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Review

Lipid–protein interactions in biological membranes: A dynamic perspective ☆

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ABSTRACT

Though an increasing number of biological functions at the membrane are attributed to direct associations between lipid head groups and protein side chains or lipid protein hydrophobic attractive forces, surprisingly limited information is available about the dynamics of these interactions. The static in vitro representation provided by membrane protein structures, including very insightful lipid–protein binding geometries, still fails to recapitulate the dynamic behavior characteristic of lipid membranes. Experimental measures of the interaction time of lipid–protein association are very rare, and have only provided order-of-magnitude estimates in an extremely limited number of systems. In this review, a brief outline of the experimental approaches taken in this area to date is given. The bulk of the review will focus on two methods that are promising techniques for measuring lipid–protein interactions: time-resolved fluorescence microscopy, and two-dimensional infrared (2D IR) spectroscopy. Time-resolved fluorescence microscopy is the name given to a sophisticated toolbox of measurements taken using pulsed laser excitation and time-correlated single photon counting (TCSPC). With this technique the dynamics of interaction can be measured on the time scale of nanoseconds to milliseconds. 2D IR is a femtosecond nonlinear spectroscopy that can resolve vibrational coupling between lipids and proteins at molecular-scale distances and at time scales from femtoseconds to picoseconds. These two methods are poised to make significant advances in our understanding of the dynamic properties of biological membranes. This article is part of a Special Issue entitled: Membrane protein structure and function.

Contents

1. Introduction . . . 172
   1.1. Voltage-gated ion channels . . . 173
   1.2. Lipid annulus of G protein-coupled receptors . . . 173
   1.3. Lipid-binding domains . . . 173
2. Spectroscopic probes of lipid–protein interactions . . . 173
3. Time-resolved fluorescence microscopy . . . 174
4. 2D IR spectroscopy . . . 175
5. Conclusion . . . 176
Acknowledgements . . . 176
References . . . 176

1. Introduction

Biological membranes are complex, two-dimensional fluids formed from amphipathic lipid bilayers and a high density of proteins and carbohydrates. The organization of these components in live cells is heterogeneous, with order observed on a range of time and length scales. At a molecular level, it is the interaction potential between specific lipids and proteins that drives this organization, and to properly describe these interactions it is essential to understand the structure and dynamics of lipid–protein complexes. This has to some extent been observed using structural biology methods, where there are a growing number of membrane protein crystal structures in which a bound lipid has been identified [1]. These crystallized lipid–protein complexes are thought to be representative of strong, long-

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lived lipid–protein interactions in the plasma membrane. In other systems, lipids exchange rapidly from sites along the perimeter of a protein into the bulk two-dimensional fluid at a rate of $10^7\ s^{-1}$ [2]. These two cases represent the range of dynamics that govern lipid protein interactions, but there is still a vast unexplored field of systems for which these dynamics have not yet been measured. One reason for this lack of data is that the interactions are very difficult to observe using traditional structural methods because of the nature of the lipid–protein environment and because of the fast dynamics of the interactions. This review will first present a few examples where direct lipid–protein interactions are central to biochemical events. It will then briefly discuss the various approaches taken to measure the interactions. Finally, it will highlight two promising methods that can resolve the specificity and dynamics of lipid–protein complexes at a range of time and length scales.

1.1. Voltage-gated ion channels

Ion channels regulate charge transport across the plasma membrane. Gating and activity in these channels depend on a balance of ion concentration and electrical gradients across the membrane. In voltage-gated potassium channels, a sensor domain on the outside of the channel induces structural rearrangements that open the pore and lower the energy cost for ions to cross through the membrane [3]. This voltage-gating has been studied extensively, and there is growing evidence that the gating mechanism is lipid dependent [4–6]. For example, inward rectifier potassium channels are thought to directly bind negatively charged $PI(4,5)P_2$ lipids, and that the channel activity is proportional to the $PI(4,5)P_2$ concentration [6]. It has also been found that KvAP channels reconstituted in a bilayer with cationic lipids lacking a phosphodiester group lose their voltage-gated behavior [4]. When phospholipids are added to the bilayer, the channels recover their voltage-gated activity with the largest enhancement resulting from anionic phospholipids. The hypothesized mechanism is that positively charged arginine side chains form hydrogen bonds with negatively charged phosphate groups in the lipid [5,7]. This is consistent with lipid composition variations and directed point mutations [8], but to date there has been no direct probe of specific lipid head group–protein side chain interactions. Anionic phospholipids are also thought to regulate the activity of the mechanosensitive ion channels like MscL [9]. It has been found that increasing concentrations of phosphatidylglycerol, phosphatidic acid, or cardiolipin increase calcein flux through the MscL channel [10], and that the effect is likely due to direct hydrogen bond formation rather than differences in spontaneous curvature [11].

