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Review

# The role of electrostatics in protein–membrane interactions

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**Abstract**

Many experimental, structural and computational studies have established the importance of nonspecific electrostatics as a driving force for peripheral membrane association. Here we focus on this component of protein/membrane interactions by using examples ranging from phosphoinositide signaling to retroviral assembly. We stress the utility of the collaboration of experiment and theory in identifying and quantifying the role of electrostatics not only in contributing to membrane association, but also in affecting subcellular targeting, in the control of membrane binding, and in the organization of proteins and lipids at membrane surfaces.


*Keywords:* Peripheral protein; Electrostatics; Protein/membrane interaction; Phosphoinositide; Computational biology; Electrostatic switch; Lateral organization

## 1. The importance of electrostatics in the membrane association of peripheral proteins

The reversible binding of proteins to membrane surfaces is critical to many biological processes and is often accomplished through lipid-interacting protein domains. The membrane association of many peripheral membrane proteins has been shown to be mediated, at least in part, by electrostatic interactions [26,58,82]. It is well established that a number of proteins, such as Src, K-Ras, and MARCKS require the nonspecific electrostatic interaction between a cluster of basic residues on the protein and acidic phospholipids in the membrane for their activity and regulation [7,20,46,51,126]. For example, removing the N-terminal basic residues of Src weakens its partitioning onto phospholipid vesicles containing acidic lipids and produces nontransforming phenotypes in biological cells [61,96,105]. Other peripheral proteins, such as secreted phospholipases A2 [44], the transducin Gβγ subunit [87] and retroviral matrix proteins [92], have basic surface patches that interact nonspecifically with acidic phospholipids. Multi-modular proteins, such as various isoforms of protein kinase C (PKC), phospholipase C-δ (PLC-δ), early endosome auto-antigen 1 (EEA1), phosphatase and tensin homolog (PTEN), and phosphoinositide 3-kinase (PI3K), are recruited to membrane surfaces by multiple lipid-interacting domains that bind to membrane surfaces through either specific interactions with a particular lipid head group, nonspecific electrostatic and hydrophobic interactions or some combination of all [23,25,30,32,66,70,97].

Some examples of peripheral proteins and peripheral protein domains are given in Fig. 1. As depicted in the figure, the proteins differ dramatically in size, shape, and, most notably, net charge, which ranges from highly positive (+10 e) to neutral to highly negative (−13 e). Since some domains that bind to acidic phospholipids can have a net negative charge, a point charge representation is clearly inadequate for characterizing both the electrostatic properties of these proteins and their propensity for favorable electrostatic interactions with negatively charged membrane surfaces [74,83]. As illustrated, some peripheral proteins are overall basic, such as the AppD49 secreted Phospholipase A2 (sPLA2, panel C) and the PTEN C2 domain (panel B), while others are quite polar, such as the Gβγ heterodimer (panel A), the Phospholipase Cδ1 Pleckstrin homology domain (PLCδ1 PH, panel D) and the Equine Infectious Anemia Virus matrix domain (EIAV MA, panel E). The way

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charge is distributed over the surface of a peripheral protein may have profound implications for its membrane targeting and/or lateral assembly functions (see below). Despite the differences in overall shape and charge distribution, in each example the region implicated in electrostatic association with the membrane surface is relatively flat. In many cases, this is likely a physical characteristic that distinguishes proteins that target the plasma membrane from those that target the nucleus and bind DNA, whose basic regions tend to possess significant curvature [54]. However, there is at least one domain, the BAR domain [127], which uses a highly curved concave surface to bind to membranes, so the distinction is not straightforward. For the proteins in Fig. 1, continuum electrostatic calculations [8,54,106], which will be introduced below, predict that most have a similar minimum electrostatic free energy of interaction with a membrane containing 33 mol % monovalent acidic phospholipid in 0.1 M monovalent salt. However, the average maximum predicted contribution, an electrostatic binding free energy of −5 kcal/mol or a related molar partition coefficient of $10^3$ $M^{-1}$, is not enough to strongly associate these proteins to the plasma membrane under physiological conditions [75]. And indeed, each of these proteins or domains employ additional membrane targeting motifs or schemes in addition to simple electrostatics for peripheral association (see Table 1).

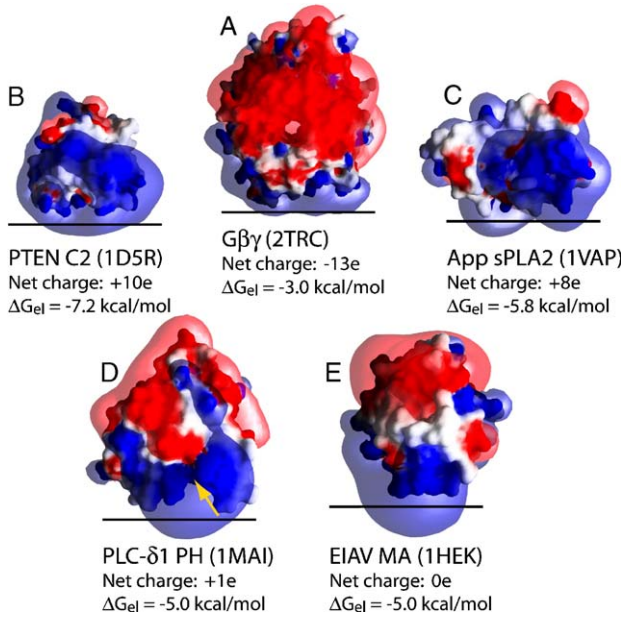


Fig. 1. The electrostatic properties of peripheral proteins can vary dramatically. Depicted in each panel is the name of the protein or protein domain, its Protein Date Bank (PDB; [11]) identifier code in parentheses, the net charge, and the electrostatic free energy calculated by the finite difference Poisson–Boltzmann method (FDPB, [40]) for the protein in its minimum electrostatic free energy orientation for 2:1 PC/PS and [KCl]=0.1 M. The black lines denote the envelope of the van der Waals surface of the membrane in the FDPB calculation. The yellow arrow in panel D indicates where $PI(4,5)P_2$ binds specifically. Electrostatic potentials were calculated and visualized in GRASP [89] for [KCl]=0.1. The electrostatic surface potentials are graded from −4 kT/e (red) to 0 kT/e (white) to +4 kT/e (blue), and the equipotential contours are fixed at −1 kT/e (red) and +1 kT/e (blue).

Table 1
Additional membrane attachment motifs/mechanisms exhibited by the proteins in Fig. 1

| Protein/protein domain | Additional membrane attachment mechanism |
|---|---|
| Fig. 1A: Gβγ | Lipid modification: prenylation |
| Fig. 1B: PTEN C2 | Nonspecific interaction with $PI(4,5)P_2$ |
| Fig. 1C: AppD49 sPLA2 | Interfacial hydrophobic residues |
| Fig. 1D: PLCδ1 PH | Specific binding to the head group of $PI(4,5)P_2$ |
| Fig. 1E: EIAV MA | Oligomerization |

In order to facilitate our description of membrane-mediated electrostatic phenomena, we will digress to briefly introduce the application of continuum electrostatics to protein/membrane interactions.

## 2. Computational approaches

The finite difference Poisson–Boltzmann (FDPB) method [31,54,104] has been widely applied to describe the electrostatic properties of proteins, nucleic acids, and membranes [8,10,82]. In a series of experimental/theoretical studies over the past decade, we and others have used this method to describe the binding of charged peptides and proteins to membrane surfaces. This work demonstrated that the FDPB method is remarkably accurate in treating electrostatic properties associated with nonspecific binding. This is because many of the relevant interactions are long range and depend only on the charge distribution of the system. The computational methodology is based on finite difference solutions to the full (nonlinear) Poisson–Boltzmann (PB) equation, an equation of classical electrostatics [40,47,52,54,103,104]. The finite difference method is a numerical procedure that allows the solution of the PB equation for biological molecules which often have complex shapes. The PB equation is as follows:

$$\nabla[\varepsilon(r)\nabla\varphi(r)] - \varepsilon_r\kappa(r)^2\sinh[\varphi(r)] + e^2/(\varepsilon_0 k_B T)\rho^f(r) = 0 \quad (1)$$

