Review

The interfacial binding surface of phospholipase A2s

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Abstract

For membrane-associated enzymes, which access substrate from either a monolayer or bilayer of the aggregate substrate, the partitioning from the aqueous phase to this phospholipid interface is critical for catalysis. Despite a large and expanding body of knowledge regarding interfacial enzymes, the biophysical steps involved in interfacial recognition and adsorption remain relatively poorly understood. The surface of the enzyme that contacts the phospholipid surface is referred to as its interfacial binding surface, or more simply, its i-face. The interaction of a protein’s i-face with the aggregate substrate may simply control access to substrate. However, it can be more complex, and this interaction often serves to allosterically activate the enzyme on this surface. First we briefly review what is currently known about i-face structure and function for a prototypical interfacial enzyme, the secreted Phospholipase A2 (PLA2). Then we develop, characterize, compare, and discuss models of the PLA2 i-face across a subset of five homologous PLA2 family members, groups IA, IB, IIA, V, and X. A homology model of human group-V is included in this comparison, suggesting that a similar approach could be used to explore interfacial function of any of the PLA2 family members. Despite moderate sequence identity, structural homology and sequence similarity are well conserved. We find that the residues predicted to be interfacial, while conserved structurally, are not highly conserved in sequence. Implications for this divergence on interfacial selectivity are discussed.

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1. Introduction

Roughly half of all proteins are membrane associated either as an embedded integral membrane protein or as a membrane-associated protein. Unlike cytosolic proteins, membrane-associated proteins interact with ligands and substrates in either the aqueous phase or from within the aggregated ligand/substrate interface. Although considerable attention has been focused on the structural and functional characterization of integral membrane proteins, a limited number of interfacial enzyme systems have been scrutinized biochemically. Here we focus on the secreted phospholipase A2 (PLA2) family of enzymes, which in humans is composed of 10 genes which code for proteins with diverse properties, tissue distributions and functions[1]. The members of the secreted PLA2 family are small (∼14 kDa) disulfide-rich proteins that catalyze the Ca-dependent hydrolysis of the sn-2 ester of glycerophospholipids.

The catalytic cycle proposed for PLA2s is summarized in Fig. 1. In this scheme, the enzyme partitions from the aqueous phase to the bilayer interface, in an E→E* step. The enzyme then undergoes allosteric activation, followed by catalysis and product release. It is thought that the enzyme shields the hydrophobic regions of the substrate from the bulk solvent, allowing for diffusion of the phospholipid into the active site[2]. The interfacial binding step is crucial to enzymatic function, as catalysis in the aqueous phase is virtually nonexistent[3,4]. Studies of PLA2s have shown that these enzymes often have comparable catalytic efficiencies for substrates under experimental conditions where interfacial binding affinity is not a factor[5,6]. This indicates that apparent rates of catalysis on
vesicles of varying composition are often heavily influenced by the affinity of the enzyme for the interface.

2. The PLA2 i-face

This crucial step is mediated by a region of the protein often referred to as the i-face. The initial structure of PLA2 [7,8] revealed a nearly flat surface which surrounded the active site opening of bovine group-IB (bGIB) PLA2. Based on a wealth of data characterizing the association with micelles and vesicles, a model emerged of how the enzyme “sits” on the phospholipid surface [9,10]. This is a planar surface on the protein composed of approximately 20 amino acids, with a surface area of about 1500 Å² [11]. The binding can be remarkably tight, with reported dissociation constants of <0.1 pM for porcine pancreatic PLA2 on anionic vesicles [10]. While the nature of the interactions between protein and bilayer has been subjects of intensive research, the deconvolution of contributions from electrostatics, structure, and mediating factors such as ions and water molecules at the interface has remained challenging. The issue is complicated by the possible involvement of other factors such as receptor or heparin sulfate proteoglycan-mediated interactions [12]. Despite a large number of crystal structures solved for the PLA2 enzymes [7,13–16], obtaining structural information for their i-face interactions has been complicated due to experimental difficulties of obtaining high resolution structural information of a protein bound to a membrane, vesicles, micelles, or even amphiphiles. Despite these difficulties, a self-consistent model of the PLA2 i-face binding has emerged from a combination of low resolution spectroscopic [17,18], mutagenesis [19–22], computational [23–25], and NMR experiments [26–29]. NMR studies of bGIB showed NOE changes in the N-terminus and C-terminus of PLA2 in the presence of SDS micelles [26]. The NMR structure of pGIB showed disordered to ordered transition for the N-terminal helix between free and micellar bound forms of the enzyme [29]. An interfacial loop surrounding residue-69 moves inward when inhibitors are bound, and forms contacts with the N-terminal helix. The anion-assisted dimer crystal structure [11], which is discussed below, mirrors the changes to the N-terminus and 69-loop, like this micelle-bound NMR structure [29].

