Steady-State Compartmentalization of Lipid Membranes by Active Proteins

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ABSTRACT   Using a simple microscopic model of lipid-protein interactions, based on the hydrophobic matching principle, we study some generic aspects of lipid-membrane compartmentalization controlled by a dispersion of active integral membrane proteins. The activity of the proteins is simulated by conformational excitations governed by an external drive, and the deexcitation is controlled by interaction of the protein with its lipid surroundings. In response to the flux of energy into the proteins from the environment and the subsequent dissipation of energy into the lipid bilayer, the lipid-protein assembly reorganizes into a steady-state structure with a typical length scale determined by the strength of the external drive. In the specific case of a mixed dimyristoylphosphatidylcholine-distearoylphosphatidylcholine bilayer in the gel-fluid coexistence region, it is shown explicitly by computer simulation that the activity of an integral membrane protein can lead to a compartmentalization of the lipid-bilayer membrane. The compartmentalization is related to the dynamical process of phase separation and lipid domain formation.

INTRODUCTION

The biological membrane is a truly nonequilibrium system, being subject to fluxes of energy and matter. These fluxes are driven by external sources or by sources associated with the membrane itself. The membrane responds to these fluxes in a variety of ways that involve both small-scale intramolecular changes as well as changes in the structural and molecular organization of the membrane on scales that range from the nanometer scale to the size of the entire cell. This is a consequence of the fact that the membrane is a complex and structured many-particle system associated with a considerable degree of cooperativity. The changes induced by fluxes may themselves induce nonequilibrium processes involving transduction of energy and matter, which ultimately couple back to the membrane. The intricate interplay of energy dissipation and recurrent structural reorganization hence lies at the very core of all membrane energetics and function.

Despite the anticipation that nonequilibrium conditions are important for membrane function, most biophysical studies of model membrane systems either proceed along the lines of thermodynamic equilibrium for the entire system or focus solely on the activity of the proteins (Blumenfeld and Tikhonov, 1994). There are three obvious reasons for this. First, the tremendous complexity involved hampers quantitative studies of the nonequilibrium organization of the full system. Second, the lipid membrane is tacitly assumed by many workers to be a featureless solvent that is not a very active player in protein function. Finally, and more important for the present paper, there is a lack of simple concepts and conceptual theoretical models that can rationalize nonequilibrium situations. In this respect, the celebrated fluid-mosaic model (Singer and Nicolson, 1972) is of little use, because it does not provide much indication of how the lipid-bilayer matrix may be structurally organized away from equilibrium.

In this paper we shall present a simple and generic model of a lipid membrane with active integral proteins that permits a study of the nonequilibrium steady-state organization of a lipid-protein array. In addition to the lipids, the model includes integral proteins that undergo conformational transitions driven by external energy sources. The model accounts for the dissipation of energy from the proteins into the lipids, the subsequent restructuring of the lipid bilayer, and the coupling back from the lipid organization to the protein conformational states. Hence the model provides a paradigm for dealing with a nonequilibrium membrane with active proteins and may, by virtue of this, provide a framework for designing new experimental model systems aimed at obtaining a deeper understanding of membranes in their functional state.

To be computationally tractable, the model proposed is rather simple. It considers a dispersion of transmembrane amphiphilic proteins integrated into a lipid-bilayer membrane. The proteins are assumed to be of cylindrical shape, characterized by a hydrophobic length. The proteins have two discrete internal conformational states, described by different hydrophobic lengths. The coupling to the lipid bilayer is assumed to be governed by the degree of hydrophobic matching between the hydrophobic lipid-bilayer thickness and the hydrophobic length of the protein (Mouritsen and Bloom, 1984, 1993; Mouritsen and Sperotto, 1992). The lipid bilayer is described by a microscopic molecular interaction model (Pink et al., 1980; Dammann et al., 1996) that is capable of describing the phase equilibria controlled by the gel-fluid phase transition. The lipid-bi-

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layer model is used in a version that accounts for the mixing properties of two phospholipid species with the same phosphatidylcholine (PC) headgroup, but with saturated acyl chains of different lengths. By considering binary lipid mixtures that mix in a way that depends only on the difference in hydrophobic acyl-chain length and by considering proteins that match these lengths, we have a very favorable and clean setting for investigating nonequilibrium properties of a lipid-protein array.

