

# Stabilization of Membrane Pores by Packing

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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 re-seal or grows without bound till to 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 sieve-like porous structure.

In addition to the area occupied by molecules of e.g., lipids or proteins, membranes incorporate also empty area or pores, and membranes can be thought of as complex structures with a varying amount of free area. The pores might assume different sizes and shapes, and therefore they must be included in the statistical treatment of membranes. Pores can form and grow in membranes in response to thermal fluctuations and external influences. Pore growth enhances the transport of biomolecules across the membranes and its biological relevance can bring new prospective biotechnological applications (see, e.g., Ref. [1, 2]). Holes appear in the membrane via a thermally activated poration process, 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 of the system (tensionless membrane with a pore of size  $n$ ) at nonzero 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],  $f(n) = f_0 + \lambda_0 l n - k_B T \ln[\omega(n)]$ , where  $f_0$  is a  $n$ -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 range. For self-avoiding walks,  $\omega(n)$  has the general form [8, 9]  $\omega(n) = \omega_0 z^n n^{\alpha-2}$  with  $\alpha = 1/2$  (self-avoiding random walks in two dimensions), where the constant  $\omega_0$  and the effective connectivity constant  $z$  of the medium are both of order 1-10 and depend on microscopic details. 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  $n > n_0$ ,

$$f(n) = F_0 + \lambda_0 \left(1 - \frac{T}{T_m}\right) l [n - n_0] + (2 - \alpha) k_B T \ln \left(\frac{n}{n_0}\right), \quad (1)$$

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  $T_m = \lambda_0 l / k_B \ln(z)$  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 numbers 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 new 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 to the situation of a membrane with an ensemble of pores (as in Ref. [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

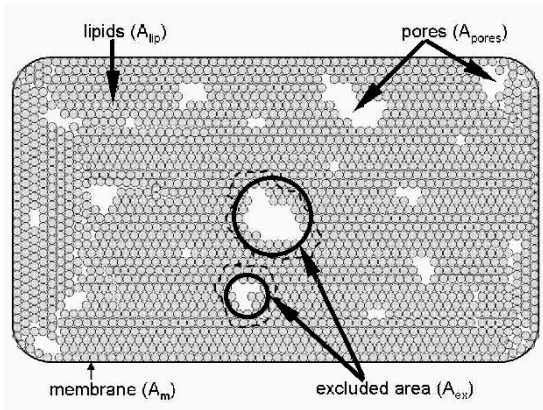


FIG. 1: Schematic depiction of a porous membrane, illustrating the different areas introduced in the text: The total surface  $A_{\text{lip}}$  covered by all lipids (gray filled circles), the total area of pores  $A_{\text{pores}}$ , and the total area  $A_m = A_{\text{lip}} + A_{\text{pores}}$ . The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

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: We first consider the 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, this is not necessary for our argument.

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

$$\Omega = \prod_n \frac{1}{[N(n)]!} \left[ \frac{A_m - A_{\text{ex}}}{a_0} \right]^{N(n)} \quad (2)$$

$$\approx \prod_n \left[ \frac{(A_{\text{lip}} - A_0) e}{a_0 N(n)} \right]^{N(n)}.$$

Here  $a_0$  is a reference area defined below,  $A_m$  is the total membrane area,  $A_m = A_{\text{lip}} + A_{\text{pores}}$ , with the area of lipids  $A_{\text{lip}}$  and the pore area  $A_{\text{pores}}$ , and  $A_{\text{ex}}$  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,  $A_{\text{ex}}$  is larger than  $A_{\text{pores}}$  as indicated in Fig. 1. Notice that  $A_{\text{lip}}$  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 rewritten the accessible area for pores as  $A_m - A_{\text{ex}} = A_{\text{lip}} - A_0$ , such that  $A_0 = A_{\text{ex}} - A_{\text{pores}} = \sum_n N(n)a(n)$ , 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  $P_{\text{eq}}(n)$  of pore sizes,

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

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,

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

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,

$$G(x) = F_0 + Q k_B T + n_0 l \int_1^x \lambda_{\text{eff}}(z) dz; \quad x \geq 1, \quad (5)$$

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

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

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 sieve-like structure of membranes. Indeed, as