where $\varepsilon(r)$ is the dielectric constant, $\phi(r)$ is the electrostatic potential, $\kappa(r)$ is the Debye–Huckel parameter and $\rho^f(r)$ is the fixed charge density of the protein and lipids; all four variables are functions of space, $r=(x, y, z)$. A protein/membrane system is represented atomistically and is mapped onto a three-dimensional lattice of points; each lattice point represents a small region of the protein or membrane ($\varepsilon=2$; to approximate electronic polarizability) or the aqueous phase ($\varepsilon=80$; to account for the highly polar nature of water molecules). Some of these quantities are schematically depicted in Fig. 2A. The solutions to the PB equation are the electrostatic potentials, $\phi(r)$, which are used to calculate the electrostatic free energy of a system, $G_{el}$ [103]. The electrostatic free energy of interaction between a protein and membrane, $\Delta G_{el}$, is calculated as the difference between the electrostatic free energy of the protein and membrane when they are close together, $G_{el}(P{\cdot}M)$, and when they are far apart, $G_{el}(P)$ and $G_{el}(M)$ [10]:

$$\Delta G_{el} = G_{el}(P{\cdot}M) - [G_{el}(P) + G_{el}(M)]. \quad (2)$$

## 3. The balance of electrostatic and non-polar contributions

Secreted Phospholipases A2 (sPL2's) have long served as a paradigm for interfacial association. sPLA2's are generally recruited to membrane surfaces through a combination of nonspecific electrostatic and hydrophobic interactions, the balance of which can be quite different for different sPLA2 groups [44]. Indeed, many peripheral proteins contain hydrophobic residues on or near their basic surfaces that have been shown to penetrate the membrane interface. The experimental interfacial hydrophobicity scale developed by Wimley and White [122] indicates that Trp, Phe, Tyr and Leu contribute most favorably to the membrane penetration of small host peptides. These residues are often found on the membrane-interacting loops or surfaces of peripheral proteins. This information can be incorporated into a computational model to calculate the non-polar contribution from the simple expression:

$$\Delta G_{np} = \gamma \Delta A, \tag{3}$$

where $\gamma$ is the surface tension coefficient of 0.013 kcal/mole/Å$^2$ as determined from the interfacial hydrophobicity scale, and $\Delta A$ is the change in solvent accessible surface area upon association.

An approximation to the total nonspecific free energy of membrane association of a peripheral protein can be taken as the sum of $\Delta G_{el}$ (from Eqs. 1 and 2) and $\Delta G_{np}$, so that

$$\Delta G = \Delta G_{el} + \Delta G_{np} \tag{4}$$

Eq. (4) defines the "FDPB/SA" method [55] (the equivalent of the PBSA and GBSA methods used in protein simulations; SA for surface area) which partitions binding phenomena into separate electrostatic and non-polar contributions. In such methods the electrostatic contribution is obtained from the PB equation or from some other approach (e.g. GB or Generalized Born, which is an effective approach to calculate charge–charge interactions in the presence of continuum solvent [37]), while the non-polar contribution is obtained from free energy–surface area relationships such as that given in Eq. (3). Fig. 2 describes how the additivity of electrostatic and non-polar interactions qualitatively explains membrane binding behavior. Long-range electrostatic attraction (red curve) increases the probability of a protein/membrane interaction and helps orient the protein. As

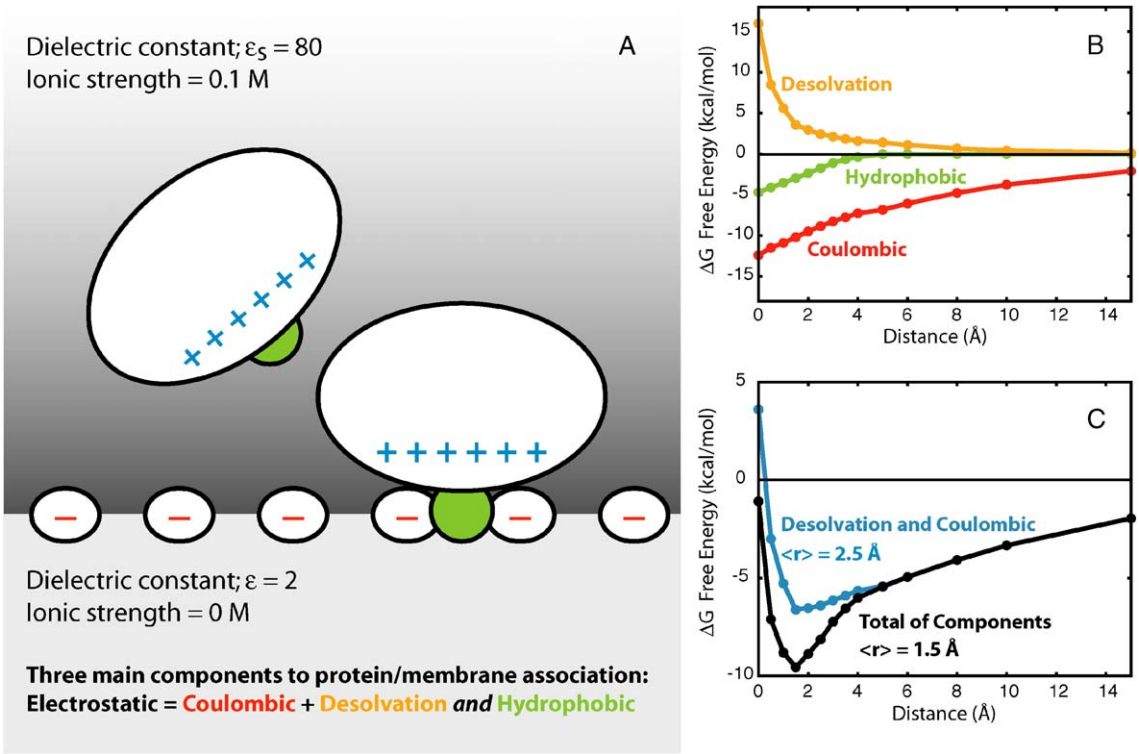


Fig. 2. Simplified representation of the nonspecific forces involved in the association of peripheral proteins with PC/PS membranes. (A) PS lipids in the membrane are depicted by their headgroups as ovals with red minus signs. PC lipids are implicitly included. The counterions in the aqueous phase are shown, by gray shading, to be more concentrated at the membrane surface and to assume the bulk value (white) far from the membrane surface. The protein (large white oval) has a basic patch (blue plus signs) and a phenylalanine residue (Phe; green) in the center of this patch. Electrostatic attraction helps orient the protein and steers it towards the negatively charged membrane surface. If the electrostatic attraction and nonpolar contribution due to the interfacial insertion of the Phe are more favorable than the desolvation of the both the protein and membrane upon association, the protein will adsorb approximately as depicted in the right side of the panel. (B) The interplay of electrostatic attraction ("Coulombic", red curve) and desolvation ("Desolvation", gold curve), as calculated by the FDPB method, and non-polar contributions ("Hydrophobic", green curve), as calculated empirically from an interfacial hydrophobicity scale, (1) is different for each situation, (2) depends on both the protein and the environment (membrane and aqueous phase), and (3) determines the membrane binding behavior. (C) If the incorporation of an attractive component in addition to "Coulombic", e.g. "Hydrophobic", out-competes the unfavorable desolvation penalty, a deeper and narrower energy well (black versus blue curve) may result, and the average position for the protein will, thus, be closer to the membrane surface.

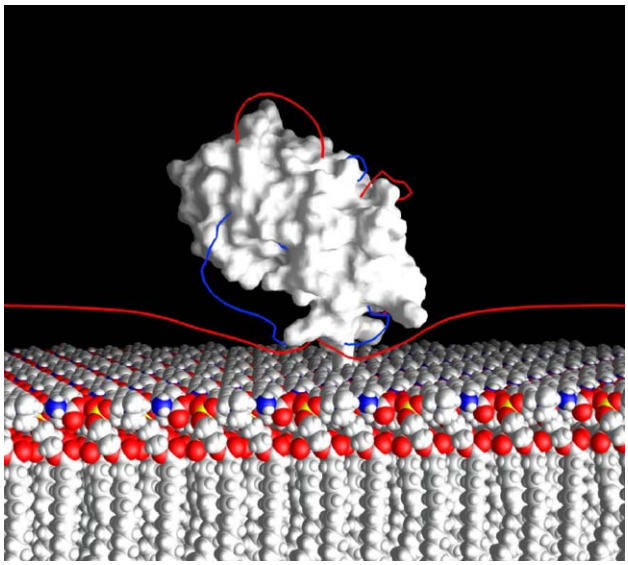

Fig. 3. Membrane interaction of the C2A domain of Synaptotagmin I as predicted by the FDPB method. The calcium-bound C2 domain (PDB identifier, 1BYN) is depicted by its molecular surface and is shown docked at the surface of a 2:1 PC/PS bilayer in 0.1 M KCl in it minimum free energy orientation. Phe 234 is predicted to penetrate the membrane interface. The red and blue lines represent the −1 kT/e and +1 kT/e two-dimensional equipotential contours calculated by the FDPB method [40] and visualized in GRASP [89]. The orientation is consistent with that determined experimentally by Frazier et al. [39]. In the bilayer, oxygen atoms are colored red, nitrogens are colored blue and phosphorus is colored yellow.

the protein approaches the membrane surface, there are two competing short-range interactions: (1) the (repulsive) desolvation of both the protein and membrane as both, which contain charged and polar groups, begin to lose their favorable interactions with the polar aqueous solvent (yellow curve); and (2) the favorable non-polar partitioning of hydrophobic groups into the membrane interface as described by the interfacial hydrophobicity scale (green curve). In addition to the qualitative insights it provides, the FDPB method, as suggested by Fig. 2B, allows us to examine quantitatively how the combination of favorable electrostatic attraction, repulsive desolvation, and favorable nonpolar interactions leads to different membrane binding behaviors. Each case must be considered separately since the relative contribution of each of these energetic terms will depend on the structure and composition of the membrane/protein system. In the example depicted in Fig. 2, the "hydrophobic" component is significant enough to both significantly decrease the free energy of binding due to electrostatics alone and to bring the protein, on average, closer to the membrane surface.

