

## Local Unbinding of Pinched Membranes

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(Received 17 April 1995; revised manuscript received 7 August 1995)

Loosely bound membranes exhibit an unusual elastic response when pinched together by optical tweezers, locally unbinding to a large intermembrane distance. Tweezing a stack of many bound membranes produces extreme local swelling in the vicinity of the tweezing point. We introduce a model that incorporates bending elasticity, fluctuations, and intermembrane interactions to calculate the membrane profiles subject to a local pinch. Theoretically, we find strongly overshooting profiles in agreement with experiment. We predict scaling behavior of the overshoot with the pinch strength and size.

PACS numbers: 82.65.Dp, 68.10.Et, 87.22.Bt

Adhesion of biological membranes involves both the attraction of two homogeneous surfaces [1] and the interactions of specific molecular binding sites [2,3]. Experiments suggest that binding sites are dominant locally. However, the overall binding of two membranes with localized “chemical stickers” is strongly influenced by the elastic response and fluctuations of the embedding bilayer [4]. To study the overall membrane response to a single binding site, we study a simplified model system by locally pinching together two membranes with optical tweezers [5,6]. This system can also model “local collisions,” which are important in the study of interacting, flexible membranes and the understanding of their unbinding transition [7,8].

Previously, we showed that laser tweezers remove area from the membrane, inducing tension in membranes with limited area and thus driving shape transitions. In the present work the tweezers are used primarily as a *mechanical* tool, clamping at a point two large planar membranes, which effectively have unlimited area and allow only a negligible buildup of tension. We studied bilayers composed of dimyristoyl-phosphatidylcholine (DMPC) (Sigma) produced by a standard technique [9]. Our experimental setup is described elsewhere [10,11]. Typical working conditions were 30 °C and laser intensities from 30 up to 150 mW. We selected sets of two or more nearly flat membranes, which were sections either of huge vesicles or of lamellar sheets that extended from the bottom of the cell to its top. We chose sections of membranes that appeared to be bound or loosely bound, at a distance of  $\sim 1 \mu\text{m}$ . The membranes are pinched by the laser tweezers in the  $\sim 0.5 \mu\text{m}$  region of the trap. Regardless of the dynamic development of the pinching process, once the two membranes are pinched together, a steady state profile is reached with a large overshooting “lip” centered around the pinch. This state, shown in Fig. 1, can be maintained for several minutes and shows stability against observable thermal fluctuations. Eventually the structure is destabilized by the occasional trapping of surrounding vesicles or due to the escape of the membranes from the trap.

The experiments indicate that the overshoot size grows with tweezing intensity. When tweezed at extremely high intensities (over 150 mW) the profile structure may change qualitatively, and more complex, budding structures develop.

We emphasize that the lip profile of Fig. 1 is obtained in steady state. The kinetic pathways in reaching this steady state are varied: (1) A moderate symmetric overshoot develops upon tweezing. (2) An asymmetric pinch develops prior to the lip profile. (3) The membranes first separate upon tweezing (no lip profile), then the two membranes are pulled back into the laser trap and the characteristic lip profile develops. In (3) only one membrane is caught in the trap at the first stage, while the other fluctuates freely thus increasing the intermembrane distance.

Our theory predicts membrane profiles which closely resemble those that are experimentally observed. The

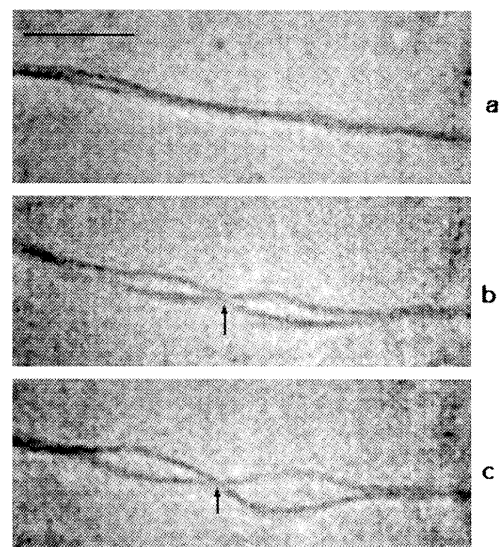


FIG. 1. (a) Two loosely bound bilayer membranes prior to tweezing. (b) Tweezing (at arrow). (c) Increased laser intensity. The bar represents  $10 \mu\text{m}$ .

