Cooperative Effects Induced by Adsorbed Polypeptides in Mixed Membranes

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Adsorbed polypeptides such as peripheral proteins disturb the homogeneity of the lipid mixture which forms membranes. The induced concentration deviations produce an enhanced adsorption of the polypeptides on the membrane surface and an attractive force between them. This substrate mediated force supports the formation of clusters or even domains with different lipid concentrations and adsorbate densities.

1. Introduction

The lipid matrix of biological membranes consists of a mixture containing various lipid compounds. Under physiological conditions, the lipid molecules move freely in the membrane plane forming a two-dimensional fluid. In several cases, the investigation of structural and thermodynamic properties of multicomponent membranes revealed a nonideal behavior of lipid mixtures even in the fluid state.[1] Cluster formation and demixing processes in lipid membranes have attracted much attention in the last years,[2] because domains can influence many properties of biological membranes. Phase diagrams of binary lipid mixtures show frequently a peritectic behavior of the fluid−solid transition.[3] The lack of miscibility in the solid phase suggests that there exist also some deviations from an ideal mixture in the fluid state. In this case, the nonideal behavior of the fluid mixture should be accompanied with an enhanced correlation length of thermal concentration fluctuations. Furthermore, it is well-known that nonideal mixing of phospholipids occurs in systems composed of lipids with a large difference in the length of the alkyl chains.[4,5] The interaction of the lipid headgroups can also produce a nonideal thermodynamic behavior. For example, altering the charge of the headgroups in lipid mixtures is a possibility to control thermodynamic properties. Increasing the content of calcium ions in binary phosphatidylcholine−phosphatidic acid bilayers leads to an increased tendency to domain formation.[6] If a spinodal or a critical demixing point is approached, the correlation range of concentration fluctuations increases considerably. For a binary phospholipid mixture (DMPC/DSPC), correlation lengths up to 50 nm were found by small-angle neutron scattering experiments.[3,7] In most practical cases, the correlation length is expected to be much smaller but can markedly exceed the cross-sectional diameter of lipid molecules.

Apart from spontaneous thermal fluctuations, the lipid mixture is disturbed by systematic forces. If there is a tendency toward demixing, the membrane responds sensitive to external disturbances which couple to the concentration. Disturbances of the local concentration distribution can be produced by peripheral proteins and peptides such as antimicrobials adsorbed on the membrane surface.[8] Many polypeptides are bound to membranes by hydrophobic and screened electrostatic forces.[9,10] The homogeneous mixture becomes perturbed by these adsorbed molecules, which have different affinities to the lipid components. Lipid species that interact more favorably with the adsorbed macromolecule migrate toward the contact zone, repelling the less favorably interacting lipids. For example, peripheral basic proteins attract the acidic lipid compounds of the membrane and push away zwitterionic species.[9] Although this effect is not necessarily accompanied with the formation of macroscopic domains, the rearrangement of the lipid mixture is accompanied with an enhanced adsorption of peptides or proteins on the membrane surface. In this case, the surface density of the adsorbate should be higher than for a homogeneous membrane of frozen lipids, which is formed below the liquid−gel transition temperature. An enhanced adsorption has been experimentally found and theoretically confirmed by considering a lattice model[11] and electrostatic models for the adsorption of charged proteins on mixed membranes.[9]

Experimental observations suggest that there are also cooperative effects resulting from the response of the lipid mixture to the adsorbate. Using florescence microscopy[12] and atomic force microscopy,[13,14] it was shown that adsorbed peptides and proteins can aggregate into macroscopic domains. Domain formation includes both the demixing of the lipid substrate and a phase separation of the adsorbate (Figure 1). Experimental results support the assumption that domain formation is not always a consequence of direct attractive interactions between the polypeptides.[12] On the contrary, the electrostatic repulsion between equally charged proteins should be an obstacle to adsorbate aggregation.[15] A possible mechanism causing domain formation is based on an indirect force between the adsorbate molecules induced by the lipid mixture which forms the substrate. Adsorbed polypeptides locally change the lipid concentrations by attracting preferred lipid species. If these disturbed regions superimpose with decreasing distance between the adsorbed molecules, the total area where the local lipid concentration is altered becomes smaller. As in this case the free energy reduces, the inclusions should attract each other. The range of the indirect attractive force is comparable to the correlation length for the concentration fluctuations in the mixture. In the vicinity of a spinodal, the interaction radius should diverge.

We use the linearized version of the Cahn−Hilliard[16] theory to investigate the response of mixed membranes to adsorbed

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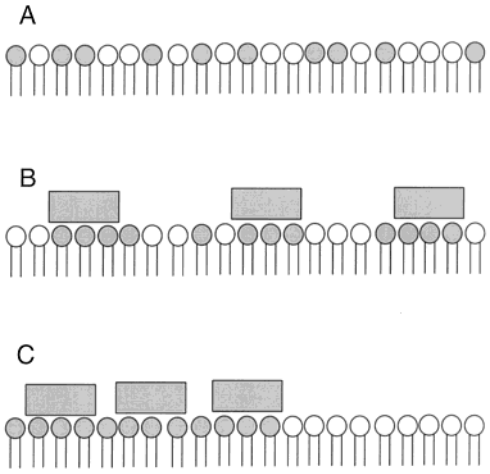

Figure 1. Rearrangements of the lipid molecules take place if the peripheral proteins have different affinities to the components (after Figure 1 in ref 11). (A) Random lipid mixture. (B) The favored component migrates to the peripheral proteins. (C) The indirect attraction interaction can enforce a phase transition producing domains with different lipid composition and substrate densities.

polypeptides. The adsorbate disturbs the homogeneous lipid mixture. After evaluating an effective potential energy for the lipid−protein interaction, some implications resulting from the disturbance of the concentration field are considered. In agreement with other theoretical models,[11] we find an enhanced adsorption on fluid membranes in comparison to frozen ones. The model presented in this paper predicts an additional effect for fluid mixed membranes. An indirect force between the adsorbate particles should occur if the correlation length for lipid concentration fluctuations markedly exceeds the cross-sectional radius of the lipid molecules. It will be shown that the substrate mediated interaction is strong enough to support the formation of domains with increased adsorbate densities.

