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Evaluation of the Defay-Prigogine model for the membrane interphase in relation to biological response in membrane-protein interactions

Edgardo A. Disalvo[1*], *Axel Hollmann*[1,2], *Liliana Semorile*[2] *and María Florencia Martini*[3]

[1]Laboratory of Biointerfaces and Biomimetic Systems- CITSE-University of Santiago del Estero, 4200 Santiago del Estero and CONICET, Argentina.

[2]Laboratory of Molecular Microbiology, Institute of Basic and Applied Microbiology, University of Quilmes, B1876BXD Bernal, Argentina.

[3]Department of Pharmaceutical Technology, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, 1113 Buenos Aires, Argentina.

*To whom correspondence should be addressed to: Dr. E.A. Disalvo, Email: disalvoanibal@yahoo.com.ar

ABSTRACT

Surface water activity appears as a common factor when the interaction of several aqueous soluble and surface active proteins with lipid membranes of different composition is measured by the changes in surface pressure of a lipid monolayer. The perturbation of the lipid surface caused by aqueous soluble proteins depends on the composition of the hydrocarbon phases, either modified by unsaturated bonds in the acyl chains or by inclusion of cholesterol. The cut-off (critical) surface pressure in monolayers, at which no effect of the proteins is found, is related to the composition of the head group region. The perturbation of surface pressure is produced by proteins when the area per lipid is above just 4% larger than that corresponding to the hydration shell of the phospholipid head groups found in the cut -off. This area excess gives place to regions in which the chemical potential of water changes with respect to bulk water. According to the Defay-Prigogine relation this interfacial water activity is the reason of the surface pressure increase induced by aqueous soluble proteins injected in the subphase. As predicted by solution chemistry, the increase of surface pressure is independent of the protein nature but depends on the water surface state determined by the lipid composition.

Keywords: lipid membranes; water-membrane-interphases; monolayer surface pressure; interfacial water activity; protein interaction.

INTRODUCTION

The classical view of a biological membrane is based on the Singer-Nicholson mosaic fluid model, in which the structural backbone is the lipid bilayer. The lipid membrane is usually described by a low dielectric slab with polar head groups organized at the interphases of 1 nm thickness at each side [1]. This region is located between two ideal planes: one at the carbonyl level that defines the interface between the hydrocarbon region and the polar interphase and another at the external plane along of the hydrated phosphates of the phospholipids representing the membrane interphase-bulk water interface [2-4] (Figure 1).

The interpretation of the permeation of solutes or protein/peptide penetration under the frame of this model, usually invokes the partition phenomena between the aqueous phase and the membrane hydrocarbon region, disregarding the interphase region and the hydration state of the phospholipids. Structural and dynamical properties of water at the membrane adjacencies and its influence on the dynamical behavior of biomembranes have been studied by neutron scattering technique [5]. These measurements revealed a strong interaction of a “first hydration layer” with the membrane surface and a reduced self-diffusion of aqueous solvent parallel to the membrane surface. It is concluded that protein/lipid complexes are strongly affected by the amount of solvent interacting with the lipids and the membrane proteins. In particular, the lipids and their ability to attract solvent molecules play an important role on the “hydration-induced flexibility” of biomembranes. On the basis of this statement, the impact of hydration on the function of biomembranes should be discussed in terms of the lability of the solvent structure facing membrane surfaces of different polarities (i.e. polar or non polar groups). In thermodynamic terms, the lability is related to excess free energy that is the driving force for protein insertion.

Indirect references to the state of hydration have been used as an argument to reconcile experimental results with thermodynamic foundations [6]. Mostly, different types of peptides are assayed in model lipid membranes of known composition. The phenomenological results are explained by models based on geometrical considerations of the lipid molecules postulating hypothetical intermediaries in membrane conformational arrangements in which water might be involved [7]. Although the presence of water and its peculiar structural properties have been recognized in several previous studies, no explanation in regard to its role in the thermodynamics of membrane response has been considered in those proposals [8].

The suggested deeper penetration of water into bilayers composed by unsaturated hydrocarbon chains has been correlated with the looser packing at the lipid-water interface [9]. This is immediately correlated with an area increase. However, the area creation is concomitant with the excess free energy promoted by the exposure of different kinds of groups to water and therefore it cannot be explained only in terms of free geometrical space. In this regard, the relation between the functional activities of the biological structures with the lability of the water ensembles at the lipid surfaces at different surface pressures has not received the necessary thermodynamic analysis to understand membrane response. In consequence, no general considerations can be derived from the multiple systems studied.

