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Review

The electrical interplay between proteins and lipids in membranes☆

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ABSTRACT

All molecular interactions that are relevant to cellular and molecular structures are electrical in nature but manifest in a rich variety of forms that each has its own range and influences on the net effect of how molecular species interact. This article outlines how electrical interactions between the protein and lipid membrane components underlie many of the activities of membrane function. Particular emphasis is placed on spatially localised behaviour in membranes involving modulation of protein activity and microdomain structure.

The interactions between membrane lipids and membrane proteins together with their role within cell biology represent an enormous body of work. Broad conclusions are not easy given the complexities of the various systems and even consensus with model membrane systems containing two or three lipid types is difficult. By defining two types of broad lipid–protein interaction, respectively Type I as specific and Type II as more non-specific and focussing on the electrical interactions mostly in the extra-membrane regions it is possible to assemble broad rules or a consensus of the dominant features of the interplay between these two fundamentally important classes of membrane component. This article is part of a special issue entitled: Lipid–protein interactions.

Contents

1. Introduction

The interactions between lipids and proteins have been a subject of intense study for many years (see e.g. [42]), actually for rather longer than the fluid-mosaic membrane model has been in existence. Within membranes, the nature of these interactions fall into two broad categories and it's worth defining them separately to aid the discussion. Thus we deem Type I interactions to involve an explicit interaction between the

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protein and a particular membrane lipid that may be described as a specific molecular recognition event(s) as illustrated in Fig. 1A. In many cases this would involve the lipid(s) bound relatively tightly in a 'docking' site on the protein probably within one leaflet of the membrane bilayer. The second mode of interaction defined as Type II, is probably best described as a mean-field effect in which the lipid environment has an influence on the behaviour of the membrane protein as shown in Fig. 1B. The effect of the environment however may take several different forms such as mechanical (e.g. line tension, curvature etc., see [72]) or electrical effects such as static charges or dipole fields exerting distinct effects on the protein behaviour (e.g. [55]). These definitions of course, are closely allied to the much-debated, historical descriptions of annular and non-annular lipid protein assemblies (see [42]). For the sake of clarity we prefer to use the 'Types I & II' nomenclature as there are subtle and not so subtle differences between our working definition and the *annulus* hypothesis. In particular the Type II nomenclature accommodates the interactions of lipid with proteins and with each other in the extra-membrane regions via the supporting electrolyte media as illustrated in Fig. 2. We emphasise this region particularly as a significant element of membrane behaviour.

A spectrum of particular interactions is likely to exist between each of these possibilities with both types of interaction co-existing in some protein-membrane systems. Similarly, proteins may have reciprocal effects on the lipids and their behavioural characteristics (such as phase behaviour). Rather than solely take a retrospective or historical view however we hope to use this present forum to identify some of the key questions (and new ways to address them) as well as trying to rationalize the hitherto disparate views of membrane function in a cellular context.

2. Lipid–protein interactions and membrane protein function

It will be necessary to discuss some aspects of Type I interactions further but it is the Type II category of molecular interaction that the present paper will mostly address. Nevertheless this still represents a huge body of work and even concentrating solely on the electrical interactions as the article's title indicates, necessitates consideration of a very large set of activities. This is particularly the case as it has now become apparent that local (microdomain or membrane raft) structures have a bearing on membrane function (see e.g. [24]).

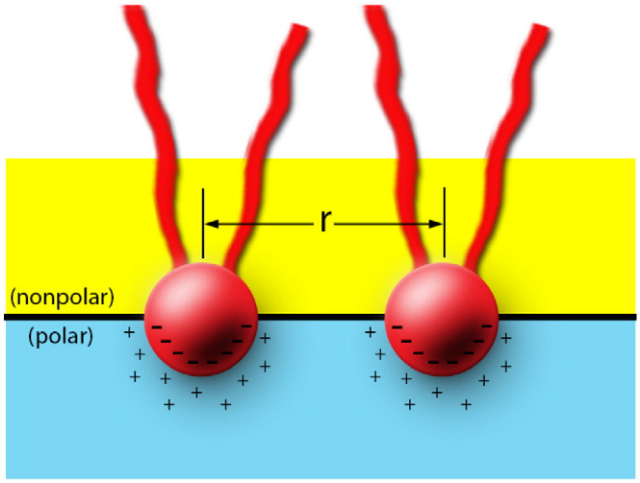

Fig. 2. Schematic of the intermolecular interactions between lipid headgroups in the aqueous (polar) and intra-membrane (non-polar) regions.

Although all molecular interactions relevant to cellular and molecular structures are electrical in nature they manifest in a rich variety of forms with their own characteristic range and influences on the net effect of how molecular species interact with each other see e.g. Fig. 3A. Collectively the distance-dependence of the attractive and repulsive interactions are embodied in the so-called DLVO theory (e.g. [35]) which has evolved to be a coarse grain or 'rule of thumb' methodology for predicting and describing the net interaction between macromolecular assemblies (e.g. colloids) as indicated in the idealised sketch in Fig. 3B. It's also worth noting that although the form of the DLVO energy-distance profile is reminiscent of the more explicit Leonard–Jones 6–12 potential profile, the formal rigour and molecular scales each formalism addresses are very different. The DLVO formalism is helpful for practical purposes as it simplifies the manifold ranges and magnitudes of the influences of 'each' of the forces outlined in Fig. 1 for macromolecular assemblies.