2. Effective Potential Energy for the Adsorbate

The theoretical model is based on the observation that the size of the adsorbed polypeptides is much larger than the headgroup area of the lipid substrate molecules. Typically, a protein resting on the membrane surface has contact with more than 10 lipid headgroups. Thus, in the framework of a mesoscopic description, the lipid matrix can be considered as a continuum, whereas the large adsorbate molecules are described as usual in classical mechanics. It is assumed that each polypeptide covers a circular region on the membrane surface. The adsorbed molecules are expected to influence the substrate, which can be considered as a two-dimensional fluid consisting of several lipid components. Disturbances of the concentration distribution in the membrane plane are generated if the interaction energies between the adsorbate molecules and the components of the lipid mixture are different. A mesoscopic description for mixtures is suitable to account for concentration fluctuations. It is assumed that the adsorbed particles have the same effect as an external field, which linearly couples to the concentration. A linearized theory should be valid as long as the concentration alterations remain small. In a first step, the Cahn−Hilliard theory[16] is used to define an effective Hamiltonian allowing to evaluate the free energy for a mixed membrane subjected to the nonhomogeneous external field produced by the polypeptides. This free energy can be considered as an effective potential, because it depends on the coordinates of the adsorbed particles. Taking into account additional contributions in the interaction potential resulting from direct forces between the polypeptides, e.g., the contribution related to repulsive forces, thermodynamic functions of the adsorbate can be evaluated using statistical mechanics. It will be shown that the redistribution of the lipids due to the protein−lipid coupling causes forces between the adsorbate molecules and influences the adsorption.

Let us consider a lipid membrane consisting of two lipid components and polypeptides adsorbed on the membrane surface. If M_A and M_B denote the particle numbers of the binary lipid mixture, its composition can be characterized by the ratios $X_A = M_A/M$ and $X_B = M_B/M$, where $M = M_A + M_B$. Concentration fluctuations are taken into account by introducing local particle number densities $\rho_A(x)$ and $\rho_B(x)$, which may depend on coordinates x defined for the membrane plane. It is a reasonable approximation to assume that the averaged cross-sectional area per lipid molecule is equal for both components. The densities $\rho_A(x)$ and $\rho_B(x)$ satisfy the normalization conditions

$$\int \rho_{\mathrm{A}}(x)\,\mathrm{d}x = M_{\mathrm{A}} \qquad \text{and} \qquad \int \rho_{\mathrm{B}}(x)\,\mathrm{d}x = M_{\mathrm{B}} \tag{1}$$

Generally, there are different affinities of lipid components to the adsorbate. As the preferred component is gathered around adsorbed polypeptides, the homogeneous distribution of the lipid mixture is disturbed. If fluctuations of the total density $\rho_L = \rho_A(x) + \rho_B(x)$ are neglected, the concentration field $\psi(x) = \rho_B(x) - \rho_B = \rho_A - \rho_A(x)$ characterizes deviations from the equilibrium densities $\rho_A = M_A/S$ and $\rho_B = M_B/S$, where S is the membrane surface area. Obviously, the field $\psi(x)$ satisfies the condition

$$\int \psi(x)\,\mathrm{d}x = 0 \tag{2}$$

It is possible to derive an analytic expression for the free energy of a heterogeneous mixture in the region where deviations from homogeneity and concentration gradients are not too large. In the spirit of a general mesoscopic approach in the fluctuation theory, the effective Hamiltonian H for the fluctuating mixture is expanded to powers of $\psi(x)$ and $\nabla\psi(x)$. The lowest order terms of this expansion can be written as[16]

$$H = \frac{1}{2}\int [A(\nabla\psi(x))^2 + B\psi^2(x) - F(x)\psi(x)]\,\mathrm{d}x + O(\psi^3) \tag{3}$$

where the symbol $O(\psi^3)$ indicates that terms of third and higher order are neglected. This approximation is reasonable if the quadratic form in eq 3 is positive definite ($A > 0$ and $B > 0$). Explicit expressions for coefficient B can be evaluated using simple model systems in thermodynamics. In appendix A, coefficient B is obtained from the regular solution model, which is widely used in the thermodynamic description of simple mixtures. For $F(x) = 0$, eq 3 allows us to investigate spontaneous thermal concentration fluctuations of an undisturbed membrane. The length scale $\xi = (A/B)^{1/2}$ corresponds to the distance below that concentration fluctuations are correlated.

The term $-F(x)\ \psi(x)$ arises when an external field linearly couples to the concentration distribution. In the present model, this field is generated by the adsorbate molecules. $F(x)$ is different from zero only in those regions where adsorbed polypeptides touch the membrane surface. Let us define the positions of N circularly symmetric adsorbed particles by a set of coordinates $\{x_1, x_2, ..., x_N\}$ for their gravity centers. Then, a linear coupling of the lipid components to the adsorbed polypeptides can be generally written as

$$F(x) = -\rho_L^{-1}\sum_{i=1}^{N}[\epsilon_A(x_i - x)\rho_A(x) + \epsilon_B(x_i - x)\rho_B(x)] \quad (4)$$

where the vector $x - x_i$ corresponds to the lateral distance between the center of gravity x_i of an adsorbed molecule and a point on the membrane surface x. We define adsorption energies ϵ_A and ϵ_B which refer to a protein bound to membranes consisting of the pure lipid components A and B, respectively. It is easily checked that the integral kernels in eq 4 must satisfy the conditions

$$\int \mathrm{d}x\, \epsilon_A(x) = \epsilon_A \qquad \text{and} \qquad \int \mathrm{d}x\, \epsilon_B(x) = \epsilon_B \quad (5)$$

Using the definition for $\psi(x)$ and eq 5, the field term in eq 3 is rewritten as

$$-\int \mathrm{d}x\, F(x)\, \psi(x) = N\epsilon_X - \int \mathrm{d}x\, \psi(x)\, P(x) \quad (6)$$

where

$$\epsilon_X = X_A\epsilon_A + X_B\epsilon_B \quad (7)$$

$$P(x) = \sum_{i=1}^{N} p(x_i - x) \quad (8)$$

and

$$p(x - x_i) = -\rho_L^{-1}[\epsilon_B(x_i - x) - \epsilon_A(x_i - x)]. \quad (9)$$