The simplicity of the model is both its advantage as well as its most severe shortcoming when it comes to modeling realistic systems. By not accounting for the energy flow in a detailed manner, by considering only a two-state protein, by involving a number of phenomenological model parameters, and by focusing on the hydrophobic mismatch interaction, it will, in most cases, fail to produce results that can be quantitatively compared to experiments. However, its simplicity provides a transparent picture of the essential biophysical principles of self-organization and active membranes, and may in this capacity serve as a conceptual tool for future developments of a more refined picture.

MODEL AND METHODS

Model

The model is a microscopic version of the mattress model of lipid-protein interactions in membranes (Mouritsen and Bloom, 1984; Zhang et al., 1993; Dammann et al., 1996), which assumes that the dominant part of the lipid-protein interaction is controlled by the hydrophobic matching of lipid bilayer thickness, d_L, and hydrophobic length, d_P, of the protein. The hydrophobic mismatch interaction is expected to be relevant when it comes to a determination of phase equilibria involving the gel-fluid phase transition, because the bilayer thickness undergoes substantial changes in the transition region. To further focus on the effects of mismatch, we shall be concerned here with binary mixtures of lipids with the same headgroups (PC), but with different acyl-chain lengths, specifically dimyristoylphosphatidylcholine (DMPC) and distearoylphosphatidylcholine (DSPC).

The model we use for the binary lipid mixture in a lamellar phase is the 10-state lattice model by Pink et al. (1980), modified to describe the phase equilibria in mixtures of lipids with different acyl-chain lengths (Risbo et al., 1995; Jørgensen and Mouritsen, 1995). The 10 internal states and the associated degeneracies of each lipid species reflect the internal conformational statistics of long hydrocarbon chains. For appropriately chosen sets of phenomenological parameters, the model faithfully describes the phase equilibria of highly nonideal lipid mixtures. In particular, it describes the broad gel-fluid phase coexistence region of the DMPC-DSPC mixture (Jørgensen and Mouritsen, 1995). In this coexistence region, the gel phase consists predominantly of the long-chain lipid, DSPC, which has the higher transition temperature, and the fluid phase is mainly

made up of the short-chain lipid, DMPC. The interaction with integral membrane proteins is taken into account (Mouritsen et al., 1996) by parameterizing the lipid-protein interaction in terms of the mismatch, $|d_L - d_P|$.

The Hamiltonian of the model can formally be written as

$$\mathcal{H} = \mathcal{H}_0 - \sum_{\langle i,j \rangle} \sum_{p,q} (J_{pq}\mathcal{L}_i^p\mathcal{L}_j^q + K_{pP}\mathcal{L}_i^p\mathcal{P}_j - K_{PP}\mathcal{P}_i\mathcal{P}_j) \\ + \sum_i E_i^P\mathcal{P}_i \tag{1}$$

where $\mathcal{L}_i^p = 0,1$ is the site occupation variable for lipid species p (= DMPC, DSPC), and $\mathcal{P}_j = 0,1$ is the site occupation variable for the protein. $\mathcal{L}_i^p$ has an implicit dependence on the conformational state of the acyl chain, and similarly, $\mathcal{P}_j$ has an implicit dependence on the internal state of the protein. $\mathcal{H}_0$ is a single-site energy including the intrachain conformational energy of the lipid acyl chains in addition to an internal pressure-area term (Pink et al., 1980). J_{pq}, $K_{pP} = K|d_L^p - d_P|$, and K_{PP} are positive interaction constants. The function J_{pq}, furthermore, depends on the conformational state of the two involved acyl chains. Details regarding values of some of the interaction constants can be found in Mouritsen et al. (1996). The interactions are restricted to nearest neighbors on a triangular lattice. The lipid composition is given by $x_p = (2C)^{-1}\Sigma_i\mathcal{L}_i^p$ and $x_q = (2C)^{-1}\Sigma_i\mathcal{L}_i^q$, with $C = \frac{1}{2}(N - \Sigma_j\mathcal{P}_j) + n_P^{-1}\Sigma_j\mathcal{P}_j$, where N is the number of sites on the lattice, each of which is occupied by a lipid acyl chain (i.e., one half of a lipid molecule) or by a fraction, n_P^{-1}, of a protein. Each protein is taken to occupy n_P lattice sites arranged in a connected compact hexagonal shape. The protein concentration is therefore $x_P = 1 - x_{DMPC} - x_{DSPC} = (n_PC)^{-1}\Sigma_j\mathcal{P}_j$. The proteins are assigned two discrete internal states, a ground state and an excited state, described by two energy levels, $E_i^P = E_g^P, E_{ex}^P$, separated by an energy gap, $E_{ex}^P - E_g^P = \Delta E_P$, and two different hydrophobic lengths, d_P^g and d_P^{ex}.

