

## Entropy-driven swelling of fluid-multimembrane assemblies with underlying colloidal interactions

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**Abstract** - In recent years, it has become apparent that condensed-fluid membranes behave similar to linear-flexible polymers. Collective-thermal motions act to randomize surface conformations; thus, restrictions posed by closed vesicle volumes and membrane-membrane binding lead to entropy-driven steric forces. For example, tension in single bilayer vesicles and weak adhesion of bilayer vesicle pairs clearly show entropy-dominated regimes and nonclassical behaviour. Further, osmotic dehydration and swelling of multilamellar lipid-water phases demonstrate significant departure (and cross-over) from underlying exponential repulsion to steric power-law character as intermembrane separations become large. To expose the effects of entropy confinement, a self-consistent field approximation has been developed which captures the nonclassical features of continuous disjoining and steric repulsion plus provides good estimates of renormalized potentials in the cross-over region.

### INTRODUCTION

Molecularly-thin membranes are of widespread interest in science: i.e. as low-dimensional condensed states of matter in physics and chemistry and as the central design employed by Nature to construct higher cellular organisms in biology. Usually, thin membranes are conceptualized as "smooth"-continuous materials with well-defined surface elastic and viscous properties (ref. 1). As "macrocolloidal" structures, membranes are expected to interact with one another - and with other condensed materials - via the classical forces involved in the stability and coagulation of colloidal dispersions: long-range van der Waals attraction and electric double-layer repulsion with solvent and surface structure forces ultimately opposing close approach (ref. 2). Colloidal attraction (and/or osmotic dehydration) often causes single membranes to assemble into multimembrane "stacks"; these assemblies are soft 1-D lattices. Studies of the swelling properties of these "soft" assemblies have exposed unconventional forces that are due to the exceptional flexibility of fluid membranes (refs. 3,7). The unexpected features arise because condensed-fluid membranes behave similar to linear-flexible polymers. Thermal fluctuations act to "roughen" the surface and randomize conformations; thus, constraints posed by lateral area restrictions or by membrane-membrane binding lead to steric effects (entropy-driven tension and long-range steric repulsion - ref. 5). When several (two or more) membranes are forced together by osmotic pressure or held together by van der Waals attraction, the underlying colloidal interactions become "renormalized" by fluctuations to enhance repulsion and reduce attraction. As such, the swelling and unbinding behavior embodied in the renormalized potential can significantly depart from the response described by the direct colloidal prescriptions.

### ENTROPY-DRIVEN TENSION IN CLOSED-MEMBRANE VESICLES

Increasing the internal volume of a vesicle with fixed number of surface molecules progressively stresses the surface as the vesicle approaches a smooth spherical shape. In the conventional mechanics of membranes, a macroscopic-size vesicle should swell with almost no internal (osmotic) pressure. Tension in the membrane should be negligible until the spherical shape is reached. Further, swelling should increase tension in proportion to the pressure as the surface dilates under direct elastic expansion. But this is not the actual behavior! Close to the spherical form, there is significant increase in vesicle pressure before the membrane is required to stretch because fluctuations become severely restricted.

Results (Fig. 1) from pipet pressurization of isolated vesicles (to produce a range in tension of four orders of magnitude) demonstrate the cross over from entropy-dominated compliance to direct stretch of the surface (ref. 6).

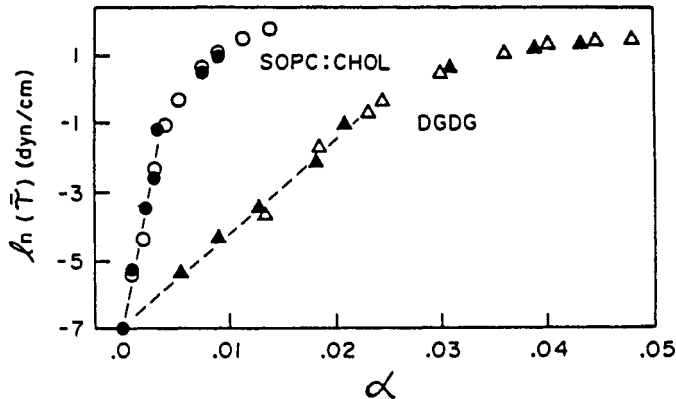


Fig. 1. Logarithm of tension  $\bar{\tau}$  produced by pressurization of a bilayer vesicle ( $\sim 20 \mu\text{m}$  diameter) versus the fractional increase in apparent surface area  $\alpha = \Delta A/A$ .