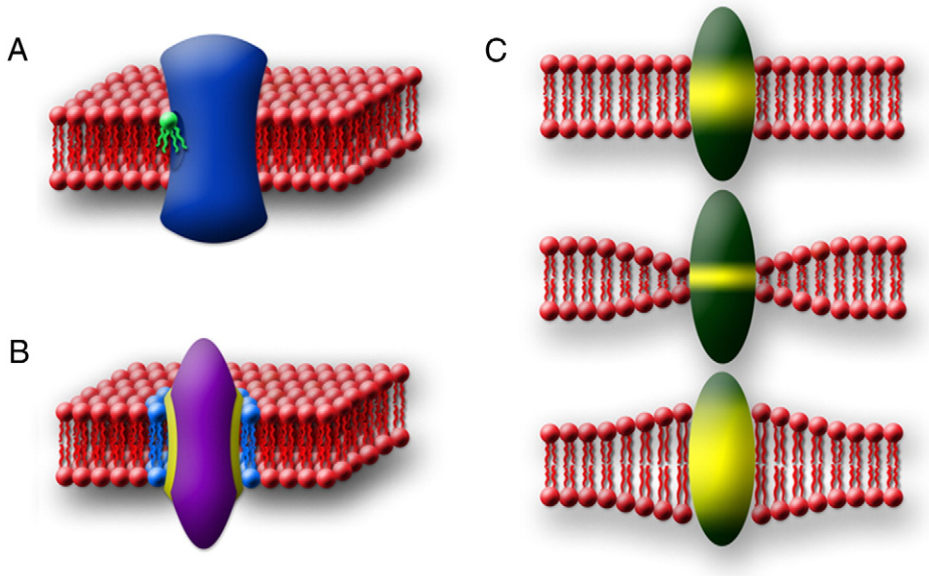

Fig. 1. Schematics of various types of lipid–protein interactions within membranes. A: Type I lipid–protein interaction i.e. illustration of a specific lipid binding or docking site on the body of the protein. B: Type II lipid–protein interaction i.e. illustration of a non-specific lipid mean-field effect of the lipid environment on a membrane protein. C: Lipid–protein mismatches in the membrane bilayer thickness with the hydrophobic regions of the membrane protein shown as the bright shaded regions (for more details see [42]).

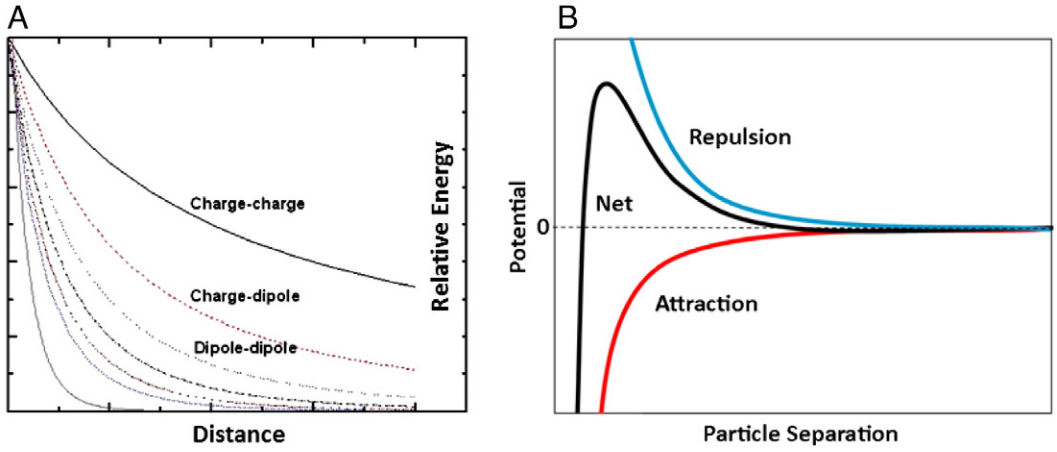

Fig. 3. Schematics of various intermolecular forces between molecular structures. A: The relative distance dependence and magnitude of various kinds of intermolecular interactions. B: The relative distance dependence and magnitude of the net interaction potential energy according to DLVO-type coarse grain models of (macro)-molecular interactions.

Computational theory and software have improved enormously since the introduction of the original DLVO approach, nevertheless they still leave much to be desired in terms of our ability to characterise fully the interactions between macromolecules with each other and other molecular species/assemblies, particularly in membrane systems. From an experimental point of view our laboratories amongst several others are able to characterise the main determinants of most types of interaction. This work includes studies with protein–protein-interactions surfaces [40,86], molecular interactions with model membranes [16,40,86] as well as cell–cell interactions [33,37]. Thus by specifically targeting particular types of intermolecular reactions it proved possible to identify the dominant mode of an interaction for protein–protein interactions and particularly molecular membrane interactions. We need to consider these properties further but before returning to the general considerations, the next section outlines some specific examples of the interplay between the lipids and membrane proteins. The explicit Type I or Type II mechanism is not emphasised but it is clear that one or both categories may underlie the catalytic responses or conformational modifications of membrane proteins to the lipid environment.

