

Tension and solute depletion in multilamellar vesicles

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We show that a metastable multilamellar vesicle (“onion”), in contact with excess solvent, can spontaneously deplete solute molecules from its interior through an unusual, entropy-driven mechanism. Fluctuation entropy is gained as the uneven partition of solute molecules helps the onion relieve tension in its lamellae. This mechanism accounts for recent experiments on the interaction between uncharged phospholipid onions and dissolved sugars.

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The richness of self-assembled structures exhibited by amphiphilic molecules (surfactants) in solution has attracted intensive research [1]. More recently, attention has been turned toward the behavior of amphiphilic structures under stress [2]. An example of such structures is the multilamellar vesicles (“onions”) that form when a phase of surfactant bilayers is subjected to shear stress [3]. Onion structures pose intriguing questions related to metastability and flow [3–5] and have possible applications, e.g., for encapsulation of solid particles and drug delivery [6]. They also appear in biology, e.g., as a key ingredient in the maintenance of the lung surfactant monolayer [7].

Some properties of onions are rather well understood, e.g., the viscoelasticity of the onion phase [8], dependence of size on shear-rate [3], and kinetics of swelling upon shear-rate change [9] or dilution [4,5]. Other important features remain unresolved, in particular, the mechanism of the dynamic lamellar-to-onion transition [10]. Here we address another aspect of onions that has emerged in recent experiments [11]—their curious behavior in the presence of dissolved molecules that do not directly associate with the bilayers. We show that, as in the case of onion dilution [5], a key role is played by surface tension appearing in the lamellae when the onion is under osmotic stress due to contact with excess solvent. The unique way in which tension affects the onion entropy may lead to a situation where molecules are depleted from the onion interior for purely entropic reasons.

Typical onions are of micrometer size, whereas the intermembrane spacing is usually of order 10 nm, and the thickness of an individual membrane is a few nanometers. Thus, a typical onion comprises a spherical stack of hundreds to thousands of loosely packed membranes. Since synthetic bilayers are usually symmetric and thus do not possess a spontaneous curvature [12], the onion structure is generally *not* a global free energy minimum; the equilibrium state of the membranes, depending on water content, is a flat lamellar (L_α) phase, a disordered “sponge” (L_3) phase, or a vesicle (L_2) phase [1,12]. Yet the path to this ultimate state requires onion disintegration or coalescence, involving breakage of hundreds of membranes per onion, and may take days [4]. Thus, an onion is in general far from equilibrium with its environment. Internally, however, the stack may relax on shorter time scales through the occasional formation of passage defects between membranes [13,9]. Here we focus on

this intermediate time regime, which may last hours to days, where the onion is in quasiequilibrium but cannot annex or release membranes. We ask how such a structure affects the distribution of small solute molecules in its vicinity.

In uncharged systems, or when electrostatic interactions are screened by salt, lamellar phases are stabilized by the Helfrich interaction [14]—an entropic, fluctuation-induced repulsion between membranes. For tensionless membranes the free energy per unit area of this steric effect is $f_{st}(d) = bT^2/(\kappa d^2)$, where d is the intermembrane spacing, T the temperature in energy units, κ the bending rigidity of the membranes, and b a numerical prefactor whose value is under debate; analytic calculations [14] give $b = 3\pi^2/128 \approx 0.2$, whereas computer simulations [15] find $b \approx 0.06$. The steric repulsion is supplemented by van der Waals attraction and shorter-range repulsion coming from hydration effects [16]. These interactions determine the equilibrium spacing d , as well as the transition point at which the stack “melts.”

When a lamellar domain coexists in equilibrium with excess solvent, the intermembrane pressure equals the external osmotic pressure. This fact was used in experiments to measure intermembrane interactions [17]. In general, however, a spherical onion in contact with solvent is out of thermodynamic equilibrium and an osmotic stress may appear. Although water molecules can permeate rather freely through the membranes on the relevant time scales, further onion swelling is hindered by the lack of additional surfactant molecules and the high barriers to membrane breakage. Mechanical equilibrium requires that any pressure difference between the interior and exterior be balanced by surface tension in the lamellae. Since tension can be transmitted between membranes through occasional passages, a tension profile should in general be established throughout the onion. Such tension profiles have been shown recently to stabilize onions against “melting” in an excessively diluted environment [5].