Fig. 3 illustrates an explicit application of the FDPB/SA method to a small protein domain, the calcium-bound C2A domain of Synaptotagmin 1 (Syt1). Calcium-induced nonspecific electrostatic interactions ("the calcium/electrostatic switch") have been clearly demonstrated to provide a major driving force for the membrane association of Syt1-C2A and other similar C2 domains [98]; this is manifested as the dramatic perturbation of the negative electrostatic potential profile (red line in Fig. 3). FDPB calculations are able to account for a wide range of experimental observations on these C2 domains [5,65,86]. For the Syt1 C2A domain, it was shown experimentally that a phenylalanine (Phe234) on one of the calcium-binding loops penetrates the membrane interface [24]. Application of the full FDPB/SA method results in the model of Fig. 3 and a predicted free energy of membrane partitioning, $\Delta G$, of −7 kcal/mol. The close agreement with the experimental value of −6.4 kcal/mol (for the same conditions) is most certainly fortuitous as, for example, the membrane model is highly simplistic in that it does not account for lipid dynamics. However, the theoretical model appears to capture the essence of the physics underlying the interaction, and, notably, the relative orientation of the domain with respect to the membrane, predicted as the minimum free energy orientation, is similar to that obtained from electron paramagnetic resonance (EPR) experiments with site-directed spin-labeled forms of Syt1 C2A from the Cafiso lab [39]. The partitioning of free energies into individual components, which is implicit in the FDPB/SA approach, is intuitively simple. While more work is required to more thoroughly develop and test its quantitative validity for peripheral proteins, it provides an excellent framework within which to rationalize the correlation between peripheral protein design and membrane binding behavior.

## 4. The effect of lipid composition on protein/membrane electrostatics

"Common" phospholipids, such as the zwitterionic, electrically neutral lipids phosphatidylcholine (PC) and phosphatidylethanolamine (PE), and the monovalent acidic lipid phosphatidylserine (PS), are relatively abundant in cellular membranes [17,37]. For example, PC and PE constitute ~60% and PS constitutes ~25% of the phospholipid in the inner leaflet of the plasma membrane [125]. Hence, the cytosolic surface of the plasma membrane carries an appreciable negative charge due to the large proportion of PS relative to other intracellular membranes. Fig. 4 summarizes experimental observations from the McLaughlin lab on how the membrane association of a basic peptide changes as a function of the composition of mole percent PS in a phospholipid vesicle [78]. The binding is almost two orders of magnitude stronger when the membrane contains 25 mol% PS versus 10 mol% PS. Electrostatic attraction between positively charged regions on proteins and monovalent acidic lipids has, thus, been suggested to contribute a driving force for the plasma membrane localization of peripheral proteins, e.g. those in Fig. 1 as well as K-Ras, MARCKS and Src, whose basic membrane binding regions carry net charges of +7e, +13e and +5e, respectively [75,84,99].

## 5. Membrane aggregation

Fig. 5A shows an atypical but very interesting example of an sPLA2, the human group IIA sPLA2 (hGIIA sPLA2), whose binding is largely driven by electrostatic interactions [108]. Its net charge is +17e and its electrostatic potential profile is almost completely positive. Vesicle binding measurements were confounded by vesicle aggregation when the acidic lipid

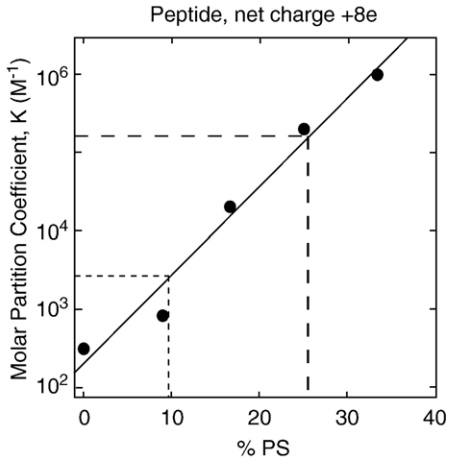


Fig. 4. Basic regions of proteins exhibit significantly higher affinity for membranes with higher anionic lipid composition. A peptide carrying a net charge of +8e binds more than two orders of magnitude more strongly to a membrane containing 33 mol% monovalent acidic lipid (phosphatidylserine, PS) than to a membrane containing 10 mol% PS. The molar partition coefficient in these experiments was measured using a sedimentation assay with sucrose loaded vesicles. PS exists on a background of the zwitterionic lipid phosphatidylcholine, PC, and [KCl]=0.1 M. The figure is adapted from McLaughlin et al. [78].

composition was higher than 25 mol% [22]. FDPB calculations predict that there are two roughly equivalent minimum electrostatic free energy orientations for hGIIA sPLA2 ([33]; Fig. 5B): 1) an interaction with the membrane surface through the enzyme's interfacial binding surface (IBS), which contains the active site, and 2) an interaction through the surface opposite the IBS. The existence of alternate binding orientations is consistent with the electropositive nature of the enzyme (Fig. 5A). Thus, membrane-associated hGIIA sPLA2 might exist in a dynamic equilibrium between these two (and perhaps other) orientations. Since only the IBS has interfacially associating hydrophobic residues, it is likely the preferred membrane binding surface as observed in EPR studies [22].

In the preferred orientation, the opposite face of the enzyme would generally be free to interact with a second membrane if the lipid concentration was sufficiently high. Such a membrane–protein–membrane interaction would bring distinct membrane surfaces into close proximity, i.e. within the distance of a diameter of the enzyme, resulting in vesicle aggregation. As shown explicitly in Fig. 5C, it is electrostatically unfavorable for two anionic membranes to be at this distance of separation from each other in the absence of enzyme. However, based on electrostatic considerations alone, even a very low surface