Considering circularly symmetric adsorbed molecules, the protein−lipid coupling term $p(x_i - x)$ depends only on the distance $|x_i - x|$. An explicit evaluation of the coupling function $p(x_i - x)$ requires the introduction of a detailed adsorption model. For example, the Poisson−Boltzmann theory[9] or the introduction of effective Yukawa potentials[15] are useful starting points if the adsorption energy is dominated by screened electrostatic forces. Taking into account eq 5, the condition

$$\int P(x)\, \mathrm{d}x = -N\rho_L^{-1}(\epsilon_B - \epsilon_A) \quad (10)$$

is found to be satisfied for $P(x)$. Combining eqs 6 and 3, the complete Hamiltonian is represented by

$$H = N\epsilon_X + \frac{1}{2}\int [A(\nabla\psi(x))^2 + B\psi^2(x)]\, \mathrm{d}x - \int (P(x) - \lambda)\psi(x)\, \mathrm{d}x \quad (11)$$

where the Lagrange parameter λ is required to satisfy eq 2 for the concentration distribution. Using this Hamiltonian, the free energy of the lipid mixture for a fixed set of protein coordinates $\{x_1, x_2, ..., x_N\}$ is evaluated by the functional integral

$$F = -kT \ln \int D\psi\, \exp\left(-\frac{H}{kT}\right) \quad (12)$$

over all possible fields ψ. This free energy is considered as an effective potential $F = U(x_1, ..., x_N)$ depending on the protein coordinates. Equation B7 obtained in appendix B can be expressed as

$$U(x_1, ..., x_N) = Ne + \sum_{i<j} u(x_i, x_j) \quad (13)$$

where

$$e = \epsilon_X + \frac{u(0)}{2} + \frac{(\epsilon_B - \epsilon_A)^2\Gamma}{2B\rho_L^2} \quad (14)$$

and $\Gamma = N/S$ is the surface density of the polypeptides. If $y_{ij} = x_j - x_i$ denotes the distance between two adsorbed molecules i and j, eq B8 for the effective pair potential $u(x_i, x_j)$ of circularly symmetric particles can be transformed into

$$u(y_{ij}) = -\int \mathrm{d}x\, p(x)[\int G(x - \bar{x} - y_{ij})p(\bar{x})\, \mathrm{d}\bar{x}] \quad (15)$$

where the integral kernel G is defined by eq B3. For $y_{ij} = 0$, the expression

$$\frac{u(0)}{2} = -\frac{1}{2}\int \mathrm{d}x\, p(x)\, G(x - \bar{x})\, p(\bar{x})\, \mathrm{d}\bar{x} \quad (16)$$

defines the self-energy of an adsorbate molecule. If the adsorbate density is low the nonhomogeneous membrane regions around the polypeptides do not overlap. In this case, the effective pair potentials $u(x_i, x_j)$ can be neglected, but the contribution Ne in eq 13 is also important at small adsorbate densities. Obviously, the contribution ϵ_X in the effective potential is equal to the average adsorption energy of adsorbate particles bound to a membrane with randomly distributed components. The remaining terms in eq 14 result from the redistribution of the lipid mixture because of the lipid−protein coupling. These terms are equal to zero if the lipid−protein coupling terms and thus the adsorption energies coincide ($\epsilon_A = \epsilon_B$). In the general case $\epsilon_A \neq \epsilon_B$, the self-energy $u(0)/2$ is smaller than zero and the condition $e < \epsilon_X$ is generally satisfied, except perhaps for very large adsorbate densities Γ. The corresponding reduction of the effective potential (eq 13) leads to an enhanced adsorption. In the case of high adsorbate densities, when the mean distances between the adsorbate particles are comparable with the correlation length ξ, the effective pair potential (eq 15) must be considered additionally. For not too large distances, i.e., in the region where the interaction is strong, the substrate induced lateral force between adsorbed molecules is found to be always attractive. If the average energy related to this lateral force markedly exceeds the mean thermal energy kT for the translational motion of adsorbed molecules on the membrane surface, the adsorbate can form clusters or even domains with increased surface density.

3. Adsorption and Indirect Interaction

3.1. Enhanced Adsorption. A detailed description of the lipid substrate and the thermodynamic behavior of the adsorbate requires the introduction of a special adsorption model to evaluate the integral kernels $\epsilon_A(x - x_i)$ and $\epsilon_B(x - x_i)$ in eq 4, which define the protein−lipid coupling. However, some results can be obtained without introducing special model assumptions. For different adsorption energies ($\epsilon_A \neq \epsilon_B$), the disturbance of the concentration distribution because of the adsorbed polymers leads to a shift of the adsorption equilibrium. The surface density of the adsorbed polypeptides should be larger than expected for the adsorption on a randomly distributed lipid mixture. This effect is expected to be very strong for large values of the correlation length ξ but does not disappear if ξ tends to zero. Recently, the increase of the surface concentration because of the rearrangement of the lipids has been studied using a molecular statistical lattice model.[11] It is also instructive to investigate this effect in the framework of the mathematical procedure based on the Hamiltonian defined by eq 11. For a correlation length ξ much smaller than the radius a_P of the cross-

sectional area of a polypeptide, an analytical treatment of the adsorption problem is possible. We consider an ideal lipid mixture with a short correlation length ($\xi \ll a_P$). If $\xi \to 0$, the interaction terms $u(x_i, x_j)$ with $i \neq j$ tend to zero, but the self-energy $u(0)/2$ has an important contribution to the effective adsorption potential. Taking into account that $G(x - \bar{x}) \to B^{-1}\delta(x - \bar{x})$ for $\xi \to 0$ and using eq 16, the self-energy is obtained from the equation

$$\lim_{\xi \to 0} \frac{u(0)}{2} = -\frac{1}{2B} \int p^2(x)\, dx \tag{17}$$

The polypeptides dissolved in the aqueous environment of the membrane are expected to form an ideal solution, because their bulk concentration is usually small. The chemical potential of the polypeptides can be written as $\mu_P' = kT \ln[C/C_0(T)]$, where C is their bulk density and $C_0(T)$ denotes a function of the temperature. Analogously, if the protein surface density $\Gamma = N/S$ is small, the chemical potential for adsorbate molecules reads $\mu_P'' = e + \Gamma(\partial e/\partial \Gamma) + kT \ln[\Gamma/\Gamma_0(T)]$, where $\Gamma_0(T)$ is a temperature function and e is defined by eq 14. The equilibrium condition $\mu_P' = \mu_P''$ leads to the Henry law limit of the adsorption isotherm, namely