The majority of previously reported PLA2 crystal structures [7,13–16] were solved from protein crystals grown using 2-methyl-2,4-pentanediol as a precipitant with few or no salts added. Several PLA2 enzymes, such as group-IB and group-IIA are known to optimally bind and function on anionic surfaces. In an attempt to obtain crystal structures of group-IB with anions bound we screened a wide range of conditions that includes various monovalent and divalent anions. Here we had a fortuitous crystallization event, which lead to a crystal form with not only anions bound, but a form which has emerged as an i-face bound mimic form of PLA2. The solution of the anion-assisted dimer form of porcine pancreatic group-IB PLA2 (pGIB) [11] revealed a structure in which two monomers of pGIB share a single inhibitor. Five anions are bound at the interface between the two monomers, and charge compensation occurs from both protein and water contacts. Perhaps most significantly, the contact surface between the two monomers very closely matches the previously-postulated i-face for the enzyme [10,18,30,31]. A comparison between the structure of the anion-assisted dimer (inter-subunit contact area ~1500 Å²) and monomeric (one molecule per asymmetric unit) forms of pGIB revealed several important structural differences. Perhaps the most noticeable change occurs in the 69-loop, so named for a Tyr-69 which is important for catalysis. This loop, which is often disordered in monomeric structures, is more ordered in the anion-assisted dimer. Structures of anion-assisted dimers of pGIB with different inhibitors bound indicate that the structure of the 69-loop is not dependent upon the species contained in the active site. In Fig. 2, we show the inter-subunit contact residues for both the anion-assisted dimer (Fig. 2A) and monomeric (Fig. 2B) pGIB as CPK (space filling) colored by residue type. Two residues of the 69-loop, Val-65 and Asp-66,
are included in this representation, and indeed significant differences in orientation are apparent. We also see different orientations for Arg-6, as well as residues in the 20–30 region. Fig. 3A shows a comparison of the RMSD of the anion-assisted dimer (PDB: 1fxf) to monomeric (PDB: 4p2p) crystal structures of pGIB. Backbone (black) and sidechain (red) atoms show elevated RMSDs in these interfacial loops. As RMSD is a measure of differences in atomic positions of homologous atoms, this indicates that the positioning of these loops are different in the two structures. Qualitatively, the selected residues in the monomer structure tend to be more disperse along the axis perpendicular to the membrane surface. To compare the RMSD deviation with conformational flexibility, we have plotted the residue by residue B-factor plot of the 0.97 Å high resolution crystal structure of bGIB PLA2 [14]. The B-factor is a measure of atomic oscillation in crystallographic models, providing information about molecular dynamics of the crystallized protein. The correlation between the RMSD deviation peaks of Fig. 3A and the B-factor peaks of Fig. 3B indicates an apparent plasticity of the surface loops of PLA2, several of which are interfacial.

Mechanistically, the anion-assisted dimer crystal structure [11] has a water coordinated to the catalytically active histidine...
which is not found in the non-dimer structures \[32\]. The anion-assisted dimer structure (PDB code: 1fxf) when compared to the monomeric crystal structure are each consistent with a sequence of anion binding, i-face desolvation, i-face loop rearrangement, and active site changes to achieve an interfacially activated enzyme. A central feature that appears to be consistent among the growing array of structural and functional data is the importance of the assisting water, \( w_7 \), which appears to be the difference between an active and inactive enzyme. This, along with other evidence reviewed recently \[32\], lends support to the postulate that the anion-assisted dimer is a reasonable analog of the interfacially-bound, or E* form of the enzyme.

To date, no other PLA2s, aside from the pGIB enzyme, have been crystallized in an analogous anion-assisted dimer manner. Several crystal structures have been reported of PLA2 in dimeric forms \[33–35\], however, the intersubunit contact of these PLA2 structures is not as extensive as the anion-assisted dimer. Furthermore, the intersubunit contact residues in the other PLA2 dimers do not match with existing models of the i-face \[9–11,18\]. In contrast, the anion-assisted dimer’s intersubunit contact residues correlate with all existing models of the PLA2 i-face. We therefore can use the anion-assisted dimer structure of pGIB as a template to predict the i-face residues of other PLA2 family members. Here we are aided by the overall structural homology of the PLA2 paralogs. For this review we have selected a subset from the 10 human PLA2 proteins. We have selected pGIB, human group-IA (hGIIA), and human group-X (hGX), as well as the (snake) Naja naja atrahuman group-IA (sGIA) due to existing crystal structures. A homology model of the human group-V was developed for this comparison; the results of which further suggest that a similar approach could be used to develop an i-face model of any homologous PLA2 enzyme.

