PIP$_2$ AND PROTEINS: Interactions, Organization, and Information Flow

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Abstract  We review the physical properties of phosphatidylinositol 4,5-bisphosphate (PIP$_2$) that determine both its specific interactions with protein domains of known structure and its nonspecific electrostatic sequestration by unstructured domains. Several investigators have postulated the existence of distinct pools of PIP$_2$ within the cell to account for the myriad functions of this lipid. Recent experimental work indicates certain regions of the plasma membrane—membrane ruffles and nascent phagosomes—do indeed concentrate PIP$_2$. We consider two mechanisms that could account for this phenomenon: local synthesis and electrostatic sequestration. We conclude by considering the hypothesis that proteins such as MARCKS bind a significant fraction of the PIP$_2$ in a cell, helping to sequester it in lateral membrane domains, then release this lipid in response to local signals such as an increased concentration of Ca$^{2+}$/calmodulin or activation of protein kinase C.

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INTRODUCTION

Phosphatidylinositol 4,5-bisphosphate, abbreviated as PI(4,5)P₂ or PIP₂, is the major polyphosphoinositide in mammalian cells. Other recent reviews (29, 30, 32, 48a, 53, 55, 56, 70, 83, 97a, 100, 106) and a monograph (28) discuss the functions of phosphoinositides in cells, as well as their production by different kinases and their breakdown by phosphatases and phosphoinositide-specific phospholipase Cs (PLCs). This review focuses on the biophysics of PIP₂ and how it interacts with proteins in the plasma membrane.

Early studies established the role of PIP₂ as the source of two second messengers in the cell (16), diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP₃), a topic that we will not consider in detail. Subsequent studies have shown that PIP₂ has a host of functions in cells related to its ability to interact with different proteins. Indeed, Cantley (24) suggests its role in IP₃-induced Ca²⁺ release in higher eukaryotes is a relatively late adaptation to its original role as a lipid anchor. It is now well documented that PIP₂ is important in the attachment of the cytoskeleton to the plasma membrane, exocytosis, endocytosis, membrane trafficking, and the activation of enzymes. This has led several reviewers to ask how one lipid can play so many different roles and to suggest that there are different pools of PIP₂ in the plasma membrane (e.g., 49, 70, 97a). New tools for visualizing phosphoinositides in living cells have revealed that PIP₂ and other phosphoinositides are in fact distributed nonuniformly in the plasma membrane.

In a perceptive recent review on targeting by membrane lipids, Hurley & Meyer (53) note that “a picture has emerged of a restless subclass of signaling proteins that spend a significant fraction of their time diffusing through the cytosol in search of binding partners.” Although many of these signaling proteins indeed move only by passive diffusion, the remarkable feature of signal transduction pathways, including those that involve PIP₂, is that the flow of information from the surface to the interior of the cell resembles an elegantly choreographed ballet rather than a collection of uncoordinated random walks. But what choreographs this diffusional “dance to the music of time” (87)? We argue that cells utilize several biophysical
mechanisms to facilitate this flow of information. We refer to these concepts as "cheap tricks," a term coined by the distinguished physiologist Knut Schmidt-Nielsen (94) to describe how countercurrent exchangers function passively (i.e., without a direct input of energy) in a number of different organ systems. This physiological cheap trick minimizes, for example, the loss of heat from the leg of a wading bird or flipper of a seal, helps the mammalian kidney to concentrate urine, and thus explains why a desert rat need never take a drink of water. We discuss cheap tricks that act at the molecular or biophysical rather than at the organ systems level.

The three tricks we describe in this review are well known to membrane biophysicists, but cell biologists or biochemists studying signal transduction may be less familiar with biophysical principles. In this spirit, we refer a reader unfamiliar with the fundamentals of diffusion theory to Berg’s pithy, lucid, and still timely introduction to this topic in Random Walks in Biology (14). Berg’s discussion of the diffusional mechanism by which a ligand revisits a surface explains, for example, why only a small fraction of a cell need be covered by receptors to effectively capture a ligand; subsequent theoretical and experimental studies extend Berg’s discussion [e.g., (62, 63) and references therein].

Cheap trick #1: Reduction of dimensionality or local concentration effect. Consider, for example, a phosphoinositide-specific phospholipase C-δ (PLCδ) enzyme (91) diffusing randomly through the cytoplasm of a cell: When its pleckstrin homology (PH) domain encounters a PIP2 in the plasma membrane, it binds to PIP2 with sufficiently high affinity and specificity to anchor the protein to the membrane (65). This confines the catalytic site of PLCδ to the surface phase, which Guggenheim defines as a thin (few nm) region of the aqueous phase adjacent to the membrane (10). In the surface phase, the catalytic site experiences a \( \approx 1000 \)-fold higher concentration of PIP2 than if it was diffusing throughout the cytoplasm of a cell, which greatly facilitates PLCδ’s ability to hydrolyze PIP2 when it is activated by an increase in intracellular Ca\(^{2+} \). This reduction of dimensionality—or more appropriately, local concentration effect—is well understood at a biophysical level (1, 15, 61, 71, 74).

Membrane anchoring and the local concentration effect are important for all major proteins in the calcium/phosphoinositide second messenger signal transduction pathway. For example, acyl and prenyl chains anchor G proteins to the plasma membrane; PH and other domains anchor PLCs; C1 and C2 domains reversibly anchor protein kinase C (PKC); and a myristate and a cluster of basic residues reversibly anchor MARCKS (myristoylated alanine-rich C kinase substrate). Of course the domains that anchor the protein to the membrane may serve other functions: The PH domain of PLCδ contributes to its preferential localization at the plasma versus internal membranes; the C1 and C2 domains on PKC are involved in the activation of the enzyme as well as membrane targeting (54). As discussed below, it is now well established that PIP2 often serves to activate as well as merely anchor proteins to the plasma membranes. In other words, it can act as a second messenger as well as a passive membrane anchor.
PHYSICAL/CHEMICAL PROPERTIES OF PIP2 AND ITS CONCENTRATION IN CELLS

Orientation of the Head Group

Figure 1 illustrates the chemical structures and space-filling models of phosphatidylcholine (PC), cholesterol, and PIP2. In PC and other common phospholipids, the glycerol backbone is approximately perpendicular to, and the head group parallel to, the plane of the membrane as illustrated here (43). The orientation of the head group of PIP2 with respect to the membrane surface is unknown. We show it as perpendicular to the plane of the membrane, in agreement with studies of the structure of phosphatidylinositol (21, 113). Note that the potentially erect structure and large size of the PIP2 head group implies that it may protrude further into the aqueous phase than a typical phospholipid.