For the sake of simplicity, we have restricted ourselves to the study of a single path in the phase diagram, $x_{DMPC} = x_{DSPC} = 0.49$, and $x_P = 0.02$ for $n_P = 7$, corresponding to a small integral protein or polypeptide with a cross-sectional area of ~ 200 Å^2. The mismatch interaction constant is taken to be $K = 0.06$ erg/Å. The protein-protein interaction constant is taken to be $K_{PP} = 6.18 \times 10^{-13}$ erg, corresponding to a repulsive protein-protein interaction. The two internal states of the protein are taken to be associated with hydrophobic lengths of $d_P^{ex} = 21.75$ Å and $d_P^g = 42.5$ Å, respectively, cf. Fig. 1. Hence the ground-state protein length is close to the hydrophobic thickness of DSPC bilayers in the gel phase, and the excited-state protein length is matched to the DMPC hydrophobic bilayer thickness in the fluid phase. The internal energy gap between these two states is taken to be $\Delta E_P = 21 \times 10^{-13}$ erg. The model is defined on a triangular lattice with $N = 100 \times 100$ acyl-chain sites, subject to periodic boundary conditions.

For comparative purposes, some simulations have also been performed at protein concentrations other than those

indicated above and for a case of very small "proteins," which occupy only one lattice site ($n_p = 1$ and $\Delta E_p = 3 \times 10^{-13}$ erg).

Method

The equilibrium properties of the model described above can readily be investigated by Monte Carlo computer-simulation methods (Mouritsen et al., 1995; Dammann et al., 1996). In contrast to membrane models described by force fields (Merz and Roux, 1996), the model in Eq. 1 has no natural dynamics of its own and is therefore implemented with a stochastic dynamics of the following type. Transitions between internal states in the lipid acyl chains are controlled by Glauber dynamics, whereas the diffusional motion of lipids and proteins is accounted for by two-particle Kawasaki exchange between nearest neighbors. Both the lipids and the proteins are therefore subject to translational motion, and the system can relax by interdiffusion of the different species. In the case of diffusion of proteins, the elementary move corresponds to a translation over the range of one lattice constant. During such a move, the displaced lipids in front of the protein are translocated behind the protein. The acceptance criterion for a dynamical move is given by the standard Monte Carlo Metropolis rate, $\min\{1, \exp(-\Delta\mathcal{H}/k_B T)\}$. This rate implies that the membrane everywhere in space is coupled to a thermal bath characterized by a temperature T. For simplicity, the time scales for the different dynamical processes involved are taken to be of the same magnitude without any loss of generality.

On top of this dynamics, we assign internal conversions within the proteins as illustrated in Fig. 1. The proteins are activated (e.g., by photons in the case of a light-sensitive protein) on a time scale τ, where a randomly chosen protein is subject to an attempt to change the internal state (Glauber dynamics). If the protein under consideration happens to be in its ground state, it becomes excited with probability 1, i.e., it is driven to the excited state. If it is already in the excited state, it will decay with a Boltzmann probability involving the energy gap, ΔE_P, as well as the change of energy due to interactions with the neighboring lipids. The strength of the drive is therefore conveniently described by the parameter $\Gamma = \tau^{-1}$, implying that the total flux of energy from the environment into the system is proportional to the number of proteins. The value of Γ is a measure of the

level of protein activity. The protein undergoes a translational diffusional motion on the same time scale as the drive. The dynamical method produces a nonequilibrium situation for the model, which after some relaxation time will settle into a steady state characterized by the value of the driving strength, Γ. The number of dynamical moves needed to bring the system into the steady state varies, depending on the value of Γ—the smaller the value of Γ, the more moves are needed. Typical values are 5×10^5 Monte Carlo steps per site. The presence of the drive makes the system open (i.e., a non-Hamiltonian system), which is related to a general class of statistical nonequilibrium systems, so-called driven-diffusive systems (Schmittmann and Zia, 1995). A simple lattice-gas version of this type of model was studied by Gilhøj (1996) and Sabra et al. (unpublished observations) to describe binary fluid mixtures with chemically reactive impurities. A related use of chemical reactivity to compartmentalize polymeric materials was investigated recently (Fredrickson, 1996; O'Shaughnessy and Sawhney, 1996).