3. Homology model of human group-V PLA2

As previously described, the overall fold of the PLA2 enzymes are highly conserved, and the primary sequences also exhibit reasonable homology. In order to include the physiologically interesting hGV PLA2 \[12\], it was necessary to construct a homology model. To do so, we initially utilized the MODELLER \[36,37\] implementation in Insight II (Accelrys) to build a model based on a previously published sequence alignment \[16\], using the structures of hGIIA and hGX as templates. Several models were constructed and compared, and we found very minor fluctuations in backbone RMSDs, typically less than 1 Å between models. We were not content with the validity of the models, however, as energy minimization using the program CNS \[38\] would frequently lead to collapse of the substrate binding site opening. In order to optimize the model, we performed a structurally-corrected multiple sequence alignment using the STAMP program \[39\] (Fig. 4) of several PLA2s, namely pGIB, hGIIA, hGV (model), hGX, and sGIA. The resultant alignment was then used to remodel hGV, with all other proteins included in the alignment as templates. The new model reached a stable energy minimum
in fewer cycles than the initial model, and was more robust in the face of energy minimization or simulated annealing. This model was reincorporated into the STAMP alignment (Fig. 4) to generate an accurate, structurally-corrected alignment for this group of PLA2s. This model was submitted to the PDB, and was assigned the accession code 2ghn. As discussed below, given the modest overall sequence homology and conserved backbone structure among the PLA2 family, a simpler modeling approach using a standard sequence alignment and limited template selection did not generate acceptable models.

4. Comparison of i-faces across the PLA2 family

To select residues that are likely to lie at the interface, we translated the corresponding residues of pGIB (from the anion-assisted dimer inter-residue contacts discussed above) according to the STAMP alignment (Fig. 4). This gave a selection of 19 residues for pGIB, hGIIA, and hGV, and (due to a gap in the alignment) 18 residues for hGX and sGIA. A list of these residues is shown in Table 1. Note that the numbering scheme used herein is continuous through the given PLA2, with no exceptions for gaps in the sequence alignment. The planar nature of the i-face can be seen in Fig. 5A, which displays the interfacial residues rendered as CPK colored by residue type, with the point of view parallel to the hypothetical bilayer. Rotation of the views shown in Fig. 5A by 90° about the x-axis yields a view of each enzyme (Fig. 5B), in which the point of view is perpendicular to the plane of the bilayer looking towards the i-face. There are limited numbers of charged residues on the interface, regardless of PLA2. This is in general agreement with findings from the anion-assisted dimer structure, in which inter-subunit contacts are mainly mediated by backbone amides and intervening water molecules as opposed to direct charge–charge interactions [11,40]. In what may be an indication of the conformational changes which occur upon interface binding, several proteins show residues that project from the plane of the i-face. This is quite evident in hGIIA, where both Phe-63 and Lys-115 are oriented towards the interface.

We had previously compared the electrostatic properties of the proposed i-faces of hGIIA and hGX [16]. Here we show electrostatic surfaces of all five PLA2s included in this comparison (Fig. 5C). It is immediately apparent that hGIIA is something of an abnormality, with a very cationic i-face. The high pl of hGIIA has been indicated in bacterial cell wall penetration, leading to the elevated antimicrobial activity of this enzyme [41]. The other PLA2s exhibit a more neutral electrostatic surface, with possibly a predilection towards anionic character in hGX and sGIA. Strikingly, pGIB, which is known to have a remarkably high affinity for anionic surfaces, does not exhibit a significant cationic surface charge. The comparison of PLA2 structures in Fig. 5C also demonstrates a clear variation of size and shape of the active site opening for each enzyme.

5. Contribution of electrostatic effects to interfacial binding

Electrostatic effects have long been considered crucial to interfacial recognition by PLA2s. The preference of many PLA2s for anionic surfaces, and the negative effects of adding salt to the reaction solution helped to verify this hypothesis [42]. However, site-directed mutagenesis studies based on charge neutralization have not always provided the expected results.