Water by itself may constitute a bidimensional domain inhomogeneously distributed along the lipid surface. Water immediately adjacent to the glycerol backbone, the side groups and the hydrocarbon chains, has a lower activity than a zone of similar size in the bulk solution. In this region, due to the exposure of acyl chains and carbonyl groups to the aqueous phase, several populations of different water species in terms of hydrogen bonding has been reported [10,11]. It has been proposed that in solid monolayers, half of the water molecules in the surface layer is

replaced by amphiphilic molecules, the whole forming a highly ordered structure [12]. However, the connection between the different water organizations with the excess surface free energy due to the membrane group-water interaction that may trigger the peptide or protein insertion has not been systematically analyzed so far.

To understand the origin of this surface free energy as a consequence of the stability of the different arrays of water around the different membrane groups, a description of the interphase in terms of physicochemical considerations is required. In this regard, it is important to take into account the proposal made by Defay-Prigogine for an interphase [13, 14]. This model allows to ascribe measurable thermodynamic properties to the lipid surface.

The region confined between the carbonyl group plane and external plane tangent to the phosphates depicted in Figure 1 is considered as a bidimensional solution in which the hydrated polar groups are imbibed in water. In consequence, the surface pressure of an insoluble monolayer is a direct measure of the surface water activity [13]. Thus, from the thermodynamic point of view, the surface tension of pure water can be defined as

$$\gamma^0 A = RT \ln\left(\frac{a_w^i}{a_w^b}\right)$$

where γ^0 is the surface tension of pure water, A is the average area per mole of water in the interphase region, a_w^i is the activity of water in the interphase of pure water and a_w^b is the water activity in the bulk phase. When a monolayer is spread on the water surface, the surface tension changes to

$$\gamma A = RT \ln\left(\frac{a_w^L}{a_w^b}\right)$$

where a_w^L is the surface water activity in the presence of lipids, i.e. in the interphase region.

Thus, the difference between the surface tension of pure water (γ^0) and surface tension of water with lipids spread on it forming a monolayer (γ), i.e. the surface pressure of the monolayer (π) is expressed as a function of the surface water activities as [15].

$$\pi = (\gamma^o - \gamma)A = RT \ln \frac{a_w^i}{a_w^L} \quad (1)$$

This equation clearly denotes that the surface pressure (π) increases when a_w^L decrease below 1 and becomes zero when $a_w^i = a_w^L$, i.e. the activity of pure water when lipids coverage is zero. In that condition: $\gamma = \gamma^0$. An important consequence of equation (1) is that the surface pressure increases with the amount of lipids at the interface at constant area. This provides a method to regulate surface pressure by adding lipids to the air-water surface [16, 17]. In the present work, the different initial surface pressures before the addition of the proteins to the subphase are adjusted by adding known amounts of lipids to an air-water surface in a Langmuir trough. This method allows to fix the initial water activity at the interphase and has an extra benefit in relation to the thermodynamic state of the monolayer as compared to that in which the surface excess and hence, the surface pressure is varied by decreasing the area at constant lipid amounts. In this last case, lipids are forced to pack by a lateral external force that may cause distortions in the head group region [18].

It is clear that with the Defay-Prigogine definition, the thermodynamic parameter of surface pressure can be related to the water organization, which is implicit in the water activity term. At equilibrium, the chemical potential of water at the interphase (μ_{wi}) will be equal to the chemical potential of water in the bulk water phase (μ_{wb}).

$$\mu_{wi} = \mu_{wb} \qquad (2)$$

When a solute from the bulk water dissolves in the interphase region, the water activity (a_w^L) changes. This decrease in water activity with respect to bulk promotes a flow of water into the interphase region. In consequence, the surface pressure increases when the water enters the interphase. The film pressure can be described as a difference in osmotic pressure, over a thickness of the bidimensional solution, between the interphase at the monolayer and the bulk phase [19].

In terms of solution chemistry, in principle, this should be independent of the particle nature that dissolves in it, at least in diluted systems according to the definition of colligative properties. Therefore, independent of the protein or peptide used, lipid membranes should give a similar response if determined water activity conditions are achieved. Most probably, deviations from the ideal behavior should be included in the activity coefficient different from 1 included in the activity term.