theoretical analysis of the pinching of two flat, bound, tensionless membranes is based on an interfacial curvature model [12], which takes into account both the bending energy [3,12,13] of the membranes and an effective intermembrane interaction that models the effects of thermal fluctuations [14]. We minimize the free energy,

$$F = \int \left( \frac{\kappa}{2} (\nabla^2 h)^2 + V(h) + Ph \right) dS, \quad (1)$$

subject to boundary conditions imposed by the pinch. Here  $h(\vec{r})$  is the local intermembrane distance,  $\kappa$  is the bending modulus,  $P$  is an external pressure arising from the mechanical constraints of other close vesicles and the walls of the cell, and  $dS$  is the area element. The intermembrane interaction potential,  $V(h)$ , can have both repulsive and attractive terms [14]. The repulsive term in the  $\mu\text{m}$  region is an effective interaction induced by the confinement of the thermal fluctuations. The loss in fluctuation entropy per membrane due to the proximity of the neighboring membrane produces an effective repulsion [15]:  $V_f = (3\pi^2/64)(k_B T)^2/\kappa h^2$ . The combination of this repulsion with the pressure produces a bound state with an equilibrium spacing  $\bar{h}$ . Alternatively, the membranes can bind due to microscopic attractive (e.g., van der Waals [16]) interactions [17] even in the absence of pressure.

The minimum energy configuration of a membrane near a pinch is given by solving the fourth order, nonlinear Euler-Lagrange equation:

$$\kappa \nabla^4 h(r) + dV/dh + P = 0. \quad (2)$$

This equation is solved, assuming axial symmetry, with two boundary conditions at the edge of the pinch,  $r = r_0$ , and two at  $r = \infty$ . At the pinch the membranes are forced to maintain a distance  $h_0 < \bar{h}$ . At infinity their distance decays back to the equilibrium value,  $\bar{h}$ . The absence of any constraint on the slope at the edge of the pinch is equivalent to imposing  $\nabla^2 h|_{r=r_0} = 0$ . Previous work [20,21] treated the limit  $h_0 \approx \bar{h}$ , where Eq. (2) can be linearized [quadratic  $V(h)$ ]. The resulting overshoot is then very small and depends linearly on  $h_0$ .

In the nonlinear case where  $h_0 \ll \bar{h}$  the behavior is striking and resembles the experimental profiles. The repulsive interaction in the vicinity of the pinch is strong, leading to a large positive slope [22] and thus a strong overshooting response. In order to minimize curvature energy the profile oscillates [20,21]. This is unique to systems governed by curvature elasticity, as opposed to lower order gradient terms (tension) that dictate a minimum of area and therefore produce monotonically decaying profiles. In Fig. 2 we show several membrane profiles for a system bound by pressure. The qualitative shape of these profiles is the same for any attractive potential that goes to zero at infinity.

There are two physical parameters of the pinch that determine the membrane profile: The strength of the pinch, modeled by  $h_0$ , and the trap area,  $r_0^2$ . As the pinch becomes

