Using the modified Frumkin approach, the adsorption of protein onto the surface of lipid monolayer has been simulated allowing for the different types of protein-lipid interactions. The results were presented in terms of the monolayer surface pressure increase induced by protein sorption (Δπ) as a function of initial surface pressure (π₀). The simulated curves were obtained under varying a range of interaction parameters. It was suggested that the trend of Δπ(π₀) plot allows to distinguish between protein interfacial localization and its penetration into lipid monolayer. The theoretical predictions are in accord with the experimental observations available in literature.

**KEY WORDS:** lipid monolayer, protein sorption, Frumkin isotherm.
structure, activity and functioning of the cells [10, 11]. Protein-membrane association has also been recognized to play a pivotal role in cell signaling [12], membrane fusion [13], functioning of blood coagulation cascade [14] and toxin attack to the cells [15]. Adsorption of peripheral proteins to membranes is a complex process implicating a range of interrelated events. Formation of electrostatic, hydrogen bonding and hydrophobic protein-lipid and protein-protein contacts is accompanied by interdependent conformational transition of polypeptide chain and structural reorganization of a lipid bilayer which consequently may affect a protein surface reactivity. A variety of model systems have been developed to analyze various aspects of protein interfacial behavior [16-20]. Among them, lipid monolayers represent the most suitable prototype of cell membranes due to their homogeneity, stability and planar geometry [21, 22]. Furthermore, lipid monolayers are one of the most convenient systems to gain insight into fundamental principles of protein membrane sorption and penetration [23-25]. Comprehensive analysis of the protein monolayer adsorption is provided by a number of theoretical studies [26-32]. Specifically, based on the Gibbs’ equation, Pethica [26, 27] and Alexander & Barnes [28] derived the expressions for the surface excess of the soluble component. However, the validity of these expressions was restricted to ideal bulk solutions and surface condensed phases and required the knowledge of relationships between the areas per molecule of insoluble component in pure and mixed monolayers. In turn, Motumura et al. [29] and Ter-Minassian-Saraga [30] proposed the equations for surface excess of soluble component implicating the slope of surface pressure concentration dependences for the mixed monolayer and soluble component alone. Another approach, developed by Fainerman and Vollhardt [31] involves the application of Butler’s equation to express the chemical potential of the surface layer component. Finally, using the Frumkin isotherm, Sundaram and Stebe suggested the equation of state for mixed monolayer [32]. In the present study the last approach was employed to elucidate how experimentally monitored surface pressure trends can be correlated with particular modes of protein-lipid or protein-protein interfacial interactions.

THEORY

The theoretical description of protein adsorption and penetration processes was performed in terms of the modified Frumkin model [32]. Contrary to Langmuir adsorption model, suggesting the absence of intermolecular interactions on the surface, Frumkin framework allows for nonideal interactions (repulsive or cohesive) between the adsorbate molecules. According to this model, the activation energies for the adsorption and desorption ($E_a$ and $E_d$, respectively) linearly depend on surface concentration of the $i$-component ($\Gamma_i$), where $i = 1, 2$ stand for lipid and protein components, respectively:

$$E_{a,d} = E_{a,d}^0 + \sum_{i=1,2} \nu_{a,d,i} \Gamma_i$$

where $\nu_{a,d,i}$ are the adsorption/desorption constants, respectively [32]. The modified Frumkin isotherm is derived by substituting eq. (1) into the expressions for adsorption and desorption fluxes which depend on the fraction of interface occupied by either lipid or protein component given by:

$$x_i = \Gamma_i / \Gamma_{xi}$$

Here $\Gamma_{xi}$ denotes the maximum surface packing of the component $i$. In addition, for calculation of the adsorption isotherms, the following parameters are introduced:

- adsorption number $a$:
\[ a = \frac{\alpha_0}{\beta_0} \exp \left( \frac{E_d}{RT} \right) \] (3)

where \( \alpha_0, \beta_0 \) are desorption and adsorption kinetic constants, respectively.