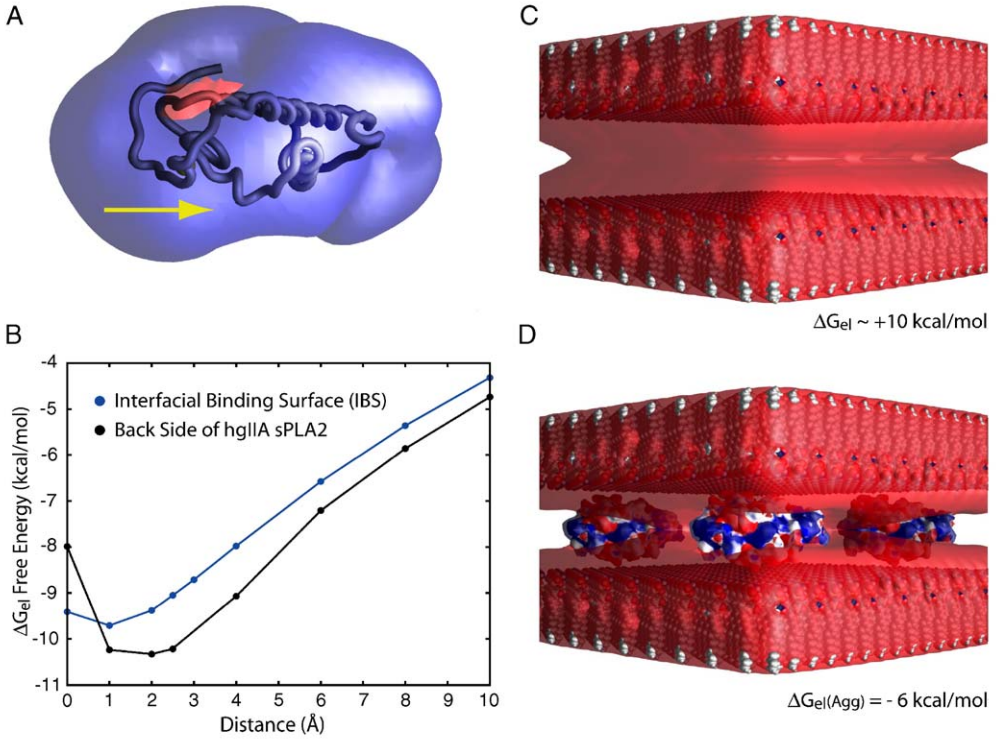


Fig. 5. Model for the membrane aggregation induced by Human Group IIA secretory phospholipase A2 (hGIIA sPLA2). (A) The equipotential contours for hGIIA sPLA2 (PDB identifier 1POD, net charge +17e) were calculated and visualized in GRASP [89] for [KCl]=0.1 M; red and blue, respectively, represent the −1 kT/e and +1 kT/e equipotential contours. The yellow arrow points to the interfacial binding surface (IBS), which contains the enzyme's active site. (B) The electrostatic free energies of interaction between two orientations of the enzyme, as a function of the minimum distance between the van der Waals surfaces of the enzyme and a PS membrane for [KCl]=0.1 M, were calculated by the FDPB method. The blue curve represents calculations for the orientation with the IBS directed toward the membrane, and the black curve represents calculations for the orientation with the opposite surface, or "back side", directed toward the membrane. (C) The −1 kT/e equipotential contours for two PS membranes separated by 40 Å was calculated by the FDPB method [40] and visualized in GRASP [89] to be highly repulsive. (D) The hGIIA sPLA2's are placed between the membranes in such a way so that they no longer repel each other and, in this way, induce aggregation.

density, e.g. one hGIIA sPLA2 per 144 $nm^2$ or 212 lipids (per each vesicle surface) is sufficient to aggregate two PS membranes, in agreement with light scattering experiments from the Gelb lab [22]. The physiological significance of electrostatically induced aggregation is not clear. One role may be related to the activity of hGIIA sPLA2 against bacteria which have a highly negatively charged surface layer [56]. Another may be related to atherosclerosis. Intriguingly, hydrolysis of low-density lipoproteins by hGIIA sPLA2 has been implicated in particle aggregation [50]. Such particle fusion is thought to promote atherosclerosis [50] and may be mediated by the mechanism outlined here. Similarly, recent observations show that the Synaptotagmin 1 C2B domain, whose electrostatic profile is also highly positive, is crucial for the ability of Synaptotagmin to simultaneous bind two membranes, thus bringing them into close apposition [6]. Such a complex may facilitate synaptic vesicle fusion with the plasma membrane and has been similarly modeled with FDPB calculations (Li and Murray, unpublished).

## 6. Discreteness of charge

A number of proteins that contain basic clusters are known to exist at high concentrations in localized regions of the plasma membrane [77]. For example, MARCKS is concentrated at nascent phagosomes in macrophages [4] and HIV-1 Gag self-assembles into lateral domains on the cytoplasmic surface of the plasma membrane of infected cells before viral budding [18,101]. Experiments and FDPB calculations with atomic models of peptides and membranes describe the electrostatic properties of membranes that contain high surface concentrations of basic peptides that mimic the basic clusters in these proteins [83]. As depicted in Fig. 6, the electrostatic potential is highly non-uniform at the surface of a membrane containing acidic lipids and basic peptides. While smeared charge theory has been shown to accurately describe membrane association as a function of the mole percent of monovalent acidic lipid, it cannot account for experimental results obtained by varying the peptide concentration. This indicates that the charges in the system cannot all be treated equally, and it is necessary to account for the *discrete* nature of the peptide to describe its membrane association at high concentrations. The "discreteness-of-charge" effect illustrated in Fig. 6 arises from the localized effect that the basic peptides have on the negative potential profile of the membrane. This discreteness accounts for a number of experimental observations, including the ability of basic peptides to reverse the charge on negatively charged phospholipid vesicles [83].

Conversely, localized regions of negative potential resulting from peptide binding could account for the co-localization of proteins whose membrane association also requires electrostatic interaction with acidic lipids. For example, protein kinase Cα (PKC) co-localizes with MARCKS in nascent phagosomes [3]; electrostatic interactions of the $Ca^{2+}$-bound C2 domain of PKC with acidic lipids contribute to its membrane localization [26,98]. Similarly, localized areas of positive potential could act as basins of attraction for multivalent acidic lipids present at trace concentrations in the plasma membrane. Specifically, multivalent phosphoinositides should partition nonspecifically into these regions because the Boltzmann relation predicts that their local concentration depends on some power, $n$, of the local potential, where $n$ is the magnitude of the net charge of the lipid. The lateral accumulation of phosphoinositides due to non-specific electrostatic interactions could act in combination with specific protein–lipid interactions to target protein domains and to organize signaling complexes [9,32,59,70].

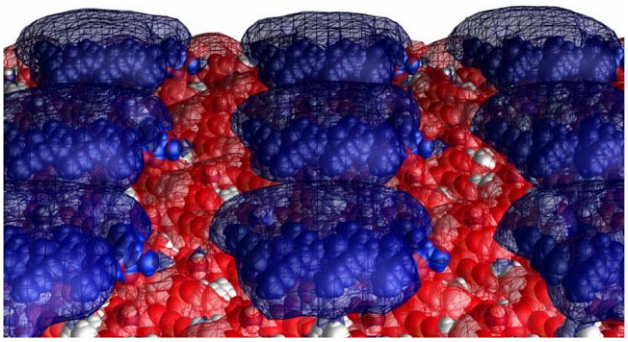

Fig. 6. Discreteness of charge effects. Experiments show and theory predicts that when enough basic peptide is bound to a 2:1 PC/PS vesicle in 0.1 M KCl to effectively neutralize the negative surface charge density of the monovalent lipids, additional peptides may still bind. As represented here, this suggests that there are localized regions of positive and negative potential (blue and red contours) on the membrane surface which may serve as basins of attraction for negatively and positively charged molecules, respectively. The electrostatic potentials were calculated with the FDPB method [40] and visualized in GRASP [89].

## 7. Phosphoinositides

Compared to other phospholipids, phosphoinositides are present in cells at very low levels [70,90,100]: Phosphatidylinositol comprises ~4% of cellular membrane phospholipid, and its phosphorylated derivatives, the phosphoinositides, together comprise ~1%. The phosphoinositides are designated according to the positions that are phosphorylated; for example phosphatidylinositol 4,5-bisphosphate, or $PI(4,5)P_2$, is the phosphoinositide obtained by phosphorylation of the inositol ring of phosphatidylinositol at the 4 and 5 positions. The levels of some phosphoinositides, e.g. $PI(4,5)P_2$ and PI(3)P, are relatively stable while others, e.g. $PI(3,4,5)P_3$ and $PI(3,4)P_2$, are dynamically regulated [28,94,100]. However, all phosphoinositides function as signals in different cellular pathways. For example, in addition to its established role as a source of second messengers [12], it is now clear that $PI(4,5)P_2$ also functions as a signal itself by directly recruiting proteins to the plasma membrane surface [73,79,124], while PI(3)P recruits proteins to endosomal membranes [26,32,59]. A number of reviews in this volume describe protein domains that interact specifically with phosphoinositides. Below, the special roles of these lipids in

altering the electrostatic properties of both proteins and membranes and the implications for the regulation of signaling processes will be discussed.

## 8. Electrostatic mechanisms for regulating protein/membrane association

Electrostatics is not only a driving force for peripheral membrane association but also a major contributor to ligand-specific mediated events, e.g. the interaction of a FYVE domain with a mixed membrane containing PC, PS and PI(3)P. Table 2, while not exhaustive, lists some of the ways in which membrane association is regulated by nonspecific electrostatic effects. The mechanisms in this table will be described throughout the rest of this review as examples of the strikingly diverse functionality of such a simple physical force. By characterizing these events in quantitative detail and separating out specific from nonspecific effects through various experimental and computational approaches, the extent to which electrostatic forces are important is beginning to be determined.

## 9. Protein phosphorylation

The membrane partitioning of a basic peptide corresponding to the N-terminal portion of the non-receptor tyrosine kinase Src, which is crucial for the function of the intact protein, was examined experimentally and computationally with the FDPB method [85]. The calculations accurately predict how the membrane binding is altered as a function of (1) the mole percent acidic lipid in the membrane, (2) the ionic strength of the solution, (3) the number of basic residues in the peptide, and (4) the phosphorylation state of the peptide. While phosphorylation of serines 12 and 17 was shown to significantly decrease the binding of the peptide to phospholipid vesicles by decreasing the net charge of this region, phosphorylation of each serine, one at a time, by Protein Kinase C and Protein Kinase A, respectively, was found not to alter the function of the intact protein in cells [85]. Perhaps phosphorylation of both serines simultaneously is required in order to illicit a physiological effect, but this remains to be established.

