

## Stabilization of membrane pores by packing

D. J. Bicout,<sup>1,2</sup> F. Schmid,<sup>1,3</sup> and E. Kats<sup>1,4</sup>

<sup>1</sup>*Institut Laue-Langevin, 6 rue Jules Horowitz, B.P. 156, 38042 Grenoble, France*  
<sup>2</sup>*Biomathematics and Epidemiology, ENVL-TIMC, B.P. 83, 69280 Marcy l'Etoile, France*

<sup>3</sup>*Fakultät für Physik, Universität Bielefeld, D-33615 Bielefeld, Germany*

<sup>4</sup>*L. D. Landau Institute for Theoretical Physics, RAS, 117940 GSP-1, Moscow, Russia*

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We present a model for pore stabilization in membranes without surface tension. Whereas an isolated pore is always unstable (since it either shrinks, tending to reseal, or grows without bound until membrane disintegration), it is shown that excluded volume interactions in a system of many pores can stabilize individual pores of a given size in a certain range of model parameters. For such a multipore membrane system, the distribution of pore size and associated pore lifetime are calculated within the mean-field approximation. We predict that, above the temperature  $T_m$ , when the effective line tension becomes negative, the membrane exhibits a dynamic sievelike porous structure.

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In addition to the area occupied by macromolecules, e.g., lipids or proteins, membranes are complex structures that incorporate also empty areas or pores in a varying amount. Of various sizes and shapes, membrane pores enhance the transport of biomolecules across the membranes and their biological relevance can bring new prospective biotechnological applications (see, e.g., Refs. [1,2]). Pores can form in membranes in response to thermal fluctuations, thermally activated poration process, or external influences, and their subsequent growth is controlled by the effective line tension (assuming a negligible small surface tension). A schematic depiction of a membrane with holes is displayed in Fig. 1. Preliminary computer simulations of coarse-grained models for lipid membranes have indicated that close to their disintegration point, membranes sometimes exhibit a perforated state where they are peppered of many pores [3]. In the present paper, we investigate a mechanism that can stabilize such a state.

We first consider a single pore formation in a membrane with zero surface tension. For simplicity, the pore edge is regarded as a closed self-avoiding path of  $n$  steps of constant length  $l$ . In the simplest approximation, the free energy (difference between the state with and that without pores) of the system (tensionless membrane with a pore of size  $n$ ) at non-zero temperature can be written as a summation of two contributions: a purely energetic part as suggested by Litster [4], plus an entropic part as modeled by Shillcock and Boal [5],  $f(n) = f_0 + \lambda_0 \ln n - k_B T \ln[\omega(n)]$ , where  $f_0$  is an  $n$ -independent energy,  $\lambda_0$  the bare line tension of the pore edge,  $k_B T = 1/\beta$  the thermal energy, and  $\omega(n)$  the number of possible conformations of the pore contour of size  $n$ . Experimental data (see, e.g., [6,7]) typically yield values of  $\lambda_0$  in the range  $10^{-7} - 10^{-6}$  erg/cm. For self-avoiding walks,  $\omega(n)$  has the general form [8,9]  $\omega(n) = \omega_0 z^n n^{\alpha-2}$  where  $\omega_0$  is a constant,  $z$  the effective connectivity constant of the medium (both  $\omega_0$  and  $z$  depend on microscopic details and are of order of 1–10), and the exponent  $\alpha = 1/2$  for self-avoiding random walks in two dimensions and  $\alpha < 2$  for any kind of pores. Let  $n_0$  be the minimal size of a pore. The above free energy can then be rewritten as  $f(n) = 0$  for  $0 \leq n \leq n_0$ , and for  $n > n_0$ ,

$$f(n) = F_0 + \overbrace{\lambda_1} \left(1 - \frac{T}{T_m}\right) l [n - n_0] + (2 - \alpha) k_B T \ln\left(\frac{n}{n_0}\right), \quad (1)$$