Charge of PIP2

Cholesterol is a neutral molecule and PC is a zwitterion that also has zero net charge, as indicated in Figure 1. The net charge on PIP2, however, depends on a number of factors such as the local pH and its interaction with proteins. NMR experiments show that the pK values for the first protonation of the 4 and 5 position phosphates of PIP2 (in a PC/PIP2 99:1 vesicle) are about 6.7 and 7.7 [(107) confirmed by A. McLaughlin & S. McLaughlin]. Thus at pH 7.0, ~1 proton is bound and the net charge on PIP2 might be expected to be −4 (Figure 1). However, the electrophoretic mobility of a PC/PIP2 vesicle formed in 100 mM KCl, pH 7.0 suggests the net charge on PIP2 is only −3 (101, 110); presumably both a K+ and a H+ ion are bound to the lipid. It seems likely the K+ ion will be displaced when PIP2 binds.
to a protein, although this must be determined in each case. The proton bound to PIP_2 may also be displaced upon binding to a protein, so the charge on PIP_2 could be $-3$, $-4$, or $-5$.

### Partial Charges on Lipids

The distribution of the partial charges on PC and other common phospholipids has been determined by ab initio quantum mechanical calculations, but similar calculations have not been performed for PIP_2. The dearth of information about both the orientation of the head group and the distribution of partial charges within the PIP_2 molecule severely limits the types of theoretical calculations that can be performed with this important lipid.

### Concentration of PIP_2 in a Typical Mammalian Cell

We assume the cell is a sphere of radius 10 $\mu$m and that PIP_2 is confined to the plasma membrane. If all the phospholipids in the plasma membrane of this hypothetical cell (e.g., phosphatidylcholine, phosphatidylethanolamine, PIP_2) were dissolved in the cytoplasm, they would be present at an effective concentration of $\approx 1$ mM. If we assume PIP_2 comprises 1% of the phospholipid in the plasma membrane of our hypothetical cell, as it does in human erythrocytes (37, 46), the effective concentration of PIP_2 in the cell is $\approx 10$ $\mu$M. The recent studies using constructs of PH domains linked to green fluorescent protein (GFP) provide more direct information about the concentration and location of PIP_2 in a mammalian cell. Fluorescent (52, 98, 99, 105, 108) constructs of the PH domain from PLC\(\delta\) are localized on the plasma membrane, whereas the PH domain from pleckstrin is found in the cytoplasm (65). In vitro the PH domain from PLC\(\delta\) binds PIP_2 with a $K_d$ of 2 $\mu$M, whereas the PH domain from pleckstrin binds PIP_2 with a $K_d$ of 30 $\mu$M (65). If we assume the $K_d$ values are the same in the test tube and the cell, then $2 \mu$M $< [\text{PIP}_2]_a < 30 \mu$M, where $[\text{PIP}_2]_a$ is the effective concentration of PIP_2 that is accessible to the PH domains. This range is consistent with our calculation of a total effective concentration of $\approx 10$ $\mu$M but, as discussed below, does not imply that all the PIP_2 in the plasma membrane is free.

The relative concentration of the other phosphoinositides in cells is considered elsewhere; PI(4,5)P_2 comprises $> 99\%$ of the doubly phosphorylated phosphoinositides in a mammalian cell according to (106). We next consider how the PIP_2 located on the cytoplasmic leaflet of the plasma membrane anchors and, in some cases, activates different proteins.

### DOMAINS/PROTEINS THAT BIND PIP_2

#### Domains of Known Structure

Figure 2a shows ribbon diagrams of three structurally diverse domains that bind PIP_2: the PLC\(\delta\)-PH domain (36), the CALM N-terminal ENTH domain (38), and the radixin FERM domain (47). Either IP_3 or the head group
of PIP$_2$ is shown in each binding pocket with the 1-phosphate facing down; the domains are thus presumably in the orientation they have when they bind to PIP$_2$ in a bilayer (e.g., Figure 1). Figure 2b illustrates how two Lys residues of PLC$\delta$-PH form hydrogen bonds with both the 4- and 5-phosphates.

PH DOMAINS As described in many recent reviews [(65) and references therein], PH domains comprise about 120 amino acids; their function is generally to bind to phosphoinositides. The PH domain of PLC$\delta$ (PLC$\delta$-PH), for example, binds to PIP$_2$ with a $K_d = 2 \mu M$, as measured with PC/PIP$_2$ vesicles using calorimetry (66), a BIAcore sensor chip (50), or a PLC$\delta$-PH-GFP construct and a centrifugation assay (A. Gambhir & S. McLaughlin, unpublished data). Intact PLC$\delta$ binds with a similar $K_d$ (26, 91), an affinity sufficiently strong to anchor the protein to the plasma membrane. The mechanism by which PLC$\delta$-PH and several other PH domains bind phosphoinositides is revealed by crystal structures of the domains with bound IP$_3$ (36, 65, 81). The 4- and 5-phosphates form several hydrogen bonds with residues in PLC$\delta$-PH, holding the lipid head group in a classical “lock and key” complex. Specifically, Lys$_{30}$ and Lys$_{57}$ form bonds with both phosphates simultaneously (Figure 2b) “clamping the 4- and 5-phosphates in the binding pocket” (36). PLC$\delta$-PH increases the surface pressure of a mixed lipid monolayer when it binds PIP$_2$, which shows it penetrates the polar head group region (K. N. J. Burger, personal communication). PLC$\delta$-PH binds about 10-fold more strongly to IP$_3$ than to PIP$_2$, which complicates the use of PLC$\delta$-PH-GFP constructs to determine the subcellular location of PIP$_2$. For example, translocation of PLC$\delta$-PH-GFP from membrane to cytosol upon activation of PLC could be due to either a decrease in the level of PIP$_2$ in the membrane or an increase in the level of IP$_3$ in the cytosol, an issue that is still unresolved (50, 105, 108).

As discussed elsewhere (65), many of the $\approx 250$ PH domains bind only weakly, and with little specificity, to phosphoinositides: They do not bind phosphoinositides with sufficient avidity to provide a stable membrane anchor on their own. How then do they anchor a protein to the membrane if this is indeed their main function? The PH domain could cooperate with additional membrane-binding sites on the protein or the protein could oligomerize so that more than one PH domain contributes to the binding; signal transduction proteins almost certainly use both schemes (65).