The fact that tension has a significant effect on membrane interactions in onions is demonstrated by a simple argument. The parameter that determines the extent of this effect is the ratio between the intermembrane spacing d and the capillary length $l \equiv (2T/\pi\sigma)^{1/2}$, σ being the surface tension [18]. If $x \equiv d/l \ll 1$, tension is negligible and the steric interaction is given by Helfrich’s tensionless expression. When $x > 1$ membrane fluctuations are strongly suppressed, leading to an exponential decay of the intermembrane repulsion [18]. Sup-

pose, by negation, that tension was negligible, $x \ll 1$. The interior pressure then would be roughly $p \approx 2b'T^2/(\kappa d^3)$, where the numerical factor b' is a certain fraction of b , i.e., of order 0.01–0.1. If there is no external pressure, the interior pressure is balanced solely by tension, $\sigma = \frac{1}{2}Rp \approx b'T^2R/(\kappa d^3)$, where R is the onion radius. This gives $x^2 \approx b'(T/\kappa)(R/d)$. Since typically $R/d \approx 10^2$ – 10^3 and $\kappa \approx (1-10)T$, one finds $x > 1$, contrary to the initial assumption [19].

We begin the analysis by recalling the effect of tension on the thermodynamics of a single membrane in a lamellar stack. The elastic Hamiltonian governing a single membrane is written in Fourier space as $\mathcal{H} = \frac{1}{2} \int d^2q (\kappa q^4 + \sigma q^2) |h_{\mathbf{q}}|^2$, where $h_{\mathbf{q}}$ is a single mode of height fluctuations. The fluctuations can be divided, using the Helfrich patch size ξ [14], into long-wavelength modes, $q < \pi/\xi$, that are affected by the confinement due to the adjacent membranes, and short-wavelength ones, $q > \pi/\xi$, that are confinement-free. The former modes give rise to a steric repulsion for which, in the case of nonzero tension, there is no exact expression. A self-consistent approximation [20] yields $f_{\text{st}}(d, \sigma) = (bT^2/\kappa d^2) \times (x/\sinh x)^2$, which adequately coincides with the tensionless expression for small $x = d/l$ and with renormalization-group calculations [18] for large x . Since tension suppresses the steric repulsion, this contribution to the free energy decreases with tension. Yet the dominant effect of tension is to *increase* the total free energy by reducing the fluctuation entropy of individual membranes. Being independent of the spacing d , this contribution, denoted here f_{σ} , is usually ignored. (It is implicitly included in the surfactant chemical potential.) In the current study, however, we need to consider it explicitly. It is calculated by tracing $e^{-\mathcal{H}/T}$ over the short-wavelength modes $|\mathbf{q}| > \pi/\xi$. The resulting integral $f_{\sigma} = \pi T \int_{\pi/\xi}^{\pi/\delta} q dq \ln(1 + \sigma/\kappa q^2)$ is dominated by the microscopic cutoff δ , which is of the order of the membrane thickness. The result is

$$f_{\sigma} = \frac{\pi^3 T}{2\delta^2} [(1+y)\ln(1+y) - y \ln y], \quad y \equiv \frac{\delta^2 \sigma}{\pi^2 \kappa}. \quad (1)$$

Let us proceed to the thermodynamics of the entire spherical stack. We employ a phenomenological, ‘‘Flory-like’’ approach [21,5], where the steric effect is taken into account accurately whereas the other interactions are incorporated in a second-virial term. Using the expressions for f_{st} and f_{σ} we obtain the free energy density of the onion as

$$f_{\text{on}}(\phi, \sigma) = \frac{bT^2}{\kappa \delta^3} \left[\frac{\phi^3 x^2}{(1-\phi)^2 \sinh^2 x} - \chi \phi^2 \right] + f_{\sigma}(\sigma) \phi. \quad (2)$$

In Eq. (2) $\phi \equiv \delta/(d + \delta) = N\delta^3/(4\pi R^3/3)$ is the surfactant volume fraction (N being the total number of surfactant molecules in the onion), and χ characterizes the net attraction balancing the steric effect. We hereafter consider, for mere simplicity, uniform concentration and tension profiles within the onion [22]. Thus, the Gibbs free energy of the entire onion is written as $G(T, p_0, N, \sigma) = (4\pi/3)R^3(f_{\text{on}} + p_0) + 4\pi R^2 \sigma$, where p_0 is an external pressure.