where we have defined  $F_0$  as the free energy required to create or initiate a minimal-sized pore,  $\lambda_1$  the entropically modified line tension of the pore edge, and  $T_m = \lambda_0 l / k_B T \ln(z)$  the disintegration temperature. In what follows, we will neglect the logarithmic term,  $(2 - \alpha) \ln(n/n_0)$ , that only slightly renormalizes the results. Simple inspection of Eq. (1) indicates that the free energy of the system monotonically increases as the pore grows larger at low  $T < T_m$  when  $\lambda_1 > 0$ , keeping the membrane stable with an unstable pore that reseals. In this case, a pore of any size has a finite lifetime and will ultimately shrink to disappearance. In contrast, when the effective line tension is negative,  $\lambda_1 < 0$ , at high  $T > T_m$ , all newly initiated pores grow without bound (i.e., with a diverging lifetime) leading hence to destabilization and disintegration of the membrane. There are a number of works that have investigated mechanisms for stabilizing membranes with a single pore. These include, for instance, membrane bending fluctuations, renormalization of linear and surface tension coefficients [10], area exchange in tense membranes [11], osmotic stress [12], hydrodynamics [6,13], orientational ordering [14], and others (see Ref. [15] for more details and references). In this paper we focus on a stabilizing effect, which is created by the presence of multiple pores in the membrane.

Indeed, as the probability of initiating several pores on a membrane increases as the temperature gets higher, we are now confronted with the situation of a membrane with an ensemble of pores (as in Refs. [16,17]). If the bare line tension is negative, the system tries to create as much pore rim as possible [18]. In that case, a membrane state with many small pores is more favorable than one with only one very large pore. In some sense, such a state is similar to a droplet microemulsion structure in amphiphilic systems, where the fluid is macroscopically homogeneous, but filled with internal interfaces on the microscopic scale [19]. To quantify this expectation, we proceed as follows: First, we consider the

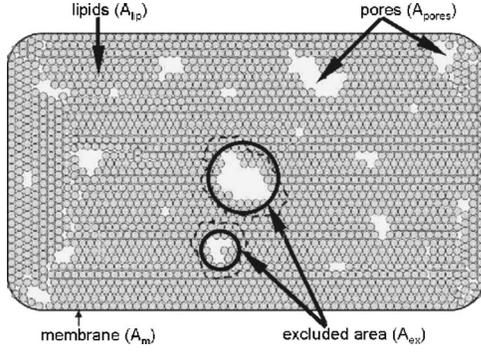


FIG. 1. Schematic depiction of a porous membrane, illustrating the different areas introduced in the text: The total surface  $A_{lip}$  covered by all lipids (gray filled circles), the total area of pores  $A_{pores}$ , and the total area  $A_m = A_{lip} + A_{pores}$ . The circle around the pore represents the area that is effectively excluded by the pore, and the total excluded area for all pores is  $A_{ex}$ . Due to the fractal shape of pores, the excluded area of pores is larger than their actual area.

free energy  $F\{N(n)\}$  of a membrane where the number  $N(n)$  of pores with contour length  $n$  is fixed (constrained). In the second step, we will relieve the constraint and minimize  $F\{N(n)\}$  with respect to  $N(n)$ . The free energy  $F\{N(n)\}$  has energetic line tension contributions and entropic shape contributions as in Eq. (1). Moreover, the pores have the translational entropy of a two-dimensional gas. However, they may not overlap, since a configuration with two “overlapping” pores would have to be replaced by a new configuration with just one, larger pore, and a different  $N(n)$ . For fixed  $N(n)$ , this restricts the translational degree of freedom of pores, as if they had excluded volume interactions. Coarse-grained molecular-dynamics simulations performed by Loison, Mareschal, and one of us (F.S.) [17] have demonstrated that the spatial distribution of pores in a model membrane is compatible with a hard-core repulsion between them. Note that these “interactions” are purely entropic. One could also introduce real repulsive interactions between pores, originating, e.g., from direct electrostatic or van der Waals forces, entropic or Helfrich interactions arising from pore shape fluctuations, or from membrane undulations [20,21]. However, it is not necessary for our argument to include those interactions, and they can be qualitatively lumped into the excluded area constraints.