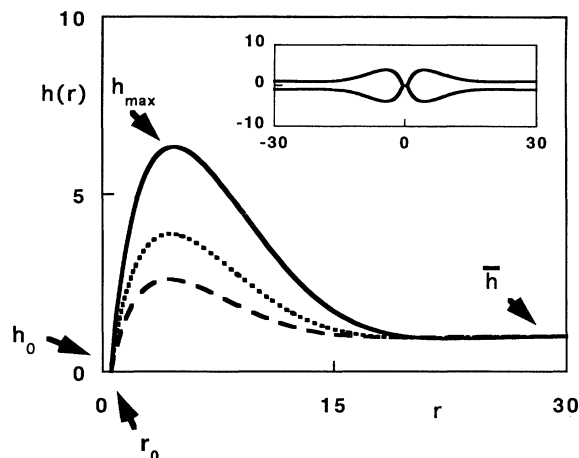


FIG. 2. Theoretical profiles for a pinch of  $r_0 \sim 1$  and several pinching strengths.  $h_0 = 1 \times 10^{-4}$ —solid curve,  $h_0 = 3 \times 10^{-4}$ —dotted curve, and  $h_0 = 1 \times 10^{-3}$ —dashed curve. The membrane height and the distance from the pinch are both measured in units of the equilibrium intermembrane distance,  $\bar{h}$ . Inset: Entire lip configuration for  $h_0 = 1 \times 10^{-4}$ . In these profiles  $\kappa \sim 10k_B T$ .

stronger ( $h_0 \rightarrow 0$ ) and its area larger the overshoot size increases.

Balancing the chemical potential of the membrane in different spatial regions, we can formulate scaling relations for the overshoot profile. We find several scaling regimes depending on both  $h_0$  and  $r_0$ , which will be presented elsewhere [23]. For simplicity we present only the scaling behavior for the region  $r_0 \gg \bar{h}$  and  $h_0 \ll \bar{h}$ , where the behavior is essentially independent of  $r_0$ .

In this quasi-one-dimensional regime we find that the overshoot height,  $h_{\max}$ , and the profile width,  $w$ , scale as  $h_{\max} \sim h_0^{-1/2}$ ,  $w \sim h_{\max}^{1/4}$  for the case with a finite external pressure, while  $h_{\max} \sim h_0^{-1}$  and  $w \sim h_{\max}^{1/2}$  for a system bound by an attractive potential. Figure 3, where we show the results of one-dimensional numerical calculations (i.e., effectively  $r_0 \rightarrow \infty$ ), confirms this scaling behavior. The theoretical profiles, which agree very well with the experimental observations, are at the edge of this scaling region. This one-dimensional regime is also interesting because of its relevance to adhesion problems where many binding molecules aggregate to form a relatively large close contact region [20,24].

To compare experiment and theory we fit the theoretical parameters to the observed experimental profiles. The Helfrich repulsion and pressure term define a potential well at  $\bar{h}$ . The pressure is rescaled so that  $\bar{h} = 1$ . The depth of the well is then given by the coefficient of the Helfrich interaction,  $(3\pi^2/64)(k_B T)^2/\kappa$  [15,25], which is  $0.05k_B T$  for this system ( $\kappa \approx 10k_B T$ ). In the theory we fit two parameters:  $h_0$  and  $\kappa$  (which defines the depth of the well). To match the observed profiles  $h_0/\bar{h}$  must be of the order of  $10^{-3}$ – $10^{-2}$ . The effective depth of the intermembrane potential well needed to fit the typical cases of Figs. 1(b) and 1(c) is  $0.05k_B T$  and  $0.08k_B T$ ,