Cheap trick #2: Two domains are better than one. Many signal transduction molecules use two or more domains/motifs to bind to membranes; the membrane-binding energies of the individual domains often simply add (binding constants multiply), as discussed elsewhere (23, 31, 44, 77). Why do signal transduction molecules use multiple domains with low membrane affinities rather than one domain with a high affinity to anchor themselves to the plasma membrane? Multiple membrane–anchoring sites offer at least three advantages. First, they may promote lateral organization or sequestration of the molecules: Proteins with $\geq 2$ saturated acyl chains, such as GAP43 (9), are concentrated in the detergent-insoluble membrane fraction (DIGs) and may be directed laterally.
to putative cholesterol-enriched rafts in the unperturbed membrane (22, 60, 95). Second, they offer greater possibilities for reversible binding: Dynamin may form oligomers where the \( n \) PH domains in the oligomer bind to \( n \) PIP\(_2\) (65). Thus membrane binding can be controlled through oligomerization without changing the level of PIP\(_2\) in the membrane. Third, multiple binding sites provide a mechanism for synergy/coincidence detection: The C1 and C2 domains of PKC require DAG and Ca\(^{++}\), respectively, to bind to membranes—concomitant membrane binding of the C1 and C2 domains removes the pseudosubstrate region of PKC from its binding site and activates the enzyme (54).

Synergy/coincidence counting can also occur when PIP\(_2\)-binding motifs act in concert with other membrane-binding domains to anchor and simultaneously activate proteins. For example, activation of the Wiskott Aldrich syndrome protein (WASP) family, which is involved in regulating the actin cytoskeleton (35, 75, 82, 86), occurs when it binds PIP\(_2\) together with the prenylated, membrane-bound, low-molecular-weight G protein Cdc42 (25). Fawcett & Pawson (35) suggest that “perhaps N-WASP is a ‘coincidence detector’ because when it binds to activated Cdc42 it becomes destabilized and primed such that only a small amount of a coincident signal—in this case PIP\(_2\)—is needed for it to become fully activated.” The factor(s) that increase the level of PIP\(_2\) in the membrane to produce the coincident signal are not well understood.

**ENTH DOMAINS** Proteins in the epsin family share a conserved region, the epsin N-terminal homology (ENTH) domain, that binds PIP\(_2\). Two recent structural studies provide details of how the ENTH domains of epsin (57) and the AP180 homolog CALM (38) may bind to PIP\(_2\) in membranes. The structure of the CALM N-terminal domain, a superhelix of nine major alpha helices, is illustrated in Figure 2 with a bound headgroup of PIP\(_2\). The PIP\(_2\)-binding site in CALM (X-ray structure) is unusual in that it consists of an exposed cluster of lysines that do not form a binding pocket and, curiously, appear to be different from the PIP\(_2\)-binding site in epsin (NMR structure).

Epsin and CALM regulate assembly of the clathrin lattice, and binding of PIP\(_2\) to epsin is necessary for endocytosis (45, 70). Several other proteins that are involved in either recruiting receptors to clathrin-coated pits (e.g., AP-2, \( \beta \)-arrestin) or the final pinching-off process from the plasma membrane (e.g., dynamin) also bind PIP\(_2\). Finally, dephosphorylation of PIP\(_2\) by synaptojanin to form PI(4)P causes disassembly of the clathrin coat. Tsujishita et al. (102) recently obtained the crystal structure of a synaptojanin and provide an excellent discussion of how the binding pocket could accommodate a phosphoinositide.

**FERM DOMAINS** Members of the ezrin/radixin/moesin or ERM family, which help link actin to the plasma membrane, contain an N-terminal FERM (4.1 and ERM) domain that binds to the C-terminal region of the protein. Upon binding to PIP\(_2\), the FERM domain is released from the C-terminal autoinhibitory domain,
allowing the protein to interact with other proteins, such as CD44, and link actin filaments to the plasma membrane (47, 103). Figure 2a shows the radixin FERM domain bound to IP$_3$ (47). The PIP$_2$-binding pocket in the FERM domain is formed from a basic cleft between two subdomains: The cleft is located on a positively charged, relatively flat surface, which should facilitate interaction of the FERM domain with the surface of a negatively charged membrane. The 4-phosphate of PIP$_2$ intrudes into the cleft, but the 5-phosphate does not; in contrast, both the 4- and 5-phosphates are buried in the pocket of the PH domain of PLC$\delta$, as shown in Figure 2b. This probably explains the ability of radixin to bind moderately strongly to PI4P as well as to PIP$_2$ (47).

OTHER PIP$_2$-BINDING DOMAINS Several other domains of known structure bind PIP$_2$: The Tubby domain is a new structure with a basic pocket that binds PIP$_2$ (93); the PX domains contained in many different proteins (75a, 81, 93a) generally bind phosphoinositides that contain a phosphate at the 3 position, but one also binds PI(4,5)P$_2$.

Several important PIP$_2$-binding proteins have clusters of basic/aromatic residues of relatively undefined structure that interact with PIP$_2$. For example, although phospholipase D (PLD) contains a PH domain, the PIP$_2$-binding site responsible for activation has been localized to a different region of the protein containing a conserved cluster of basic/aromatic residues (96); the structure of PLD is unknown. The structure of the actin-binding protein gelsolin was recently determined: Tuominen et al. (104) studied the binding of fluorescently labeled PIP$_2$ to gelsolin and to peptides corresponding to two putative PIP$_2$-binding regions that contained clusters of basic/aromatic residues. They found that PIP$_2$ bound more strongly to the native protein than to either of the two peptides. We obtained similar results with peptides corresponding to the cluster of seven basic residues in N-W ASP that apparently binds PIP$_2$ (88): A peptide corresponding to this region binds weakly to PC/PIP$_2$ vesicles (J. Wang & S. McLaughlin, unpublished data). The weak binding of the basic peptides from gelsolin and N-W ASP to PC/PIP$_2$ vesicles suggests that the structural context of the binding region is important in determining the affinity of a cluster of basic residues for phosphoinositides.

Experiments in several laboratories over the past decade with many different hydrophilic (i.e., lacking aromatic residues) peptides containing a small cluster of basic residues (<7 Lys or Arg) have failed, to our knowledge, to identify peptides where addition of 1% PIP$_2$ significantly increases (>100-fold) the partition coefficient of the peptide onto a vesicle in physiological salt solutions. Included in this list are myristoylated and nonmyristoylated basic peptides corresponding to the basic N-terminal regions of Src (23) and CAP23 (J. Wang & S. McLaughlin, unpublished data) and a peptide corresponding to the basic C-terminal region of Kras4B (67).