The changes in surface pressure at different initial surface pressures, induced by defined concentrations of peptides in the subphase, has been usually interpreted by the plots of $\Delta\pi vs\pi$ as shown in Figure 2. In this figure, we summarize published results obtained with different kinds of lipids and two different aqueous soluble proteins (protease of *Mucor miehei* and S-layer extracted from lactobacilli). It is observed that the cut-off (or critical pressure defined as the pressure at which no perturbation is observed) depends on the head group region composition and the slope on the acyl chain composition.

In this paper, we analyze these data in terms of the Defay and Prigogine model in a systematic interpretation of the protein-membrane phenomena in terms of the thermodynamic activity of water in different lipid membranes. The perturbation caused by different proteins on the surface

pressure of a monolayer can be measured considering that this is a thermodynamic parameter related to the interfacial tension and hence with the surface free energy. The connection made by the Defay-Prigogine hypothesis between the thermodynamics and the structural properties is given by the reformulation of the functionality of the surface pressure with the water activity, being this affected by the changes that proteins can do on the membrane interphase

EXPERIMENTAL METHODS

Chemicals: 1,2-dimyristoyl- sn-glycero- 3- phosphocholine (DMPC), 1,2-di-O-tetradecyl-sn-glycero-3-phosphocholine (etherPC), 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE), 1,2-di-O-tetradecyl-sn-glycero-3-phosphoethanolamine (etherPE), 1,2-dipalmitoyl- sn-glycero-3-phosphoethanolamine N-monomethylated (mmDPPE) and N,N-dimethylated (dmDPPE) were obtained from Avanti Polar Lipids, Inc (Alabaster, AL). Soybean phosphatidylcholine (PC), stearylamine (SA) and cholesterol were purchased from Sigma (St. Louis, MO, USA). The purity of lipids was checked by thin layer chromatography using a chloroform:methanol:water mixture as running solvent.

Protease Rennet from *Mucor miehei*, was from Sigma. For details see Martini et al. [20]. The S-layer protein was extracted from *Lactobacillus brevis* JCM 1059. A single protein band with an apparent molecular mass of 49.5 was observed as published in a previous work [21].

Changes on the surface pressure of lipid monolayers: The changes of the surface pressure of monolayers induced by proteins were measured in a Kibron μTrough S equipment (Kibron Inc, Espoo, Finland) at constant temperature (22 ± 0.5 °C). The surface of an aqueous solution contained in a Teflon trough of fixed area was exhaustively cleaned. Then, a chloroform solution of lipids was spread on this surface, to reach surface pressures between 20 and 42 mN/m. Similar results were obtained when the lipids were spread on the surface and the chosen pressure were attained by moving the barriers. For simplicity, the addition of lipids to a constant area trough was adapted. The monolayers were allowed to stabilize at long times in order to assure the complete evaporation of the chloroform. Several solvents with different rate of evaporation were tested and similar results were obtained in all cases provided the monolayer is allowed to

stabilize for long times (usually more than 30 minutes). Once the surface pressure was adjusted, protein solutions were injected in the sub-phase underneath the monolayer at each chosen surface pressure and the changes on the surface pressure were followed during time to reach a constant value. A control injecting the same volume of water produced no changes in the initial monolayer pressure. The difference between the final pressure obtained with the protein and the initial surface pressure before protein addition was taken as a measure of the perturbation caused in the lipid interphase ($\Delta\Pi$). This value is usually plotted vs the initial surface pressure. The same procedure was followed for all monolayer compositions. In the range of pressures used through this study the surface pressure–area isotherms of DOPC, DPhPC, DMPC, DPPC, and DMPE show that all lipids are forming monolayers when they are spread on an air-water surface [22-25].

RESULTS AND DISCUSSION

In order to interpret the surface pressure perturbation within the frame of the Defay-Prigogine model, similar experiments to those of Figure 2 A and C were carried out. In Figure 3, data of Figures 2A and C, corresponding to the perturbation induced by the proteins on the initial surface pressure of the monolayer (π), are plotted in function of the difference between the critical surface pressure (π_c) and a chosen initial surface pressure achieved by the lipid surface excess.

It is observed that the slopes are directly related to the unsaturation of the acyl chains. In addition, it is observed in Figure 3B, that the presence of carbonyl groups contributes to the hydrocarbon phase properties. The depletion of the CO group has a similar effect than the increase of unsaturation or branching in the hydrocarbon chains, that is, an increase in the slope.