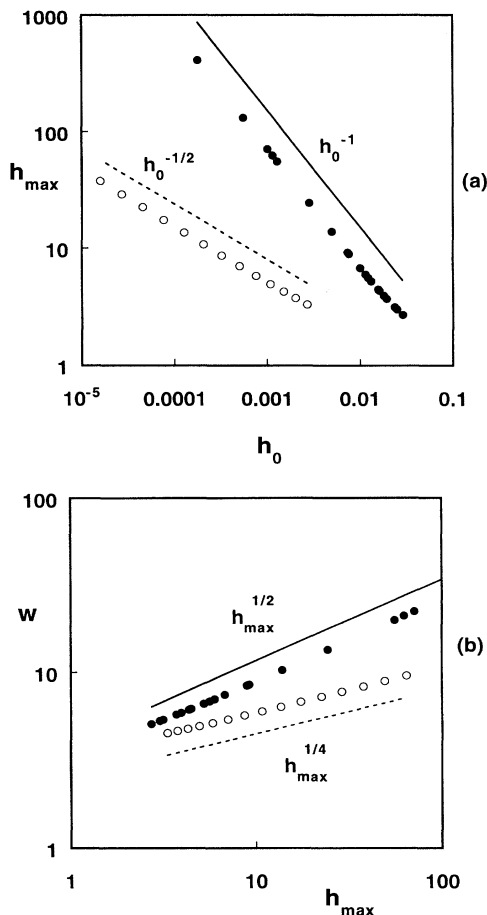


FIG. 3. (a) Maximum intermembrane separation (overshoot) as a function of the intermembrane distance,  $h_0$ , at the pinching point, for both the pressure case (empty circles) and the interaction case (solid circles). (b) Width of overshooting region as a function of the overshoot. The graphs show the scaling laws in the one-dimensional limit discussed in the text (i.e.,  $r_0 \rightarrow \infty$ ).

respectively (for  $r_0 = 0.5 \mu\text{m}$ ). This is in reasonable agreement with the predicted magnitude given above.

While the theory presented here provides a satisfactory and physically interesting explanation of the experiment, the fact that the laser is applied continuously during the entire process leads us to consider several alternative effects, which turn out to be less relevant.

(1) *Global tension.*—The main action of optical tweezers on a lipid bilayer is to attract lipid into the trap. So long as the membrane has excess area, it will accommodate the area loss with practically no tension by changing its shape. Once tension appears it can lead to shape transformations in tubes [11] and to pressurization of floppy micron size vesicles [10], but its effect is reduced in the huge lamellar structures of the present work because a significant reduction of area is needed for the buildup of high tension. The presence of fluctuations while pinching indicates that the tension induced by the laser is at

most localized near the trap and does not deviate strongly from the initial background tension, which is present due to the finite area,  $A$ , of the system and is of the order of  $k_B T/A \leq 10^{-6} \text{ erg/cm}^2$ . Theoretically we find that a strong overshoot is obtained as long as the tension is low. Above a threshold uniform tension the membrane responds monotonically to the pinch without overshooting. Estimates of the curvature and stretching energies in the overshooting area yields an estimate for this threshold of the order of  $10^{-4} \text{ erg/cm}^2$ .

(2) *Tension gradients and lipid flow.*—Even in a flat membrane with large excess area the tension near the trap is set by the laser and is high. The maximal tension,  $\sigma_{\max}$ , which can be induced in the bilayer is set by the energy flux of the laser that is  $5 \times 10^{-3} \text{ erg/cm}^2$  [10,26], for laser intensity  $I_0 = 50 \text{ mW}$ . This tension is still not high enough to actually change the intermolecular distances in the bilayer [27]. But if lipid is pulled (flowing) into the trap, there must be entrainment of water and consequently a buildup of pressure near the trap, which might drive a bulging overshoot [28]. However, the pressure and tension rapidly fall off away from the trap, as evidenced by the visible fluctuations. It is conceivable that hydrodynamics determines the response only right at the trap [28], microscopically fixing the boundary conditions used to parametrize our model. Currently,  $h_0$  is a parameter fitted by the experimental profiles to rather low values.

(3) *Heating effects.*—Heating effects due to local absorption were measured to be negligible [29]. Unbinding as a result of heating [8] is ruled out since we heated the