Formally, the driven model described by this dynamical method can be considered a model with two thermodynamic temperatures, a (lower) temperature of the bulk matrix (the lipids), and another (higher) temperature associated with mobile "hot spots" (the proteins). In the driven state, the system receives energy in the "hot spots," which in turn transduct this energy, via the molecular interactions, to the bulk matrix, from which it is finally returned to the heat bath. It should be pointed out that the assumption of a coupling to the heat bath everywhere in the membrane system is very realistic, because the lipids experience a strong and fast thermocouple to the water phase. Therefore, the present situation dealing with a lipid bilayer in water is very different from that of thermal conduction of energy in a temperature gradient or diffusion of thermal energy from a bath of high temperature to one with low temperature, where the system is isolated between the baths (Harris and Grant, 1988).

RESULTS

The upper part of Fig. 2 shows a gallery of microconfigurations typical for steady state for a series of different driving strengths, Γ (protein activities), for two different temperatures. Red and green correspond to DSPC and DMPC acyl chains, respectively. To visualize the nature of
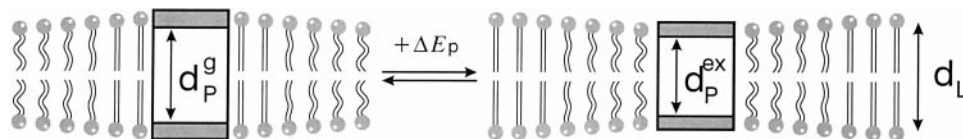

FIGURE 1　Schematic illustration of the hydrophobic matching principle for lipid–protein interactions in membranes (of hydrophobic thickness d_L) and how the driven internal transition in an active protein takes place. The transition involves two states of the protein, the group state (g) and the excited state (ex), characterized by hyrophobic lengths, d_P^g and d_P^{ex}, respectively. The transition is associated with internal energy change, ΔE_P.
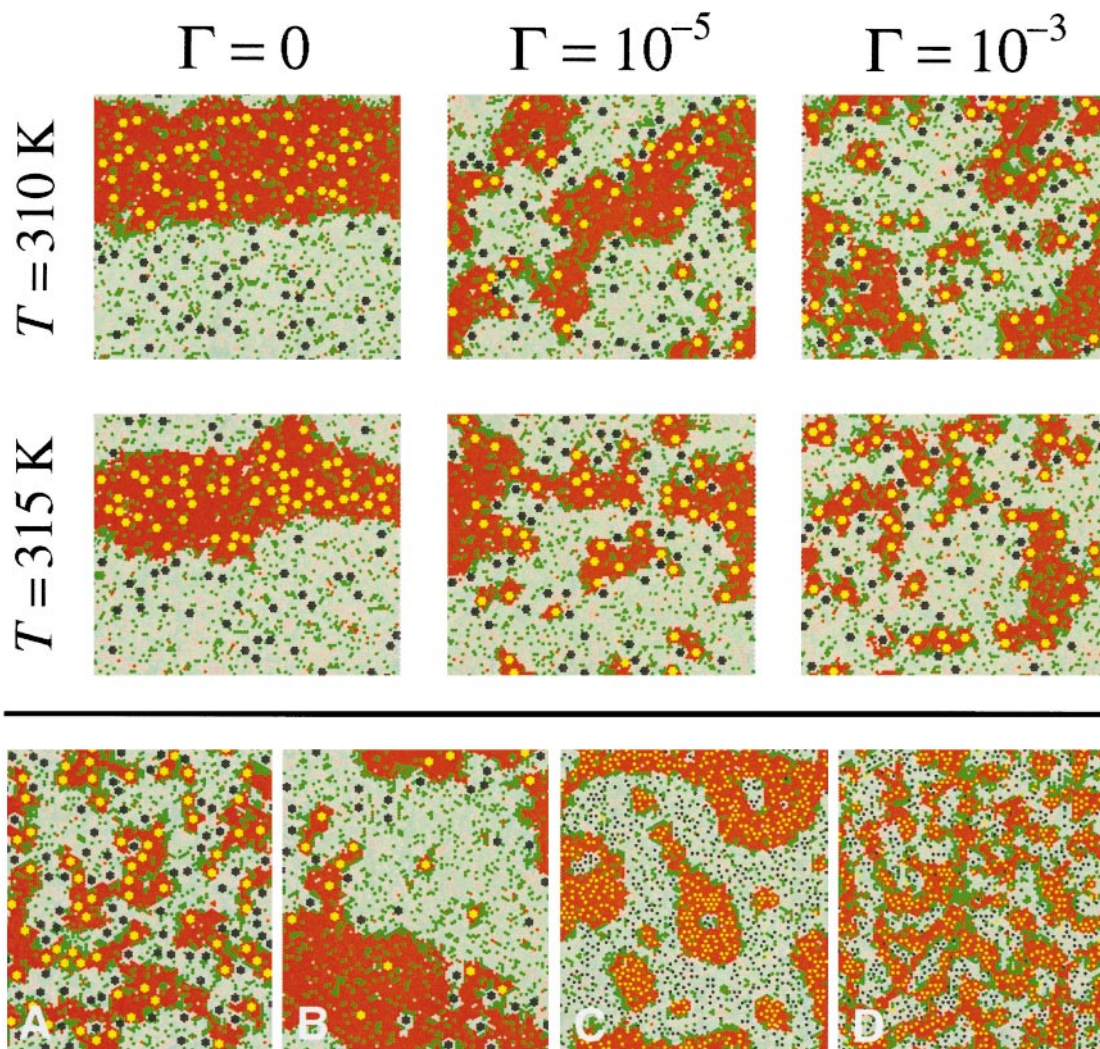
FIGURE 2 (*Upper panel*) Snapshot of steady-state domain organization of binary DMPC-DSPC lipid bilayers with active model proteins at different levels, Γ, of activity. Results are shown for two different temperatures which in equilibrium correspond to states in the gel-fluid coexistence region. The proteins occupy seven sites and occur in a concentration $x_P = 0.02$. Hexagonal seven-site symbols denote proteins in the ground state (*yellow*) and in the excited state (*black*). DMPC acyl chains are denoted by small dots of dark green (gel) and light green (fluid). DSPC acyl chains are denoted by small dots of dark red (gel) and light red (fluid). (*Lower panel*) Snapshot of steady-state domain organization of binary DMPC-DSPC lipid bilayers with active model proteins at an activity level Γ and temperature $T = 310$ K. (A) Seven-site proteins in a concentration of $x_P = 0.01$ for $\Gamma = 10^{-3}$ and $x_{DMPC} = x_{DSPC} = 0.485$. (B) Seven-site proteins in a concentration of $x_P = 0.01$ for $\Gamma = 10^{-3}$ and $x_{DMPC} = x_{DSPC} = 0.495$. (C) Single-site proteins in a concentration of $x_P = 018$ for $\Gamma = 10^{-3}$ and $x_{DMPC} = x_{DSPC} = 0.41$. (D) Single-site proteins in a concentration of $x_P = 0.18$ for $\Gamma = 1$ and $x_{DMPC} = 0.36$ and $x_{DSPC} = 0.46$.