THE ROLE OF STRUCTURE The data accumulated to date with these small hydrophilic basic peptides suggest that a structured domain, one that can form multiple hydrogen bonds with the phosphates of the phosphoinositide (e.g., Figure 2b), is
required to anchor a protein firmly to a single PIP2 in the plasma membrane. It seems even more likely that structure is required for specificity. For example, the biologically important 3-phosphorylated phosphoinositides bind with high specificity to several structured domains (e.g., PH, PX, and FYVE domains) (75a, 81, 93a). In contrast, small unstructured molecules, such as neomycin (6) or a peptide corresponding to the MARCKS effector domain that contains 13 basic residues (110), exhibit no specificity between PI(3,4)P2 and PI(4,5)P2, although they bind to PIP2-containing bilayers with high affinity.

If unstructured clusters of basic residues on proteins are located near the membrane-solution interface, however, they could well be involved in sequestering PIP2 laterally in the membrane. The process by which a basic motif binds to a membrane [reviewed in (76)], and the simpler process by which a basic motif located at the interface uses electrostatics to laterally reorganize multivalent lipids such as PIP2, involve different combinations of forces.

Unstructured Domains

THE UNSTRUCTURED BASIC EFFECTOR REGION OF MARCKS BINDS WITH HIGH AFFINITY TO VESICLES CONTAINING PIP2 The MARCKS protein is a puzzle: It is present at high concentration (≈10 µM) and is the major PKC substrate in many cell types, but its function is unknown (2, 18). The mechanism by which this unstructured protein binds to membranes containing acidic lipids is well understood (Figure 4a) and has been reviewed in detail elsewhere (7, 74). Binding requires both the hydrophobic insertion of the N-terminal myristate (orange) into the bilayer and electrostatic interaction of a conserved cluster of basic residues (blue “+” signs), termed the effector domain, with acidic lipids. As illustrated in Figure 4b, the 25 residue effector domain contains 13 basic (blue) and 5 aromatic (cyan) residues; it also contains the 3 serine (purple) residues phosphorylated by PKC and binds with nM affinity to calcium/calmodulin (Ca++/CaM) (7, 74). The myristate alone cannot anchor the protein to the plasma membrane, so abrogation of the electrostatic interaction with the membrane by binding to Ca++/CaM or phosphorylation by PKC translocates MARCKS from membrane to cytosol in many cell types. This myristoyl/electrostatic switch mechanism (7, 74) has been demonstrated in both phospholipid vesicles and cells, most directly with GFP-MARCKS (80). Although the physiological concentration of monovalent lipids in the plasma membrane provides sufficient electrostatic attraction to anchor the protein, recent work has shown the effector domain binds PIP2 with high affinity. Physiological concentrations of both the intact protein and a peptide corresponding to the effector domain inhibit PLC-catalyzed hydrolysis of PIP2 in phospholipid vesicles. The inhibition occurs because the effector domain sequesters PIP2 away from the catalytic domain of PLC, as shown by direct binding measurements (110). Incorporating 1% PIP2 into PC vesicles increases the binding of the effector domain peptide by four orders of magnitude. For comparison, 100-fold higher concentrations of PC/PIP2 vesicles are required to bind the same fraction of the PH domain of PLCδ.
HOW DOES THE UNSTRUCTURED BASIC EFFECTOR DOMAIN OF MARCKS BIND WITH SUCH HIGH AFFINITY TO PIP₂ ON A BILAYER SURFACE? It uses cheap trick #2, binding several (probably three) PIP₂ to form an electroneutral complex (110) as demonstrated most directly by recent electron spin resonance (ESR) experiments with spin-labeled PIP₂ (D. Cafiso, personal communication). Small basic hydrophilic peptides with <7 basic residues presumably do not bind with high affinity to PC/PIP₂ membranes because they cannot form complexes with >1 PIP₂. Several lines of evidence support the conclusion that local electrostatic interactions drive this high-affinity binding of the MARCKS effector domain: Raising the salt concentration screens the binding; PI(4,5)P₂ and PI(3,4)P₂ bind with the same affinity, indicating it is independent of the chemical nature of the phosphoinositol; and peptides with 13 Lys or 13 Arg residues bind with the same affinity as the MARCKS effector domain peptide (110).

FUNCTIONS OF PIP₂

Figure 3 summarizes some of the well-established functions of PIP₂; we discuss these only briefly because they have been considered in detail in other reviews (29, 30, 32, 53, 55, 56, 70, 83, 100, 106).

Production of Second Messengers

Receptor-mediated activation of PLC catalyzes hydrolysis of PIP₂ to produce the second messengers DAG and IP₃ (16). IP₃ releases Ca⁺⁺ from intracellular stores (17, 27), whereas DAG remains in the membrane and helps activate PKC by binding to its C₁ domain (54). More recently it has been recognized that PIP₃, which can be formed from phosphorylation of PIP₂ by a PI₃ kinase, is also an authentic second messenger that functions as a membrane anchor for a number of proteins (32). It is easy to understand how the products of PI₃ kinases, such as PIP₃, function as second messengers. Their level in a quiescent cell is low (106); signaling to a

Figure 3 Functions of phosphatidylinositol 4,5-bisphosphate (PIP₂). See text for discussion.
PI3 kinase can thus produce a large increase in the level of the 3-phosphorylated lipid in the membrane, which can be recognized by a binding domain with a high specificity for that lipid. In contrast to DAG, IP₃, and PIP₃, where the level of messenger can increase dramatically, the overall concentration of PIP₂ in the plasma membrane is unlikely to increase significantly, making it less clear how PIP₂ itself acts as a second messenger (53) to activate actin-binding proteins such as the WASP and ERM families, and enzymes such as PLD.

**Membrane Targeting**

In some cases it is not necessary for the concentration of PIP₂ to increase for it to function effectively as a membrane anchor. The PH domain of PLCδ was the first PIP₂-binding domain to be understood in atomic detail, and the local concentration effect (cheap trick #1) explains why targeting is important with this enzyme. The enzyme is activated not by translocation to the membrane but by an increase in the intracellular [Ca²⁺]: Membrane anchoring simply facilitates interaction of the catalytic domain of PLCδ with its membrane-bound substrate PIP₂ (91). TUBBY, in contrast, is apparently anchored to the plasma membrane to prevent it from interacting with its target molecules in the nucleus: Hydrolysis of PIP₂ produces translocation of TUBBY from membrane, allowing it to diffuse to the nucleus (24, 93). In many other cases, PIP₂ acts as a second messenger and activates proteins.

