of (16), an integral over all the states with pores having contour L, and including the static defect must be carried out. The result of the integration cannot be presented in a simple form, analogous to (19), because the limits of the integration with respect to the direction of the pore, including the static defect, depend on the mutual disposition of the centres of the static defect and of the simultaneous pore, as well as on the size of this pore. For each contour form proper calculations of Z_{def} must be carried out. As a result, equations similar to (20) and (21) will appear, but corrected with « form factors » specific for each contour; the dependence of the edge energy on the derivatives of the curvature along the edge will be included as well.

On obtaining (14) we assumed that N_0 and N_1 are large enough for the Stirling formula and the results of the statistical mechanics, valid for large number of particles to be used. Consequently, the connection which we found between the radius of the pores, the edge energy for this radius and the numbers of pores can be used for interpreting experimental data on membranes with large enough areas — for example the permeability of the membranes of giant vesicles, the resistance of macroscopic artificial lipid bilayers, when ions with considerable dimensions are the charge carriers, etc.

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