To proceed we adopt a simple van der Waals approach and approximate the free energy  $F\{N(n)\}$  by  $F\{N(n)\} = \sum_n N(n)f(n) - k_B T \ln[\Omega]$ , where  $f(n)$  is the free energy of a single pore as given in Eq. (1), and the total translational entropy  $\Omega$  of the pores is given by

$$\begin{aligned} \Omega &= \prod_n \{1/[N(n)]!\} [(A_m - A_{ex})/a_0]^{N(n)} \\ &\approx \prod_n [(A_{lip} - A_0)/a_0 N(n)]^{N(n)} \exp\{N(n)\}. \end{aligned} \quad (2)$$

Here  $a_0$  is a reference area defined below,  $A_m$  is the total membrane area,  $A_m = A_{lip} + A_{pores}$ , with the area of lipids  $A_{lip}$  and the pore area  $A_{pores}$ , and  $A_{ex}$  is the area that is effectively inaccessible to a test pore due to the presence of the other

pores (the excluded area). Due to the fractal nature of the pores,  $A_{ex}$  is larger than  $A_{pores}$  as indicated in Fig. 1. Notice that  $A_{lip}$  is constant since it is proportional to the number of lipids. In writing the rightmost expression in Eq. (2), we have used the approximation,  $N! \approx N^N e^{-N}$ , and have rewritten the accessible area for pores as  $A_m - A_{ex} = A_{lip} - A_0$ , such that  $A_0 = A_{ex} - A_{pores} = \sum_n N(n)a(n)$ , where  $a(n)$  is the difference between the excluded and the actual areas of a pore of size  $n$ . Since the contours of the pores have self-avoiding walk statistics,  $a(n)$  scales like  $a(n) = a_0(n/n_0)^{2\nu}$  with the Flory exponent  $\nu \approx 3/4$  [22]. This defines  $a_0$ . Now, inserting Eq. (2) into the free-energy expression, and minimizing with respect to  $N(n)$ , yields the normalized equilibrium distribution  $P_{eq}(n)$  of pore sizes,

$$P_{eq}(n) = N(n) \left/ \sum_{n=1}^{\infty} N(n) \right. = \frac{\exp\{-\beta G(n)\}}{Q}. \quad (3)$$

Here we have defined the effective free energy,  $G(n) = f(n) + k_B T Q (n/n_0)^{2\nu}$ . The partition function  $Q$  can be regarded as a packing density of pores, and is given self-consistently as

$$Q = \frac{a_0}{(A_{lip} - A_0)} \sum_n N(n) = \int_1^{\infty} e^{-\beta G(x)} dx. \quad (4)$$

Defining  $x = n/n_0$  and using  $\nu = 3/4$ , the effective free-energy  $G(x)$  of a pore in a membrane containing an ensemble of pores (in gas phase) reads as  $G(x) = 0$  for  $0 \leq x < 1$ , and

$$G(x) = F_0 + Q k_B T + n_0 l \int_1^x \lambda_{eff}(x') dx'; \quad x \geq 1, \quad (5)$$

where the effective line tension  $\lambda_{eff}$  is defined as

$$\lambda_{eff}(x) = \lambda_0(1 - T/T_m) + (3Q k_B T / 2n_0 l) x^{1/2}. \quad (6)$$