$$\ln \frac{\Gamma}{\Gamma_0(T)} = \ln \frac{C}{C_0(T)} - \frac{\epsilon_X}{kT} - \frac{u(0)}{2kT} \tag{18}$$

where a small term proportional to Γ has been omitted. The last term in eq 18 is always positive and produces an increased adsorbate concentration in comparison to a homogeneous substrate with spatially constant lipid concentration. A further evaluation of this effect requires the introduction of a special adsorption model. In this paper, we assume that the range of the adsorption force of a polypeptide is short and mainly restricted to the membrane area $s_P = \pi a_P^2$ covered by the adsorbed particle. Otherwise, if this condition is not satisfied, the radius a_P could be chosen as a fit parameter, which characterizes the range of the lipid−protein coupling. Thus, taking into account eq 5, the lipid−protein coupling is defined by

$$\epsilon_{A(B)}(x_i - x) = \begin{Bmatrix} \epsilon_{A(B)}/s_P & \text{for} & |x_i - x| < a_P \\ 0 & & \text{else} \end{Bmatrix} \tag{19}$$

Then eqs 9, 17, and 19 yield the self-energy

$$\frac{u(0)}{2} = -\frac{(\epsilon_B - \epsilon_A)^2}{2 s_P B \rho_L^2} \tag{20}$$

For an ideal lipid mixture, the relation $B = (X_A X_B \rho_L)^{-1}\, kT$ (appendix A) holds, and the equilibrium condition (eq 18) results in

$$\frac{\Gamma}{\Gamma_0(T)} = \frac{C}{C_0(T)} \gamma \exp\left(-\frac{X_A \epsilon_A + X_B \epsilon_B}{kT}\right) \tag{21}$$

where

$$\gamma = \exp\left[\left(\frac{\epsilon_B - \epsilon_A}{kT}\right)^2 \frac{X_A X_B}{2 s_P \rho_L}\right] \tag{22}$$

The factor $\gamma > 1$ determines the increase of the protein surface density resulting from the rearrangement of the lipid molecules. Equation 21 indicates how the surface density Γ of the adsorbate depends on the composition of the lipid mixture.

For extending the description of adsorption isotherms to higher surface densities, direct interactions between adsorbed polypeptides should be included. There are repulsive forces due to the excluded volume effect and attractive forces, e.g., van der Waals forces, between the adsorbate molecules. As an approximation, we assume that these direct forces are pairwise additive and can be obtained from a pair potential $w(x_i, x_j)$. Taking into account that $u(x_i, x_j) \to 0$ for $\xi \to 0$, eq 13 is replaced by

$$U(x_1, ..., x_N) = Ne + \sum_{i<j} w(x_i, x_j) \tag{23}$$

In particular, the excluded volume effect restricts the adsorption efficiency.[19,20] Considering hard circularly symmetric adsorbate particles (hard disks), the pair potential $w(x_i, x_j)$ is infinity for $|x_i - x_j| < 2a_P$ and zero for $|x_i - x_j| > 2a_P$. It is useful to express the free energy of the adsorbate as a function of the surface density $\theta = \Gamma s_P$, which is equal to the area fraction of the membrane covered by adsorbate molecules. Then the free energy for a system of N particles adsorbed on the membrane surface is written as $F_P = N[e + f(T, \theta)]$, where $f(T, \theta)$ is the free energy per particle for the two-dimensional hard particle fluid. Equal thermodynamic potentials for adsorbed and dissolved proteins require that

$$\ln \frac{C}{C_0(T)} = \frac{e + f(T,\theta)}{kT} + \frac{\Pi s_P}{\theta kT} \tag{24}$$

where the surface pressure Π of the adsorbate is defined as

$$\Pi = \frac{\theta^2 \partial(e + f(T, \theta))}{s_P \quad \partial\theta} \tag{25}$$

An excellent approximation for the pressure Π of a fluid consisting of disklike particles results from the scaled particle theory.[21] In this case, the free energy per particle can be written as

$$f(T, \theta) = \left(\ln \frac{\theta}{1-\theta} + \frac{\theta}{1-\theta} - 1\right) kT \tag{26}$$

Using this free energy and the corresponding pressure

$$\frac{\Pi s_P}{kT} = \frac{\theta}{(1-\theta)^2} \tag{27}$$

the equilibrium condition (eq 24) reads

$$\frac{C}{C_1(T)} = \frac{\theta}{1-\theta} \exp\left[\frac{X_A \epsilon_A + X_B \epsilon_B}{kT} - (1 - 2\theta)\left(\frac{\epsilon_B - \epsilon_A}{kT}\right)^2 \frac{X_A X_B}{2 s_P \rho_L} + J(\theta)\right] \tag{28}$$

where

$$J(\theta) = \frac{\theta(3 - 2\theta)}{(1-\theta)^2} \tag{29}$$

and $C_1(T) = C_0(T)[\Gamma_0(T)\, s_P]^{-1}$ is a temperature function. This modified Volmer isotherm allows us to investigate how the density of adsorbed molecules depends on the lipid composition. In agreement with results of Heimburg et al.,[11] Figure 2 demonstrates a markedly enhanced adsorption on fluid mixed

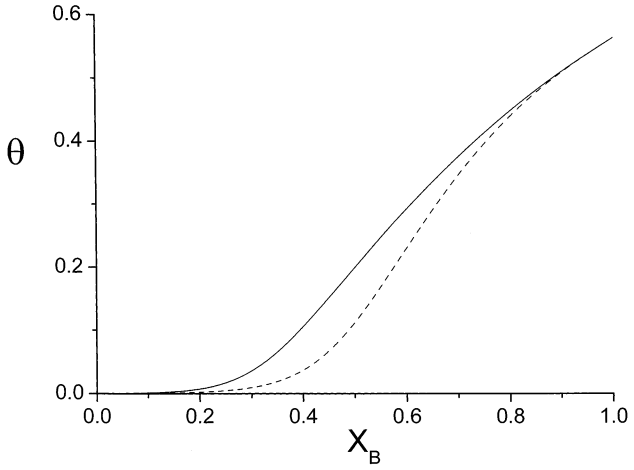

Figure 2. Plot of the polypeptide surface density θ versus the particle number fraction X_B of the lipid mixture. The continuous curve is evaluated by eq 28 for a fluid substrate assuming that $\epsilon_A = 0$, $\epsilon_B = -nkT$, $n = 15$ ($n = \rho_L s_P$), and $C/C_1(T) = 10^{-4}$. For a frozen substrate with fixed lipid molecules, the surface density is lower (dashed curve).

membranes in comparison to lipid substrates which are in a gellike state.