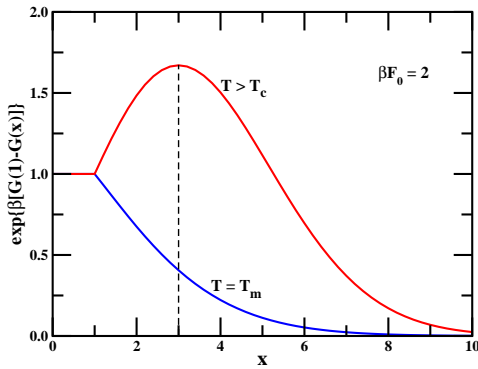


FIG. 2: Reduced equilibrium distribution,  $P_{\text{eq}}(x)/P_{\text{eq}}(1) = e^{-\beta[G(x)-G(1)]}$ , of pore size  $x$  for  $\beta F_0 = 2$ ,  $Q_0 = 4$  and two temperature regimes.  $G(x)$  is given in Eq.(5) with  $Q = 0.215$  for  $T = T_m$  and  $Q = 8/(9\sqrt{3}) = 0.513$  for  $T > T_c$ 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

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

$$\lambda_{\text{eff}}(x_1) = 0 \Leftrightarrow \sqrt{x_1} = \left(\frac{Q_c}{Q}\right) \left(\frac{T_c}{T}\right) \left(\frac{T - T_m}{T_c - T_m}\right) \quad (7)$$

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

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

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

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

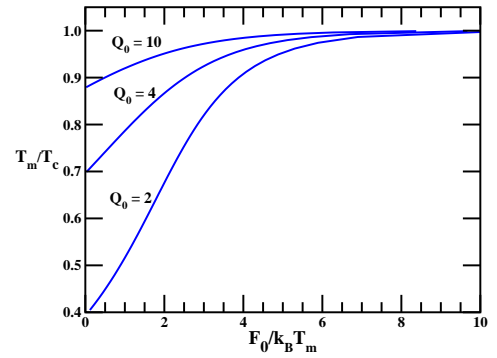


FIG. 3: Ratio  $T_m/T_c$  in Eq.(8) as a function of the reduced energy of pore formation.

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 the membrane should be highly permeable in this regime. The nature of the pores is dynamic, and they are crucial for any dynamic process in membranes (lateral diffusion of lipids in the bilayers, or diffusion of various solute molecules within or across membranes). Indeed, to diffuse, a solute particle needs, in addition to a sufficient activation energy, a large enough empty space to jump to. In the mean-field approximation, the diffusion coefficient depends on the ratio of the close-packed area and the average free area per particle. Of course these simple mean-field arguments can not be used for quantitative predictions. Understanding all its limitations, we nevertheless hope that our crude mean-field theory captures the essential elements of pore formation and dynamics, and when properly interpreted, that it can yield reasonable estimates for the pore size distribution and the multipore structure, which may be used further on for a semi-quantitative description of permeation and diffusion in membranes. However, apart from the number and size of pores, another quantity determining the 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.

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 coa-

lesce 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]

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

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

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

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 on average because of the drift towards  $x = 1$ . In the stabilized regime above  $T_c$ , the pore size diffuses towards the 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 allows 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 nano-porous membrane state where the membrane has a sieve structure with long-lived holes of finite size. Owing to the diversity of biological systems (all

the more artificial lipid bilayers) and wide range of accessible parameters, we expect such stable multipore membranes in the

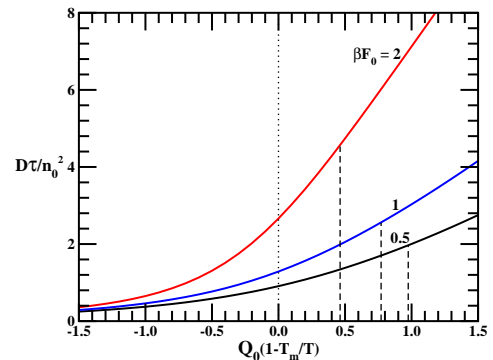


FIG. 4: Reduced pore shrinking time in Eq.(9) as a function of the reduced temperature  $Q_0(1 - T_m/T)$  for different  $\beta F_0$ . The dotted vertical line indicates  $T = T_m$  above which the line tension becomes negative. Dashed vertical lines indicate the crossover points  $T = T_c$  [i.e., 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

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 diversity 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 sieve-like membrane structures to be more likely in thin membranes. although other mechanisms do exist, for example, in mixed membranes or membranes with additives, that lead to reduction of the line tension. Another option could be to play with the effect of a constant transmembrane potential  $U$  which (as it was shown by Winterhalter and Helfrich [27]) modifies the bare line tension as

$$\lambda(U) \rightarrow \lambda_0 - \frac{\epsilon_w U^2}{2\pi},$$

where  $\epsilon_w \simeq 80$  is the dielectric constant of water.

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