3. The Type I lipid–protein interactions involving an electrical interplay

Lipid–protein interactions are fundamental to many physiological responses and can be influenced by the membrane electrostatic potential/charge. Annexins for example, are a family of Ca^{2+}-dependent phospholipid-binding and membrane-binding proteins [64], important physiologically due to their roles in calcium buffering, membrane structure and cytoskeleton remodelling [6]. Some members of the Annexin family have been shown to bind preferentially to charged phospholipids. Annexin V, for example, binds only weakly to bilayers containing phosphatidylcholine or sphingomyelin but binds more strongly to bilayers which contain anionic phospholipids [2,42]. The strength of this binding interaction is dependent upon the lipid constituents decreasing in the order phosphatidic acid > phosphatidylserine > phosphatidylinositol [42]. Similarly, large conductance, Ca^{2+}-activated K^+ channels are critical to the negative feedback mechanism that opposes vasoconstriction allowing arterial tone to be regulated [81]. The Ca^{2+} sensitivity of these channels is modified by the auxiliary β1 subunit, a process which has been shown to require negatively charged lipids in the membrane [81].

Inositide lipids bind readily with many proteins through interactions with a variety of binding motifs including Pleckstrin homology (PH), FYVE, PX, ENTH, CALM, PDZ, PTB and FERM domains [4]. The presence of a PH domain in Phospholipase C δ1 (PLCδ1), for example, mediates its binding to Phosphatidylinositol(4,5)*P*2 (PIP2) directing its membrane localisation and providing an indirect regulatory mechanism of its enzymatic activity [76]. The affinity of the binding interaction between PLCδ1 and PIP2 can be influenced by membrane charge with phosphatidylserine having been shown to induce a conformational change in the PH domain structure of PLCδ1 and lead to a reduction in the membrane binding affinity of PLCδ1 [76]. Membrane concentrations of phosphatidylserine are known to fluctuate during physiological processes [43] indicating one route by which Phospholipase C isozymes and their downstream signalling pathways may be regulated [76]. Phosphoinositides have also been shown to have an involvement in SNARE complex-mediated vesicle fusion [44]. PIP2 and Phosphatidylinositol(3,4,5)*P*3 (PIP3) can induce cholesterol-independent clustering of syntaxin-1A, one of the major components of the SNARE complex. This clustering is partly due to the phosphoinositides acting as 'charge bridges' (see also [42]) to bring syntaxin-1A molecules together, and partly due to the reduction in the energy barrier for protein/lipid clustering that occurs following neutralisation of the charges on syntaxin-1A and the phosphoinositides when binding between the two occurs [44].

4. Mean-field examples of lipid–protein interactions involving an electrical interplay — The Type II condition

Tony Lee, in a lucid review in this journal about a decade ago, outlines a number of mechanisms by which lipids may affect membrane protein function (see [42]). This area remains both complicated and clearly still in need of much more work however, before a deep understanding of how recent advances in lipid behaviour (see e.g. [72]) can be reconciled with the behaviour of proteins in membranes with a view to a better understanding of cell biology. This is a major subject in cell biology and as well as the excellent review mentioned above by Lee [42] others for example by White and Wimley [80] are also recommended. The following text focuses on examples of tractable systems that may allow a better understanding of the mechanisms involved.

The influence of the lipid environment is described comprehensively by Lee [42] who develops this narrative by illustrating how a hydrophobic mismatch illustrated in Fig. 1C may stress the protein or the lipid (or both) and lead to alterations in the behaviour of either or both. The importance of other aspects of lipid composition on the efficacy of drug transport by the p-glycoprotein for example has been described by Bellamy [9], and Romsicki and Sharom [69]. The p-glycoprotein is an ATP-binding cassette protein comprising two nucleotide-binding domains which bind and hydrolyze ATP and two transmembrane domains also acts as the drug-binding sites [71]. Many substrates of p-glycoprotein are hydrophobic and partition into the bilayer prior to

interaction with p-glycoprotein. As a consequence the lipid environment becomes an important factor in the transport process and it has been demonstrated that several drugs bind with varying affinity to p-glycoprotein when in a neutral rather than charged lipid environment [69]. Some of our own work (see e.g. [3]) also sheds some light on the specific electrical nature of the Type II lipid–protein interactions that operate within the p-glycoprotein system. It is to the more explicit electrical interactions that we turn to next as these are manifold and very influential in membrane function.

The p-glycoprotein is particularly interesting to us as it has emerged that the membrane microdomain environment itself appears to modulate the protein structure such that the behaviour of (e.g.) receptor systems may be altered by their localisation within rafts as compared to the FM membrane (see [24,82]). This appears to be achieved through the agency of the membrane dipole potential, a relatively recently understood membrane property which is markedly different in rafts compared to the fluid membrane and has been demonstrated to affect protein structure within membranes [14,54] and modulate receptor function in cells [3] as well as having effects on the properties of more macroscopic membranous assemblies [83]. Thus this would be defined by a Type II mechanism, although recent work (see [46,57]) indicates that the differences between these Types I and II mechanisms are not as marked as our above definition would imply. In order to develop the context of this view further we outline the electrical properties of membranes in a little more detail in the next section.