**Enzyme Activation**

We consider only one example, a major PIP₂ synthesis pathway in mammalian cells. The PI4P 5-kinases (PIP kinases) produce PIP₂ mainly by phosphorylating PI₄P; they are strongly activated by phosphatidic acid (PA) (5, 40, 49, 52). PA is produced by PLD, which requires PIP₂ for activation (68). Thus the potential exists for a positive feedback loop. Actually, several complicated positive and negative feedback loops involving PIP₂ control the activation of PIP kinases (32, 34, 89). There is also evidence the enzymes involved in these control mechanisms may be concentrated together in specific regions of the plasma membrane, such as membrane ruffles (52).

**Cytoskeletal Attachment**

Many actin-binding proteins bind to PIP₂ and are activated by this lipid. We mentioned that PIP₂ induces conformational changes in N-WASP and the ERM proteins [more extensive lists are considered elsewhere (49, 97)]. The possibility that a local increase in the free concentration of PIP₂ acts as a signal for anchoring actin has been discussed widely, and the importance of PIP₂ in cytoskeletal attachment was demonstrated directly by elegant experiments using laser tweezers (90). Decreasing the level of PIP₂ produced a dramatic release of the cytoskeleton from the membrane (90).
Exocytosis, Endocytosis, and Membrane Traffic

This topic has been reviewed recently (30, 70, 97a) and there is no lack of candidate PIP2-binding molecules that could be involved in exocytosis and clathrin-mediated endocytosis. Several investigators have stressed the importance of understanding the lateral organization of PIP2 in the membrane and the role it might play in these functions. With respect to exocytosis, Martin notes that immunocytochemical studies from his lab using PC12 cells reveal “plasma membrane rafts of PIP2 that colocalize with secretory granules” (70). With respect to endocytosis, “the focal assembly of clathrin lattices implies that there may be PIP2 rich patches in the plasma membrane” (45). The mechanism(s) by which these putative PIP2-enriched rafts or patches are assembled and maintained is unclear.

Other Functions

Space limitations prevent us from discussing the many other functions of PIP2 [e.g., regulation of ion channels (48a), binding of scaffolding proteins]. How does one simple lipid do all these different jobs? We consider the possibility there are separate pools of PIP2 in the plasma membrane.

EVIDENCE FOR THE LATERAL ORGANIZATION OF PIP2 IN THE PLASMA MEMBRANE

The older literature contains hints that much of the PIP2 in the plasma membrane is not free. For example, microvesicles released from erythrocytes after a variety of treatments contain only about half the fraction of polyphosphoinositides (mainly PIP2) seen in the original erythrocyte membranes (46). Other studies suggest different metabolic pools of PIP2 may exist in cells [see discussion in (49, 108)]. PIP2 is concentrated in the noncaveolar DIGs fraction of the plasma membrane (85, 111), which suggests an association with putative cholesterol-rich rafts in the unperturbed membrane (22, 60, 95). Because PIP2 has a polyunsaturated chain (Figure 1), it is unlikely it partitions spontaneously into rafts by itself (22, 33).

Recently, researchers have developed new tools to study the location of PIP2 in living cells more directly (11, 98, 108). Botelho et al. (20) used fluorescent chimeras of PH domains to show that PIP2 is concentrated in the nascent phagosomes of macrophages. This elegant, tightly reasoned paper showed the localized accumulation of PLCδ-PH-GFP is transient; the chimera dissociated rapidly once the phagosomes pinched off from the membrane. Several factors could, in principle, decrease the level of PIP2, but the concomitant appearance of the hydrolysis product DAG (detected by a fluorescent C1 domain chimera) indicates that PLC-catalyzed hydrolysis is at least partially responsible for reducing the concentration of PIP2 in the phagosomes. Are these transient changes in the level of PIP2 important? Their observation that neomycin or overexpression of PLCδ-PH inhibits phagocytosis suggests these transient changes in the level of PIP2 are required for phagocytosis. Botelho et al. (20) discuss the (still imperfectly understood) roles that PIP2,
PLC, PKC, and other enzymes could play in phagocytosis. They also showed that a PIP kinase involved in the synthesis of PIP\(_2\) migrates to the nascent phagosome, then dissociates shortly after completion of phagocytosis. In a subsequent report, they used GFP constructs of PH domains that bind PIP\(_3\) (e.g., Akt) to show PIP\(_3\) also accumulates in and is sharply restricted to the phagosomal cup (69). The accumulation is transient: PIP\(_3\) disappears within 2 min of phagosomal sealing, probably because of the action of the phosphatase SHIP1.

Two other groups have used fluorescent constructs of PLC\(\delta\)-PH to show transient increases in the concentration of PIP\(_2\) in a different region of cells, membrane ruffles. When HeLa cells were stimulated with epidermal growth factor, PLC\(\delta\)-PH-GFP first translocated to the ruffles then dispersed as the membrane ruffles disappeared (52). The localization of PLC\(\delta\)-PH-GFP at the ruffles coincided with the presence of a PIP kinase (52), as was observed with the phagosomes. A PLC\(\delta\)-PH-EGFP chimera has also been shown to be concentrated in the membrane ruffles of NIH-3T3 fibroblasts (99).

The PH-GFP chimeras, of course, are not perfect indicators of PIP\(_2\) (11). Holz et al. (51), for example, minimized potential artifacts by using two PIP\(_2\) indicators, fluorescent neomycin (6) and PLC\(\delta\)-PH-GFP, in their study of exocytosis. Fortunately, they produced similar results. Thus the available evidence strongly suggests that PIP\(_2\) is concentrated in nascent phagosomes and membrane ruffles, but how?

**MECHANISMS THAT COULD CONCENTRATE PIP\(_2\) IN THE PLANE OF THE PLASMA MEMBRANE: SYNTHESIS OR SEQUESTRATION?**

We first note that the concentration of PIP\(_2\) in a submicroscopic region of the plasma membrane is unlikely to change significantly in response to enhanced local synthesis of PIP\(_2\) by a PIP kinase. Put simply, PIP\(_2\) will diffuse away faster than it can be produced. Consider, for example, a lateral domain of diameter 100 nm with a single PIP kinase enzyme at the center producing PIP\(_2\) at a rate of 100 per sec. The Einstein relation (14), \(t = x^2/4D\), gives the average time \(t\) to diffuse a distance \(x\) in the two-dimensional surface, where \(D\) is the diffusion coefficient. For PIP\(_2\), \(D\) should be \(\approx 10^{-8}\) cm\(^2\) s\(^{-1}\) (48, 109), so a newly synthesized PIP\(_2\) will diffuse away from the domain in <1 ms: Synthesis cannot keep up with diffusion over short distances. More specifically, it is unlikely that local synthesis could produce an accumulation of PIP\(_2\) in the putative cholesterol-enriched rafts if they have dimensions <100 nm.

