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. 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 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 [3], plus an entropic part as modeled by Hillcock and Boal [4],

$$ f(n) = f_0 + \lambda_0 \ln n - k_B T \ln [\omega(n)], $$

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 $n$. For self-avoiding walks, $\omega(n)$ has a general form [4, 7]

$$ \omega(n) = \omega_0 z^n n^{n-2}, $$

where $\omega_0$ is a constant, $z$ the connectivity constant of the medium, 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 the pore, the above free energy can be rewritten as

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

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 shrinks 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 [8], area exchange in tense membranes [3], osmotic stress [10], hydrodynamics [11, 12], orientational ordering [13], and others (see Ref. [14] 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 [16]. 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 [17]. 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 min-
imize $F\{N(n)\}$ 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. 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. 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

$$\Omega = \prod_n \frac{1}{[N(n)]!} \left[ \frac{A_m - A_{ex}}{a_0} \right]^{N(n)} \approx \prod_n \left[ \frac{(A_{lip} - A_0) e^{a_0 N(n)}}{a_0 N(n)} \right]^{N(n)}.$$  

Here $a_0$ is an area constant 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 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 $\nu \approx 3/4$. 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.

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

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

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

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

$$G(x) = F_0 + Q k_B T + n_0 l \int_1^{x_{eff} - 1} \lambda_{eff}(-x) dx ; x \geq 1 ,$$  

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

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

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 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. 1 admits a minimum at $x = x_1$ such that

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

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

$$2 Q(1 - y) 3 = e^{-y \beta_m F_0} \times \int_1^{\infty} dx \exp \left\{ Q_0 \left( 1 - y \right) \left[ \left( x - 1 \right) - \frac{2}{3} x^{3/2} \right] \right\}.$$
with $Q_0 = n_0 \ln(z)$ and $y = T_m/T_c$. Interestingly, Eq. 7 states that the sizes corresponding to a vanishing line tension is that of stable pores.

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 $P_{eq}(n)$ of pore size drops monotonically as a function of $n$. (ii) At intermediate temperatures, $T_m < T < T_c$, the line tension is negative, $P_{eq}(n)$ 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 $P_{eq}(n)$, i.e., pores have a most probable size. Figure 2 shows the reduced distribution of pore size, $P_{eq}(x)/P_{eq}(1)$, for two temperatures below and above $T_c$, with a maximum at $x = 3$ (or $n = 3n_0$) for $T > T_c$. This regime 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 it should have peculiar permeability characteristics. 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 amplitude characteristics. 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 am

\[ \tau = \frac{n_0^2}{D} \int_1^\infty dx \exp\left[\beta G(x) - \beta G(1)\right] \left( \int_x^\infty e^{-\beta G(y)} dy \right)^2. \]

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

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

This $\tau_0$ quadratically increases with temperature and diverges with the membrane disintegration as $T$ approaches $T_m$. As displayed in Fig. 3, 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

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

[1] I. G. Abidor, V. B. Arakelyan, L. V. Chernomyrdik, Y. A. Chizmadzhev, V. A. Pastushenko, and M. R. Tarasevich, Bioelectrochem. Bioenergy 6, 37 (1979).
[2] S. A. Safran, T. L. Kuhl, and J. N. Israelachvili, Biophys. J. 81, 850 (2001).
[3] I. Cooke, M. Deserno, and K. Kremer, 2004, poster presented at the NIC winter school in Computational Soft Matter.
[4] L. Litster, Phys. Lett. A 53, 193 (1975).
[5] J. C. Shillcock and D. H. Boal, Biophys. J. 71, 317 (1996).
[6] D. S. McKenzie, Phys. Rep. 27, 35 (1976).
[7] U. Glaus, J. Stat. Phys. 50, 114 (1988).
[8] O. Farago and C. D. Santangelo, J. Chem. Phys. 122, 044901 (2005).
[9] P. Sens and S. A. Safran, Europhys. Lett. 95, 43 (1998).
[10] Y. Levin and M. A. Idiart, Physica A 331, 571 (2004).
[11] J. D. Moroz and P. Nelson, Biophys. J. 72, 2211 (1997).
[12] D. V. Zhelev and D. Needham, Biochim. Biophys. Acta 89, 1147 (1993).
[13] V. L. Golo, E. I. Kats, and G. Porte, JETP Lett. 64, 575 (1996).
[14] Structure and Dynamics of Membranes (Elsevier, Amsterdam, 1995).
[15] C. Loison, M. Mareschal, and F. Schmid, J. Chem. Phys. 121, 1890 (2004).
[16] We assume that the membrane is still stable or at least metastable. Obviously, an alternative, competing state is a fluid of membrane patches. We assume that this state is suppressed for some reason - either kinetically or thermodynamically. For example, it will be prevented at high lipid concentration, since the lipids can then be packed more efficiently in a membrane stack (smectic phase) than in a fluid of aligned bilayer patches (nematic phase).
[17] G. Gompper and M. Schick, Self-Assembling Amphiphilic Systems (Academic Press, London, 1994).
[18] M. Goulian, R. Bruinsma, and P. Pincus, Europhys. Lett. 22, 145 (1993).
[19] V. I. Marchenko and C. Misbah, Eur. Phys. J. E 8, 477 (2002).
[20] 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 $(1 - 10^4)$ s and thus presumably much larger than the typical life time (msec) of unstable or metastable pores.
[21] A. Szabo, K. Schulten, and Z. Schulten, J. Chem. Phys. 72, 4350 (1980).
[22] H. Risken, The Fokker-Planck Equation (Springer, Berlin, 1989).
[23] D. J. Bicout and A. Szabo, J. Chem. Phys. 106, 10292 (1997).