3.2. Attraction Force between the Adsorbate Molecules. *Multipole Expansion.* In the case of dense adsorbates, the substrate-mediated interaction becomes important. If the correlation length ξ is comparable to or even larger than the polypeptide radius a_P, the substrate mediated interaction between adsorbate molecules can be evaluated by using a multipole expansion. Starting from eq 15 and using the notation $r = |y_{ij}|$, the multipole expansion reads[22]

$$u(r) = -p_0^2 G(r) - \frac{1}{2}p_0 p_2 \nabla^2 G(r) - \frac{1}{32}(p_0 p_4 + 2p_2^2)(\nabla^2)^2 G(r) - ... \quad (30)$$

where $\nabla^2 = r^{-1}\partial/\partial r + \partial^2/\partial r^2$, and the moments of the coupling function 9 are defined by

$$p_n = \int s^n p(s) 2\pi s \, ds \qquad n = 0, 2, 4, ... \quad (31)$$

with $s = |x|$. Using eqs 9 and 5, the simple result $p_0 = -\rho_L^{-1}(\epsilon_B - \epsilon_A)$ is obtained for $n = 0$. Inserting the Green's function (eq B3) into eq 30, the leading term of the multipole expansion for the effective pair potential is found to be

$$u(r) = -\frac{(\epsilon_B - \epsilon_A)^2}{2\pi B \xi^2 \rho_L^2} K_0\left(\frac{r}{\xi}\right) \quad (32)$$

where K_0 is the modified Bessel function.[18] This quite general result does not depend on the special choice of the lipid−protein coupling model. The effective potential for the substrate mediated interaction has a direct relation to adsorption energies ϵ_A and ϵ_B, which are experimentally accessible in calorimetric measurements. If the critical demixing point or the spinodal is approached, B tends to zero, whereas the correlation length ξ tends to infinity. The coefficient A in eq 11 has no singularity at the critical point. Therefore, the product $B\xi^2 = A$ in the denominator of the pair potential (eq 32) can be considered as a constant. When the critical demixing point or the spinodal is approached, the amplitude of the pair potential remains approximately unchanged, whereas the range of the substrate mediated interaction becomes very large ($\xi \to \infty$). However,

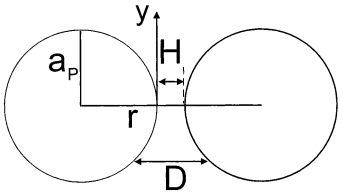

Figure 3. Definition of geometrical parameters.

the region where a linearized theory is valid reduces with decreasing values of B (appendix C). To obtain quantitative results, which are valid in the vicinity of the critical point, higher order terms in the expansion of the Hamiltonian (eq 3) must be considered additionally.

Deryaguin's Approximation. The multipole expansion does not converge well if the correlation length ξ is smaller than the polypeptide radius a_P. If this radius is large ($a_P \gg \xi$), Deryaguin's approximation[23] for the interaction between macrobodies is applicable. In a first step, the substrate-mediated interaction potential is evaluated for a modified reference model, which depends only on a single coordinate. We assume that two adsorbed particles on the membrane surface cover the area of two parallel strips with length L and width $2a$. If the distance between the midlines of the strips is equal to r, the locations of the covered areas can be chosen as $-a \le x \le a$ and $-a + r \le x \le a + r$. As $L \gg \xi$, edge effects at the corners of the strips can be neglected and the system can be replaced by a one-dimensional model. Solving the one-dimensional version of the nonhomogeneous Helmholtz-equation (eq B2) in appendix B yields the Green's function[18] $G(x - \bar{x}) = (2B\xi)^{-1} \exp(-|x - \bar{x}|/\xi)$. Combining eqs 9, 19 and 15 leads to the following interaction potential per unit length L:

$$\frac{\hat{u}(r)}{L} = -\frac{(\epsilon_B - \epsilon_A)^2}{2B\xi(\rho_L s_P)^2} \int_{-a}^{a} dx \left[\int_{-a}^{a} \exp(-|x - \bar{x} - r|/\xi)\, d\bar{x}\right] \quad (33)$$

We note that the product $\rho_L s_P = n$ in eq 33 is equal to the number of lipid-polypeptide contact sides per adsorbed polypeptide. Using the notation $h = r - 2a$ for the gap between both stripes, we obtain

$$\frac{\hat{u}(h)}{L} = -\frac{(\epsilon_B - \epsilon_A)^2 \xi}{2B(\rho_L s_P)^2}\left[1 - \exp\left(-\frac{2a}{\xi}\right)\right]^2 \exp\left(-\frac{h}{\xi}\right) \quad (34)$$

and for $2a \gg \xi$ this result is simplified to

$$\frac{\hat{u}(h)}{L} = -\frac{(\epsilon_B - \epsilon_A)^2 \xi}{2B(\rho_L s_P)^2} \exp\left(-\frac{h}{\xi}\right) \quad (35)$$

Equation 35 can be used to evaluate the interaction energy for large polypeptides. According to Deryaguin's method, the pair potential for two circular particles with large radii $a_P \gg \xi$ is obtained from the integration[23]

$$u(H) = \int_{-\infty}^{\infty} dy \left(\frac{\hat{u}(D)}{L}\right) \quad (36)$$

where, in a parabolic approximation, $D = H + y^2/a_P$ is equal to the distance between opposite points at the protein borderlines and $H = r - 2a_P$ is the gap between the particles (Figure 3). Finally, the integral in eq 36 yields the pair potential

$$u(H) = -\frac{(\epsilon_B - \epsilon_A)^2 \xi \sqrt{\pi a_P \xi}}{2B(\rho_L s_P)^2} \exp\left(-\frac{H}{\xi}\right) \qquad (37)$$

for the indirect attractive force.