In Figure 4, the plot of data in Figure 2B and D are shown. In panel A, the slope remains unchanged with the addition of stearylamine (SA) maintaining the cholesterol ratio constant. In panel B, it is clearly shown that the slope decreases with the increase of cholesterol in a monolayer of constant PC/SA ratio.

Finally in Table I, the values of the slopes for the different membrane compositions are shown.

The plots of Figure 3 and 4 can be phenomenologically described by

$$\Delta\pi = k(\pi_c - \pi) \qquad (3)$$

where $\Delta\pi$ defines the perturbation of the initial surface pressure of the monolayer induced by the protein addition to the subphase. The initial surface pressure is related to the water activity at the interphase, according to equation (1), and is modulated by the amount of lipids added to the water surface at values below π_c

The value of the slope *k* is clearly a function of the acyl chain composition including the presence of carbonyl groups, according to data in Figure 3 and 4. Specifically, the increase in branching or unsaturation and the depletion of cholesterol and carbonyl groups increases the slope (Table I). A direct conclusion could be that the magnitude of the perturbation is related to the kinks formation due to the rotational isomers of the acyl chains and the cooperativity [26, 27]. However, since those membrane conformers imply water penetration [9], it is plausible to analyze these results in terms of the effects that those lipid components may cause on the water activity of the surface, following the hypothesis of Damoradan [13] and the formalism of Defay Prigogine [19] described in the introduction.

The physical meaning of *k* in equation (3) is clearly related to phase state of the monolayer. It is interesting to observe the effect of cholesterol. In natural systems, the liquid condensed phase is physiologically relevant. Cholesterol, mainly found in the plasma membranes of eukaryotic cells [28, 29] is considered a passive modulator of membrane physical properties [30]. Biophysical studies in phospholipid:cholesterol model systems have been carried out in the range of 20% molar ratio [30-32].

In the liquid condensed phase, molecules have high diffusivity parallel to the plane of the membrane and undergo rapid rotational diffusion about the axis perpendicular to the plane of the membrane. In the absence of cholesterol, this enhanced diffusion is always accompanied by the onset of conformational freedom of the acyl chains, i.e., low orientational order, so that the normal fluid phase of pure lipid systems is appropriately described as the 'liquid expanded' (ld) phase.

The departure of the surface pressure values with respect to the critical surface pressure denoted in the abscissa can, in principle, be related to area changes concomitant to the onset of conformational freedom of acyl chains (Figures 3 and 4). The area per lipid molecule corresponds to the area excluded by the lipid head group and the immobilized hydration shell. This area, calculated from monolayers studies and from X-ray diffraction is around 64 $Å^2$ for DOPC [16, 34]. Note that this area is larger than that reported for collapse monolayers, and corresponds to lipids at the water interphase at saturation without compression (i.e. constant area)

The excess area beyond that occupied by a lipid molecule with its hydration shell at saturation is difficult to justify by the ideal formalism of an increase in geometrical space. Several equations of state have been proposed to consider the non-ideal behavior of lipid monolayers, introducing

the co-volume of the lipid head groups and the intermolecular interactions [33, 35]. Thus, changes in the area per lipid lead to a membrane state that is affected by the protein perturbation.

Therefore, the increase in surface pressure promoted by the proteins cannot be interpreted with the simple geometrical criterion in which the protein intercalates with the lipids and therefore increases the surface pressure. The water molecules beyond the hydration shell of the phospholipids and the phospholipid themselves defines the thermodynamic state of the interphase. The monolayer expansion gives place to surface sites, in terms of surface excess free energy, reactive for the amino acid residues of the proteins. The perturbation can thus be expressed by the difference of free energy between the final state of the monolayer with protein and the free energy of the initial state of the monolayer (prior to protein addition).

The resolution of *k* in terms of equation (1) can now be done considering that the surface pressure produced by the protein in the monolayer is

$$\pi = RT \ln \frac{a_w^i}{a_w^p}$$

where a_w^p is the water activity of interphase after protein addition

Thus, considering that π_p as a surface pressure of monolayer in the final state after protein addition, the pressure perturbation ($\Delta\pi$) can be written as

$$\Delta\pi = \pi_p - \pi = RT \ln \frac{a_w^L}{a_w^p} \quad (6)$$

This equation denotes that there is no perturbation when $a_w^L = a_w^p$. This condition is achieved when the whole surface is occupied by the lipids, i.e. π_c is reached. In addition, $\Delta\pi > 0$ when $a_w^p < a_w^L$. That is, the protein insertion reduces the water activity at the interface.