Note in Fig. 1: the weak logarithmic dependence of the membrane tension on area persists over a large range in tension (values up to 0.5 dyn/cm). This entropy-driven feature is unexpected because macroscopic fluctuations (displacements on the order of the wavelength of light) disappear from view at very low tensions ( $> 10^{-3}$  dyn/cm) to leave an optically-smooth spherical image. In the low tension regime, the slope of the logarithm of tension versus projected area is governed by the elastic bending modulus  $k_c$  scaled by temperature (i.e.  $8\pi k_c/kT$ ).

### ENTROPY-DRIVEN UNBINDING OF ADHERENT VESICLES AND SWELLING OF MULTILAMELLAR PHASES

In the conventional view of adhesion and disjoining by colloidal forces, it is expected that inverse-power law attraction should dominate at large distance over an exponentially-decaying repulsion. Consequently, neutral surfactant membranes and moderately charged membranes in electrolyte solutions are predicted to cohere without external forces to push them together. However, a different scenario is observed for charged membranes in electrolyte solutions: i.e. rapidly weakening adhesion and finally no attraction at low charge densities (ref. 7). This nonclassical "unbinding" appears as a precipitous departure from the conventional colloid treatment (Fig.2). The departure from standard theory is consistent with the anticipated "softening" and expansion of repulsion by entropy confinement.

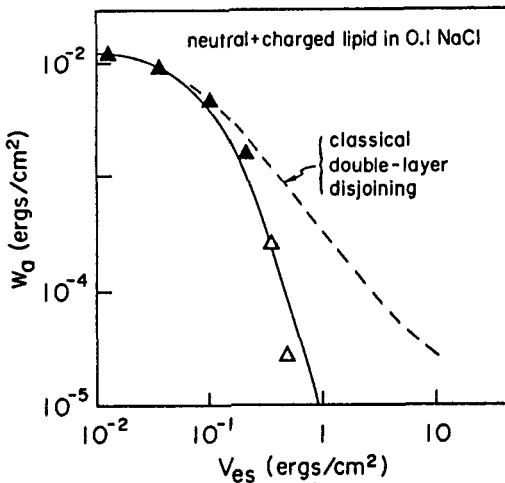


Fig. 2. Measurements of adhesion energy carried-out in 0.1 M NaCl with pairs of vesicles made from neutral phosphatidylcholine lipids plus small amounts of negatively-charged phosphatidylserine (0 to 7 charges per 100 lipids). [The solid-triangle symbols are direct measurements derived from micropipet control of vesicle tensions; the open-triangle symbols are values of adhesion energy derived from the constitutive relation for entropy-driven tension and contact geometry. The dashed curve is the adhesion energy predicted by superposition of electric double-layer repulsion plus an attractive potential derived from the adhesion energy for neutral-lipid bilayers.]

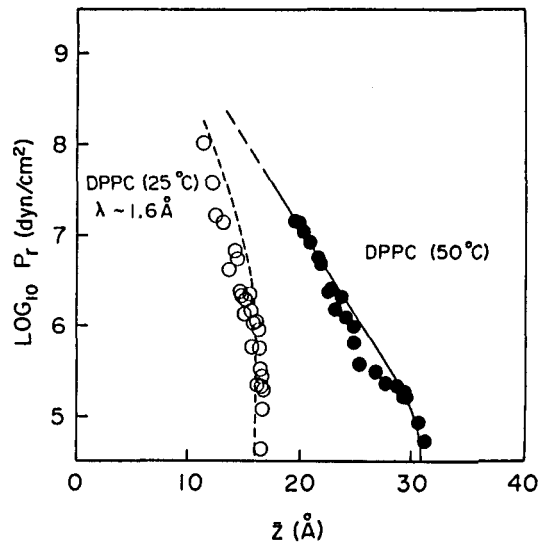


Fig. 3. Osmotic pressure  $P_r$  versus bilayer separation  $\bar{z}$  (average aqueous space) computed from x-ray diffraction and gravimetric data for multibilayer arrays of DPPC lipids in water (replotted from Rand and Parsegian - ref.2).