4. Discussion

In the final discussion, we want to estimate the magnitude of the substrate-mediated force and its influence on the structure and the thermodynamics of adsorbates. The model contains two essential parameters A and B entering into the Hamiltonian 11. Parameter B varies between $4\rho_L{}^{-1}kT$ (ideal mixture, $X_B = 0.5$) and 0 (critical point) for binary mixtures with a tendency toward demixing. The value of B can be obtained from the $T - X$ phase diagram and the mixing enthalpy. Then, relation $A = B\xi^2$ determines parameter A, where the correlation length ξ is accessible by scattering methods.[3,7] Unfortunately, systematic experimental investigations to fix both parameters A and B seem to be rather time-consuming. However, some conclusions do not depend on the precise numerical values of these parameters.

In comparison to frozen membranes below the gel transition temperature, liquid membranes respond more sensitive to the disturbance produced by the adsorption. The redistribution of the lipid mixture results in an enhanced adsorption of the polypeptide from the aqueous solution on the membrane surface. This effect can be rather strong, especially as long as the surface density of the adsorbate remains relatively low. Let us roughly estimate the magnitude of the factor γ (eq 22), which characterizes the magnification of the adsorption due to the rearrangement of an ideal lipid mixture. The number of lipid molecules which are in contact with an adsorbed polypeptide is obtained from the relation $n = s_P\rho_L$. As this number is usually large ($n > 10$), the adsorption can be rather strong, even if the contact energies ϵ_A/n and ϵ_B/n per lipid molecule are relatively small. For $X_A = X_B = 0.5$ and for a moderate difference $|\epsilon_B - \epsilon_A|/n \simeq kT$, we obtain $\gamma \simeq \exp(n/8)$. If we assume reasonable values for n ($n \simeq 8-25$), the magnification factor γ is found to be markedly larger than one. It should be noted that this estimation for γ refers to a very short correlation length ξ of concentration fluctuations. For larger values of ξ the magnification of the adsorption should be even more pronounced.

Domain formation due to attraction forces between the polypeptides leads to a further increase of the adsorbate density. Apart from direct interaction, i.e., van der Waals attraction, the redistribution of the lipid substrate produces an indirect attractive force between the adsorbed polypeptides. For large and small correlation lengths ξ, the pair potentials for this indirect force is expressed analytically by eqs 32 and 37, respectively. The indirect force is essential if its pair potential has a large magnitude exceeding the mean thermal energy kT for the translational motion of an adsorbed particle on the membrane interface. Let us estimate this magnitude choosing a small value for the adsorption energy difference $|\epsilon_B - \epsilon_A|/n$ so that the linear theory is valid (appendix C). For an ideal lipid mixture and $X_{A(B)} = 0.5$, the relation $B = 4\rho_L{}^{-1}kT$ is satisfied (appendix A). If there is a tendency toward demixing of the lipid components, the lipid−lipid interaction parameter w (Appendix A) is positive, and thus, the value of B becomes smaller. For an estimation, we chose the values $B = \rho_L{}^{-1}kT$, $a_L \simeq 0.4$ nm, $\rho_L \simeq (\pi a_L{}^2)^{-1} \simeq 2$ nm^{-2}, and $\xi \simeq 2.5$ nm. A cross sectional area of $s_P \simeq 5$ nm^2 for a protein is compatible with the assumption that $n \simeq 10$ lipids are in contact with an adsorbed peptide. If $|\epsilon_B - \epsilon_A|/n \simeq kT$ the interaction energy $|u(r = \xi)| \simeq 0.5\ kT$ results from eq 32. For higher values of the adsorption energy difference $\epsilon_B - \epsilon_A$, the interaction energy should be comparable to or larger than the mean thermal translational energy kT per adsorbed peptide.

In the case of short correlation lengths ξ or large particle radii a_P ($a_P/\xi \gg 1$) Deryaguin's method provides an estimation of the interaction energy. As previously, we chose $B = \rho_L{}^{-1}kT$ and assume again a moderate value of the adsorption energy difference $|\epsilon_B - \epsilon_A| \simeq nkT$. Then, eq 37 is transformed into $u(H) = -(kT/2)\rho_L\xi(\pi a_P\xi)^{1/2} \exp(-H/\xi)$. For example, if $\xi \simeq 1$ nm, $a_P \simeq 4$ nm, and $\rho_L \simeq 2$ nm^{-2}, the absolute value of the interaction energy $|u(H = 0)| \simeq 4kT$ for two particles in contact is larger than the mean thermal translational energy kT per adsorbed molecule.

Obviously, the substrate-mediated attraction force supports the formation of clusters or even large domains of the polypeptides. It would be interesting to decide whether this attraction is strong enough to cause a phase transition to a high-density adsorbate without the help of direct attractive forces between the polypeptides. The tendency toward macroscopic domain formation increases with increasing values of ξ and $|\epsilon_B - \epsilon_A|/n$. In particular, the efficiency of the substrate-induced attraction should increases dramatically if a critical demixing point or a spinodal is approached, because in this case the interaction radius diverges. Unfortunately, for large correlation lengths ξ and large adsorption energy differences $|\epsilon_B - \epsilon_A|$, a linear mathematical model does not provide reliable quantitative results (Appendix C). In this case, a more advanced nonlinear theory which includes higher order terms in the Hamiltonian (eq 11) should be used. However, the simple linear approach provides an insight into the qualitative effects produced by the lipid redistribution in the field of the adsorbed polypeptides.

In conclusion, different affinities of the lipid membrane components to the adsorbed polypeptides facilitate the adsorption and produce an attraction force between the adsorbed molecules. The magnitude of this force depends strongly on the adsorption energy of the polypeptides, whereas the interaction range is equal to the correlation length of thermal concentration fluctuations. For moderate adsorption energies and correlation lengths, the attraction is found to be sufficiently strong to support the formation of cluster or even domains with enhanced density of the polypeptide molecules.