In contrast, phosphorylation of three serines within the basic effector region of the myristoylated alanine-rich C kinase substrate, MARCKS, causes the desorption of this protein from the plasma membrane [102,114]. Experiments with the MARCKS protein and peptides based on the effector region have established that the dissociation from phospholipid vesicles is due to a simple electrostatic mechanism, termed an "electrostatic switch", in which the net charge decreases from +13e to +7e [63,64,75]. These observations and the cell studies indicate that electrostatics plays an important regulatory role under physiological conditions. Most recently, a similar electrostatic switch has been proposed for K-Ras due to PKC phosphorylation of serines near its C-terminal cluster of basic residues [13].

Table 2
Control of peripheral association by electrostatic mechanisms

| Regulatory mechanism | Representative protein |
|---|---|
| Phosphorylation of serines by PKC | MARCKS, K-Ras |
| Protein/Protein dissociation | Gγβ from Gα |
| Effector binding | Phosducin to Gγβ |
| Protonation of ionizable residues | AppD49 sPLA2 |
| $Ca^{2+}$ binding | C2 domains |
| Specific phosphoinositide binding | FYVE domains |
| Oligomerization | Retroviral MA/Gag |
| Calcium-bound calmodulin | MARCKS, EGFR juxtamembrane region |

## 10. Partner and effector association/dissociation

The basic patch on Gβγ depicted in Fig. 1A is largely occluded when the heterodimer is bound to the Gα subunit in the transducin heterotrimer during the resting state of a cell [67]. Activation of a G protein-coupled receptor signaling pathway and GTP-induced dissociation of Gα from Gβγ, uncovers this basic feature, which is adjacent to the site of prenylation on the Gγ subunit [109]. Likely, the basic patch serves mainly to orient Gβγ at the membrane surface for productive effector interactions [87] as many of its known effectors have been shown to interact with residues that are located on the lower half of the protein [38], as it is oriented in Fig. 1A. Interestingly, the co-crystal structure of Gβγ and phosducin, a phosphoprotein that regulates Gβγ activity by removing it from the plasma membrane, revealed that rather than covering the basic patch or interacting with the prenyl group, that phosducin introduces a large lobe of negative potential immediately adjacent to the membrane binding surface of Gβγ [42]. It is thought that charge–charge repulsion between phosducin and the negatively charged plasma membrane contributes to the translocation of the Gβγ/phosducin complex from the plasma membrane to cytosol [87].

## 11. Protonation of interfacial glutamates and histidines

The desolvation of a protein upon membrane association (Fig. 2) can change the electrostatic character of the protein just as explicitly as phosphorylation. The effect is dependent on the close apposition of protein and membrane so that the dielectric and/or electrostatic environment of the protein is significantly altered. Desolvation is a surface or interfacial phenomenon that always opposes binding so that there must always be an attractive membrane binding motif on the protein to provide the bulk of the binding energy. The proximity of ionizable residues to a membrane surface can have dramatic effects on their p$K_a$'s. Since desolvation effects involve the removal of groups from the aqueous phase, they will always favor a shift in p$K_a$'s that will tend to destabilize the ionized forms of any titratable group, e.g. the p$K_a$ of glutamate with be shifted up and that of lysine will be shifted down [104]. Such effects are well known in proteins [2,123]. However the interaction with acidic phospholipids will favor p$K_a$ shifts in the upward direction for all groups since such interactions will always favor the protonated species. As an example, consider again hGIIA sPLA2 (Fig. 5A), which has a strong electrostatic component to its membrane association, and a number of hydrophobic residues on its IBS, as well. Two ionizable residues also exist on the IBS, a glutamate and a

histidine. FDPB calculations predict large positive shifts in the p$K_a$'s for both residues when the enzyme is docked onto a membrane containing 33 mol% acidic phospholipid, indicating that both are likely protonated: Specifically, (1) neutralization of the glutamate decreases charge–charge repulsion with the negatively charged membrane as well as the desolvation of the protein; and (2) protonation of the histidine increases charge–charge attraction with the membrane surface, presumably to a greater degree than the desolvation repulsion is increased.

Calculations of p$K_a$ shifts due to the interactions with membranes of ionizable groups on sPLA2's were first done for bee venom sPLA2 in order to address the paradoxical observation that a form of the enzyme in which five basic residues on or near the IBS were substituted to glutamates (E5) binds almost equally well to anionic membranes as does the native enzyme [45]. Of course one would expect that the native enzyme would bind much more tightly. In subsequent work, theory and experiment were in agreement that, for membrane-associated bv-sPLA2, the two glutamates nearest the membrane interface are likely protonated in the E5 form [15]; the other three residues were shown by EPR to be more than a Debye length away from the membrane surface [71]. Thus, the *balance* of "Coulombic", "Desolvation" and "Hydrophobic", described in the context of Fig. 2, is roughly equivalent for these two forms of the enzyme. The individual components, however, are different between the two forms. For native bee venom sPLA2, the major driving force for membrane association is "Hydrophobic"; while the native basic residues contribute a "Coulombic" component, they also produce a large desolvation penalty. At the surface of a PS membrane, interfacial glutamates are easily protonated. Hence, for the E5 form, the lack of a "Coulombic" component is compensated for by a significantly depressed "Desolvation" repulsion. This example illustrates again that utility of partitioning a problem in terms of the three main nonspecific contributions to peripheral association.

Membrane composition significantly affects the protonation of ionizable residues, i.e. it takes less energy to protonate a glutamate at the surface of a PS membrane than at a PC membrane (when everything else is equal), and may, thus, play a role in membrane targeting. This was proposed by Cornell and co-workers who observed that glutamates on an amphipathic α-helix of Cytidylyltransferase regulate membrane association by becoming protonated and inducing membrane association only in the presence of acidic lipids and that this helix exhibits selectively for membranes that contain more acidic lipid [60]. A physiological role for this phenomenon remains to be established, but it suggests an intriguing mechanism for fine-tuning electrostatic control and how a protein may be directed to a specific membrane, i.e. a motif like this amphipathic helix would be expected to target intracellular membranes that are more negatively charged, such as the plasma membrane, where protonation of the interfacial glutamates is the least energetically costly.

## 12. Ligand-induced electrostatic switches

The term "switch" was originally coined in regard to C2 domains to describe how calcium binding increases attractive electrostatic interactions with acidic phospholipids [98]. The meaning of this "calcium/electrostatic switch" was expanded to include C2 domains from cytosolic phospholipase A2 [86] and 5-lipoxygenase [65], which have a large negative potential surrounding a cluster of hydrophobic residues. As suggested in Fig. 7A and B for the C2 domain from 8R-lipoxygenase, calcium ions neutralize the negative potential (red) produced by coordinating aspartates; the subsequent decrease in desolvation penalty associated with the interfacial penetration of the hydrophobic clusters (green residues) is predicted to allow for membrane insertion. Experimental/computational collaborative work on C2 domains demonstrates the ability to match the results of electrostatic calculations to both biochemical and cellular assays: To a first approximation, the electrostatic properties of C2 domains appears to correlate with the negative charge density of intracellular membranes, as expected from Fig. 4 (see Ref. [5,36,65] for more detail).