On the other hand, the difference of the surface pressures with respect to the critical one (π_c) can be expressed as

$$\pi_c = RT \ln \frac{a_w^i}{a_w^{Lc}}$$

From which

$$\pi_c - \pi = RT \ln \frac{a_w^L}{a_w^{Lc}} \qquad (7)$$

Equation (7) makes clear that for $\pi_c - \pi > 0$, the water activity at the interphase for any lipid concentration should be higher than at the critical a_w^{Lc}, given by the limit of packing of the lipids with its hydration shells.

Thus, dividing member by member (6) by (7) we have:

$$k = \frac{\left(\ln a_w^L - \ln a_w^p\right)}{\left(\ln a_w^L - \ln a_w^{Lc}\right)} \qquad (8)$$

The increase of the slopes due to the increase of unsaturation or the depletion of cholesterol, as shown in the Figures and in Table 1, means that the protein insertion depends on the difference in a_w^L with respect to a_w^P, for a given departure from a_w^{Lc}.

Multiplying and dividing by RT and knowing that chemical potential (μ) can be defined as:

$$\mu = \mu^0 + RT \ln a$$

$$k = \frac{(\mu_w - \mu_{wp})}{(\mu_w - \mu_{wc})} \qquad (9)$$

Assuming that there are no significant differences between the standard chemical potentials in the different conditions, equation (9) clearly denotes that the process is driven by the difference in the chemical potential of water in the different states of the interphase.

For a given value of $\mu_w - \mu_{wc}$, the perturbation increases with the unsaturations and cholesterol depletion, which is reflected in a greater difference between the chemical potential of water at the pure lipid interphase and that with proteins that grows with the increase in water spaces.

A change in surface pressure in 6-8 mN/m is equivalent to an energy change for protein adsorption of 6 kJ/mol, which amounts the energy of one H-bond and is 6 times higher than a dispersion force. This surface pressure change is, according to the equation (9) a change in the chemical potential of water most probably related to hydrogen bonds between water molecules. This energy is near to the free energy reported for Cytochrome interaction with PC bilayers which is around ~10 kJ/mol [35]. These numbers are indicative that the changes in surface pressure (surface tension) are energetically comparable with reported values for protein-membrane interaction. These interactions would take place within the water-accessible region of the membrane, that is, about three methylene groups of the lipid acyl chains. The energy of the interaction calculated for 100 $Å^2$ amounts an equivalent of two CH_2 groups. Thus, the energy changes measured by surface tension are related to the lipid membrane groups exposed to water that determines the water activity at the interphase, according to its coordination degree. The

value agrees with literature data on hydrocarbons and amphiphiles where the group contributions per methylene of $\Delta\Delta G_0$ were two chains, burying 20% of the surface [35].

Thus, the k values are related to chain conformation and packing on the interaction enthalpy and serves to explain a variety of effects reported on membrane binding. The structural counterpart of these responses is given by changes in the saturation/unsaturation ratio, presence or absence of cholesterol and carbonyl depletion (ether vs esther phospholipids). In other words, an excess of free energy can be obtained in relation to water organization around the lipids being these modulated by metabolic factors affecting membrane composition.

Conclusions

Surface water activity appears as a common factor in relation to the interaction of several aqueous soluble and surface active proteins with lipid membranes of different composition. Under the thermodynamic approach of the Defay-Prigogine, protein perturbation can be measured by changes in the surface pressure of lipid monolayers at different initial water surface activities. As predicted by solution chemistry, the increase of surface pressure is independent of the particle nature that dissolves. Therefore, the membrane response is given, in terms of determined surface states, by water activity independent of the protein or peptide.

In real systems, in which area and lipids are maintained constant, the excess of free energy necessary for peptide or protein insertion can be produced by fluctuations in curvature and packing according to the viscoelasticity of the membrane system.

The link between the thermodynamics of lipid interfaces with the hydration state of the membrane to explain the interactions of aqueous soluble protein should be established by

demonstrating the relationship of the water activity values with the organization of water at the different regions of the membrane, which deserves further studies.

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FIGURE LEGENDS

Figure 1

Polar head groups organized at the interfaces in a 1 nm thickness located between one ideal plane at the carbonyl levels (A) and another at the external face of the hydrated phosphates (B) of the phospholipids.