the phase of the lipid bilayer, the dark colors (red and green) denote the lipid acyl-chain states that are characteristic of the gel phase, and the light colors denote the chain states that are fluidlike. The proteins are shown in yellow and black, corresponding to the ground and the excited state, respectively. For the sake of comparison, the equilibrium states ($\Gamma = 0$) are included in the upper part of Fig. 2. The top row of Fig. 1 corresponds to a temperature, $T = 310$ K, deep within the equilibrium gel-fluid coexistence region, ~8 K below the liquidus line. The equilibrium situation is a phase-separated state with almost the same amount of gel and fluid phase, and with an interface between the gel and fluid phase that is well defined but which has some thermal roughening. It is seen that to mediate the interface and to

lower the interfacial tension, the DMPC lipid chains in the thin fluid phase wet the interface to the thick gel phase by stretching out into their gel conformational state. The non-equilibrium-driven configurations in the upper part of Fig. 2 clearly show that the effect of the protein activity in steady state is to break down and reorganize the gel-fluid phase-separated state into domains characterized by a finite length scale. The corresponding average domain size (area), A, can be calculated from the full domain size distribution function. In addition to reducing the domain size, increasing values of Γ lead to a more ramified domain picture. Hence the protein activity leads to a structural reorganization of the binary lipid mixture. A particular aspect of this reorganization is the generation of more gel configurations for the

short-chain lipid, which will mediate the mismatch between the proliferating gel and fluid domains (Jørgensen and Mouritsen, 1995).