5. Electrical properties of membranes

Membrane potentials represent some of the most ubiquitous but also the most complex manifestations of physical forces that appear in living systems. They have an enormous variety of roles ranging from important intra-cellular processes, physiological behaviour such as brain function through to inter-organismal interactions. An understanding of the electrical sophistications of biological membranes has been extended over the last few years as it is now recognised there are three electrical potentials associated with membranes [55,56]. These electrical potentials are a ubiquitous feature of all living cells and play many different roles in manifold cellular processes. Each type is involved in a number of different biological processes that exploits the different physical characteristics of the potential. The three distinct membrane potentials known to exist include the transmembrane potential difference, the surface electrostatic potential and the membrane dipole potential. A comprehensive and rigorous outline of their physical origins and differences together with their biological implementations of each of these membrane potential can be found in O'Shea [55]. The transmembrane potential is perhaps the best known and characterised membrane potential and has its origins in active energy-dependent processes. In the present review however we focus on the membrane surface and the membrane dipole potentials. These potentials arise respectively from static net charges or gradients of electric charge over sub-molecular dimensions. Both are affected significantly by different lipid types respectively from the obvious such as phosphatidylserine conferring excess negative surface charge (electronegative surface potential) to the more subtle effects of different lipid types on the membrane dipole potential [3,17,66]. There is a growing awareness that this potential is as influential and important as the transmembrane potential particularly as this may also include aspects of molecular stereospecificity in their properties as reported by Bandari et al. [5]. This latter work was relatively recent but promises to be very important in our understanding of membrane function.

6. Water at the membrane-solution interface

The interactions of the membrane-associated electric charges of the phospholipid head groups as shown in Fig. 2 would in the simplest terms is governed by Coulombs law. These charges are located in an aqueous environment ostensibly with a dielectric permittivity of around 80 however this value is known to be a significant overestimation. Thus, although it is fairly well understood that the interactions of water in the presence of phospholipids are essential to maintain the structure of the membrane, the membrane's effect on the adjacent (local) water structure has been considered for some time (e.g. [28]) but is less clear. There is consensus that the mutual interaction of water with the membrane leads to an environment that exhibits a dielectric constant rather less than that of bulk water (e.g. [28,78]). The water molecules at the membrane surface are also thought to constitute part of the dipole potential associated with the lipid bilayer and the complex water layers appear to project a significant distance from the molecular surface of the membrane [66]. We show elsewhere [67] using atomistic molecular modelling that the ordering of water on the membrane lipid head-groups contributes to the significant membrane dipole potential and the gradient of dielectric permittivity from the bulk phase water to the membrane interior. We showed in this work that the ordering of water on the lipid head-groups contributes to the large peak in the membrane dipole potential, and also for the gradient of dielectric permittivity from the bulk water to the molecular surface of the membrane. Similarly the electrical properties of these regions including the dipole potential partially within membrane are also interesting [55,56]. Thus there is an electrical gradient from the molecular surface of the membrane to the bulk phase water that depends on a number of parameters such as the membrane net excess surface charge density and the nature (e.g. valency) and concentration of the surrounding electrolyte.

The non bulk-water structure adjacent to the molecular surface of the membrane therefore may have substantial effects on the lipid–lipid and/or lipid–protein intermolecular interactions on and within the membrane. Accordingly, as one denominator of Coulombs law is the Dielectric constant, changes from around 80 as in bulk water to say around 2 in the membrane interior means that single charge–charge interactions taking place in either of the environments could be affected by as much as a 40-fold difference depending on their location.

These considerations are also complicated by the possibility that a lateral gradient of dielectric constant may also exist. Important work from Jarvis et al. [73] (see also [27]) indicated the water structure adjacent to the membrane surface was quite different over lateral dimensions adjacent to membrane microdomains compared to the much more fluid regions of the membrane. Around the same time we identified from separate atomistic computational and experimental spectroscopic studies that gradients to the membrane normal from bulk water to around 12–20 on the membrane surface [67] was also quite different adjacent with different lipid assemblies (see also [72]). Thus lateral gradients of water structure on the membrane surface seem to exist depending on the nature of the local lipid structures. Lateral Coulombic interactions will be affected very significantly (see e.g. Fig. 3) therefore as well as other parameters that 'feel' the water structure. This also suggests that a lateral force arising from the hydrophobic effect is present and could well underlie some aspects of phase behaviour in membranes underlying local lipid partitioning. Clearly the latter would complicate explanations and formal modelling (e.g. [65]) of microdomain formation in membranes. We are currently attempting to calculate the thermodynamics of these possible mechanisms for membrane partitioning to determine how significant they may be.

7. Lipid–protein interactions and membrane organisation

It has long been known that protein dispositions within cell membranes exhibit striking heterogeneities both in terms of large-scale aggregates with surface diameters of the order of μm and smaller scale aggregations of less than 100 nm in diameter. Examples of the former include the densely packed plaques of gap junctions [26], the distributions of ion channels and coupled ion transport systems and the purple patches

in the membranes of the halophile archeobacteria [75]. Similarly, the long known lymphocyte 'capping phenomenon' (e.g. [53,62,84]) was an interesting phenomenon with the recent understanding of the existence of the immune-synapse as a well-studied and instructive example [21] that relates directly to cell function. Smaller scale heterogeneities within cell membranes have also attracted attention from cell biologists and is the subject of enormous worldwide efforts as such localisation of activity are thought to reflect recognition or signalling processes and thus underlie control within cell signalling. In all cases, however, the underlying mechanisms which promote transitions between the various organisational regimes still remain obscure. Over many years a number of mechanisms have been proposed which include active and passive processes [7,10,19,53,84] up to the more recent concepts involving specific membrane lipids such as cholesterol (see e.g. [63]) and larger structures such as membrane rafts (see e.g. [51,59]) but there remains many questions.