Figure 2

Protease interaction with DMPC and DMPE membrane (A); S-layer protein interacting with PC-cholesterol membranes with and without stearyl amines (B); Protease interaction with PC membranes of different acyl chains (C); S layer proteins interacting with PC/SA membrane for different cholesterol ratios (D). A and C adapted from ref [20] and B and D from ref [36].

Figure 3

Effect of chain composition on the perturbation of PC lipid monolayers at different excess surface pressure by aqueous protease.

Figure 4:

Effect of cholesterol on the perturbation of PC/SA lipid monolayers at different excess surface pressure by S-layer proteins.

FIGURE 1

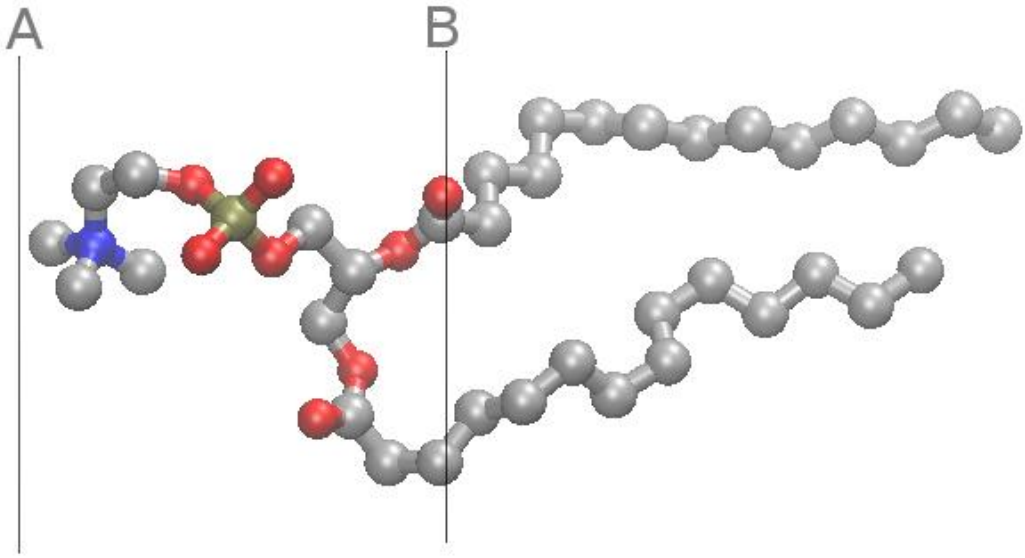


FIGURE 2

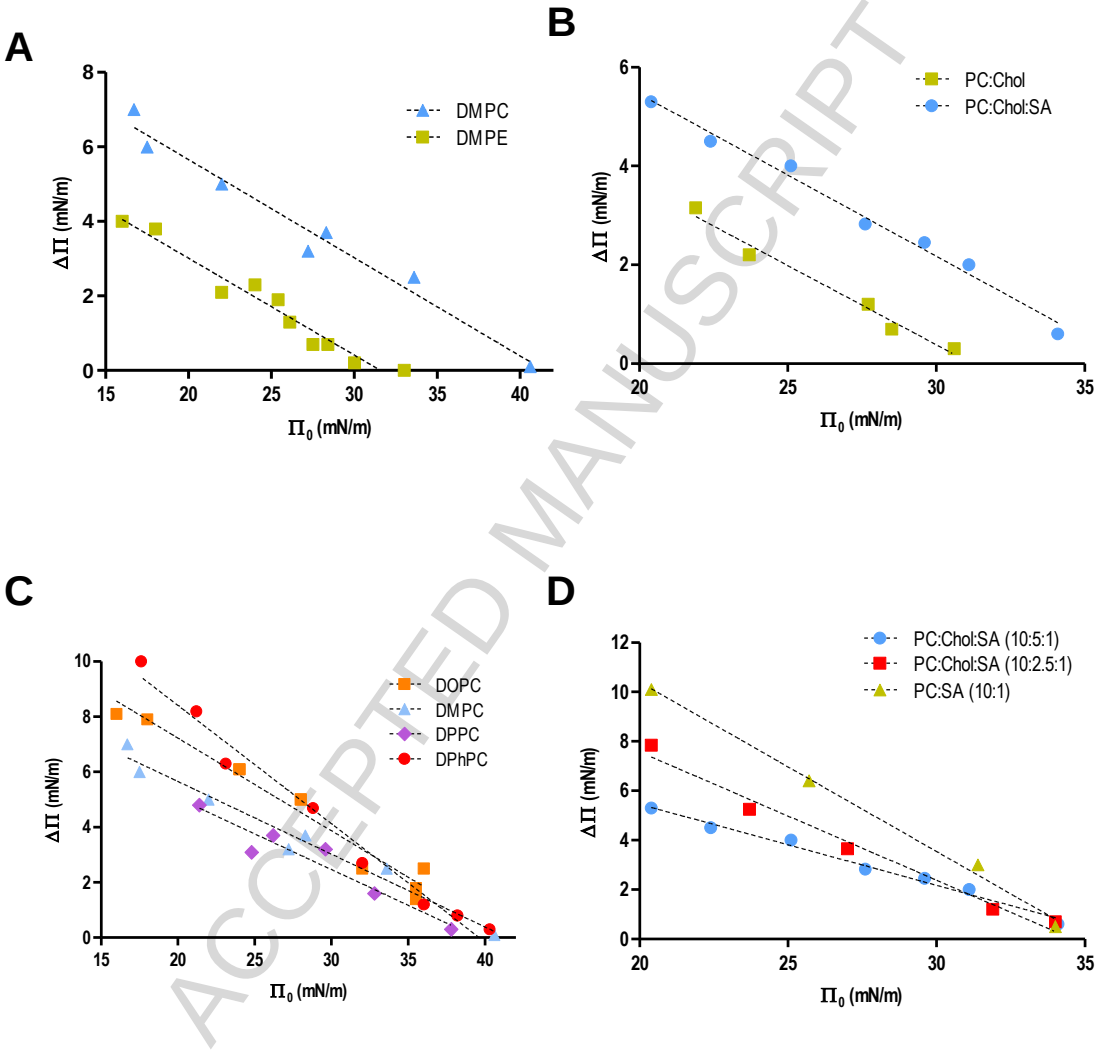
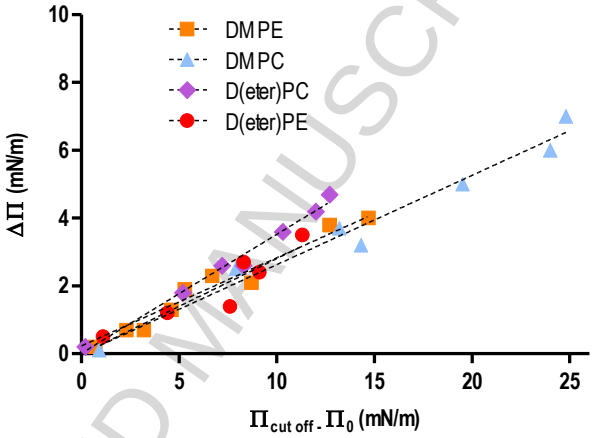


