P H Y S I C A L   R E V I E W   L E T T E R S

Adhesion of Membranes with Active Stickers

Bartosz Różycki,[1,2] Reinhard Lipowsky,[1] and Thomas R. Weikl[1]

[1]*Max-Planck-Institut für Kolloid- und Grenzflächenforschung, 14424 Potsdam, Germany*
[2]*Instytut Fizyki Teoretycznej, Uniwersytet Warszawski, Hoża 69, 00 681 Warszawa, Poland*
(Received 1 July 2005; published 31 January 2006; publisher error corrected 1 February 2006)

We consider a theoretical model for membranes with adhesive receptors, or stickers, that are actively switched between two conformational states. In their "on" state, the stickers bind to ligands in an apposing membrane, whereas they do not interact with the ligands in their "off" state. We show that the adhesiveness of the membranes depends sensitively on the rates of the conformational switching process. This dependence is reflected in a resonance at intermediate switching rates, which can lead to large membrane separations and unbinding. Our results may provide insights into novel mechanisms for the controlled adhesion of biological or biomimetic membranes.

Introduction.—Fluid lipid bilayers are the fundamental building blocks of biological membranes and, as such, have been studied extensively [1]. As a consequence, many equilibrium properties of lipid membranes are now well understood. However, biological membranes are often out of equilibrium, driven by embedded active components or by the coupling to the dynamic cytoskeleton. These active processes affect the membrane behavior, e.g., by enhancing membrane shape fluctuations [2–4] or lateral diffusion [5]. The enhancement of shape fluctuations has been theoretically predicted for membranes with ion pumps as active force centers [2], and experimentally verified for giant lipid vesicles with embedded bacteriorhodopsin pumps [3]. Other active membrane proteins have been suggested to couple to the local thickness [6] or curvature [7] of the membranes.

In this Letter, we consider another type of active membrane process. The active components embedded in these membranes are adhesion molecules, or "stickers," that can be switched between two states: an "on" state in which the stickers interact with a second membrane, and an "off" state in which this interaction is negligible (see Fig. 1). An important biological example of actively switched adhesion molecules are integrins. In one of their conformations, the integrin molecules are extended and can bind to ligands present in an apposing membrane [8]. In another conformation, the molecules are bent and, thus, deactivated. The transitions between these conformations are triggered by signaling cascades in the cells, which typically require energy input, e.g., via adenosine triphosphate (ATP). In biomimetic applications, conformational transitions of appropriately designed stickers may also be triggered by light [9].

In an equilibrium situation, the adhesiveness of a biomembrane only depends on the concentration and binding energy of the sticker molecules [10]. We show here that the adhesiveness of membranes with active stickers also depends strongly on the switching rates of the stickers.

Model.—Before considering extended membranes with active stickers, let us first focus on a single sticker. In the simple example shown in Fig. 2, the sticker can only attain two different separations from its ligand. The sticker is within binding range of the ligand at close separation, and out of binding range at a larger separation. At both separations, the sticker can be in either of its two conformations, on or off. In this one-sticker model, we assume that the change in elastic energy of the membrane, which is associated with the change of separation, is negligible. The energy of an off sticker is then independent of the separation, which implies that the rate for the transition from the larger to closer separation is identical to the transition rate for the reverse process. In the dimensionless units used in this example, the two transition rates are equal to 1. An on sticker, in contrast, gains the dimensionless binding energy u when bound to the ligand. If we adopt standard Metropolis dynamics, the dimensionless unbinding rate of
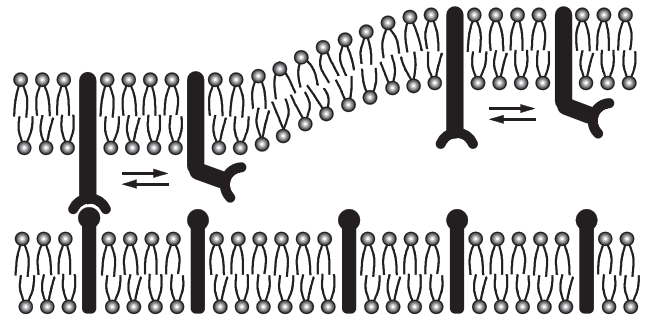