We now apply the quasiequilibrium assumptions mentioned above. The mechanical equilibrium condition is obtained from the requirement $(\partial G/\partial R)_{T, p_0, N, \sigma} = 0$, leading to a Laplace equation $p - p_0 - 2\sigma/R = 0$, with

$$p(\phi, \sigma) = \frac{bT^2}{\kappa \delta^3} \left[2 \left(\frac{\phi}{1-\phi} \right)^3 \frac{x^3 \cosh x}{\sinh^3 x} - \chi \phi^2 \right]. \quad (3)$$

In addition, the assumption of internal equilibration implies a single, uniform chemical potential,

$$\begin{aligned} \mu(\phi, \sigma) &= \delta^3 (\partial f_{\text{on}}/\partial \phi)_{T, p_0, \sigma} \\ &= \frac{bT^2}{\kappa} \left[\frac{\phi^2 x^2 [(1-\phi)\sinh x + 2x \cosh x]}{(1-\phi)^3 \sinh^3 x} - 2\chi \phi \right] \\ &\quad + f_{\sigma}(\sigma), \end{aligned} \quad (4)$$

which, as expected, is an increasing function of σ .

Unlike systems that can only shrink in response to external pressure, onions have the option to release tension instead. For example, if N and R are constrained so that ϕ is fixed, one can still increase the pressure, as seen from Eq. (3), making the tension decrease in response. One way to introduce external pressure in practice is to add large molecules (e.g., polymers) that cannot be accommodated in the intermembrane spacings and thus remain in the surrounding solution, exerting an osmotic pressure on the onion [17,23]. The current paper, however, concerns solute molecules that *can* dissolve in the intermembrane water layers. In this case the molecules will partition between the interior and exterior, and the question is whether their distribution will be even or not. Systems exhibiting depletion usually involve steric constraints (as for the polymers mentioned above) or repulsive interactions. We consider a case where neither of these is significant; the molecules can comfortably dissolve in the intermembrane layers while being neither attracted to nor repelled from the membranes. Here we encounter again a delicate consideration of relaxation times. We assume that permeation of the molecules through the membranes is kinetically hindered, such that during the intermediate regime of interest they can be viewed as impinging on the membranes and exerting pressure. This holds, e.g., for sugar molecules, whose diffusion through an onion stack may take days [11]. One might wonder then how the distribution of molecules can relax. This indeed poses a severe experimental difficulty; it has been overcome, nonetheless, by techniques of freeze drying and rehydration [23], where a relaxed distribution equivalent to several weeks of molecular diffusion can be achieved.

Let us introduce a dilute reservoir of solute molecules of volume fraction ψ_0 . We add to the free energy of the onion a contribution $G_s = (4\pi/3)R^3 f_s$, with

$$f_s = (T/a^3) [\psi \ln(\psi/\psi_0) - (\psi - \psi_0)], \quad (5)$$

where $\psi = Ma^3/(4\pi R^3/3)$ is the volume fraction of the solute molecules inside the onion (M and a being their total number and molecular size, respectively). Naturally, G_s by