Rotational and lateral diffusion of proteins within cell membranes, has also received widespread attention and a number of well documented reviews have been available for some time [13,36,45,50]. Diffusion events are linked with the functional role of membranes such as signal processing [48], energy transduction [34], pathology and growth & differentiation [53,85]. These processes are linked to the interactions between proteins and the lipids, thus, the rotational and lateral motion of membrane components are also intimately linked to the overall architecture of the membrane. More recent technologies offering spectacular resolution (e.g. [11,48]) have helped enormously but this aspect of membrane behaviour also remains an area of contention with much debate about common mechanisms [23,39,48]. It is necessary in all the deliberations of how proteins move within membranes to consider their electrical interactions with the membrane lipids. The following sections therefore, develop this dialogue in more detail.

8. The membrane dipole potential: role in modulating the behaviour of microdomain-located membrane proteins

The presence and biological roles of cell membrane microdomains (often referred to as membrane rafts) and briefly discussed above has grown enormously since their conceptual introduction into the cell biology research community more than a decade ago, although it must be conceded that this idea has been around for rather longer and remains contentious [38]. Nevertheless in our hands we clearly observe these structures in both artificial model systems (e.g. [29,65]) and the membranes of living cells [24,54]. We published the first theoretical model of the possible mechanisms of assembly and disassembly of these structures [65] and firmly believe they are a feature of living cell membranes. The growing list functions of associated with microdomains revolves around their ability to act as local platforms for endocytosis/exocytosis or to localise reactants whether they be small ligands or proteins [45,50]. In this way reactants are localised in the raft aiding their interaction or they are sequestrated from the more fluid membrane preventing their interaction with potential partners resident in the fluid membrane. Both these processes, however, are conceptually analogous.

We considered that membrane microdomains may exhibit a quite different membrane dipole potential to that of the fluid phase membrane due to their different lipid packing and complement of sterols and lipids (etc.). We demonstrated this was indeed a function of the various lipids present (see [3]) then showed that this parameter had a significant effect on membrane protein conformation [3,14]. We also demonstrated with representative ligand–receptor systems that this behaviour may alter the behaviour of such receptor systems depending on whether they were resident in the rafts or in the fluid phase regions of the membrane [3,15] (also see [74]).

9. Does the membrane dipole potential modulate protein function via a Type I or Type II mechanism?

The interaction of membrane lipids with membrane proteins is clearly reciprocal but in terms of the lipid modulation of a catalytic or signalling process, the likelihood is that they would take place through the Type I or Type II mechanism defined above. Some lipids such as cholesterol however may exhibit both mechanistic types (perhaps even both with the same protein system). One common physical quantity associated with membrane function is the membrane dipole potential and this parameter seems may play a role in both Type I and Type II mechanisms (see e.g. [57]).

Phospholipid membrane components include moieties such as the $C^{\delta+} = O^{\delta-}$ and $O^{\delta-}$–$P^{\delta+}$ exhibit polarisation. The membrane dipole potential ϕ_d has its origins in the dipole moments of polar groups from the lipidic components of the bilayer; it seems likely that the water molecules at the molecular surface of membrane also make a contribution [67]. The organisation of the membrane components that contribute to this potential have been verified from neutron diffraction studies and NMR spectroscopy [8]. These dipolar groups are oriented such that the potential located towards the hydrophobic interior of the membrane is positive with respect to the pole located towards the external aqueous phases, and ϕ_d has a magnitude of several hundred millivolts (see e.g. [46,67] and references therein).

The question of whether a particular membrane lipids exerts an effect via a Type I or Type II mechanism (or both) is interesting and for lipids such as cholesterol generate much debate (see e.g., [63,68]). Cholesterol does seem to operate via both Type I and Type II mechanisms and no doubts further examples of both mechanisms will emerge. It's worth emphasising some recent work from the group of Chattopadhyay (see [5]), however as they offer some exciting evidence of the possibility that chiral selective interactions based on different optical isomers. This is could be enormously important as it may offer a profound change in our understanding of the nature of many types of lipid-protein interactions associated with the membrane dipole potential.

10. Visualising molecular interactions in membranes

Major industries have sprung up over the years that allow measurement of molecular interactions with and within membranes that are important for both basic science questions and in pharmacology as part of the commercial pharmaceutical industry. In our own laboratories for example we have pioneered technologies that contribute to studies in both areas (see e.g. [16,24,78,79]). As many of these techniques involve fluorescence detection and, as discussed above spatial heterogeneity is important, imaging technologies have become enormously important in the study of cell biology.

Thus, the huge strides in identifying the positions of proteins in membranes from the now established super-resolution techniques (reviewed by [58]) to single molecule identifications of protein disposition have been important and have certainly made a huge impact on membrane studies. The interplay dynamics involving Type II systems however are rather less easy to study as many interactions are transient and challenge the temporal resolution capabilities of many imaging systems. This is particularly relevant to those proteins which are reversibly localised about the cell membrane during for example signalling processes, the various phases of differentiation or as cells undergo more pathogenic changes. Likewise, many other proteins appear to undergo similar phase transitions (i.e. intra-membrane aggregation), although the functional rationale is rather less clear. There are methodologies however that may not demand that dynamical measurements are made of protein dispositions in membranes, simply that the disposition is measured in order to then determine the interaction potential energies that arise from electrical interactions between the membrane components.