The underlying-repulsive interaction between lipid bilayers has been exposed by osmotic dehydration of multibilayer phases in water. As described by Rand and Parsegian (ref. 2), correlation of x-ray diffraction (lamellar repeat spacing) with gravimetric (weight ratio of lipid:water) data provide values for average water separation  $\bar{z}$  between bilayers as a function of osmotic stress  $P_r$ . In Fig. 3, data for dipalmitoyl phosphatidylcholine DPPC lipids in water demonstrate the difference between osmotic dehydration properties of these neutral multibilayers above - and below - the acyl chain crystallization temperature ( $\sim 41^\circ\text{C}$ ).

The dashed curve in Fig. 3 is a superposition of exponential repulsion and van der Waals attraction which is taken as the direct potential "reference". In the melted state, separations between lamelli increase which is reflected in a larger "apparent" decay length  $\lambda$  for repulsion (from  $\lambda \sim 1.6 \text{ \AA}$  in the gel state to  $\lambda \sim 2.2 \text{ \AA}$  in the fluid state). Also, there is clear evidence for a strongly-bound state at low temperature ( $\bar{z} \sim 16-18 \text{ \AA}$  separation) driven by van der Waals attraction. But at high temperature, cohesion is weak with a much larger stress-free separation around  $31 \text{ \AA}$ . As will be outlined next, multilayer expansion in the fluid state is consistent with predictions for entropy-driven augmentation of the underlying repulsion and weakening of cohesion.

### RENORMALIZATION OF MEMBRANE INTERACTIONS BY ENTROPY CONFINEMENT: SCMF APPROXIMATION

Thermodynamic theory of collective shape fluctuations is formulated on the basis that the likelihood of a particular shape is proportional to a Boltzmann probability derived from the elastic energy required to displace the contour from equilibrium. Free energy of the membrane is derived from a partition function that is the sum (integral) over these configuration probabilities. Cumulation over all surface conformations leads to a functional integral that, without further simplification and approximation, is extremely difficult to analyze in most situations. Rigorous approaches have involved recursive perturbation schemes (renormalization group methods - ref. 8) or random-walk (Monte Carlo - ref. 9) computer simulations neither of which is easily implemented. Thus, similar to the practical (and successful) approach developed in polymer thermodynamics, a self-consistent mean-field SCMF approximation has been developed to provide a tractable method for estimation of free energies and forces (refs. 7, 10). The principles of the SCMF method are guided by a "polymer-membrane metaphor". Like polymers, the lateral correlation lengths of undulations in a membrane surface are reduced and "screened" by excluded volume effects: i.e. confinement in a potential field. Although there will be a distribution of correlation lengths, it is assumed that on the average the surface breaks up into N statistically-separate sections with a size given by an "effective" correlation length. Based on this concept of independent sections, the density of configurations is defined by the convolution of probability densities for the positions of membrane sections (governed by the potential) and the joint probability density for distribution of small fluctuations within each section. Evolution of unnormalized distributions follows "diffusion-like" equations where a mean field restricts fluctuations in the position of each independent section. The underlying "physics" is that the positions of statistically-independent sections are confined by the mean field whereas smaller-scale fluctuations wander throughout the potential, encouraged to drift towards the minimum away from repulsive boundaries. With the probability density for membrane configurations and "effective" correlation length, an upper bound to the free energy is calculated which is then minimized with respect to parameters in the self-consistent field to obtain an optimum value.

Continuous "unbinding" has been shown to be a second order transition (ref. 8) with no coexistence between bound (adherent) and unbound (completely separate) states. The conclusion is very important since it predicts that no energy barrier opposes approach of a membrane to an adherent configuration. Similarly, results from the self-consistent mean-field SCMF approximation demonstrate continuous "unbinding" as a function of temperature and repulsive potential as shown in Fig. 4. In Fig. 4, the results predicted for equilibrium separations  $\bar{z}/\lambda$  are plotted as functions of potential coefficients scaled by a characteristic energy  $E_H$  (the Helfrich energy - refs. 5,7) which is proportional to temperature T. The energy-density for exponential repulsion (decay length  $\lambda$ ) is expressed as,

$$\bar{v}_r e^{-\bar{z}/\lambda} \qquad \bar{v}_r \equiv v_r \cdot \lambda^2 / E_H$$

van der Waals attraction is specified to follow an inverse power-law form which ranges between,

$$\bar{A}_H \equiv A_H / E_H$$

$$\bar{A}_H \lambda^2 / 12\pi \bar{z}^2 > |\bar{v}_a| > \bar{A}_H (d_{B\ell} \lambda)^2 / 2\pi \bar{z}^4$$