As a result of the renormalization of Eq. (1) by the excluded area constraints due to other pores, the effective line tension becomes a function of the pore size. This is the origin of the packing stabilization mechanism that leads to a sievelike structure of membranes. Indeed, as already mentioned above, a single pore destabilizes the membrane at high temperature  $T \geq T_m$  as the effective line tension becomes negative. In a multiple pore system, however, the membrane remains stable even beyond  $T_m$ . For temperatures such that  $T_m < T_c \leq T$ ,  $G(x)$  in Eq. (5) admits a minimum at  $x = x_1$  such that

$$\lambda_{eff}(x_1) = 0 \Leftrightarrow \sqrt{x_1} = (Q_c T_c / Q T) [(T - T_m) / (T_c - T_m)], \quad (7)$$

where  $Q_c = Q(T = T_c)$  and  $T_c$ , the critical temperature at which  $x_1 = 1$ , is given by  $3Q_c = 2Q_0(1 - T_m/T_c)$ , i.e.,

$$\frac{2Q_0(1-y)}{3} = e^{-y\beta_m F_0} \int_1^{\infty} dx \exp\{Q_0(1-y)[(x-1) - \frac{2}{3}x^{3/2}]\}, \quad (8)$$

with  $Q_0 = n_0 \ln(z)$  and  $y = T_m/T_c$ .

Two conclusions can be drawn out of these results. First, the excluded area constraints between pores can stabilize a membrane even in parameter regions where the effective line

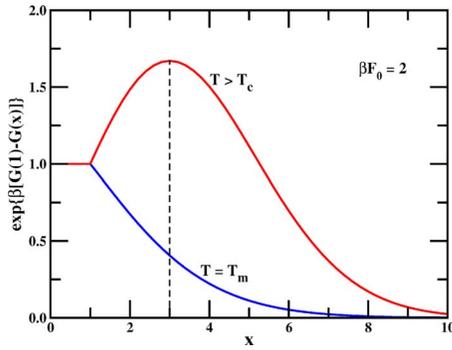


FIG. 2. (Color online) Reduced equilibrium distribution,  $P_{\text{eq}}(x)/P_{\text{eq}}(1) = e^{-\beta[G(x)-G(1)]}$ , of pore size  $x$  for  $\beta F_0 = 2$ ,  $Q_0 = 4$ , and two temperature regimes.  $G(x)$  is given in Eq. (5) with  $Q = 0.215$  for  $T = T_m$  and  $Q = 8/(9\sqrt{3}) = 0.513$  for  $T > T_c$  (i.e., for  $T = 3T_m/2$  and  $T_c = 1.082T_m$ ). The dashed-vertical line indicates the maximum  $x_1 = 3$  for  $T > T_c$ .

tension of pores is negative. Second, the distribution of pore size in the case of negative effective membrane line tension may have a maximum at nonzero contour length as illustrated in Fig. 2. Three regimes can be distinguished: (i) At low  $T < T_m$ , the line tension is positive and the distribution  $P_{\text{eq}}(n)$  of pore size drops monotonically as a function of  $n$ . (ii) At intermediate temperatures,  $T_m < T < T_c$ , the line tension is negative,  $P_{\text{eq}}(n)$  still drops monotonically, but the pores are now stabilized due to the presence of the others. (iii) At high  $T > T_c$ , a maximum emerges in  $P_{\text{eq}}(n)$ , i.e., pores have a most probable size. Figure 2 shows the reduced distribution of pore size,  $P_{\text{eq}}(x)/P_{\text{eq}}(1)$ , for two temperatures below and above  $T_c$ , with a maximum at  $x = 3$  (or  $n = 3n_0$ ) for  $T > T_c$ . The onset of this regime, which corresponds to a “porous membrane state,” sensitively depends on the bare pore free energy,  $F_0$ , required for creation of a minimal-sized pore. This is illustrated in Fig. 3, where  $T_c$  decreases towards  $T_m$  (i.e.,  $T_m/T_c$  increases towards 1) when increasing either  $F_0$  or the effective smallest pore size  $Q_0$ .