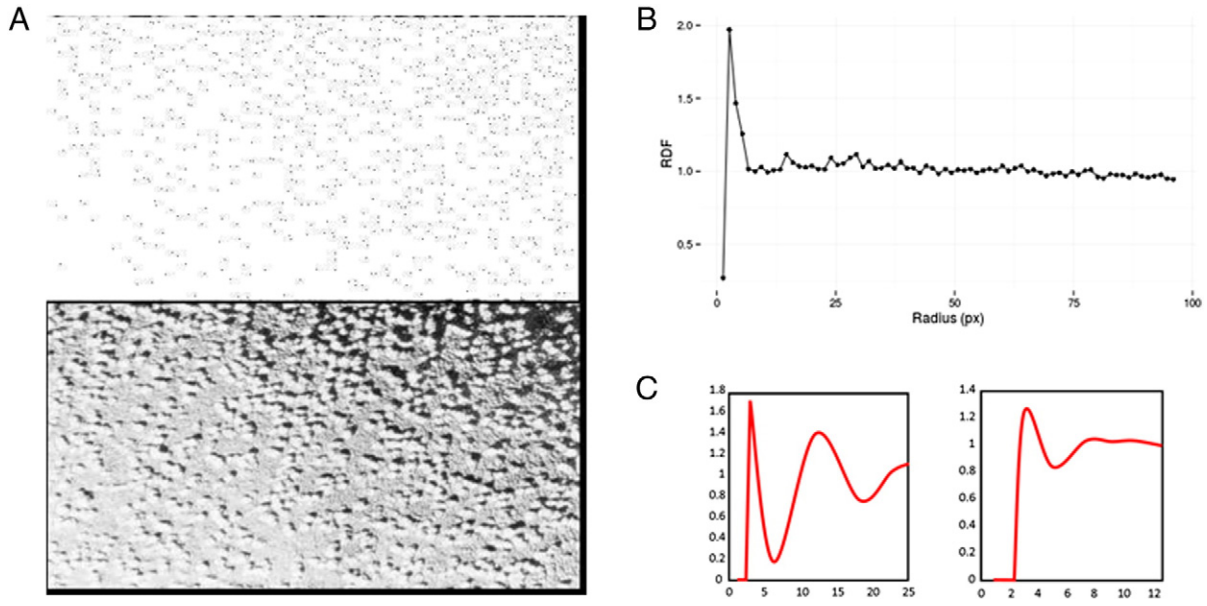

Fig. 4. Distributions of membrane proteins in model membrane systems. A: Computer-based recognition of particle disposition based on the lower freeze-fracture micrograph of a single type of membrane protein (we are grateful to Tony Watts for providing us with some of these images). B: Radial distribution function (RDF) of A with the abscissa in units of pixels (px). C: An idealised representation of this distribution will change with different observed aggregation patterns.

We would like to use the opportunity of the platform presented by the present paper to suggest some ways forward to address these problems. Thus once visualised, by any means that is as hopefully non-invasive as possible, protein dispositions within cell membranes may be analysed by a number of procedures. Acquisition of a disposition of proteins in a defined lipid membrane system as shown in Fig. 4 may be analysed so that it makes no *a priori* assumptions about the disposition both dynamically or in terms of the nature of the molecular interactions. All that is needed is the means to determine the interaction potential energies based on the patterns. Thus the aim of the measurement would be to identify the pattern or associated distribution functions and from that determine the energetics. Roos et al. [70], for example, assessed the degree of clustering by statistical means but with little analysis of the underlying physical mechanisms. Other techniques are available which are based more within the realms of statistical mechanics, however, and yield explicit thermodynamic information. Earlier work in this area involved the determination of distribution functions and the use of the Percus–Yevick equation (e.g. [47]). Perelson [62] and Middlehurst and Parker [49] added further refinements so that curved membranes and deviations from spherical geometry could be dealt with. Pearson et al. [60] included angular correlations of many-particle interactions in the erythrocyte membrane. Similarly, techniques for boundary corrections during data collection have been reported [25]. Most of these studies, however, have tended to ignore the mean potential forces between particles, emphasising instead, the distribution functions. More importantly, however, there have been few attempts to relate specific values or ranges of particular interaction potential energies to parameters of physiological relevance [62]. More recent studies bring to bear a number of different techniques (e.g. [41]) that may offer a way forward by using inverse problem solutions to determine protein–protein interaction energies in different membrane systems. These require nanoscopic localisation (preferably nanometre or less) of many proteins in real time to determine the energetics in this manner.

Assuming that transitions between the various aggregated states of membrane proteins depends upon the value of their interaction potential energies with each other or the membrane lipid and that these interactions are likely to be electrical, a study of absolute values, the factors which affect these parameters, together with measurements of how the former may vary have not yet been undertaken. This is an important question although equally, there may be many other circumstances where these inherent assumptions may not hold.