FIGURE 3

A



B

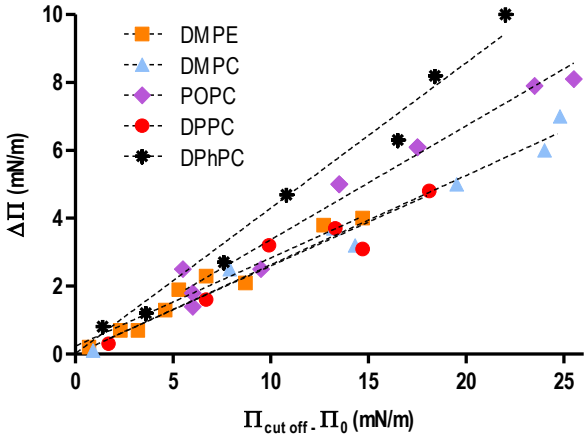


FIGURE 4

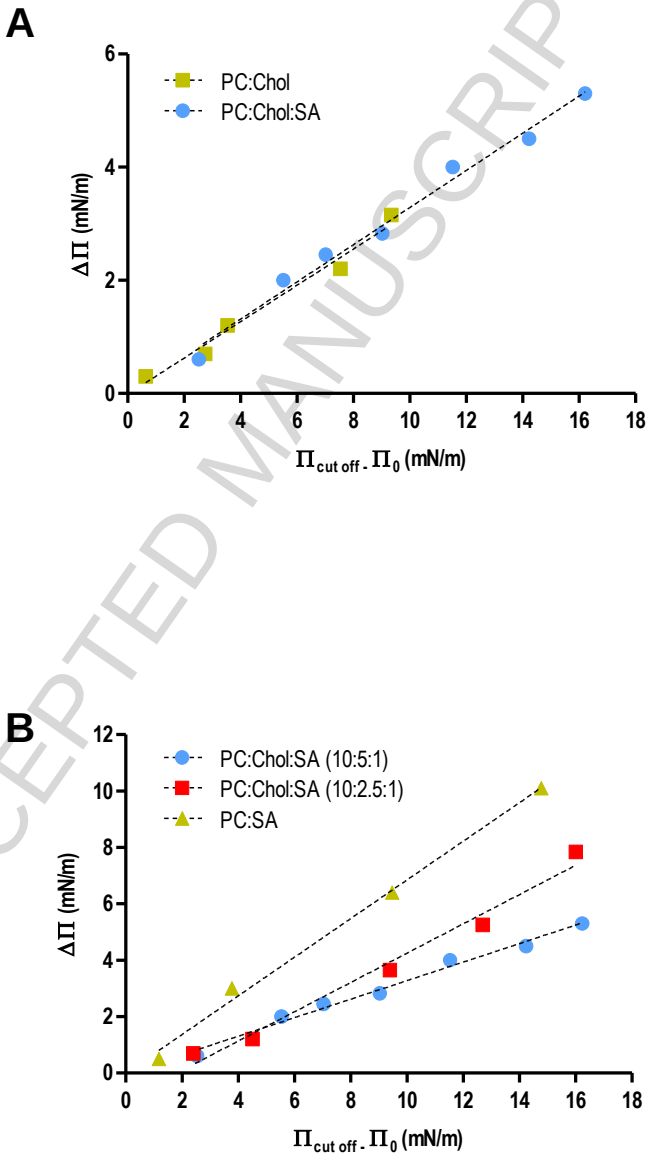
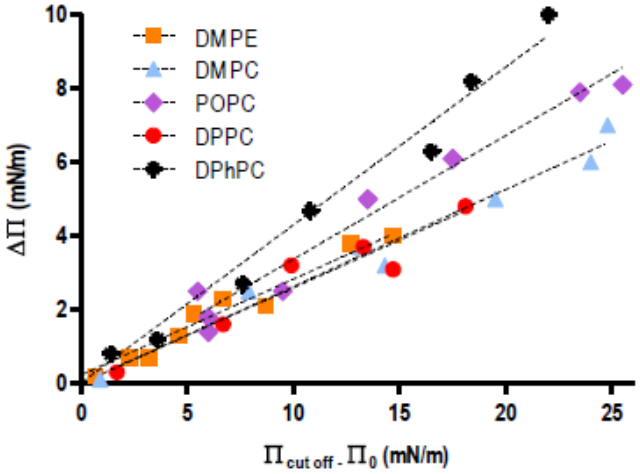


TABLE I

VALUES OF SLOPES (k) AND CUT-OFF FOR DIFFERENT PROTEIN-MEMBRANE SYSTEMS

Membrane composition	**k**	**Cut off**	**Protein**
DMPC	0.264	41.5	Aqueous protease
DMPE	0.266	30.8	Aqueous protease
Di(ether)PC	0.351	31.8	Aqueous protease
Di(ether)PE	0.282	29.4	Aqueous protease
DPPC	0.259	39.5	Aqueous protease
DOPC	0.336	41.5	Aqueous protease
DPhPC	0.428	39.6	Aqueous protease
PC:SA (10:1)	0.685	35.18	Bacterial S-layer
PC:Chol:SA (10:2.5:1)	0.519	34.6	Bacterial S-layer
PC:Chol:SA (10:5:1)	0.328	36.64	Bacterial S-layer



$$\Delta\pi = k(\pi_c - \pi)$$

$$k = \frac{\left(\ln a_w^L - \ln a_w^p\right)}{\left(\ln a_w^L - \ln a_w^{Lc}\right)}$$

Graphical Abstract

Highlights

Surface water activity determines protein interaction with membranes.

Hydrocarbon phases and cholesterol modulates surface properties.

Perturbation occurs at 4% of area increase beyond the lipid hydration shell.

Interfacial water activity relates to the surface pressure increase induced by proteins.