- dimensionless bulk concentration of a protein (or scaled concentration) \( k = C_2/a \), where \( C_2 \) is the molality of the protein,
- interaction parameters:

\[ K_i = \frac{(v_{ai} - v_{di}) \Gamma_{\alpha_i}}{RT} \] (4)

where \( K_{12} \) and \( K_{22} \) denote the interaction parameters for lipid-protein or protein-protein interactions, respectively. Negative values of interaction parameters describe the cohesive interactions, while the positive ones stand for the repulsive contacts.

The general expression for the adsorption isotherm within Frumkin framework can be written as:

\[ \frac{x_2}{1 - x_1} = \frac{k}{k + \exp \left( K_{12} x_1 + K_{22} x_2 \right)} \] (5)

where \( K_{12} \) and \( K_{22} \) are assigned non-zero values to take into account the lipid-protein or protein-protein interactions or both of them.

The corresponding change in surface pressure of a lipid monolayer (\( \Delta \pi \)) is given by:

\[ \Delta \pi = -RT \Gamma_{\alpha_2} \ln \left( \frac{1 - x_1}{1 - x_2} - K_{12} x_1 x_2 - \frac{K_{22}}{2} x_2^2 \right) \] (6)

where \( \Gamma_{\alpha_2} \) is the maximum surface packing of the protein taken as \( 4.6 \times 10^{-6} \) mol/m² [33]. In the simulation procedure the signs and absolute values of \( K_{12} \) and \( K_{22} \) were allowed to vary in the wide limits to account for variations in the nature and magnitude of protein-lipid and/or protein-protein interactions.

### RESULTS AND DISCUSSION

**Lipid-protein interactions**

Shown in Figs. 1 and 2 are the families of \( x_2(\log(k)) \) dependencies and corresponding \( \Delta \pi(\pi_0) \) curves calculated at varying \( K_{12} \) and \( K_{22} = 0 \), i.e. for the case when protein molecules do not interact with each other. It can be seen that the isotherms are characterized by S-like shape and reducing values of \( x_2 \) with increasing \( x_1 \). The behavior of \( \Delta \pi(\pi_0) \) plots appeared to be more interesting. Specifically, for all values of \( K_{12} \) (except for \( K_{12} = 0 \)) \( \Delta \pi \) profiles are characterized by sigmoidal shape with the steepness increasing with \( K_{12} \) modulo. Negative values of \( K_{12} \) correspond to cohesive interactions between the soluble (protein) and insoluble (lipid) components of the system, while positive ones describe repulsion between protein and lipid molecules. Interestingly, in the case of cohesive interactions the examined curves are ascending; whereas repulsive interactions give rise to the opposite behavior of \( \Delta \pi(\pi_0) \) plots. Physically, the decrease in \( \Delta \pi \) with elevating \( \pi_0 \) values (as observed for \( K_{12} > 0 \)) is explained by tighter packing of lipid molecules in more compressed monolayers with higher \( \pi_0 \). This would prevent the adsorbate penetration into lipid film, resulting in the
decreased $\Delta \pi$. These considerations suggest that lipid monolayer plays the surface blocking role in the protein penetration process [32, 33]. Evidently, in the case of $K_{12} > 0$ the contribution of surface blocking effect of lipid monolayer is higher than contribution of repulsive contacts, and the behavior of $\Delta \pi(\pi_0)$ curves follows the above expectations. However, when $K_{12}$ is negative, cohesive interactions prevail over steric hindrance imposed by lipid molecules, thereby resulting in the enhancement of protein binding with increasing $\pi_0$. Apparently, in this case multilayer sorption of protein molecules onto the surface of lipid monolayer is facilitated.

![Graph A](image1.png)

![Graph B](image2.png)

**Fig. 1.** Modified Frumkin adsorption isotherm for 1-2 cohesion (A) and repulsion (B)

In this context it seems of interest to discuss our simulation results within the theoretical framework suggested by Calvez et al. [34, 35]. Briefly, these authors presented the changes in monolayer surface pressure as $\Delta \pi = a\pi_0 - \pi_0 + b$, where $a$ is a so-called synergy factor proportional to the slope of $\Delta \pi(\pi_0)$ curves, and $b$ is the y-intercept of these curves.