FIG. 1. A membrane with active stickers (top) adhering to an apposing membrane. In one of their two conformations (stretched), the stickers are on and can bind to ligands in the second membrane. The stickers are actively switched between their conformations, with on and off rates that are independent of their binding state. Switching off a bound sticker (left) requires an energy input equivalent to the sticker binding energy, which keeps the system out of equilibrium.
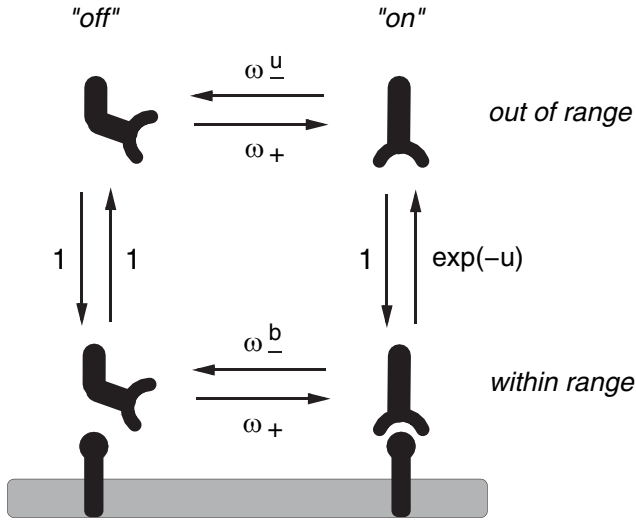
FIG. 2.　A simple four-state model of an active sticker. In this model, the sticker can attain only two different separations from its ligand, and two conformational states, on or off. At close separation, an on sticker is bound to its ligand with binding energy $U = uk_BT$. The transition rates between the four states are given in units of $1/\tau$, where τ is a typical time scale for the local membrane relaxation between the two separations. The dimensionless rates ω_-^b and ω_-^u for switching a sticker off may in general depend on whether the sticker is bound or unbound, whereas the on rate ω_+ is taken to be independent of the separation.

this sticker is $\exp(-u)$, whereas the rate for the reverse process is 1.

　Let us now consider the transition rates between the on and off state. The rate ω_+ for switching the sticker on is independent of the membrane separation if we assume that this transition is thermally activated and that the corresponding energy barrier is not affected by the presence of the ligand. However, the off rates ω_-^b for a bound sticker and ω_-^u for an unbound sticker in general can be different. In equilibrium, the transition rates between the states have to obey detailed balance, which implies that the total rate $\omega_-^b \omega_+$ for passing through the four states in a clockwise direction has to be equal to the total rate $\exp(-u)\omega_-^u \omega_+$ for the counterclockwise cycle. This leads to the equilibrium condition $\omega_-^b = \exp(-u)\omega_-^u$.

　In the following, we will consider a situation in which the stickers are switched off by an active process that violates detailed balance and, thus, the equilibrium condition above. The active process requires energy input, e.g., via ATP hydrolysis. The energy that is set free by the hydrolysis of an ATP molecule typically is significantly larger than the binding energy of an adhesion molecule. Therefore, it seems not unreasonable to assume that the rate of an ATP-driven off-switching process is independent of whether the sticker is bound or not. We then have $\omega_-^b = \omega_-^u \equiv \omega_-$ [11]. At small ATP concentration, the off rate ω_- should be proportional to this concentration.

　Let us now consider a simple, discrete model for a membrane with active stickers that interact with ligands in an apposing membrane. In this model, a "reference plane" parallel to the membranes is discretized into a square lattice with lattice spacing a. At each site i, a sticker molecule is present that can be in the state on ($n_i = 1$) or off ($n_i = 0$). The sticker distribution within the membrane thus is approximated by a square-lattice distribution. The conformations of the membranes are described by the local membrane separation $l_i \geq 0$ at the sticker sites i. The membrane separation is nonnegative since the membranes cannot penetrate each other.

　The configurational energy of the membranes at time t has the form

$$H\{l, n(t)\} = \sum_i \left[\frac{\kappa}{2a^2}(\Delta_d l_i)^2 + n_i(t)V(l_i) \right]. \quad (1)$$