From a practical point of view, the porous membrane state is interesting because one may expect that the membrane be highly permeable in this regime. Therefore, the dynamical nature of pores is crucial for any dynamical process in membranes (e.g., lateral diffusion of lipids in the bilayers, or diffusion of various solute molecules within or across membranes). Indeed, to diffuse, a solute particle needs both a sufficient activation energy and an empty space large enough to jump to. In the mean-field approximation, the diffusion coefficient depends on the fraction of the free area or cavities per particle, the rearrangement time of free cavities, and their lifetimes. Although limited, the mean-field picture nevertheless captures the essential features of pore formation and dynamics, and when properly interpreted, it yields reasonable estimates for the pore size distribution and the multipore structure which can be used further for a semiquantitative description of permeation and diffusion in membranes. Accordingly, apart from the number and size of pores, another key quantity that determines the membrane permeability is the lifetime of pores, i.e., how long a pore stays open once created. We now discuss briefly how the lifetime of pores is affected by the presence of the other pores.

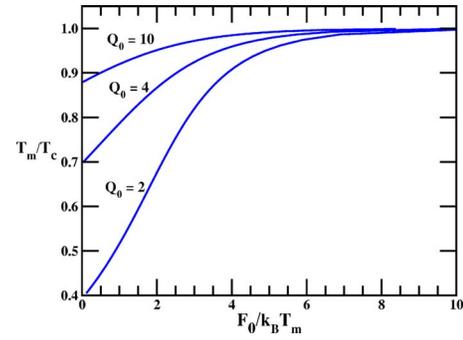


FIG. 3. (Color online) Ratio  $T_m/T_c$  in Eq. (8) as a function of the reduced energy of pore formation.

Six processes contribute to the dynamical evolution of the pores: Pore opening and closing, pore growth and shrinking, pore coalescence and splitting. The pore opening and closing is mainly controlled by the potential barrier that must be overcome to create a pore: the amphiphiles must change their orientation, and the free energy in the intermediate state is different from the corresponding energy when a pore already exists. The characteristic time scale of this process does not depend on the pore packing and shall not be considered here. Amphiphile rearrangements also take place when pores coalesce and split, hence these processes are rate driven and the characteristic time scale depends on the height of a potential barrier. For simplicity, we shall assume that this height is very large, i.e., we shall neglect coalescence and splitting events. Hence we are left with the characteristic time  $\tau$  for the growing and shrinking of a pore. Our main interest in this quantity is to allow comparison on the pore lifetime in two distinct situations: a single pore in a membrane versus a pore in a gas phase ensemble of pores in a membrane.

Neglecting hydrodynamic effects [23], the growth and shrinking dynamics of the pore can fairly be described by a diffusion process with a diffusion constant  $D$  (assumed here independent of  $x$ ) in the potential  $G(x)$ . Then,  $\tau$  is given by the mean time that an already existing pore takes to first reach the minimum pore size  $x = 1$  (or,  $n_0$ ). According to the first passage time theory, the lifetime  $\tau$  can be estimated from the relation [24–26]

$$\tau = (n_0^2/D) \int_1^\infty dx e^{\beta G(x)} \left[ \int_x^\infty e^{-\beta G(y)} dy \right]^2 / \int_1^\infty e^{-\beta G(x)} dx. \quad (9)$$

In the case of a single pore in a membrane, Eq. (9) reduces to the pore lifetime  $\tau_0$  as

$$\tau_0 = (n_0^2/D) [Q_0(T_m/T - 1)]^{-2}; \quad T < T_m. \quad (10)$$

This  $\tau_0$  quadratically increases with temperature and diverges with the membrane disintegration as  $T$  approaches  $T_m$ . As displayed in Fig. 4, in the case of an ensemble of pores, the pore lifetime  $\tau$  begins to increase considerably at  $T_m$  but without diverging and thus, with no membrane disintegration. At  $T$  below  $T_c$ , a pore of any size will ultimately shrink to disappearance because of the drift towards  $x = 1$ . In the stabilized regime above  $T_c$ , the pore size diffuses towards the