Recently, the term "electrostatic switch" has been applied to the phosphoinositide-mediated desolvation effects that allow for the nonpolar partitioning of hydrophobic groups on the membrane-binding loops of phosphoinositide-binding domains [26,112]. In contrast to calcium, these ligands, i.e. phosphoinositide head groups, are negatively charged. As depicted in

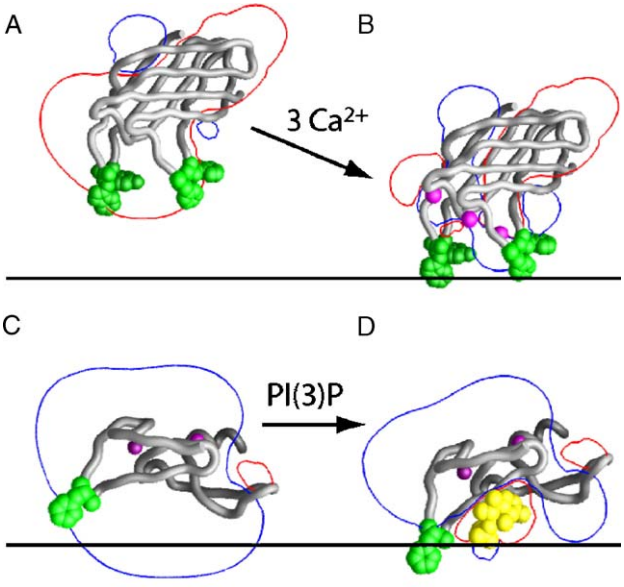


Fig. 7. Membrane insertion induced upon ligand binding through the neutralization of negative or positive electrostatic potential shrouding hydrophobic motifs (green residues). (A and B) Calcium induced. (A) The C2 domain from 8R-lipoxygenase (PDB identifier, 2FNQ) has a negative contour (red, −1 kT/e) surrounding four tryptophan and phenylalanine residues (green) on the calcium binding loops that disappears when conserved aspartates coordinate three calcium ions (magenta spheres), as depicted in B, i.e. the desolvation penalty associated with interfacial penetration is significantly reduced upon binding calcium. (C and D) Phosphoinositide induced. (C) The FYVE domain from Endofin (a homology model constructed based on high similarity to the Hrs FYVE domain; PDB identifier, 1DVP) has a positive contour (blue, +1 kT/e) surrounding a phenylalanine (green) on the turet loop that disappears when a conserved basic motif binds the phosphoinositide 3-phosphate head group ($IP_2$, yellow space filling), as depicted D, i.e. the desolvation penalty associated with interfacial penetration is significantly reduced upon binding the phosphoinositide headgroup. The figure was produced in GRASP [89] with [KCl]=0.1 M.

Fig. 7C and D for the Endofin FYVE domain, the PI3P head group (yellow) is predicted to facilitate the insertion of the hydrophobic group (green) into the membrane interface by neutralizing the *positive* potential (blue) produced by the basic motifs that specifically binds the PI3P [14]. Phosphoinositides are signals in their own right, but they are membrane-embedded rather than soluble. Hydrophobic groups on most protein domains that bind these lipids do not penetrate the membrane in the absence of the specific protein/phosphoinositide interaction. This is consistent with observations that phosphoinositide binding is a prerequisite for hydrophobic partitioning, and that mutating these hydrophobic residues to alanines can dramatically reduce membrane affinity without affecting phosphoinositide specificity.

## 13. FYVE domains: a combination of nonspecific and specific components of electrostatic control

FYVE domains contain a basic motif responsible for ligand binding and a small hydrophobic motif adjacent to the ligand binding site [35,80,81]. Most FYVE domains bind only PI(3)P [19,43,93], which is predominantly found in endosomal compartments. Hence, many FYVE domain-containing proteins function in the endocytic pathway. FYVE domains provide a nice example of how the collaboration of experiment and theory has been able to delineate the contribution of nonspecific electrostatic interactions to membrane binding function at several levels: membrane recruitment, membrane-associated orientations, interfacial penetration, and nonspecific affinity determinants.

The structure of the Hrs FYVE domain was determined crystallographically in the context of a dimer [72]. The previously determined structure for the Vps27p FYVE [80] domain suggested that the long axis of the domain is oriented perpendicular to the membrane surface so that the headgroup of PI(3)P binds to the basic motif while the hydrophobic motif penetrates into the membrane interface. In contrast, the Hrs FYVE structure suggested that the FYVE domains are compulsory dimers, and that each monomer is oriented parallel to the membrane surface so that the hydrophobic motif (F173) forms part of the dimer interface, rather than penetrating into the membrane interface. FDPB calculations [34] predicted, for 2:1 PC/PS and 0.1 M KCl, that $\Delta G_{el} \sim -3.5$ kcal/mole for both the Vps27p FYVE *monomer* and the Hrs FYVE *dimer*, i.e. the Vps27p monomer was equally effective in the electrostatic component to membrane binding as the Hrs FYVE dimer. Calculations of $\Delta G_{el}$ were carried out for many different orientations of the Hrs monomer and a minimum electrostatic free energy orientation (with $\Delta G_{el} \sim -5.0$ kcal/mol) was found that was significantly different from and more favorable than that suggested by the crystal structure. Subsequently, the structure of the highly similar EEA1 FYVE domain [35] was solved with the headgroup of PI(3)P bound to the basic domain. Its predicted membrane-associated orientation is identical to that predicated computationally for Hrs-FYVE. FDPB calculations predict that the orientation of Vps27p-FYVE is less oblique because of its more polarized electrostatic potential, in agreement with the model based on the original structural studies.

The predicted membrane-associated orientation for FYVE domains is consistent both with PI(3)P binding and with the penetration of the hydrophobic motif into the membrane interface [81,112]. This suggests that both non-specific electrostatic and non-polar interactions are optimized in the phosphoinositide-bound state of the domains. As discussed above for Fig. 7, FDPB calculations predicted that the positive potential due to the basic motif, which is responsible for PI(3)P binding, introduces a large desolvation barrier which opposes the membrane insertion of the conserved hydrophobic motif. This barrier is reduced upon PI(3)P binding. Cho and co-workers observed that the substitution F173A in the Hrs FYVE domain decreased membrane association by 20-fold [112]. FDPB calculations predict that upon binding PI(3)P, the insertion of F173 into the membrane interface is more favorable by 2.0 kcal/mol (or the membrane partitioning of F173 is more favorable by 30-fold) [34]. This and other favorable comparisons provide strong support for a "phosphoinositide/electrostatic switch". The experimental and theoretical work on FYVE domains represents the first example of a lipid second messenger inducing membrane penetration of a protein. A similar mechanism appears to be of equal physiological importance for PX and ENTH domains [110,111,113].

Different FYVE domains use different combinations of electrostatic and nonpolar interactions to adsorb to PI(3)P-containing membranes (see Fig. 2). Based on the biophysical analysis of structures and models, experiments with a range of residue-substituted FYVE domains were tested for membrane binding function [14]. For example, the effects of substituting residues outside the PI(3)P binding pocket to alanine on binding to membranes containing 15 mol% PS and 2 mol% PI3P were examined. Alanine substitution of the hydrophobic residue (Fig. 7C, (Phe 762, green) decreased the membrane affinity of the Endofin-FYVE 10-fold as did changing a nearby lysine residue, confirming the computational prediction).

## 14. Lateral co-localization at membrane surfaces

$PI(4,5)P_2$ is the source of two second messengers in the cell, diacylglycerol (DAG) and inositol 1,4,5-trisphosphate ($IP_3$) [12], and is crucial for a wide variety of cellular functions [73,79]. $PI(4,5)P_2$ has been shown to be distributed non-uniformly in the plasma membranes so that the existence of distinct pools have been suggested to help organize its functionality. Nonspecific electrostatic sequestration mediated by membrane-associated MARCKS is one mechanism by which not only phosphoinositides, but also phosphoinositide-bound protein domains may be laterally accumulated at the plasma membrane surface [77].

MARCKS and peptides based on MARCKS(151–175) inhibit phospholipase C-catalyzed hydrolysis of $PI(4,5)P_2$ [48]. The basic effector of MARCKS region sequesters $PI(4,5)P_2$ away from the catalytic domain of PLC, thus shielding the lipid from hydrolysis, consistent with the idea of a "pool" of $PI(4,5)P_2$ "organizing" its functionality.

Interestingly, $Ca^{2+}$/CaM or PKC release this inhibition by causing the desorption of the MARCKS protein or the effector region from membranes; PI(4,5)$P_2$ is then accessible for hydrolysis by PLC [117]. Since MARCKS exists in some cells (e.g.,brain cells and activated macrophages) at concentrations comparable to that of PI(4,5)$P_2$, it has been suggested that MARCKS may act as a buffer for much of the PI(4,5)$P_2$ in these cells [68,77]. Recent experiments and complementary FDPB calculations reveal that membrane-associated MARCKS(151–175) binds three to four PI(4,5,)$P_2$ with high affinity through *non-specific* electrostatic interactions [41,95,117–119]. This suggests a mode of PI(4,5)$P_2$ binding that does not depend on structural detail and need not be 1:1. Rather, in this case, there may be a "many to one" interaction between several PI(4,5)$P_2$ lipids and one of many possible basic sequences. Hence, these observations support the existence of an interaction between peripheral proteins and phosphoinositides that is fundamentally different from that observed for phosphoinositide-specific binding domains.