In contrast, local synthesis can and does produce significant gradients of 3-phosphorylated phosphoinositides in the plasma membrane as a whole, and a number of recent reports demonstrate these gradients are important in chemoreception [see references in (48)]. Haugh et al. (48) demonstrated the existence of these gradients in fibroblasts using evanescent wave microscopy and a GFP construct of the Akt PH domain; they also provided a theoretical model that accounts for the gradients.
The accumulation of PIP$_2$ (and PIP$_3$) in lateral membrane domains of intermediate size (e.g., nascent phagosomes, ruffles; size 1–10 µm) by a local synthesis mechanism is certainly possible theoretically, but as discussed critically by Marshall et al. (69), it is difficult to reconcile a simple local synthesis model with the experimental observation that PIP$_3$ is confined sharply to the phagosomal cup. They considered a more complicated synthesis model in which phosphatases were concentrated at the rim of the cup to hydrolyze escaping PIP$_3$, but they were unable to obtain experimental support for this model.

What other mechanisms might account for the enhanced local concentration of phosphoinositides in ruffles and phagosomes? One alternative possibility is that specific proteins can act as “buffers,” binding and passively concentrating PIP$_2$ (and PIP$_3$) in these lateral membrane domains. Such proteins would have to satisfy three criteria: be concentrated in the domains, be present at a sufficiently high concentration to sequester PIP$_2$ (≥ 10 µM), and have a high affinity for PIP$_2$. One protein that satisfies these criteria is MARCKS: It is concentrated in the ruffles of fibroblasts (78) and the forming phagosomes of macrophages (4) possibly because of its interaction with actin. Its concentration is comparable to that of PIP$_2$ (3, 18), and its basic effector domain binds PIP$_2$ with high affinity (8, 110). [As an aside, we note that the PIP$_2$ electrostatically associated with the effector domain of MARCKS (e.g., Figure 4f) is accessible to PLC$\delta$-PH-GFP. Concentrations of the MARCKS effector domain peptide that bind >90% of the PIP$_2$ in a PC/PIP$_2$ vesicle, have no significant effect on the binding of a PLC$\delta$-PH-GFP construct (A. Gambhir & S. McLaughlin, unpublished observation).

Cheap trick #3: Electrostatic sequestration. A well-understood example of electrostatic sequestration is the binding of charged solutes to membranes because of their nonspecific electrostatic accumulation in the diffuse double layer adjacent to a charged surface. Helmholtz clearly appreciated the qualitative aspects of the phenomenon in the nineteenth century (and the electrostatic double layer is still often approximated as a capacitor with a thickness equal to the Debye length: $1/\kappa = 1$ nm for a 0.1 M solution). Gouy and Chapman combined the Poisson and Boltzmann equations to describe the double layer quantitatively in the early twentieth century. Their model, which predicts the electrostatic potential falls with distance $x$ from the surface as $\exp(-\kappa x)$, describes accurately the electrostatic sequestration of small ions adjacent to phospholipid bilayers (72, 73). For example, the Boltzmann relation predicts, and experiments with membrane-bound pH indicators confirm, that the concentration of a monovalent cation (i.e., a H$^+$ ion) in the aqueous phase at the surface is enhanced 10-fold over its value in the bulk solution if the surface potential is −60 mV. The effect is much more dramatic for multivalent ions, such as charged basic peptides: Their nonspecific accumulation can be calculated from the first principles of physics using detailed atomic-level models of membranes and peptides in conjunction with the Poisson and Boltzmann relations (12, 13). One calculates the Gibbs surface excess, the integral of the excess concentration of the charged peptide...
over distance from the surface (76). This is the number of peptides bound per unit area, which can be compared with experimental data. There is satisfactory agreement between theoretical predictions and experimental results, as reviewed elsewhere (76).

We consider the hypothesis that MARCKS uses cheap trick #3, nonspecific electrostatic sequestration, to accumulate laterally a significant fraction of the PIP2 in a cell. Specifically, the cluster of basic residues in the MARCKS effector domain produces a significantly positive local electrostatic potential, which enhances the local two-dimensional concentration (i.e., number per unit area) of a trivalent lipid such as PIP2 by the Boltzmann factor (see cheap trick #3). Figure 4 illustrates various features of this model. Figures 4a,b show the orientation of the MARCKS effector domain with respect to the bilayer. Monovalent acidic lipids [e.g., phosphatidylserine (PS)] comprise about 20%–40% of the phospholipids on the cytoplasmic leaflet of a plasma membrane in a mammalian cell. Figure 4c shows the −25 mV electrostatic equipotential profile adjacent to a 2:1 PC/PS membrane in 100 mM monovalent salt (76, 84). Although the discrete nature of the monovalent fixed charges (i.e., PS) produces a slight undulation in the potential profile, to a good approximation the −25 mV surface may be regarded as flat and located about 1 nm from the surface, exactly as predicted by much simpler Gouy-Chapman theory. Figure 4d shows how binding of the MARCKS effector domain modifies this potential. Although the positive charges on the peptide are screened by the counterions in the aqueous solution and the monovalent acidic lipids in the membrane, they nevertheless produce a positive potential in the neighborhood of the peptide, as illustrated by the +25 mV equipotential profile shown in blue. It is apparent from Figure 4d that a cluster of basic residues confined to the membrane-solution interface can act as a basin of attraction for multivalent acidic lipids.