![Graph C](image3.png)

**Fig. 2.** Surface pressure change as a function of initial surface pressure upon protein sorption

It was postulated that positive slope (i.e. positive values of $a$) describes the positive synergy between lipid monolayer and protein molecules, while $a < 0$ reflects the negative synergy. Remarkably, our results are consistent with the proposed theory. Specifically, in the case of cohesive lipid-protein interactions $\Delta \pi(\pi_0)$ plots have positive slope reflecting the positive synergy between lipids and adsorbing protein. This implies that lipid monolayer provides an environment favorable for protein sorption despite the steric restrictions imposed by increased lipid packing density with elevating $\pi_0$. This positive synergy may manifest itself in relocating the site of protein binding from lipid hydrocarbon tails to the headgroup region. Theoretical calculations predict that the energy of
hydrophobic interactions is about 8-15 kJ/mol, while the energies of electrostatic and H-bond contacts occurring at lipid/water interface reach the magnitudes of 20-40 kJ/mol [36,37]. It seems likely that loose lipid packing at low initial surface pressure would favor protein penetration into lipid monolayer and formation of hydrophobic contacts with lipid acyl chains of relatively low energy. In turn, high \( \pi \) produces the tighter lipid packing, and it would be energetically favorable for the adsorbate to reside onto lipid monolayer surface. Strong cohesive lipid-protein interactions of high energy would favor protein monolayer adsorption resulting in the \( \Delta \pi \) increase.

**Protein-protein interactions**

In analyzing the case of protein-protein interactions \( K_{12} \) was fixed at zero value, while \( K_{22} \) was varied from -4 to 4. Shown in Fig. 3 are the adsorption curves for different \( x_1 \) as a function of \( k \). It can be seen that for cohesive interactions \( x_2 \) experiences rapid increase with \( k \) and then reaches the plateau, while repulsive contacts result in slower increase in \( x_2 \), almost without saturation in the plots. Fig. 4 represents the \( \Delta \pi(\pi_0) \) profiles corresponding to different \( K_{22} \) values. Since 1-2 (lipid-protein) interactions are not taken into account, lipid monolayer can be considered only from the viewpoint of its surface blocking role [32], and \( \Delta \pi \) is expected to decrease with elevating \( \pi_0 \), as predicted by the above simple physical rationales.

![Fig. 3. The isotherms of protein adsorption onto the surface of lipid monolayer for the case of protein-protein attraction (A) and repulsion (B)](image_url)

However, this behavior was observed only at negative \( K_{22} \) values (cohesive protein-protein interactions). For positive \( K_{22} \) (repulsive protein-protein interactions) \( \Delta \pi \) was found to increase with initial surface pressure. This may be the case, for instance, when charged amino acid residues do not form clusters, but distribute uniformly over the surface of protein molecule. Such proteins do not possess exposed hydrophobic patches that would facilitate the intermolecular contacts between protein molecules and favor the adsorbate monolayer penetration.
Thus, it would be energetically favorable for the proteins to reside on the lipid surface. Zuckermann and Heimburg hypothesized that adsorbed proteins form a two-dimensional gas on the bilayer surface which generates a lateral pressure on the membrane [38], thereby producing local changes in its curvature. The concomitant membrane “squeezing” near the area of protein-lipid contact may perturb the lipid order, manifesting itself in the general $\Delta \pi$ tendency to increase. Again, the steepness of $\Delta \pi(\pi_0)$ profiles increases with increasing the magnitude of either cohesive or repulsive protein-protein interactions.

\textbf{Lipid-protein and protein-protein interactions}

When both lipid-protein and protein-protein interactions take place in the examined system, the behavior of $x_2(\log(k))$ and $\Delta \pi(\pi_0)$ plots depends on relative contributions of $K_{12}$ and $K_{22}$ parameters. To exemplify, for $K_{12} = 1$, $K_{22} = -3$ (repulsive lipid-protein and cohesive protein-protein interactions), the $\Delta \pi(\pi_0)$ curve has negative slope, indicating that the magnitude of surface pressure change decreases with increasing $\pi_0$. In turn, the values $K_{12} = -2$, $K_{22} = 4$ (cohesive lipid-protein and repulsive protein-protein interactions) yield the ascending curve, mirroring the opposite effect (Fig. 5).