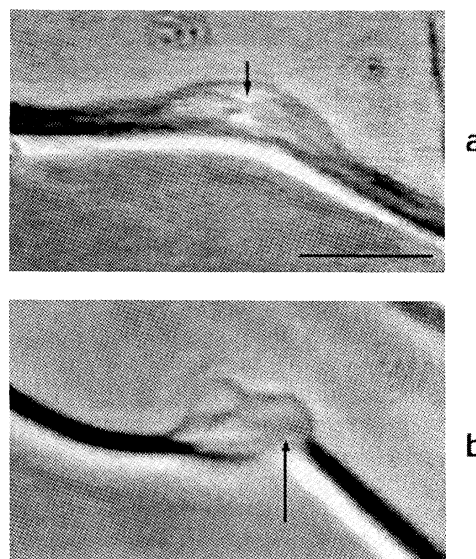


FIG. 4. (a) A stack of about 12 bound bilayers (part of a huge multilamellar vesicle) pinched at a point. Only a few bilayers are actually pinched. (b) Tweezing a stack of about 20 bilayers, the membranes continuously swell to a large intermembrane distance. Violent fluctuations appeared during this swelling. The membranes relax to their initial state after tweezing ends. The bar represents  $10 \mu\text{m}$ .

sample to 20 °C above the working temperature and observed no unbinding.

Finally, as an experimental extension of the two membrane profiles, we show in Fig. 4 the effect of tweezing on multilamellar stacks. The response of the stack to the pinching of a few of the lamellae within it is complex, leading to a continuous dynamic evolution of the stack's profile.

We have studied both experimentally and theoretically the local unbinding of pairs of membranes. These local defects are interesting because they are manifestations of the highly nonlinear interplay between interactions and curvature energy of the membranes, and are of importance to the understanding of the nontrivial unbinding transition of membranes.

We thank E. Bar-Ziv, N. Dan, and T. Frisch for useful discussions. We are especially grateful to P. Pincus and P. Nelson for illuminating insights. E.M. would like to acknowledge the support of the Minerva Center for Nonlinear Science and the Klutznick Fund for Research. S.A.S. would like to acknowledge the support of the Ministry of Science and the Arts and the Israel Science Foundation.

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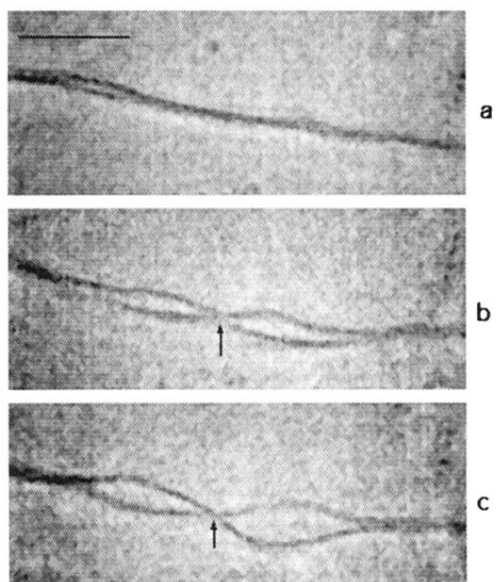


FIG. 1. (a) Two loosely bound bilayer membranes prior to tweezing. (b) Tweezing (at arrow). (c) Increased laser intensity. The bar represents  $10 \mu\text{m}$ .

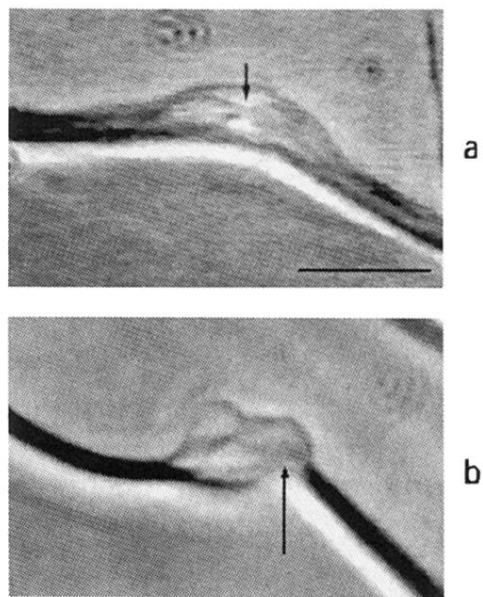


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