## 15. Lateral sequestration of protein domains due to nonspecific electrostatics

Similar to Fig. 6, the electrostatic profile of a membrane surface containing PI(4,5)$P_2$, monovalent acidic lipids and adsorbed basic sequences is expected to be quite complex and may produce driving forces for many types of lateral interactions. As an example, the panels in Fig. 8 illustrate how the electrostatic profiles of PLCδ PH domains change upon binding the headgroup of PI(4,5)$P_2$ [107]. The δ1 PH domain becomes overall negatively charged, while the δ3 PH domain remains highly positively charged. The electrostatic profiles suggest that the PI(4,5)$P_2$-bound, membrane-associated form of the δ1-PH is targeted laterally to different regions of the plasma membrane than the δ3 isoform. As described above, PI(4,5)$P_2$ is laterally sequestered by membrane-adsorbed basic peptides by nonspecific electrostatic attraction [118]. In addition, PI(4,5)$P_2$ co-localizes in membrane ruffles and nascent phagosomes with the MARCKS protein [16,53,68,115]. Because of its negative character (Fig. 8B), the PI(4,5)$P_2$-bound δ1 PH domain should be laterally directed, through favorable electrostatic interactions, to regions enriched in MARCKS, whose membrane-adsorbed effector domain constitutes a positively charged basin of attraction for negatively charged molecules (this can be imagined by examining Fig. 7). Indeed, experiments show that the interaction of a peptide based on MARCKS(151–175) with PI(4,5)$P_2$ does not displace PI(4,5)$P_2$-bound δ1-PH from the membrane [41,118]. A possible interpretation is that both PI(4,5)$P_2$ and the PI(4,5)$P_2$-bound PH domain are laterally sequestered by membrane-adsorbed MARCKS(151–175) because both species are negatively charged [107]. Similarly, the PI(3,4,5)$P_3$-bound γ1 PH domain, which also acquires a strong negative character (not shown), should be localized to these regions as well. Although highly speculative, this electrostatic sequestration provides a mechanism whereby PLCδ1 and PLCγ1 (when PI(3,4,5)$P_3$ is present) are localized to regions of membrane that are enriched in their substrate, PI(4,5)$P_2$. Conversely, PLCδ3-PH should be excluded from these regions due to its overall basic character (Fig. 8D) [107]. The visualization of green fluorescent protein constructs of the δ1, γ1 and δ3 PH domains expressed in cells would provide a test of these predictions [57,62,100].

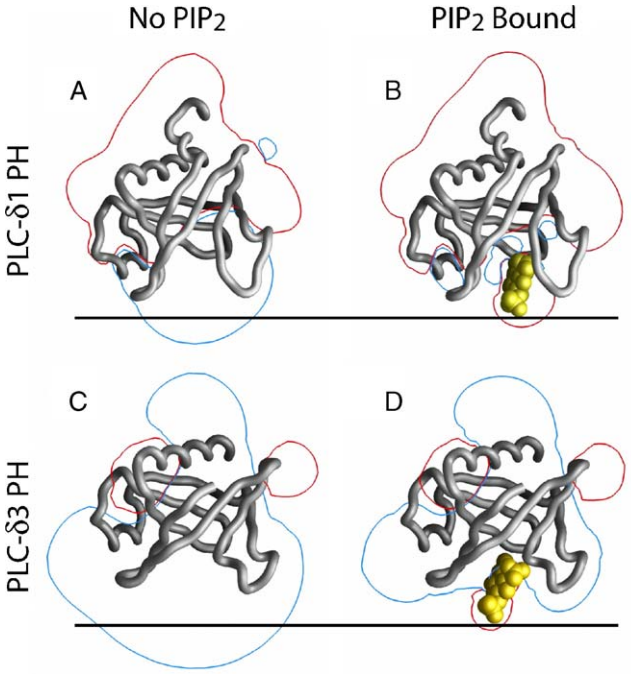


Fig. 8. Lateral targeting may be determined by the different electrostatic properties of molecules at the membrane surface. Isoforms 1 and 3 of the Phospholipase Cδ (PLCδ) Pleckstrin homology (PH) domains both bind PI(4,5)$P_2$ specifically. PLCδ1 PH is represented by a crystal structure (PDB identifier, 1MAI) while PLCδ3 PH, which has high similarity to PLCδ1 PH, is represented by a homology model. The two-dimensional contours (red, −1 kT/e and blue, +1 kT/e) were calculated for 0.1 M KCl and visualized in GRASP [89]. (A) PLCδ1 PH is highly polar. (B) Upon binding the head group of PI(4,5)$P_2$ (yellow space filling), the PH domain assumes an overall negative character. (C) In contrast, PLCδ3 PH is highly basic. (D) Upon binding the head group of PI(4,5)$P_2$ (yellow space filling), the PH domain remains basic in character.

## 16. Retroviral assembly: oligomerization

Retroviruses are enveloped viruses that cause a wide range of diseases in humans and animals [49,116]. Newly assembled viruses acquire their lipid coats by budding through the plasma membrane of host cells. Gag directs the assembly of new virions and is targeted to the inner leaflet of the plasma membrane by its matrix domain (MA) [126]. It is unclear how Gag is targeted to the host cell plasma membrane during viral assembly, but many studies implicate specific motifs in MA (see e.g., [29,92,126]). The MAs of virtually all retroviruses have a positively charged surface patch, which is proposed to interact electrostatically with acidic phospholipid headgroups [27,88]. The membrane association of MA and Gag may be regulated both by the oligomerization of Gag, which may increase membrane partitioning by producing a larger, composite basic surface [1] and by phosphoinositides, which may stabilize particle structure, promote assembly [21] and determine the site of viral assembly in cells [91].

FDPB calculations predict that nonspecific electrostatic interactions may contribute up to −5 kcal/mol to the membrane binding energy of Human Immunodeficiency Virus Type 1 (HIV-1) MA (or Gag) [88]. Notably, FDPB calculations explain the relative membrane binding of Rous Sarcoma Virus MA, which was examined systematically in biochemical assays [29]; similar results have been obtained for HIV-1 MA (Vogt and Murray, private communication). The calculations further predict that the minimum electrostatic free energy for HIV-1 MA with a membrane containing 1 mol% $PI(4,5)P_2$ (i.e., 67:32:1 PC/PS/$PIP_2$) is −7.5 kcal/mol (as compared to the value of −5 kcal/mole in the absence of $PI(4,5)P_2$). This increase in membrane affinity is due solely to nonspecific electrostatic interactions. These calculations pertain to the interaction of a single MA with the membrane surface. However, during assembly, ~1500 to 3000 Gag molecules co-localize at the plasma membrane [18].

Fig. 9A depicts the membrane-associated form of the HIV-1 MA trimer, which is one of a number of possible subunits that may form an extended MA/Gag array at the plasma membrane. (Electron microscopy studies suggest the existence of both trimers and dimers of Gag in the assembling particle; models based on FDPB calculations are consistent with these observations [1,120,121].) $\Delta G_{el}$ for the trimer form of MA is predicted to be more favorable than the monomer form (−9 kcal/mol versus −5 kcal/mol; not shown is the dimer form, with $\Delta G_{el}$ ~−8 kcal/mol). Hence, Gag oligomerization favors plasma membrane association as it increases the electrostatic component of binding. Fig. 9B and C depict a model of a network of trimers. In contrast to Fig. 9A where the membrane is horizontal and orthogonal to the page, the view in panels B and C is from above, looking down on the membrane surface. Panel B provides some characteristic dimensions of the model system, and panel C shows the equipotential contours 2 Å above the membrane surface as calculated with the FDPB model for the network associated with a 2:1 PC/PS membrane. As in Fig. 7, there are pockets of both positive and negative potential. We posit that the positive potential regions may provide nonspecific binding sites for $PI(4,5)P_2$. The calculations support two mutually consistent models: $PI(4,5)P_2$ may direct Gag to the plasma membrane by enhancing membrane association and/or membrane-bound MA may laterally sequester $PI(4,5)P_2$ in the plasma membrane, either to stabilize membrane association or to facilitate the targeting of cellular factors that are required for budding and fission [56]. Either way, the discrete