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Appendix A

Regular Solution Model. In many cases, the regular solution model provides a proper approximation for the free energy of binary mixtures.[26] Consider a two-component system with M_A and M_B particles occupying completely a two-dimensional lattice. The interaction energy between nearest neighbor particles is assumed to be w_{ij} (components i and $j = A$ and B). A proper approximation for the free energy of this system is

$$F_X = (M_A + M_B)kT \left(X_A \ln X_A + X_B \ln X_B + \frac{wz}{2kT} X_A X_B\right) \qquad (A1)$$

where $w = 2w_{AB} - w_{AA} - w_{BB}$ and z denotes the number of nearest neighbors of any molecule in the lattice ($z = 6$ for a hexagonal lattice). The surface densities defined in section 2 and the particle number fractions are related by $\rho_A = X_A\rho_L$ and $\rho_B = X_B\rho_L$. Then, the free energy is expressed as

$$F_X = SkT\left(\rho_A \ln \rho_A + \rho_B \ln \rho_B + \frac{wz}{2\rho_L kT}\rho_A\rho_B\right) \quad \text{(A2)}$$

where a constant term is omitted. Permitting fluctuations of the concentration, we expand the free energy to powers of $\psi(x) = \rho_A(x) - \rho_A = \rho_B - \rho_B(x)$ up to quadratic terms and add a gradient term. The result is written in the form

$$\hat{F} = C_0 + \int_S\left[C_1\psi(x) + \frac{kT}{2}\left(\frac{1}{\rho_A} + \frac{1}{\rho_B} - \frac{wz}{kT\rho_L}\right)\psi^2(x) + \frac{1}{2}A(\nabla\psi(x))^2\right] \mathrm{d}S \quad \text{(A3)}$$

where C_0 and C_1 do not depend on $\psi(x)$. Because of eq 2, the term $C_1\psi(x)$ in eq A3 can be omitted. Omitting also the constant term C_0, we arrive at

$$F = \int_S\left[\frac{kT}{2}\left(\frac{1}{\rho_A} + \frac{1}{\rho_B} - \frac{wz}{kT\rho_L}\right)\psi^2(x) + \frac{1}{2}A(\nabla\psi(x))^2\right] \mathrm{d}S \quad \text{(A4)}$$

Finally, replacing $\rho_{A(B)}$ by $X_{A(B)}\rho_L$ and comparing eqs 3 and A5 leads to the relation

$$B = \left(\frac{1}{X_A X_B} - \frac{wz}{kT}\right){\rho_L}^{-1}kT \quad \text{(A5)}$$

In the special case of an ideal mixture ($w = 0$) with equal concentrations $X_A = X_B = 0.5$, we obtain $B = 4{\rho_L}^{-1}kT$.

Appendix B

Evaluation of the Effective Pair Potential. Because eq 12 contains a Gaussian functional H in the exponent, the integral can be evaluated straightforwardly.[17] The result is

$$F = F_0 + Ne_X - \frac{1}{2}\int [P(x) - \lambda]G(x - \bar{x})[P(\bar{x}) - \lambda] \,\mathrm{d}x \,\mathrm{d}\bar{x} \quad \text{(B1)}$$

where F_0 is a function of the temperature. The integral kernel $G(x - \bar{x})$ is the Green's function satisfying the nonhomogeneous Helmholtz equation

$$-\nabla^2 G(x - \bar{x}) + \xi^{-2}G(x - \bar{x}) = \frac{1}{A}\delta(x - \bar{x}) \quad \text{(B2)}$$

where δ is Dirac's function. Taking into account the relation $A = B\xi^2$, the solution of eq B2 is written as

$$G(x - \bar{x}) = \frac{1}{2\pi B\xi^2}K_0\left(\frac{|x - \bar{x}|}{\xi}\right) \quad \text{(B3)}$$

where K_0 is the modified Bessel function.[18] Combining eqs 11, 12, and 2, it is easily checked that

$$\partial F/\partial\lambda = \left\langle \int \psi \,\mathrm{d}x \right\rangle = 0 \quad \text{(B4)}$$

where the brackets $\langle\rangle$ indicate the statistical average. Thus, using eq B1, we obtain the Lagrange parameter λ from the formula

$$\lambda = \frac{\int P(x)G(x - \bar{x}) \,\mathrm{d}x \,\mathrm{d}\bar{x}}{\int G(x) \,\mathrm{d}x \,\mathrm{d}\bar{x}} \quad \text{(B5)}$$

Taking into account eq 10, a straightforward evaluation results in $\lambda = (\epsilon_B - \epsilon_A){\rho_L}^{-1}\Gamma$, where $\Gamma = N/S$ is the surface density of the adsorbed polypeptides. Combining eqs B1 and B5 yields

$$F = F_0 + N\epsilon_X - \frac{1}{2}\int G(x - \bar{x}) \left[P(x)P(\bar{x}) - \left(\frac{(\epsilon_B - \epsilon_A)\Gamma}{\rho_L}\right)^2\right] \mathrm{d}x \,\mathrm{d}\bar{x} \quad \text{(B6)}$$

The temperature function F_0, which is independent of the system configuration and the number N of adsorbed polypeptides, will be omitted in further calculations. Inserting eq 8 for $P(x)$, the free energy F is expressed as a function of the protein coordinates $\{x_1, x_2, ..., x_N\}$. Using the notation $U_0(x_1, ..., x_N) = F - F_0$, we obtain the effective potential energy

$$U_0(x_1, ..., x_N) = N\left[\epsilon_X + \frac{(\epsilon_B - \epsilon_A)^2\Gamma}{2B{\rho_L}^2}\right] + \frac{1}{2}\sum_{i=1}^{N}\sum_{j=1}^{N}u(x_i, x_j) \quad \text{(B7)}$$

where

$$u(x_i, x_j) = -\int \mathrm{d}x \,\mathrm{d}\bar{x}G(x - \bar{x})p(x_i - \bar{x})p(x_j - x) \quad \text{(B8)}$$

For $i \neq j$, eq B8 defines an effective pair potential for the proteins, whereas for $i = j$, the corresponding expression $u(0) = u(x_i, x_i)$ is twice the self-energy for an adsorbed polypeptide.