Notably, when both interaction parameters are negative (i.e. cohesive lipid-protein and protein-protein interactions), the $\Delta \pi(\pi_0)$ plots are upward sloping, while for both positive interaction parameters (repulsive lipid-protein and protein-protein contacts) the curves are downward sloping. These findings suggest that the contribution of lipid-protein interactions in the overall $\Delta \pi$ trend prevails over that of protein-protein interactions. Interestingly, $x_2(\log(k))$ curves retain the same sigmoidal shape (data not shown) as for 1-2 or 2-2 interactions (see above). The steepness of the plots was found to be higher in the case of cohesive lipid-protein and repulsive protein-protein interactions.

\textbf{Comparison with the experimental results}

In the following it seems of importance to compare the simulation results presented here with the experimental data. Although the majority of monolayer studies provided typical downward sloping $\Delta \pi(\pi_0)$ profiles, there exist some indications that $\Delta \pi$ behavior can be
more complex. In particular, atypical biphasic $\Delta \pi(\pi_0)$ plots were observed by Burger et al., while investigating the interactions of influenza virus hemagglutinin with lipid monolayer [39]. This finding was explained by the dependence of protein surface activity on the initial surface pressure of lipid monolayer, and lipid-induced conformational transitions of polypeptide chain. A nonlinear relationship between $\Delta \pi$ and $\pi_0$ was revealed by Kirat et al. who examined the interactions of phospholipase D with lipidic activators – diacylglycerol and phosphatidic acid [40]. The upward Langmuir-like experimental curve has been rationalized in terms of the surface enzyme-lipid interactions with no or shallow insertion into acyl chain region.

Unexpected trend in $\Delta \pi(\pi_0)$ diagram has also been reported by Zhao and co-workers upon the binding of anticancer drug adriamycin to lipid monolayer containing polyethylene glycol (PEG) [41]. It has been postulated that nonlinearity of $\Delta \pi(\pi_0)$ plots reflects the PEG-mediated structural reorganization of lipid monolayer, resulting in specific interfacial orientation of the drug. Such an orienting effect of lipid monolayer promotes the ring-stacking interactions between adriamycin molecules and imparts positive cooperativity to the drug-lipid binding, manifesting itself in hyperbolic-like $\Delta \pi(\pi_0)$ dependencies. Furthermore, the same authors revealed nonlinear $\Delta \pi(\pi_0)$ profiles while exploring the penetration of cytochrome c into the neutral and negatively charged lipid monolayers. To explain these findings, it was assumed that conformational state of the bound protein and the mode of cytochrome c – lipid association depends on the initial surface pressure of lipid monolayer.

The nonlinear changes in monolayer surface pressure observed upon the interactions of prion protein (PrP) with lipid monolayers were interpreted as arising from the existence of threshold initial surface pressure for protein-lipid binding [42]. Accordingly, it was hypothesized that at $\pi_0 < 30$ mN/m the protein virtually does not penetrate the lipid film, while the opposite effect is observed at $\pi_0 > 30$ mN/m. The monolayer association of PrP was supposed to require the densely packed raft-like arrangement of lipid molecules.

The above examples clearly demonstrate that $\Delta \pi$ vs. $\pi_0$ relationship exhibits a versatile behavior reflecting a complex interplay between lipid-protein and protein-protein interactions, as well as structural and functional changes involved in these interactions.

**CONCLUSIONS**

The results presented here revealed that $\Delta \pi(\pi_0)$ profiles are strongly influenced by different modes of lipid-protein and protein-protein interactions. Specifically, it was hypothesized that surface protein localization on the lipid film results in the untypical ascending trend of $\Delta \pi(\pi_0)$ curves, while adsorbate monolayer penetration yields common $\Delta \pi$ decrease with elevating initial surface pressure. The theoretical predictions are in a pretty good harmony with the experimental observations available in literature. The outlined findings may prove of significance in solving the problems associated with both fundamental and applied aspects of protein sorption.

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