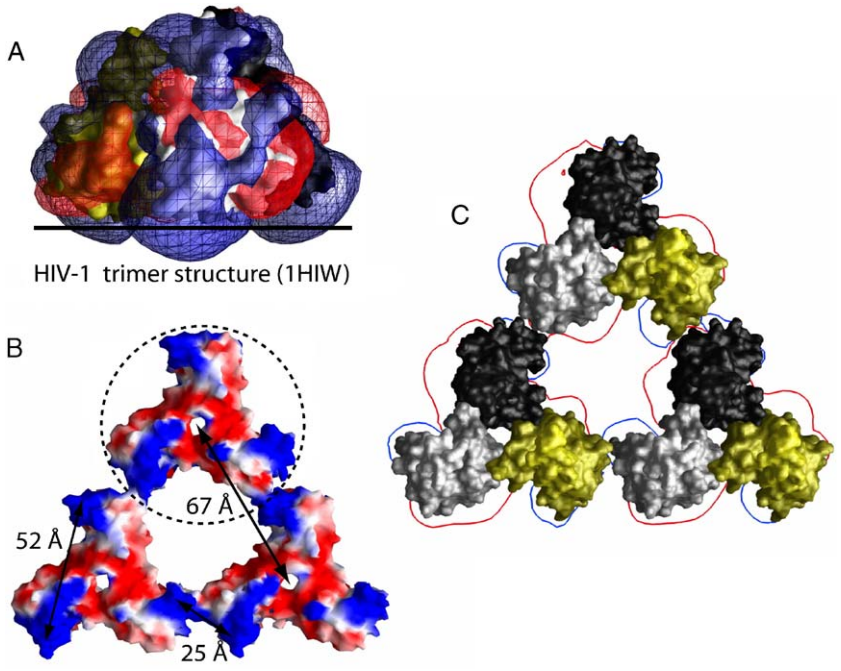


Fig. 9. Electrostatic interactions play multiple rolls in HIV-1 assembly. (A) The HIV-1 N-terminal domain of the Gag polyprotein, the matrix domain or MA, was crystallized as a trimer (PDB identifier, 1HIW), and the trimer has been shown to be a relevant multimer in Gag assembly [121]. Each MA monomer is depicted by its molecular and colored differently: white, yellow and black. The electrostatic component to the association of the trimer structure with a 2:1 PC/PS membrane in 0.1 M KCl is predicted by FDPB calculations to be strong ($\Delta G_{el}$=−9 kcal/mol) [88]. The line depicts the location of the membrane envelope. (B) The membrane binding surface of an extended network of MA trimers. One trimer is circled for clarity and for correspondence with the structure shown in panel A; this view is rotated 90° about the horizontal axis towards the viewer. The electrostatic potential of each trimer is mapped to the molecular surface and graded continuously from −4 kT/e (red) to 0 kT/e (white) to +4 kT/e (blue). Some characteristic distances among MA's are depicted; for reference, the Debye length is ~10 Å in 0.1 M KCl. (C) The network shown in B is now facing down into the plane of the page so that the membrane is below. The color scheme is the same as in A. The two dimensional equipotential contours (−1 kT/e, red; and +1 kT/e, blue) are drawn 2 Å above the membrane surface to show the discreteness of charge effect that was manifest in Fig. 7. Here, the membrane was left out for clarity. The pockets of positive potential (blue lines) may be responsible for the interaction of MA with $PI(4,5)P_2$ during assembly (see text). The calculation was performed with the FDPB method [40] with a 2:1 PC/PS membrane and 0.1 M KCl. All panels were visualized in GRASP [89].

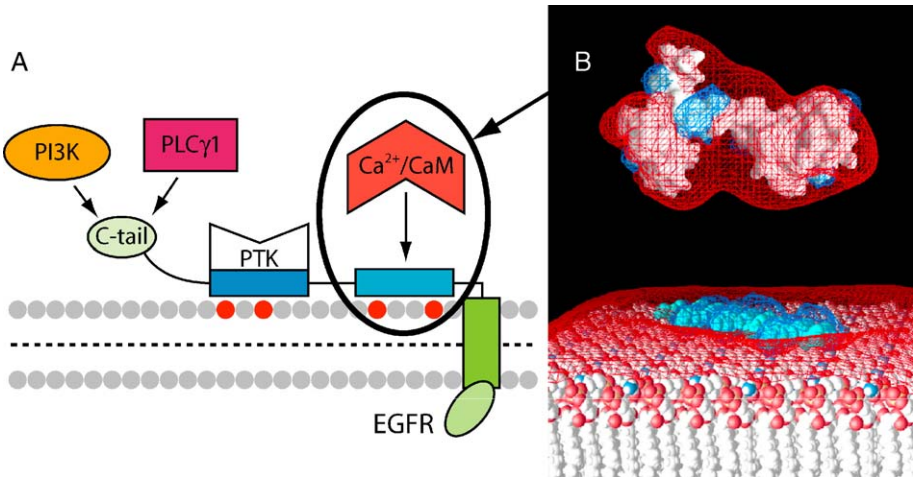


Fig. 10. Lateral organization of signaling molecules at membrane surfaces. (A) Cartoon representation of the sequestration of $PI(4,5)P_2$ (red circles) by the juxtamembrane region of the epidermal growth factor receptor (EGFR; cyan bar). (Other phospholipids are collectively colored gray.) Activation of the receptor leads to the phosphorylation of tyrosine residues on its C-terminal tail and subsequent recruitment of the enzymes Phospholipase Cγ1 (PLCγ1) and Phosphoinositide 3-Kinase (PI3K) to the membrane surface. Both PLCγ1 and PI3K use $PI(4,5)P_2$ as a substrate. The increase in calcium upon EGFR activation leads to the desorption of the juxtamembrane region from the membrane surface through its interaction with calcium-loaded Calmodulin (red), which releases $PI(4,5)P_2$ in the vicinity of the enzymes which act upon it. (B) A molecular model showing the electrostatic properties of the interaction of $Ca^{2+}$/CaM with the EGFR juxtamembrane peptide (circled in panel A). The calculation was performed with the FDPB method [40] with a 2:1 PC/PS membrane and 0.1 M KCl and visualized in GRASP [89].

localized nature of the charge distributions of the membrane-adsorbed proteins and multivalent phosphoinositides need to be considered explicitly in any model of this process.

## 17. Multi-component signaling complexes at the plasma membrane surface

Fig. 10A schematically depicts an interesting system in which a number of phenomena based on nonspecific electrostatic interactions stressed throughout this review are manifested. Binding of EGF to the exterior of the receptor (light and dark green, respectively) produces dimerization, which leads to trans-autophosphorylation (not shown). Ligand binding produces a rapid increase in the free intracellular $Ca^{2+}$ level [69]. Model peptide studies in the McLaughlin and Smith labs indicate that the transmembrane helix breaks at the interface and that the basic/hydrophobic juxtamembrane region (cyan cylinder) is bound to the cytosolic leaflet of the plasma membrane. Moreover, these studies show that peptides corresponding to the juxtamembrane basic/hydrophobic cluster laterally sequester $PIP_2$ (red circles in the bilayer) and bind strongly to $Ca^{2+}$/CaM (red figure in the cytosol, upon increased $[Ca^{2+}]$), which releases the $PIP_2$ [78].

The C-terminal tail region of the auto-phosphorylated EGFR (green) binds two enzymes that use $PI(4,5)P_2$ as a substrate, PLCγ1 (magenta) and PI3 kinase (gold). Thus, EGFR may function as a scaffolding protein that binds $PI(4,5)P_2$ and, upon activation, releases it to enzymes that use $PI(4,5)P_2$ as a substrate [77,78]. Nonspecific electrostatic interactions come into play as follows: (1) the adsorption of the juxtamembrane region to the plasma membrane; (2) the lateral sequestration of $PI(4,5)P_2$ by the juxtamembrane region; (3) components of the interaction of both PLCγ1 and PI3K with the plasma membrane surface; and (4) the steering of $Ca^{2+}$/CaM to the membrane-adsorbed juxtamembrane region. The electrostatic properties of this latter interaction are illustrated in more detail in Fig. 10B. The highly negatively charged $Ca^{2+}$/CaM will be electrostatically repelled from most regions of the plasma membrane, but will be attracted to the punctuate, positively charged, basins of attraction set up by membrane-adsorbed calmodulin ligands such as the juxtamembrane region of the EGFR and the basic effector region of MARCKS [76].

## 18. Conclusions

There is a significant body of experimental work that points to a central role of electrostatics in mediating the interactions of proteins with membrane surfaces. Calculations on atomistic models of proteins and membrane using implicit solvent models provide a unifying description of the structural and energetic origins of these interactions. The results of these calculations yield a wide range of quantities in agreement with experiment. More importantly, they form the basis of a general atomic-level description of complex protein/membrane systems from which a great deal of mechanistic insight may be derived. The fruitful exchange between theory and experiment has been crucial in bootstrapping these models to the current level of understanding. Future work lies beyond examining equilibrium phenomena with static models and, rather, involves working towards matching kinetic measurements from techniques such as surface plasmon resonance experiments with computational methods such as Brownian dynamics. In addition, it will be important to model the detailed structural and dynamical information on lipids and interfacial association obtained from NMR and EPR measurements with molecular dynamics simulations. Much remains to be done, but it is very likely that the combined application of modern experimental and

computational techniques will continue to significantly advance our understanding of the biophysical basis of the interactions of peptides and proteins with membrane surfaces.

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