The effect of temperature on the domain pattern is seen by comparing the top row and the second row of Fig. 2. The second row corresponds to a higher temperature, $T = 315$ K, closer to the equilibrium phase boundary, ~3 K below the liquidus line. At this higher temperature, the fluid fraction is larger than the gel fraction. As is well known for the phase-separated state in equilibrium, the interfaces between the phases are rougher at higher temperatures because of increased compositional fluctuations (Jørgensen et al., 1993). Turning on the protein activity at this higher temperature also leads to a break-up of the phase coexistence region. The drive is more effective at the higher temperature, given the same value of Γ, because the interfacial tension is lower, corresponding to rougher interfaces (Risbo et al., 1995).

The microconfigurations shown in Fig. 2 correspond to moderate activities in the sense that the drive is sufficiently slow to allow the neighboring lipids to adapt during the average time lapse between successive attempts to change protein conformation. This implies that the shorter proteins in the excited state accumulate in the thinner fluid lipid domains to which their hydrophobic length is best matched, and that the longer proteins in the ground state prefer to be dissolved in the thicker gel-phase domains. This is a consequence of the values chosen for the hydrophobic lengths of the protein states. As the protein activity is further increased, the proteins and the lipids have less time to adapt to the hydrophobic matching condition before a new conformational transition may take place. In addition to producing smaller domains, this also leads to a preferential location of the proteins near the interfaces between the gel and fluid regions (cf. the microconfigurations for $\Gamma = 10^{-3}$ shown in Fig. 2). In this case the active proteins will act like interfacially active agents, which is another way of describing the ability to break down the phase coexistence, in much the same way as a soap can emulsify oil-water mixtures. In the case of a very strong drive, the lipids do not have enough time to reorganize the domains before the proteins change state again. Within this limit, the proteins will effectively act as mobile inactive impurities with some average hydrophobic length, and the lipid-protein mixture will behave as an equilibrium system with macroscopic phase separation.

The effect of the protein concentration is shown in Fig. 2, *A* and *B*. For the same level of protein activity, decreasing protein concentration leads to larger lipid domains in the steady state. In fact, for the low protein concentration in Fig. 2 *B*, the state of the system seems to be one of macroscopic phase separation. This may be a finite-size effect in the sense that larger system sizes (outside the range of the present simulations) may show that the domain structure actually breaks up at length scales larger than the size of the system in Fig. 2 *B*.

The effect of the size of the active proteins is illustrated in Fig. 2, *C* and *D*. For the same level of protein activity and

protein-to-lipid mass, the smaller proteins lead to a less ramified domain morphology. The main reason for this is the relatively higher mobility of the smaller proteins, which are capable of diffusing away from the interface. Therefore, they are less effective in lowering the interfacial tension, leading to more regular interfaces. When the gel and fluid fractions are similar, small proteins, for the same reason, are capable of producing effectively connected structures, as shown in Fig. 2 *D*. In such structures, both phases are dynamically percolated.

A quantitative measure of the restructuring of the binary lipid mixture in the presence of active proteins is provided in Fig. 3, which shows the average domain area, $A(\Gamma)$, as a function of Γ in a double-logarithmic plot. It is seen that the data, to a good approximation, scale as a power law in Γ, i.e.,

$$A(\Gamma) \approx \Gamma^{-n} \qquad (2)$$

with an exponent value, $n \simeq 0.20 \pm 0.02$, for a wide range of Γ values. We shall return to a discussion of this exponent value and provide an explanation of the cross-over for large values of Γ.

The corresponding results for the average domain size in steady state in the case of the smaller, single-site proteins are shown in Fig. 4. Again we find that the data for a wide range of protein activities conform to the power law (Eq. 2) with a similar exponent value, $n \simeq 0.19 \pm 0.02$. Hence it appears that this exponent is a rather robust quantity that does not depend on temperature or the size of the protein.

The data in Fig. 3 clearly show the effect that a very high protein activity leads to a cross-over toward larger domain sizes and eventually to global phase separation. In the limit of this strong drive, the steady-state situation corresponds to a phase-separated lipid binary mixture in thermodynamic equilibrium with proteins that are effectively in an average internal state and hence are not able to break down the macroscopic phases.