An experiment in which isosteric mutation of three cationic residues (K53, K56, and K120) in bovine GIB led to an increase in the affinity of the enzyme for the zwitterionic interface [22]. The structure of the triple mutant shows a large amount of sidechain reorganization at the interface. These results may not be applicable to other PLA2s due to the unique role of Lys-56 in the affinity of the enzyme [41]. The other PLA2s exhibit a more neutral electrostatic surfaces of all five PLA2s included in this comparison (Fig. 5C). It is immediately apparent that hGIIA is something of an abnormality, with a very cationic i-face. The high pl of hGIIA has been indicated in bacterial cell wall penetration, leading to the elevated antimicrobial activity of this enzyme [41]. The other PLA2s exhibit a more neutral electrostatic surface, with possibly a predilection towards anionic character in hGX and sGIA. Strikingly, pGIB, which is known to have a remarkably high affinity for anionic surfaces, does not exhibit a significant cationic surface charge. The comparison of PLA2 structures in Fig. 5C also demonstrates a clear variation of size and shape of the active site opening for each enzyme.

Table 1

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<th>i-face residues of PLA2 family members a</th>
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a The i-face residues of hGIIA, hGV, hGX, and sGIA were modeled based on the predicted i-face residues of the anion-assisted dimer structure of pGIB [11] (see text). These five structures were structurally aligned with chain A of the anion-assisted dimer of pGIB using the program STAMP [39]. The predicted interfacial residues listed are structurally aligned with inter-subunit contact residues of the anion-assisted dimer.

b Aligned PLA2 structures were taken from the protein data bank and the PDB accession code is given. In the case of hGV P2, the homology model presented in this work has been submitted to the PDB under the code 2ghn.
Fig. 5. A structural comparison of the i-faces of pGIb, hGIIA, hGV, hGX and sGIA PLA2. The PLA2 family members are compared in a side view (column A) and a view rendered following a 90° rotation (column B), which orients the i-face and active site opening to the viewer. The interface contacting residues were rendered in space filling CPK with non-polar residues in grey, polar in green, basic residues in blue and acidic residues in red. The models shown in columns A and B were rendered using MOLSCRIPT [60], POVSCRIPT [61] and POVRAY (www.povray.org). Column C of each PLA2 family depicts the electrostatic surface potentials of each enzyme's i-face. The active sites shown in the center of each structure have noticeably different sizes and shapes. The potential surface is displayed color coded onto a van der Waals surface using the program GRASP [63], where red and blue represent a net negative and positive charge, while white represents overall neutral positions, respectively. The overall GRASP electrostatic potential surface charge is displayed on a scale of (−6, 0, 6).

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Intriguing results have been obtained from simulations of hGIIA PLA2 at the interface [23,24]. These studies indicate that the overall desolvation is minor, occurring at a ring of hydrophobic residues surrounding the active site opening. We do indeed see a cluster of hydrophobic residues near the active site openings of the PLA2s shown in Fig. 5B. The overall electrostatic charge on the surface serves to orient the protein so that the active site opening is pointing towards the substrate layer, and to aid recruitment of the enzyme to anionic interfaces. In fact, charge-repulsion is seen for zwitterionic PC interfaces, indicating a possible mechanism for selection against activity on mammalian cell membranes. Following close approach to the interface and proper orientation, hydrophobic sidechains on the i-face are able to partition into the interface, completing interfacial binding and excluding water from the region surrounding the active site, presumably permitting diffusion of the substrate into the active site for catalysis. In an interesting observation that may shed light on some of the charge-neutralization mutants discussed above, Diraviyam and Murray [23] note that the positive nature of hGIIA may lead to significant pK\textsubscript{a} shifts or reorientation for glutamates near the regions of desolvation, while those farther out on the periphery will not be greatly effected. It is also unclear how well these results would translate to more neutral PLA2s, but the authors note that a decrease in repulsion would allow energetic effects from hydrophobic residue insertion to dominate. Another simulation [25], in which “partially hydrolyzed” bilayers were modeled indicate that the electrostatic potential at the interface is complicated, and that it is likely to change during the course of hydrolysis.