The first term in this configurational energy represents the bending energy of the membranes. Here, $\kappa = \kappa_1\kappa_2/(\kappa_1 + \kappa_2)$ is the effective bending rigidity of the membranes with rigidities κ_1 and κ_2, and the discretized Laplace operator $\Delta_d l_i = \Delta_d l_{x,y} = l_{x+1,y} + l_{x-1,y} + l_{x,y+1} + l_{x,y-1} - 4l_{x,y}$ captures the local mean curvature of the separation field l. The second term is the time-dependent interaction energy of the membranes. If the sticker at site i is on at time t, then $n_i(t)$ is 1 and the membranes interact with the short-ranged sticker potential $V(l_i)$. For simplicity, we model the short-ranged sticker interaction by the square-well potential $V(l_i) = -U$ for $0 \leq l_i \leq l_r$ and $V(l_i) = 0$ for $l_i > l_r$ with characteristic binding energy $U > 0$ and potential range l_r. To reduce the number of parameters, we use the rescaled separation field $z_i = (l_i/a)\sqrt{\kappa/(k_BT)}$ in our simulations. In the following, the rescaled potential range $z_r = (l_r/a)\sqrt{\kappa/(k_BT)}$ of the stickers has the fixed value $z_r = 0.1$.

　The dynamics of the model here is simulated via a master equation with discrete time steps τ. In a time step τ, the rescaled separation z_i at each lattice site i is shifted to a value z_i' with standard Metropolis transition probabilities: the "new" value z_i' is adopted with probability 1 if the new conformation is lower in energy, and adopted with probability $\exp[-(H'-H)/(k_BT)]$ if the energy H' of the new conformation is higher than the energy H of the "old" conformation. Below, we choose $z_i' = z_i + \zeta\rho$ where ρ is a random number between -1 and 1, and $\zeta = 0.5$ is the step size.

　To ensure consistency with versions of the model that are continuous in time [12], the sticker interaction energy is averaged over each time step τ. If the sticker was always on in the interval τ, the interaction energy is $V(z_i)$. If the sticker was always off, the interaction energy is 0. If it was on during a fraction q of the interval τ, the interaction energy is $qV(z_i)$. The membrane separation field thus evolves in a stochastic sticker potential that is averaged over the time steps τ.

Results.—The attractive membrane interaction mediated by the on stickers acts against a steric repulsion of the membranes caused by shape fluctuations. If the sticker-mediated attraction is weak, the membrane fluctuations will lead to large membrane separations, and eventually to an unbinding of the membranes. The average membrane separation in the steady state and the location of the unbinding transition therefore reflect the adhesiveness of the membranes. Clearly, the adhesiveness should increase with the sticker binding energy U and the average fraction of on stickers $X = \omega_+/(\omega_+ + \omega_-)$. However, even for a constant binding energy and a constant fraction of on stickers, the average membrane separation depends sensitively on the switching rates. In Fig. 3, the average membrane separation in the steady state is shown as a function of the mean switching rate $\omega = \frac{1}{2}(\omega_+ + \omega_-)$. The fraction of on stickers is $X = 0.5$, which implies $\omega_+ = \omega_-$. The average membrane separation exhibits a resonance: it is maximal at intermediate switching rates ω, and considerably smaller at high or low switching rates. This resonance of the membrane separation can be understood from an associated resonance in the escape rate of a single bound sticker molecule [13].

The resonance of the membrane separation can lead to a reentrant unbinding transition, see Fig. 4. For specific values of the fraction X and binding energy U of on stickers, the membranes then first unbind with increasing switching rate ω, and later rebind. The unbinding transitions of the active membranes are continuous. At an unbinding point, the average separation $\bar{z}$ continuously tends to infinity [14], and the contact probability P_b, the average fraction of lattice sites with $z_i < z_r$, continuously tends to zero.
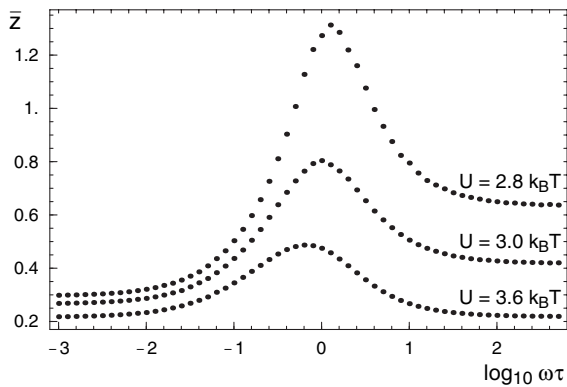
The steady-state phase behavior of the membranes can be characterized by unbinding lines, see Fig. 5. The resonance at intermediate switching rates is reflected in minima of the inverse critical binding energy $1/U_c$. This resonance effect increases with decreasing fraction X of on stickers. In the asymptotic limits of small and large switching rates, the unbinding behavior of the active membranes can be related to the behavior of "equilibrium membranes." In the limit of small switching rates, the active membranes are equivalent to membranes with a concentration X of stickers with "permanent" interaction potential $V(z)$. The effective adhesion potential V_{ef} of such membranes can be determined exactly by integrating over the stickers degrees of freedom in the partition function [10]. Close to the unbinding point, this effective potential is given by $\exp[-V_{\text{ef}}(z_i)/k_BT] = X\exp[-V(z_i)/k_BT] + 1 - X$. Hence, the critical sticker binding energy is