[based on theory (ref. 2) for discrete layers of thickness  $d_{B1}$ ]. For a pair of

membranes, the Helfrich energy scale is approximately,

$$E_H \equiv (kT)^2 / 16\pi^2 k_C c$$

where  $c \approx 0.1$ . On the other hand, multimembrane arrays are characterized by an energy scale that is a factor of two larger simply because fluctuations in multilayer spacings are twice the mean-square displacement of a single layer (ref. 7, 10). Thus, multimembrane arrays can be viewed as existing at effectively higher temperatures (by a factor of  $\sqrt{2}$ ) than single membranes adjacent to stiff substrates. As such, a single membrane may bind to an immobilized membrane under conditions where a multilamellar array will swell indefinitely!

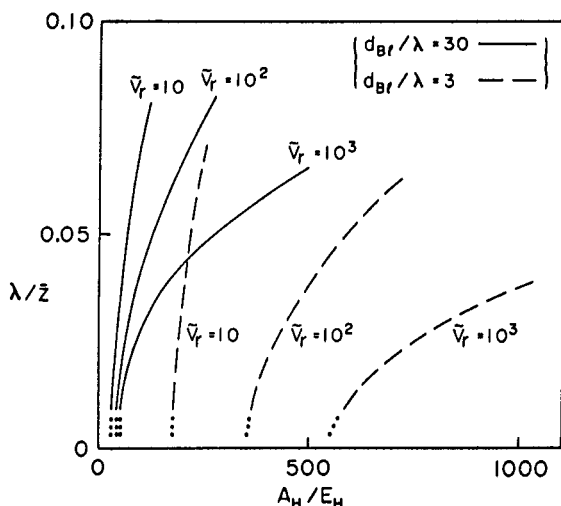


Figure 4. Predictions for continuous unbinding of multimembranes as functions of the underlying-exponential repulsion  $V_r$ , power-law attraction  $A_H$ , and bilayer thickness  $d_{B1}/\lambda$ . Equilibrium separations  $\bar{z}$  are scaled by the decay length  $\lambda$  of the repulsive field.

The power-law feature and quantitative form for the Helfrich-steric repulsion have been confirmed for multilamellar arrays well above the critical point (ref. 11). However, transitions from bound-to-unbound states have only recently been examined in relation to theoretical predictions (ref. 7,9,10). The data in Figs. 2 and 3 provide tests for the predictions in Fig. 4. To make comparisons, it is necessary to know the membrane bending stiffness  $k_C$  and the characteristics  $A_H$ ,  $V_r$ ,  $\lambda$ , of the underlying interaction. For the phospholipid bilayers involved in these studies, the bending modulus is known to be about 24 kT ( $\sim 10^{-12}$  erg). Thus, the Helfrich energy scale is set at about 0.0027 kT ( $1.1 \times 10^{-16}$  erg for a single bilayer at a temperature of 23°C) and about 0.0054 kT ( $\sim 2.2 \times 10^{-16}$  erg for multibilayer arrays). Next, based on osmotic dehydration properties of multilamellar arrays in the low temperature gel state, the scaled prefactor  $V_r$  for repulsion is found to be of order  $10^3$ . Since the bilayer thickness is about 35 Å, the critical value  $A_H^*$  of the van der Waals coefficient for neutral multibilayer cohesion is predicted to be  $1 \times 10^{-14}$  erg (or  $0.5 \times 10^{-14}$  erg for binding a single neutral bilayer to a stiff-bilayer substrate). [Note: the critical value of attraction coefficient for unbinding is a factor of 2-3 larger when  $d_{B1}/\lambda \approx 10$  which agrees well with results from renormalization-group and Monte-Carlo calculations - ref. 8.] Finally, the van der Waals coefficient  $A_H$  is estimated to be  $5-6 \times 10^{-14}$  erg based on the measured value of adhesion energy ( $10^{-2}$  erg/cm<sup>2</sup>) for neutral bilayer vesicles. Taking these values to represent the "bare" potential, predictions of osmotic dehydration of multibilayers in the fluid state are plotted as the solid curve in Fig. 3. Likewise, including the electric double-layer repulsion associated with the charges added to the neutral bilayer, predictions of disjoining of adherent vesicle pairs are plotted as the solid curve in Fig. 2. These correlations demonstrate both the expansion of exponential-like repulsion plus reduction in cohesion driven by entropy confinement.

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