To illustrate this more quantitatively, we ask the following questions. What are the average potentials the 3 negative charges on PIP2 experience when the lipid is far from the peptide and when it is close to the peptide? These charges, located about 0.5 nm from the surface of the bilayer (Figure 1 and Figure 4f), are within the −25 mV equipotential surface (shown in Figure 4c). They experience a potential more negative than −25 mV when the lipid is far (i.e., >1 nm laterally) from the peptide. The 3 charges on a PIP2 located within the blue mesh surrounding the peptide (Figure 4d) experience a potential more positive than +25 mV. For simplicity, we assume there is a neighborhood around the basic peptide where the potential experienced by PIP2 is uniformly +25 mV and that outside this region the PIP2 experiences a potential of −25 mV as shown in Figure 4e. If we also assume (incorrectly) that the PIP2 does not perturb the potential in the neighborhood of the peptide when it is sequestered, the Boltzmann relationship predicts PIP2 will be concentrated laterally by a factor of \( \exp\left(-\frac{ze\Delta\psi}{kT}\right) \), where \( \Delta\psi \) is the difference in potential (50 mV), \( z \) is the valence on the PIP2 (−3), and \( kT/e = 25 \text{ mV} \).

Thus, the simple electrostatic sequestration model predicts that PIP2 is concentrated by a factor of \( \exp(6) = 400 \) in this neighborhood of the peptide. As
MARCKS and PIP$_2$ are present at similar concentrations, and PIP$_2$ comprises about 1% of the phospholipids, this order of magnitude calculation predicts a significant fraction of the PIP$_2$ in the membrane should be sequestered by MARCKS. Work in progress on a more realistic theoretical treatment takes into account the perturbation of the potential by PIP$_2$. Figure 4 shows preliminary calculations for PIP$_2$ (yellow) sequestered adjacent to a single MARCKS effector domain peptide (green) in an otherwise electrically neutral PC membrane, showing local regions of negative potential around the PIP$_2$ and local regions of positive potential around the basic residues.

The simple electrostatic sequestration model illustrated in Figure 4 is consistent with the available experimental data obtained by both biophysicists and cell biologists. Physiological levels of MARCKS (10 µM) or its effector domain peptide are predicted to sequester a significant fraction of the PIP$_2$ in a phospholipid vesicle, even in the presence of a large excess of monovalent acidic lipids. Biophysical experiments on model membranes over the past few years show that this concentration of protein or peptide does in fact inhibit PLC-catalyzed hydrolysis and that this inhibition is due to the electrostatic sequestration of PIP$_2$ by the effector domain (8, 110). Cell biology experiments show colocalization of MARCKS and PIP$_2$ antibodies in fixed cells (64). Cell biology experiments provide other important indications that MARCKS sequesters a significant fraction of the PIP$_2$ in a cell: Overexpression of MARCKS produces a concomitant increase in the level of PIP$_2$ in the cell, which is the expected response if the cell maintains a constant concentration of free PIP$_2$ (64). The main caveat here is that the degree to which electrostatic sequestration occurs with MARCKS may depend on the cell type; the relative concentration of MARCKS and PIP$_2$ has not been determined accurately in most cells.

One interesting feature of the electrostatic sequestration mechanism sketched in Figure 4 is that MARCKS can bind PIP$_2$ globally and release it locally. Binding of Ca$^{++}$/CaM or phosphorylation by PKC releases the basic effector domain from the membrane (7, 74, 80). It is easy to demonstrate in phospholipid vesicles that translocation of the effector domain from membrane to solution by PKC or Ca$^{++}$/CaM releases bound PIP$_2$: Specifically, it reverses the inhibition of the PLC-catalyzed hydrolysis (76, 110). It is much harder to demonstrate that PIP$_2$ is reversibly sequestered with MARCKS in a living cell: Laux et al. (64) provided the best evidence to date by showing colocalization of MARCKS and PIP$_2$ in fixed cells using PIP$_2$ antibodies, but experiments on living cells with some of the new technologies discussed below are obviously necessary to test critically the reversible sequestration hypothesis. A corollary of the reversible sequestration model, if it can be confirmed in living cells, is that local increases in [Ca$^{++}$], which are controlled tightly (17), could produce local increases in the free concentration of PIP$_2$ in the membrane. Specifically, the increase in local [Ca$^{++}$/CaM] would produce a local release of PIP$_2$ sequestered by the MARCKS effector domain.

It remains to be determined experimentally whether local synthesis or electrostatic sequestration is more important in concentrating PIP$_2$ in nascent phagosomes and membrane ruffles. Some experiments (69) strongly suggest that local synthesis and degradation is important in the appearance/disappearance of phosphoinositides.
in phagosomes, e.g., the “appearance of PIP₃ coincides with the equally localized disappearance of PIP₂ from the base of the cup.” But these authors also discuss why local synthesis alone does not account for several features of the accumulation of the phosphoinositides in phagosomes (69). Because both PIP kinases and MARCKS are concentrated in nascent phagosomes and membrane ruffles, both mechanisms might be in play. What is the advantage of combining local synthesis and local sequestration? For a given rate of PIP₂ synthesis in a lateral membrane domain, the analysis of Haugh et al. (48) shows that a 10-fold-larger concentration gradient will be obtained if the diffusion coefficient is lowered 10-fold. If 90% of the PIP₂ in the lateral domain is bound to MARCKS or other proteins, the effective local diffusion coefficient of PIP₂ will be 10-fold lower (e.g., 59), and 10-fold more PIP₂ will be accumulated in the lateral domain.

The MARCKS effector domain is not unique: Other proteins have basic regions with similar sequences, and some are also present at high concentrations. For example, MacMARCKS (2, 18); adducin (58), which may function in ruffle formation (41); a *Drosophila*-scaffolding protein, DAKAP200 (92); and the N-methyl-D-aspartate (NMDA) receptor (112) all have basic regions similar to the MARCKS effector domain. The net positive charge in these effector regions is 12, 11, 10, and 9, respectively. Peptides corresponding to these regions bind PIP₂ with significant affinity: The binding affinity to a PC/PIP₂ (99:1) vesicle correlates with charge on the peptide and decreases 100-fold as the net charge on the peptide decreases from 13 for MARCKS to 9 for the NMDA peptide (J. Wang & S. McLaughlin, unpublished data). Additional work is needed to determine if these proteins also bind PIP₂ in the cell and, if so, whether this binding is biologically important. The NMDA receptor is particularly interesting because it functions as a Ca²⁺ channel, and Ca²⁺/CaM binds to the effector region when Ca²⁺ flows through the channel (112).

GAP43, MARCKS, AND CAP23: DOES THE GMC GANG HANG OUT IN RAFTS?