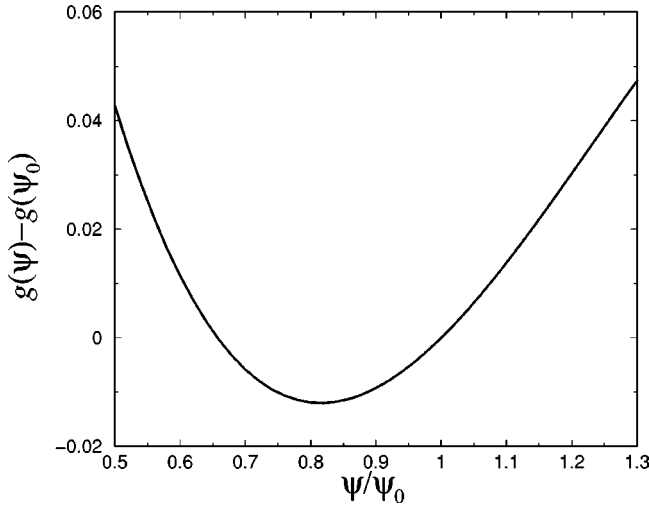


FIG. 1. Dependence of free energy on solute volume fraction. The minimum is obtained for $\psi < \psi_0$, i.e., solute depletion is favorable. Parameter values match the conditions of Ref. [11]: $\phi = 0.59$ (in the absence of solute), $\kappa/T = 10$, $\delta/R = 10^{-3}$, $a = \delta$, $b = 3\pi^2/128$, $\psi_0 = 0.05$, $\chi = 7.57$ (10% off the binodal; see text). The free energy per unit volume is given in units of $2bT^2/(\kappa\delta^3)$.

itself is minimum for an even partition, $\psi = \psi_0$. Taking the variation of $G_{\text{tot}} = G + G_s$ with respect to R we get, however, an expected contribution to the pressure, $p_s = (T/a^3)(\psi_0 - \psi)$, which generates a coupling to the onion variables σ and ϕ through the Laplace equation. We are thus required to find the value of ψ that minimizes the total free energy G_{tot} , given that the modified Laplace equation $p(\phi, \sigma) - p_s(\psi) - 2\sigma/R = 0$ is satisfied. (For simplicity we take $p_0 = 0$, i.e., assume that p_s is the sole source of osmotic pressure.) In order to examine changes in the system (e.g., tension release) as the solute content ψ is progressively increased, an additional global constraint must be supplied. One simple limit is to assume that the surfactant volume fraction ϕ remains fixed. In this case the surfactant chemical potential μ is bound to decrease with increasing ψ (since σ becomes smaller while ϕ is constant). Another simple limit is to constrain μ . In that case ϕ increases (i.e., the onion shrinks) with increasing ψ to compensate for the decrease in σ . [In practice, neither of these two constraints is expected to be strictly accurate; the system may choose another path over the $\mu(\phi, \sigma)$ surface.]

Figure 1 shows the total free energy per unit volume, $g \equiv G_{\text{tot}}/(4\pi R^3/3)$, as a function of ψ for a given external solute content ψ_0 , keeping μ fixed. The parameter values (ϕ for $\psi_0 = 0$, κ/T , δ/R , ψ_0) have been chosen to match the experimental system of Ref. [11]. The value of χ is taken close to the binodal point of the stack, $\chi = 0.9\chi_{\text{bin}}$, $\chi_{\text{bin}} \equiv \phi/(1-\phi)^3$, mimicking a situation where the bare onion is not so far from equilibrium coexistence with the surrounding solvent. As seen in the figure, the favorable solute distribution is uneven, with a considerable depletion of about 20%.

The dependence of the favorable ψ and the corresponding tension release on ψ_0 is depicted in Fig. 2(a), demonstrating how the effect is enhanced as more solute molecules are added. Finally, we have plotted in Fig. 2(b) the dependence