The foregoing discussion, therefore, has framed the broad question to be addressed in the following manner: given that the control of protein–protein interactions in cell membranes is important for a large number of cellular events, what kind of values might we expect for the energetics of inter-protein interactions that may underlie some of these membrane condensation processes? Values determined to be greater than KT is likely to lead to aggregation is likely whereas the opposite would indicate that passive intermolecular interactions would be unlikely to underlie the aggregation phenomenon and other factors must be coming into play.

11. Inverse problem solutions for quantification of inter-protein interactions in membranes

One approach to the foregoing question that as yet, is relatively unexploited, involves visualisation of the patterns of protein disposition within cell membranes followed by a global inverse analysis leading to deductions of the requisite forces necessary to generate such patterns. In other words, rather than attempt explicit calculations based on atomic resolution information (attempted in the past by e.g. [22]) in order to ascertain whether proteins may aggregate (or not) or with some coarse-grain approaches such as a 2-dimensional DLVO approach in mind, solution to the 'inverse problem' could be sought. Fortunately, there are now accepted strategies for successful (i.e. unique) solutions to 'inverse problems' which find increasing use in the physical and life sciences; applications vary from atomic physics to physiology [30]. Satisfactory solutions to the appropriate 'inverse problem' will involve the collection of information regarding the disposition of individual proteins (>1000) within cell membranes. As far as is known, membrane freeze-fracture examined with the electron microscope is the only technique currently available which combines ease of use and low cost with a sufficiently high spatial resolution [10]. Freeze-fracture does not appear to perturb the protein disposition to any significant extent and results in fairly unequivocal views of the phospholipid or cell membrane [34]. Following the preparation of suitable electron micrographs, the distribution functions describing particle dispositions within membranes

can be determined and processed. This of course does not yield dynamic information but the purpose of the present discussion is to consider the proof of concept for future studies. It's also worth emphasising that a similar approach was taken by Abney and Owicki [1].

The use of higher order distribution functions such as the Yvon–Born–Green equation (YBG) appear to be more satisfactory because they facilitate the identification of relationships between the mean particle forces and experimentally accessible parameters such as particle dispositions and distribution functions [32,52]. The YBG equation relates the mean force represented by the pair potential of a membrane particle, the triplet distribution function and the particle's radial distribution function.

This relationship has been formerly well established within the discipline of fluid mechanics [32] but to a much lesser extent within membrane biophysics. The YBG equation appears to possess the most valuable attributes for this study as it can handle several distribution functions and provides a direct relationship between them and the parameter of most interest, namely the interactive forces between membrane proteins. Croxton and McQuarrie [19] have utilised the YBG equation in an effort to describe the electrical double layer adjacent to a charged surface. Meanwhile, others [10] considered it that it may be the most appropriate choice for determining the effects of particle–particle interactions on lateral diffusion within membranes. It remains to be demonstrated however, that such a system may be utilised to describe protein–protein interactions within cell membranes.

The YBG equation emphasises two contributions to the mean force exerted on a specific particle [32,35]. Firstly, the force exerted by any other specific particle and secondly the mean force integral of all other particles upon the particle in question. Values for the mean forces between integral membrane particles, therefore, may be obtained from the YBG equation provided some values are included for the distribution functions, which are accessible from analysis of freeze-fracture electron micrographs of cell membranes (in planar polar co-ordinates). The distribution functions are obtained, therefore, in a similar manner to that reported by Duniec et al. [25], although this was somewhat limited by interrupted views of the membrane preparation.

12. Inverse solution for the determination of interaction potential energies of membrane proteins

The preliminary and simplest premise that membrane proteins are free to diffuse without let or hindrance and that their observed distributions result from purely inter-protein forces. There are a number of caveats accompanying this assumption but for the development of the analytical system we start with the possibility that the random equilibrium disposition will be attained. The strategy employed for inverse solutions to inter-particle interactions for studies within fluid mechanics, relies on the determination of various distribution functions followed by their incorporation into the YBG equation [32]. This yields the mean potential force between the particles together with their interaction potential energies. Unfortunately, structural information regarding solute dispositions within liquids (solutions) is simpler to obtain than similar particle dispositions of membrane proteins. The latter may be obtained from freeze-fracture electron micrographs of appropriate membrane preparations but there is still the problem of digitising the information and more importantly for the recognition of the particles in order for the distribution functions to be determined. Even at this primitive level, particle recognition of ca. 1024 particles, yields distribution functions which have the anticipated form (Fig. 4) for a 2-D fluid.

The distribution functions are processed by the YBG analysis in the following manner: The integral Yvon–Born–Green equation relates the particle–particle pair potential (uij), the radial distribution function (g r12) and the triplet distribution function (gt). The mean potential force (Fim) on a given particle by another specified particle together