Caroni and colleagues have pointed out striking parallels between MARCKS and two neuronal proteins, GAP43 (a growth-associated protein) and CAP23 (a cytoskeletal-associated protein). They coined the term GMC family for these proteins. Although the GMC proteins have no sequence similarity, they share important physical traits (39). For example, they lack any α-helix structure and resemble extended rods, a structure consistent with their highly acidic nature; their N-termini contain either a myristate or two palmitates (GAP43) that insert into the bilayer; and they have a conserved basic region that is phosphorylated by PKC and can bind Ca²⁺/CaM (CAP23 and MARCKS) and actin. The proteins share biological as well as physical properties. Frey et al. (39) used knockin mice to show that GAP43 can largely rescue the phenotypical abnormalities caused by the absence of CAP23. CAP23 and GAP43 together can induce spinal axon regeneration, so they may have interesting clinical applications (19). Based on their experimental
observations that PIP$_2$ colocalizes with GMC proteins in micrometer-size domains when cells are fixed in certain ways, Laux et al. (64) recently proposed a provocative, and potentially important, hypothesis: All GMC proteins are localized in small cholesterol-enriched domains or rafts in unfixed cells, all bind PIP$_2$, and thus localize PIP$_2$ to the putative rafts.

The two saturated chains on GAP43 should help localize it to cholesterol-enriched rafts, and previous work has shown that it is concentrated in the detergent-resistant or DIGs fraction of the plasma membrane (9). Proteins with a single saturated acyl chain (i.e., on CAP23, MARCKS, or a mutant GAP43 with one palmitate), however, are not generally present in DIGs, and a single acyl chain is generally considered incapable of targeting a protein to the putative rafts (22). The Laux et al. (64) experiments can be reconciled with the previous DIGs/raft work by noting that they were done under conditions where the GMC proteins were presumably chemically cross-linked into supramolecular assemblies that effectively had multiple saturated acyl chains. Recent work by Dietrich et al. (33) showed that cross-linking a saturated phospholipid analog indeed increases its partitioning into large cholesterol-enriched domains that are visible in a supported monolayer.

The biological significance of the results emerges if one postulates actin, rather than chemical, cross-linking of GMC proteins in specific regions of the cell (e.g., MARCKS in the nascent phagosome and membrane ruffles): The cross-linked GMC proteins might nucleate the formation of cholesterol-enriched rafts in those regions. The recent observation that the diffusion coefficient of a disaturated lipid analog was reduced in the phagosome is consistent with this scenario (69). The postulated connection between the GMC proteins and cholesterol-enriched rafts (64), in our opinion, is important and needs to be evaluated using new tools on living cells. The suggestion that all the GMC proteins sequester PIP$_2$ and thus act as pipmodulins (64) is also interesting. There is good biophysical evidence that MARCKS can sequester PIP$_2$ in model systems, but the interaction of PIP$_2$ with the neuronal proteins CAP23 and GAP43 has not been similarly investigated in any detail. In contrast to MARCKS, overexpression of these two proteins does not produce a heightened level of PIP$_2$ in cells, which implies they may be less important than MARCKS in acting as buffers to control the level of free cellular PIP$_2$ (64).

NEW TECHNOLOGIES

Many new techniques are available to examine the location of PIP$_2$ in living cells and model membranes. GFP constructs of domains that bind with high affinity to specific phosphoinositides are now used widely by cell biologists and should continue to provide important new information. Fluorescent and spin-labeled analogs of PIP$_2$ have been important tools for model membrane studies, but hydrolysis of these molecules would be a problem in cells; a nonhydrolyzable fluorescent analog of PIP$_2$ would be extremely useful for examining more directly the distribution of PIP$_2$ in living cells. Fluorescence resonance energy transfer (FRET) microscopy
with GFP-PH domain constructs has already provided useful information in cells (105) and has great potential for examining the possible localization of PIP₂ in submicroscopic domains such as rafts. Evanescent wave or total internal reflection fluorescence microscopy (TIRFM) [see references in (109)] has already been used to examine gradients of phosphoinositides in cells (48). We suspect that single molecule fluorescence measurements on living cells will prove to be increasingly valuable in phosphoinositide research, and the technique has been used to provide evidence for rafts in cell membranes (95). Laser tweezer measurements allow the direct determination of the force by which PIP₂ attaches the cytoskeleton to the plasma membrane (90). With respect to model membrane systems, computational studies (76) and biophysical measurements on PIP₂ are now in progress in different laboratories using laser tweezers (42), NMR, ESR, fluorescence correlation spectroscopy, and other approaches. They should help tease out the physical mechanisms by which proteins interact with phosphoinositides. Thus we are looking forward to the next several years of phosphoinositide research, as they are sure to be exciting ones.

CODA

Of course we have not answered the most important question we posed in the Introduction: How does order emerge from diffusional chaos in the many signal transduction systems that exist in a cell? We hope, however, that the biophysical cheap tricks we have described will help cell biologists to discover how PIP₂ acts as its own second messenger and choreographs the diffusional dance of so many other signal transduction molecules; in Yeats’ words, to “know the dancer from the dance.”

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Figure 2  (a) Ribbon diagrams of three domains showing their interaction with IP$_3$ or the head group of PIP$_2$. Helices, blue; $\beta$-sheets, green; loops, cyan; oxygens on IP$_3$, red. (b) Detail of the binding pocket of the PH domain of PLC$\delta$ showing the two hydrogen bonds that K30 and K57 each make with the 4- and 5-phosphates.
Figure 4  (a) Cartoon of MARCKS bound to a bilayer. The myristate (orange) inserts hydrophobically into the membrane, and the 13 basic residues (blue “+” signs) in the effector domain interact with acidic lipids (3 red balls indicate PIP2). Five Phe residues (cyan) insert into the bilayer. Phosphorylation of 3 Ser (purple) by PKC or binding of Ca++/calmodulin displaces the effector domain from the bilayer. (b) Molecular model of the effector domain of MARCKS bound to a bilayer. Basic residues, blue; Ser phosphorylated by PKC, purple; Phe, cyan. (c) Electrostatic equipotential profile (−25 mV = kT/e profile shown in red) adjacent to a PC/PS (2:1) bilayer in 100 mM monovalent salt as calculated from the nonlinear Poisson-Boltzmann equation (84). (d) Electrostatic potential profiles produced by the binding of a MARCKS effector domain peptide to a PC/PS 2:1 bilayer. The −25 mV equipotential profile is shown in red, the +25 mV potential profile in blue. (e) Simple model to illustrate electrostatic sequestration of PIP2 by MARCKS. (f) MARCKS effector domain adsorbed to a PC/PIP2 bilayer showing that it captures electrostatically several PIP2 (yellow; two are visible on the front of the peptide). The +25 mV equipotential profile is shown in blue, the −25 mV equipotential profile in red.