An interesting effect observed at low temperatures is that the total energy is found to be lower when the drive is on than when it is off. This somewhat counterintuitive effect is due to the fact that the proteins are wetted by the short
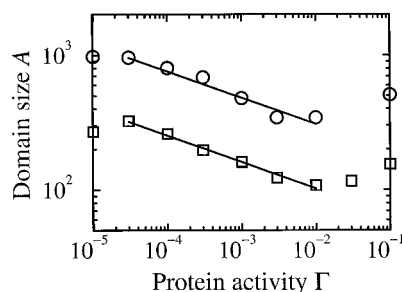

FIGURE 3 Average domain size (area), $A(\Gamma)$ (in units of number of lattice sites), for binary DMPC-DSPC lipid bilayers with active seven-site proteins at activity level Γ. Results are shown for two different temperatures. □: $T = 310$ K; ○: $T = 315$ K.
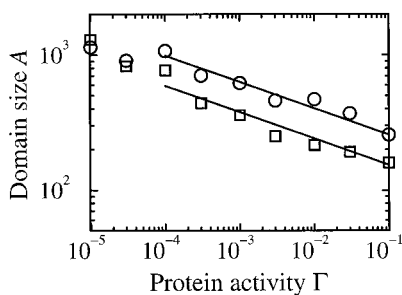
FIGURE 4 Average domain size (area), $A(\Gamma)$ (in units of number of lattice sites), for binary DMPC-DSPC lipid bilayers with active single-site proteins at activity level Γ. Results are shown for two different temperatures. $\square$: $T = 310 \ K$; $\bigcirc$: $T = 315 \ K$.

DMPC lipid chains in their gel conformation. This lowers that part of the total energy that is related to the intramolecular conformational energy. This effect becomes particularly important when a wetting layer is formed between two adjacent proteins via capillary condensation (Gil et al., 1997).

The data reported above constitute the main result of the present work. The results are generic in the sense that they are due to the fundamental nonequilibrium condition built into the model via the active proteins. The details of the results will depend, however, on the actual model parameters chosen. We have made some preliminary investigations for variations of these parameters and shall briefly describe the results obtained.

For the active proteins to have an effect on the lipid organization, it is essential that the energy gap, ΔE_{P}, is comparable to the energy representing the lipid-protein interaction. If the gap is too small or too large, most of the proteins are in the excited or ground state, respectively. In either case, the activity just serves to shift the phase equilibria toward the fluid or gel phase, respectively.

The value of the mismatch interaction constant, K, influences the rate of decay into the steady state. The larger the value of K, the faster the approach to steady state. However, the length scale of the steady-state pattern does not seem to be significantly influenced by the value of K.

The values of the hydrophobic lengths, d_{P}, of the protein conformational states relative to the average hydrophobic lengths (the bilayer hydrophobic thickness) of the lipid acyl chains determine the effect of the protein activity on the type of reorganization in steady state. The more distinct the different values of d_{P}, and the closer these match the gel and fluid bilayer thicknesses, the more dramatic the effect of breaking down the coexistence region is expected to be. Furthermore, the closer the values of d_{P} are tuned to lengths of the individual lipid species, the more the protein activity will act to locally separate the two species.

DISCUSSION AND CONCLUSIONS

We have proposed a new type of nonequilibrium model designed to describe the steady-state organization of lipid-protein membranes driven by input of energy from external sources. We have shown that the protein activity introduces a new length scale and therefore can be used as a means of compartmentalizing multicomponent membranes, which, under equilibrium conditions, would be subject to macroscopic phase separation. Such a principle of steady-state compartmentalization may be of substantial interest because it suggests a mechanism by which biological membranes can set up the compartments needed to steer enzymatic reactions on the membrane surface, without having to cope with fully isotropic random diffusion of the reactants (Melo et al., 1992). Of particular interest in this context is the possibility of forming percolating structures in the membrane (cf. Fig. 2 D), in which case the protein activity has led to two disjoint reaction compartments, each of which is effectively connected. This, in turn, may provide for a long-range communication between remote parts of the membrane structure. It is also of interest in this context to point to the possible coupling between the protein activity and the membrane curvature (Prost and Bruinsma, 1996), which we have left out of the present simple modeling.

The model proposed and the results presented in this paper should be considered in the general context of self-organization of membranes and of how this organization may serve to support function (Kinnunen, 1991; Mouritsen and Biltonen, 1993; Mouritsen and Kinnunen, 1996). A substantial amount of evidence of lipid-domain formation in membranes is currently being compiled from a number of experimental (Edidin, 1992; Tocanne, 1992; Bergelson et al., 1995; Lehtonen et al., 1996; Pedersen et al., 1996; Mouritsen and Jørgensen, 1997; Gliss et al., unpublished) and theoretical (Pedersen et al., 1996; Mouritsen and Jørgensen, 1994) studies of membrane systems. This evidence points to a heterogeneous lateral membrane organization on many different length scales (Bergelson et al., 1995). Furthermore, the activity and binding characteristics of certain enzyme systems, e.g., phospholipase A_2 (Hønger et al., 1996), protein kinase C (Dibble et al., 1996), and cytochrome c (Mustonen et al., 1987), have been suggested to be controlled by the microheterogeneity of lipid bilayers.