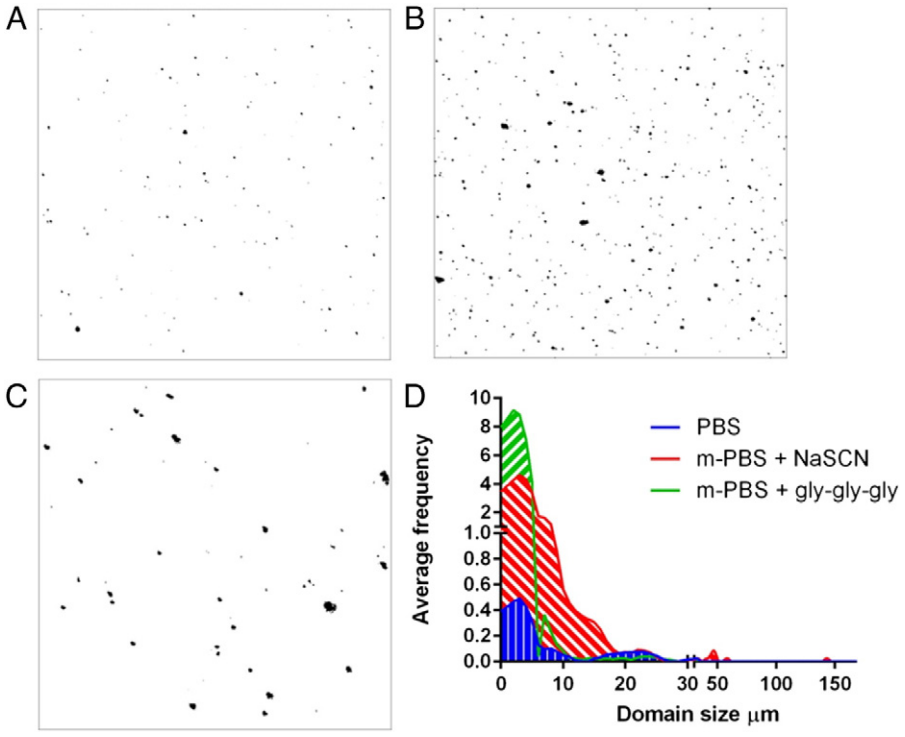

Fig. 5. Modulation of interlipid interactions by rational targeting of intermolecular forces in model membrane preparations as revealed by fluorescence imaging of membranes. Representative fluorescent microscopy images of 70% phosphatidylcholine/30% cholesterol supported bilayers (collected according to earlier studies; for more details see [24] — note these PC preparations contain 2 major types of fatty acyl chains) in media configured with the following reagents (the rationale of construction of these media are outlined in [40]). A) Phosphate buffered saline (PBS) identified as PBS. B) Modified PBS with 100 mM NaCl replaced with 100 mM NaSCN. C) Modified PBS with 100 mM NaCl, replaced with 200 mM Glycylglycylglycine. D) Analysis of particle domain size with averages (n = 9).

with all the other forces of all other particles; the pair-force is then the mean force at large dilution. Thus, if the distribution functions are known, the linear integral may be solved directly for the pair-force to yield a pair potential. The particle–particle pair potential (uij), obtained in this way indicates that there is an energy minimum of about KT which would promote protein aggregation but at longer range, a slight energy barrier amounting to about 0.5 KT exists. Thus, because of the closeness of these values to KT we expect that the equilibrium disposition of the proteins would be fairly random and they would exist singularly. In Fig. 4 this was the observation, other systems which are aggregated due to various environmental conditions exhibit widely different distribution functions and inter-particle forces are greater than KT. Thus one way forward is systematically to vary the membrane lipid types and the surrounding electrolyte to modulate the inter-particle forces and determine if this can be identified using the Inverse Problem approach outlined briefly in the current paper.

13. Modulation of molecular interactions in membranes by targeting specific intermolecular forces: effects on microdomain formation

The effect of varying the supporting electrolyte concentration on the behaviour of membrane phospholipids is fairly well understood. In the simplest terms, screening of the repulsive electrostatic interactions by electrolytes at various concentrations correlates with phase-effects on certain types of charged phospholipids (e.g. [12,44]). This behaviour more or less follows the expectations of the Debye–Hückel and Poisson–Boltzmann relations and manifests as effects on the familiar phase diagrams of ternary mixtures of phospholipids. As emphasised in Fig. 3A however, there are other possible types of intermolecular interaction but these too may be targeted independently of the electrostatic effects. This was the basis of a recent publication [40] from our laboratories in which intermolecular interactions of added molecular species with membranes were reduced or augmented by supplementing the aqueous media surrounding the membrane with reagents designed to target various physical interactions. As well as affecting the molecular interactions of molecules added to the membranes it is also possible to target reactions within the membranes in both the water phase as well as the lipid phase. In work about to be published we show that modulating the nature of the media around the membrane also leads to changes of the membrane patterning in a controlled fashion. A representative example is shown in Fig. 5 in which the electrostatic nature of the supporting media is kept fairly constant by replacing the dominant anion in phosphate-buffered saline with SCN^-. The virtue of SCN^- however is that is possesses a large entropy of hydration and the effect of this is to promote the formation of membrane microdomains. The latter are visualised using the fluorescent technologies we have described previously (see e.g. [24]). Similarly, replacing the SCN^- with triglycine leads to a quite different pattern of microdomain formation. These are plotted as the number-density of the domains versus the size of the domains (i.e. as bin size). It was found that the number and sizes of the various fractions was found to vary with the presence of these reagents under circumstances when the difference of the electrostatic interactions between the membrane components was kept fairly constant. A more detailed exposition of this analysis will appear in due course but it is clear that the electrical interplay between membrane components is affected by more than simple coulombic interactions. No doubt Nature exploits these properties in different and hitherto unrealised ways and we hope in the near future to define these more explicitly and most particularly in cellular systems.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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