$$U_c(\omega = 0) = -k_BT \ln\frac{X}{\exp[U_c^{\text{hm}}/k_BT] + X - 1}, \quad (2)$$

where U_c^{hm} is the critical binding energy of a homogeneous membrane in a square-well potential with the same rescaled potential range $z_r = 0.1$. From Monte Carlo simulations, we obtain $U_c^{\text{hm}} = 1.26 \pm 0.01$. In the limit of fast sticker switching, the critical binding energy behaves as

$$U_c(\omega = \infty) = U_c^{\text{hm}}/X \quad (3)$$

since the membranes then move in the average potential $XV(z_i)$.

Discussion and conclusions.—We have shown that active switching processes of stickers can have a strong impact on the separation and unbinding behavior of membranes. The adhesiveness of an "equilibrium sticker" is characterized by its binding energy U. In contrast, the adhesiveness of an active sticker depends also sensitively
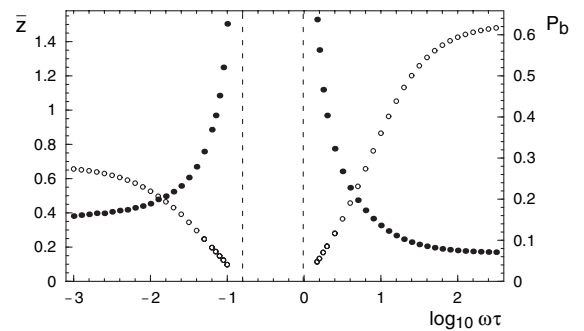
FIG. 3. The averaged rescaled separation $\bar{z}$ of the membranes as a function of the mean switching rate ω for the average fraction $X = 0.5$ of on stickers. The membrane separation has a maximum at intermediate switching rates ω, and decreases with increasing sticker binding energy U. The data points are obtained from Monte Carlo simulations with up to 10^7 steps per lattice site on lattices with up to 140×140 sites. Statistical errors are smaller than the symbol sizes.

FIG. 4. Average membrane separation $\bar{z}$ (solid circles) and contact probability P_b (open circles) as a function of the mean switching rate ω. The fraction of on stickers here is $X = 0.2$, and the sticker binding energy is $U = 10k_BT$. With increasing switching rate, the membranes first unbind and then rebind at the critical rates $\log\omega_c\tau = -0.79 \pm 0.07$ and $\log\omega_c\tau = -0.02 \pm 0.05$ (dashed lines), which are obtained from extrapolation to $P_b = 0$ and $1/\bar{z} = 0$.
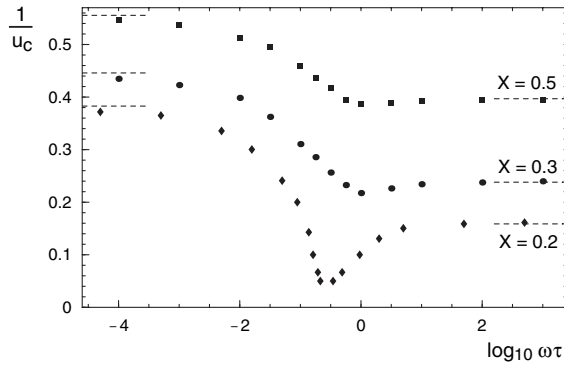
FIG. 5. Unbinding lines obtained from Monte Carlo simulations for several values of the fraction X of on stickers. Here, $u_c \equiv U_c/k_BT$ is the dimensionless critical binding energy of the stickers, and ω is the mean switching rate. At a given value of X, the membranes are bound for sticker binding energies U larger than U_c and, thus, for $1/U < 1/U_c$, and unbound for $1/U > 1/U_c$. The resonance at intermediate switching rates is reflected in small values of $1/U_c$ and most pronounced at $X = 0.2$. The dashed lines indicate the asymptotic limits given by the Eqs. (2) and (3).

on the transition rates of the conformational switching process.