As mentioned in the Introduction, most studies relating membrane structure to function are invariably concerned with systems in thermodynamic equilibrium. However, some evidence has been reported of very slow reorganizational phenomena in binary lipid mixtures (Jørgensen et al., 1996; Sperotto and Mouritsen, 1993) and of domain organization in binary lipid mixtures with and without proteins (Sankaram et al., 1992; Schram and Thompson, 1997). Protein activity of the type discussed in the present paper adds a new dimension to membrane organization and to how it may influence function. The function of many integral membrane proteins seems to be rather insensitive to the interactions with lipids, in the sense that the molecular events associated with the protein activity are not influenced by the lipids. Bacteriorhodopsin appears to be a well-known example of this type, although the aggregational state of bacteriorhodopsin is dependent on the lipids in the mem-

brane, and the protein's immediate lipidic environment is most certainly influenced by the protein (Piknová et al., 1993; Sperotto and Mouritsen, 1993; Dumas et al., 1997; Sternberg et al., 1992). It has been reported that rhodopsin function (Brown, 1994), particularly the transition from the meta-I to the meta-II state (which is of importance for the visual process in the retina), is very sensitive to certain types of lipids that are capable of adapting to the lipid-protein interface during the transition. The two rhodopsin states have different hydrophobic lengths, and it is possible that the hydrophobic matching condition and the coupling between lipid organization and protein activity, as studied in this paper, may be of some relevance for studies of lipid-rhodopsin recombinants where the external drive is provided by a light source to which rhodopsin is sensitive.

Using active proteins as a means of compartmentalizing lipid membranes is, from a physics point of view, conceptually related to halting a phase-separation process in a steady state by coupling the dynamics of the moving phase boundaries to a competing process (e.g., a chemical reaction or the production of an appropriate surfactant; Glotzer et al., 1994, 1995; Toxvaerd, 1996; Fredrickson, 1996; O'Shaughnessy and Sawhney, 1996; Christensen et al., 1996). It has been shown that this coupling will introduce a new length scale into the system. Theoretical analyzes based on a linearized version of the Cahn-Hilliard equation for spinodal decomposition (Glotzer et al., 1995; Christensen et al., 1996) have suggested that the steady-state linear length scale, R, scales with the rate $\bar{\Gamma}$ of the reaction as a power law, $R(\bar{\Gamma}) \approx \bar{\Gamma}^{-p}$, with $p = 1/3$ for low rates and a cross-over to a lower exponent value, $p = 1/4$, at higher rates. The finding of a low exponent value, $\pm \sim 0.20–0.25$, was reported in a couple of numerical simulation studies of simple models (Glotzer et al., 1994; Toxvaerd, 1996). To perform a comparison with the results of the present paper, it should be observed that $A \approx R^2$, i.e., one would expect that $n = 2p$. In our model, the level of protein activity, Γ, plays a role equivalent to the reaction rate, $\bar{\Gamma}$. The results in Figs. 3 and 4 for $A(\Gamma)$ are supportive of a power-law relation in an extended range of Γ, although with a smaller exponent value, i.e., $n \simeq p$. We have recently found that $n \simeq 2p$ for a simple lattice-gas model driven in the same way as the one studied in the present paper, but without the spectrum of internal states of the lipids (M. C. Sabra, H. Gilhøj, and O. G. Mouritsen, unpublished observations). Hence it appears that the internal conformational states characteristic of the lipids make the drive less effective in reducing the domain size, possibly because the internal states of the lipids themselves act as interfacial agents and thereby compete with the active proteins for access to the interfacial regions.

In closing it should be pointed out that the model approach proposed in the present paper is of a generic and general nature, and it should not be expected to compare quantitatively with experimental data. The algebraic relation between the domain size and the level of protein activity in Eq. 2, however, seems to be robust to details of the system, and the exponent value found may therefore be directly compared with experimental data when available. It is hoped that the simple conceptual picture put forward by the modeling in the present paper will be useful in the design of new biophysical experiments. Furthermore, the model theoretical approach can readily be refined and extended, e.g., to proteins with more conformational states, to systems with an electrostatic component of the lipid-protein interactions, as well as to models with more detailed interaction potentials.

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