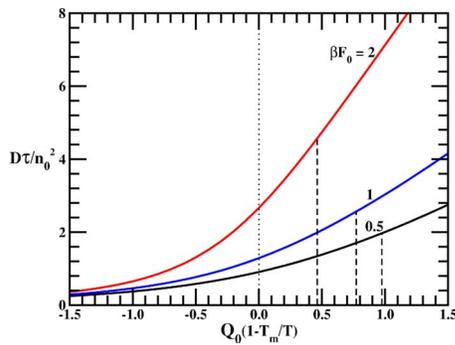


FIG. 4. (Color online) Reduced pore shrinking time in Eq. (9) as a function of the reduced temperature  $Q_0(1-T_m/T)$  for different  $\beta F_0$ . The dotted vertical line indicates  $T=T_m$  above which the line tension becomes negative. Dashed-vertical lines indicate the crossover points  $T=T_c$  [i.e.,  $Q_0(1-T_m/T_c)=0.975, 0.771, 0.463$  for  $\beta F_0=0.5, 1, 2$ , respectively] where  $P_{eq}(x)$  starts to exhibit a maximum as in Fig. 3.

minimum  $x_1 > 1$  of  $G(x)$ , and  $x=1$  can be reached *via* an escape process over the energy barrier,  $\Delta=G(1)-G(x_1)$ , such that  $\tau$  scales as  $\tau \propto \exp(\beta\Delta)$ . As a result, the pore remains open much longer in the packing stabilized regime without membrane disintegration.

To summarize, we have analyzed the statistics of multiple pore formation in membranes without surface tension within a classical van der Waals approach. We have shown that the exclude area interactions between pores allow membrane stabilization against disintegration even when the effective line tension becomes negative at high temperatures. In a certain

range of parameters, this leads to a nanoporous membrane state where the membrane has a sieve structure with long-lived holes of finite size. Owing to the rich diversity of biological systems (all the more artificial lipid bilayers) and wide range of accessible parameters, we expect such stable multipore membranes to exist at physiological conditions with pore sizes in the range of (1–10) nm. This invites speculations on possible applications of such structures, e.g., membranes with selective permeability for controlled drug delivery, or to promote biomolecule translocation.

Our result illustrates the rich variety of membrane structures that can form by chemically or physically tuning the line tension  $\lambda$ . As a crude approximation, we have  $\lambda \propto \gamma_0 h$ , where  $\gamma_0$  is the free energy per unit area between coexisting regions of hydrophobic and hydrophilic molecules, and  $h$  the membrane thickness. Such an estimation is valid only for the so-called hydrophobic pores, while for hydrophilic pores, the scaling is  $\lambda \propto \kappa/h$ , where  $\kappa \propto h^2$  is the bending modulus and  $1/h$  the membrane pore curvature. As  $\lambda \propto h$  in any case, one would expect sievelike membrane structures to be more likely in thin membranes. There are, however, other mechanisms, such as in mixed membranes or membranes with additives, that also lead to reduction of the line tension. Another option could be the effect of the applied voltage  $U$  that, as shown by Winterhalter and Helfrich [27], modifies the bare line tension  $\lambda_0$  of the membrane as,  $\lambda(U) \rightarrow \lambda_0 - \epsilon_w \epsilon_0 U^2 / 2\pi$ , where  $\epsilon_0$  is the vacuum electric permittivity and  $\epsilon_w \approx 80$  the dielectric constant of water. Nevertheless, it is worthwhile to emphasize that if the line tension reduction is one thing, the formation of sievelike structures requires in addition a pore-size-dependent line tension.

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 [23] The hydrodynamic time scale  $\tau_h \propto \eta h^2 / \lambda$  ( $\eta$  is the effective viscosity, including the membrane and the surrounding liquid,  $h$  is the membrane thickness, and  $\lambda$  the line tension), which roughly demarcates the crossover from the diffusion regime to the hydrodynamic regime, is of the order of  $(1-10^2)$  s and thus presumably much larger than the typical life time (msec) of unstable or metastable pores.  
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