At low switching rates, a bound sticker can unbind either by (i) "hopping" out of the potential well, or by (ii) diffusing out of the potential range after being switched off, since the probability for being switched on in the subsequent time steps is small. The typical time scales for these processes are (i) the intrinsic unbinding time $t_{ub} \sim \tau \exp[U]$, and (ii) the inverse off rate $1/\omega_-$. For $\omega_- \ll 1/t_{ub}$, the adhesion behavior is nearly unaffected by the sticker switching. The impact of the active switching becomes clearly noticeable for switching rates with $\omega_- \simeq 1/t_{ub}$. For biological adhesion molecules such as integrins or selectins, the typical dissociation times t_{ub} are of the order of seconds [15]. Switching processes on second or subsecond time scales therefore should allow the control of the adhesion behavior of biological or biomimetic membranes.

For a given concentration X of on stickers, the adhesion strength in our model is minimal at resonant mean switching rates $\omega \sim 1/\tau$, see Figs. 3 and 5. The characteristic time scale τ corresponds to the typical relaxation time of membrane segments with linear size a, the distance between the stickers. If hydrodynamic damping dominates, this relaxation time scales as $4\eta a^3/(\pi^3\kappa)$ [16], where η is the viscosity of the surrounding solvent. For the typical

bending rigidity $\kappa = 10k_BT$, an average lateral sticker distance of 180 nm, and the typical value $\eta = 0.06$ poise for the cytoplasm viscosity of a cell, we obtain a relaxation time scale of 0.1 ms. On this length scale, intermonolayer friction leads to the same estimate for the relaxation time [17].

B. R. thanks Marek Napiórkowski for stimulating discussions.

[1] *Structure and Dynamics of Membranes*, edited by R. Lipowsky and E. Sackmann, Handbook of Biological Physics Vol. 1 (Elsevier, Amsterdam, 1995).
[2] J. Prost and R. Bruinsma, Europhys. Lett. **33**, 321 (1996).
[3] J.-B. Manneville, P. Bassereau, D. Levy, and J. Prost, Phys. Rev. Lett. **82**, 4356 (1999).
[4] S. Ramaswamy, J. Toner, and J. Prost, Phys. Rev. Lett. **84**, 3494 (2000).
[5] R. Granek and S. Pierrat, Phys. Rev. Lett. **83**, 872 (1999).
[6] M. C. Sabra and O. G. Mouritsen, Biophys. J. **74**, 745 (1998).
[7] H.-Y. Chen, Phys. Rev. Lett. **92**, 168101 (2004).
[8] J. Takagi, B. M. Petre, T. Walz, and T. A. Springer, Cell **110**, 599 (2002); M. Kim, C. V. Carman, and T. A. Springer, Science **301**, 1720 (2003).
[9] G. Möller, M. Harke, H. Motschmann, and D. Prescher, Langmuir **14**, 4955 (1998); S. Abbott, J. Ralston, G. Reynolds, and R. Hayes, Langmuir **15**, 8923 (1999); K. Ichimura, S.-K. Oh, and M. Nakagawa, Science **288**, 1624 (2000).
[10] R. Lipowsky, Phys. Rev. Lett. **77**, 1652 (1996); T. R. Weikl and R. Lipowsky, Phys. Rev. E **64**, 011903 (2001).
[11] More generally, a violation of detailed balance is obtained for $\omega_-^b = \exp(-cu)\omega_-^u$ with $c \neq 1$. We consider here the special case $c = 0$, but expect very similar results for any $|c| \ll 1$.
[12] B. Różycki, T. R. Weikl, and R. Lipowsky (unpublished).
[13] See EPAPS Document No. E-PRLTAO-96-148605 for an appendix. This document can be reached via a direct link in the online article's HTML reference section or via the EPAPS homepage (http://www.aip.org/pubservs/epaps.html).
[14] R. Lipowsky and S. Leibler, Phys. Rev. Lett. **56**, 2541 (1986).
[15] X. Zhang, E. Wojcikiewicz, and V. T. Moy, Biophys. J. **83**, 2270 (2002); E. Evans, A. Leung, D. Hammer, and S. Simoni, Proc. Natl. Acad. Sci. U.S.A. **98**, 3784 (2001).
[16] F. Brochard and J. F. Lennon, J. Phys. (Paris) **36**, 1035 (1975).
[17] U. Seifert and S. A. Langer, Europhys. Lett. **23**, 71 (1993).