1.2. Lipid annulus of G protein-coupled receptors

G-protein coupled receptors are seven-transmembrane-helix proteins that participate in numerous signal transduction pathways. They play an essential role in vision, brain function and locomotion, and are the targets of over half of pharmaceutical treatments. The function of several GPCRs is cholesterol dependent [12–15], but one unanswered question is whether the interactions are specific, involving tight binding to well-defined protein regions, or if the effect is through physical changes to the surrounding membrane [16]. To ask this structural question is to ask about the time-scale and geometry of the cholesterol GPCR interactions. Cholesterol has been observed in crystal structures of rhodopsin and the β2 adrenergic receptor [13,17,18]. In the case of the β2 adrenergic receptor there is evidence of a cholesterol recognition amino acid consensus (CRAC) motif [18], which is a proposed cholesterol docking site [19]. It may be that these cases are unique, and that the larger dependence of GPCR function on cholesterol can be attributed to its modulating the physical properties of the membrane. However, it is clear from the literature that there is no consensus on the composition of the lipids adjacent to the protein and their respective time-scale of interaction.

Molecular dynamics simulations have been used to approach this problem for the nicotinic acetylcholine receptor (nAChR) [20]. The function of nAChR is dependent on the presence of the anionic phospholipid, phosphatidic acid (PA) [21], and is stabilized by cholesterol [22]. The simulations found that for lipids within 1 nm of the protein, PC and PA bilayers have the same thickness and order-parameter value, but that the PA lipids form a stable micro-domain around the protein [20]. This is evidence that the time-scale of lipid–protein interactions plays a key role in protein function, and argues for the development of new experimental approaches to directly test these hypotheses.

1.3. Lipid-binding domains

Integral membrane proteins are not the only class of proteins that interact specifically with membrane lipids. Cytosolic proteins make use of a variety of structural motifs to anchor them to lipid membranes [23,24]. For example, some proteins have amino acid sequences that encode for enzymatic attachment of lipid moieties that target the protein to a lipid bilayer [24,25]. These are often used in signaling pathways to regulate localization of the protein to the plasma membrane [24,25], and have also been proposed to localize the protein to specific functional membrane domains [26–28]. In spite of these observations, the physical interactions and the dynamic associations of these lipidated proteins are still poorly understood. For example, the lymphocyte cell kinase (Lck) protein is anchored to the membrane via two palmitoyl and a single myristoyl lipid modifications that are thought to target the protein to cholesterol-rich membrane regions [29,30]. However, there is evidence from biophysical studies that Lck partitions heterogeneously fluid domains in giant plasma membrane vesicles [26], which leaves open questions about the physical forces driving the organization of these proteins.

Another structural motif used to bind proteins to biological membranes is the inherent secondary and tertiary structures of the protein [23]. Such lipid binding structural motifs include target-specific domains like the C1 domain that binds diacylglycerol, the pleckstrin homology (PH) domain that binds phosphoinositides like PIP_2 and PIP_3, and FYVE domains that bind PIP_3. Other domains like PKC C2, annexin, BAR and F-BAR bind to anionic phospholipids non-specifically. While there is growing understanding of the protein–lipid binding kinetics, there are still core questions about secondary interactions. For example, PH domain binding to phosphoinositides is strengthened by insertion of hydrophobic amino acids into the bilayer as well as non-specific interactions with negatively charged lipids [23]. These interactions can be thought of as a two-dimensional analogue of co-solvent effects long studied in aqueous solutions. Central to the debate is how charge–charge interactions manifest themselves at the protein–lipid interface. Do anionic phospholipid head groups bind specifically to positively charged amino acid side chains, or is it better described as a non-specific co-solvent effect? Questions like this mirror those asked about the interaction of salts and osmolytes with proteins in aqueous solution [31]. To date, however, there has been almost no direct investigation of this effect in biological membranes.

2. Spectroscopic probes of lipid–protein interactions

Direct measures of lipid–protein interactions are difficult to make under physiologically relevant conditions. High resolution structural methods have begun to yield substantial insight. For example, several protein crystal structures have identified bound lipid molecules [1], including cholesterol bound to a GPCR as cited above [13,17,18], and a phorbol ester (a diacylglycerol analogue) bound to a C1 domain [32]. These crystal structures provide evidence for strong lipid–protein