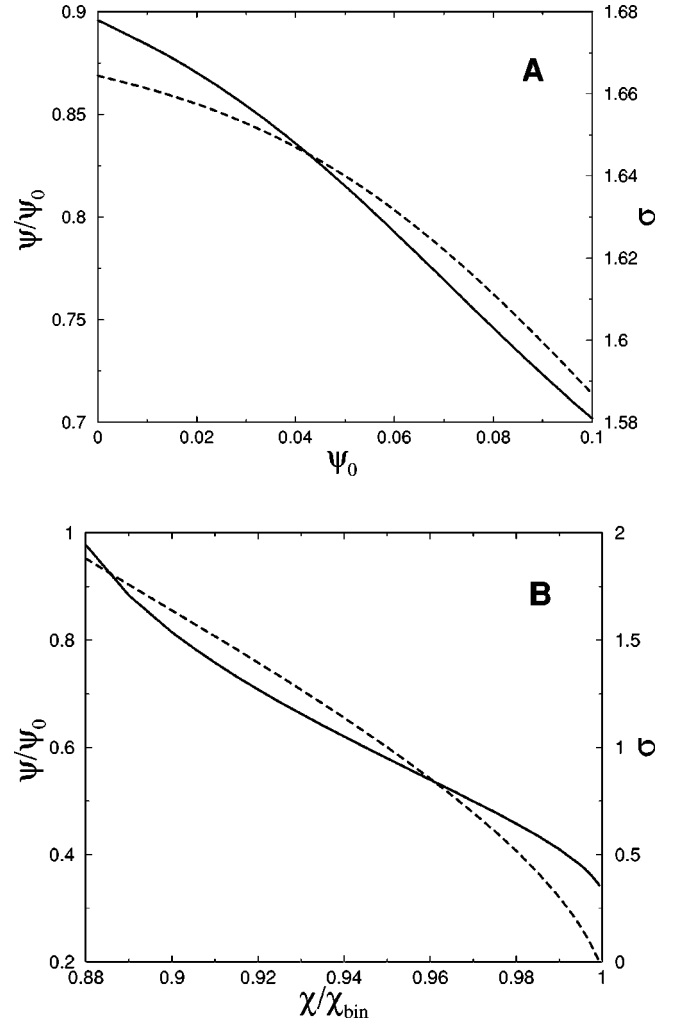


FIG. 2. Dependence of depletion (solid curve, left ordinate) and tension (dashed curve, right ordinate) on the external solute content ψ_0 (a) and attraction parameter χ (b). The depletion effect is enhanced as solute molecules are added and as χ approaches its binodal value χ_{bin} . Parameter values are the same as in Fig. 1. Tension is given in units of T/δ^2 .

on the attraction parameter χ , showing that depletion is in fact enhanced as one approaches the binodal. The reason is that the tension in the bare stack becomes lower and the onion is thus more susceptible to the solute perturbation. This finding implies that a strong depletion effect can be achieved by slight deviation of the bare stack from equilibrium coexistence with the excess solvent. Sufficiently close to the binodal, the bare tension is so low that the depletion annuls it, and further increase of χ produces no effect. For the parameter values of Fig. 2(b) this occurs extremely close to the binodal.

Intriguing experimental results have been reported recently by Demé *et al.* on the interaction of uncharged phospholipid onions with dissolved hydrocarbonates (small sugars) [11]. Using small angle neutron scattering and exploiting the distinct contrast match points in the presence and absence of sugar, they determined that the sugar volume fraction inside the onions was tens of percent lower than its external value.

In small angle x-ray scattering the peak corresponding to the lamellar periodicity was observed to broaden upon adding sugar, indicating an increase in lamellar fluctuations. The author attributed the enhanced fluctuations to strong reduction in bending rigidity due to the added sugar, yet mentioned that there was no known mechanism for such an effect. Indeed, it is hard to see how molecules that are well dissolved in the water layers and do not associate with the membranes could affect their rigidity. The mechanism suggested here provides a straightforward explanation for the increased fluctuations, as well as a relation to the observed depletion—the enhanced floppiness of the membranes is caused by tension release rather than reduced rigidity.

There is an important aspect that is not captured by the current model—the onions were found in the experiment to swell with increasing sugar content. One possible, previously studied swelling mechanism invokes the sugar effect on the dielectric constant of the solvent, which modifies the van der Waals attraction between membranes [17]. (This would translate in our notation to a ψ -dependent χ .) Note that the depletion mechanism presented here is quite general and should apply, in principle, to other solute molecules such as dissociated counterions, provided that their permeation

through the membranes is not too fast. We have considered, however, neutral molecules whose direct interactions are weak; in the case of charged molecules strong electrostatic interactions will set in and require a more complicated model.

We have treated the interplay between various ingredients of this complex system on a basic, phenomenological level. In particular, we have assumed all quantities (surfactant and solute volume fractions, membrane tension) to be uniform throughout the onion which, in fact, cannot be strictly correct [22]. The next natural step would be to consider nonuniform profiles and see, e.g., whether the solute forms a nontrivial profile inside the onion. It would be interesting to check also whether the unusual depletion mechanism suggested here is exploited in biological systems, such as the lamellar bodies in the lung.

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