Appendix C

Region where the Linearized Theory Is Justified. A linear model for the lipid membrane mixture is applicable when the deviations from the reference state $\psi(x) = 0$ are sufficiently small. It is interesting to consider the implication of this requirement for the lipid−protein coupling. The average concentration distribution $\langle\psi(x)\rangle$ is defined as

$$\langle\psi(x)\rangle = \frac{\int D\psi\psi \exp[-H/kT]}{\int D\psi \exp[-H/kT]} \quad \text{(C1)}$$

Looking at eqs 12 and 11, we find that $\langle\psi(x)\rangle = -\delta F/\delta P(x)$ and

$$\langle\psi(x)\rangle = \int G(x - \bar{x})(P(\bar{x}) - \lambda) \,\mathrm{d}\bar{x} \quad \text{(C2)}$$

Deviations from the unperturbed state $\psi(x) = 0$ are small if the condition $\max |\langle\psi(x)\rangle| \ll \rho_L$ is satisfied. The magnitude of concentration fluctuations decreases with increasing adsorbate density. In the borderlinecase, if a complete coverage of the membrane interface by adsorbate molecules is approached, the lipid−protein interaction becomes equal everywhere on the interface area, and thus, any redistribution of the lipid mixture ceases. Therefore, for estimating the upper limit $\max |\langle\psi(x)\rangle|$, we consider a membrane surface with zero adsorbate density ($\Gamma = 0$) except for a single adsorbed molecule with center of gravity at $x = 0$. In this case, we obtain $\max |\langle\psi(x)\rangle| = |\langle\psi(0)\rangle|$ and eq C2 is simplified to

$$|\langle\psi(0)\rangle| = \left|\int \mathrm{G}(x)p(x) \,\mathrm{d}x\right| \quad \text{(C3)}$$

where $p(x)$ is defined by eq 9. Considering the adsorption model defined by eq 19 and taking into account eq B3, the condition $|\langle\psi(0)\rangle| \ll \rho_L$ can be written as

$$\frac{|\langle\psi(0)\rangle|}{\rho_L} = \frac{|\epsilon_B - \epsilon_A|}{n\rho_L B}Q\left(\frac{a_P}{\xi}\right) \ll 1 \quad \text{(C4)}$$

where

$$Q(R) = \int_0^R K_0(y) y \, dy \tag{C5}$$

and $n = \rho_L a_P$ is equal to the numbers of lipid molecules which are in contact with the adsorbed particle. $Q(R)$ is a monotonic function of $R \geq 0$ with lowest value $Q(0) = 0$ and upper limit $Q(\infty) = 1$. Let us first discuss the implications of condition C4 for the adsorption energy difference $|\epsilon_B - \epsilon_A|$ of an ideal lipid mixture ($B\rho_L = 4kT$). Because the correlation length ξ of an ideal mixture is small, the ratio a_P/ξ is large and thus $Q(a_P/\xi) \simeq 1$. We obtain for equal concentrations $X_A = X_B = 0.5$ the condition

$$\frac{|\epsilon_B - \epsilon_A|}{4nkT} \ll 1 \tag{C6}$$

which is safely satisfied if the reduced adsorption energy difference $|\epsilon_B - \epsilon_A|/n$ is not much larger than the mean thermal energy kT. However, the linear theory is not applicable in the region very close to a critical demixing point where the correlation length ξ diverges. There the function $Q(a_P/\xi) \propto a_P\xi^{-1} \ln(\xi/a_P)$ tends to zero, whereas $B^{-1} = A^{-1}\xi^2$ diverges. Then, the product $B^{-1}Q(a_P/\xi) \propto a_P\xi \ln(\xi/a_P)$ tends to infinity and condition C4 is always violated when the critical demixing point is approached. In this case, higher order terms in the expansion of the Hamiltonian (eq 3) must be included to describe critical fluctuations and substrate mediated forces.

References and Notes

(1) Mouritsen, O. G.; Jorgensen, K. *Curr. Opin. Colloid Interface Sci.* **1997**, *7*, 518.

(2) Hinderliter, A. K.; Huang, J.; Feigenson, G. W. *Biophys. J.* **1994**, *67*, 1906.

(3) Sackmann, E. Physical Basis of Self-Organization and Function of Membranes: Physics of Vesicles, in Structur and Dynamics of Membranes. In *Handbook of Biological Physics*; Lipowsky, R., Sackmann, E., Eds.; Elsevier Science B.V.: Amsterdam, 1995; Volume 1A, pp 213−304.

(4) Inoue, T.; Nibu, Y. *Chem. Phys. Lipids* **1995**, *76*, 171.

(5) Inoue, T.; Nibu, Y. *Chem. Phys. Lipids* **1995**, *76*, 181.

(6) Garidel, P.; Blume, A. *Langmuir* **2000**, *16*, 1662.

(7) Knoll, W.; Schmidt, G.; Sackmann, E. *J. Chem. Phys.* **1983**, *79*, 3439.

(8) Matsuzaki, K. *Biochim. Biophys. Acta* **1999**, *1462*, 1.

(9) May, S.; Harris, D.; Ben-Shaul, A. *Biophys. J.* **1999**, *79*, 1747.

(10) Ben-Tal, N.; Honig, B.; Peitzsch, R. M.; Denisov, G.; McLaughlin, S. *Biophys. J.* **1996**, *71*, 561.

(11) Heimburg, T.; Angerstein, B.; Marsh, D. *Biophys. J.* **1999**, *76*, 2575.

(12) Denisov, G.; Wanaski, S.; Luan, P.; Glaser, M.; McLaughlin, S. *Biophys. J.* **1998**, *74*, 731.

(13) Mueller, H.; Butt, H.-J.; Bamberg, E. *J. Phys. Chem. B* **2000**, *104*, 4552.

(14) Haverstick, D. M.; Glaser, M. *Biophys. J.* **1989**, *55*, 677.

(15) Oberholzer, M. R.; Lenhoff, A. M. *Langmuir* **1999**, *15*, 3905.

(16) Chaikin P. M.; Lubensky, T. C. *Principles of condensed matter physics*; Cambridge University Press: Cambridge, 1997.

(17) Binney, J. J.; Dowrick, N. J.; Fisher, A. J.; Newman, M. E. J. *The Theory of Critical Phenomena*; Clarendon Press: Oxford, 1992.

(18) Arfken, G. B.; Weber, H. J. *Mathematical Methods for Physicists*; Academic Press: San Diego, CA, 1995.

(19) Chatelier, R. C.; Minton, P. *Biophys. J.* **1996**, *71*, 2367.

(20) Minton, A. P. *Biophys. J.* **1999**, *76*, 176.

(21) Rosenfeld, Y. *Phys. Rev. A* **1990**, *42*, 5978.

(22) Schiller, P.; Mögel, H.-J. *Phys. Chem. Chem. Phys.* **2000**, *2*, 4563.

(23) White, L. R. *J. Colloid Interface Sci.* **1983**, *95*, 286.

(24) Davis, H. T. *Statistical Mechanics of Phases, Interfaces and Thin Films*; Marcel Dekker: New York, 1996.