6. Contribution of aromatic residues to interfacial binding

Recently, an important role for aromatic residues, particularly tryptophan, has been postulated for some PLA2 family members [40,45–47]. Specifically, those proteins which are known to bind well to zwitterionic surfaces (such as cobra venom sGIA PLA2s), tend to have one or more tryptophans located on their interfacial surfaces. Indeed, the sGIA from Naja naja atra included in this review has two Trp residues included in the i-face subset. The preference of aromatic residues to reside in the region of the lipid headgroup appears to be a general property of both integral and interfacial membrane proteins [48,49]. Studies of tryptophan analogs have found that a balance between overall hydrophobicity and unfavorable entropy for membrane insertion causes these residues to insert only to the glycerol region of the bilayer [50,51], as opposed to the interpolation between the hydrocarbon chains of the lipids. The energy gained for partitioning of tryptophan into cyclohexane is on the order of 5 kcal/mol [51], which is slightly less than the energetic contribution of electrostatic effects for PLA2s calculated from molecular dynamics [23]. Studies on hGIIA in which one or more surface residues were mutated to tryptophan have generated variants that exhibit similar activity to hGV on DOPC vesicles [47]. In the Beers et al. paper, 8 single tryptophan mutants and a double mutant (V3,31W) were generated. All 8 of these residues are selected as interfacial according to the criteria we have used, although Beers et al. report significant alterations in catalytic activity on DOPC vesicles for only V3W, V31W, and the double mutant. It is unclear why only these mutations improved activity on DOPC vesicles, although it is intriguing that these are the only two valine residues defined as interfacial here.

The issue of surface aromatic residues and affinity for zwitterionic interfaces is somewhat confounded by hGX. This protein, the human enzyme which is most active on zwitterionic surfaces, has no interfacial tryptophan residues, and has the same number of interfacial aromatic residues as hGIIA. A report in which an expanded definition of the i-face was utilized determined that mutation of tryptophan 67 to alanine in hGX altered binding to DOPS/DOPC vesicles [46].

7. hGV model validation

Ours is not the first comparative model of group V PLA2 to be reported [23,45,52]. Previous models have typically been assembled by sidechain replacement or automated servers, based on a limited number of templates, and have not been subjected to extensive refinement. Our initial models, based on two templates and generated using a standard sequence alignment, generated conformations which at first analysis appeared to be adequate from the gross structural standpoint. Only upon attempted model refinement (via energy minimization or simulated annealing) did issues arise, namely the collapse of the substrate binding pocket opening on the enzyme surface. Because of this, we chose to utilize a structural sequence alignment, as well as additional template structures, to generate a more robust model. This energy-minimized model has been described and analyzed in this review. To test the limits of model stability, we performed a series of slow-cooling simulated annealing steps using the program CNS [38], from 500 K. Annealing from 500 K generated a structure very similar to that of the minimized model, with a very minor reorganization of some sidechains around the active site opening. The overall RMSD between the annealed and minimized models was 1.67 Å.

Overall, the electrostatic surface appears as one would anticipate for a protein which is active on zwitterionic surfaces—mainly neutral, with scattered charges in peripheral regions. The active site opening is relatively large, as might be presumed for a protein which must accommodate the bulky headgroup of phosphatidyl choline, or an arachidonyl group at the sn-2 position, as would be anticipated for an enzyme involved in arachidonic acid signaling [12,53,54]. It is important to note, however, that this is a homology model, and that structural differences are almost sure to be found once high-resolution data are collected. It is also relevant that PLA2 enzymes show little headgroup specificity once bound to the interface, indicating that active site conformations do not play a large role in substrate specificity.

In addition to in silico validation, we can examine the agreement of this model with reported data from the literature. A recent inhibition study [52] provides data for a library of compounds screened against a range of PLA2s, which includes...
many of those discussed here (with the exception of sGIA). One key residue for Me-Indoxam binding seems to be Phe/Leu-5. The authors postulate that, for several of the PLA2s, Phe-5 can form a favorable interaction with the indole moiety of the inhibitor. hGX, for which Me-Indoxam and its derivatives are relatively poor inhibitors, contains a leucine at this position. hGV also contains a Leu-5, but tends to be well inhibited by Me-Indoxam and derivatives. A visual inspection of our model for hGV versus the structure of hGX reveals that the hGV Leu-5 side chain is in a slightly different conformation, and does not protrude so far into the active site channel. This may alleviate any unfavorable interactions. Smart et al. [52] also mention Trp-29 of hGV as a candidate for potential interaction with Me-Indoxam. In our model of hGV, this residue is oriented towards the substrate binding pocket, with the edge of the ring system pointing towards the pocket itself. This is consistent with the hypothesis of Smart et al. [52] for inhibition of hGV by their inhibitor library. In the final analysis, of course, there is simply no substitute for a high-resolution structure of the protein.

Even for the PLA2 enzymes, where the backbone fold is highly conserved, and multiple templates are available, it is clear that there are still ambivalences in the modeling process. Specifically, the overall conservation of the backbone fold means that alignment and side chain placement are of the utmost importance. Because of the level of homology involved, standard pairwise alignments of the PLA2 enzymes are not satisfactory. The need for proper side chain replacement is alleviated by the inclusion of multiple templates, which contribute similar residues for much of the model sequence, minimizing any rotamer searching. Finally, the model must be refined, as even minor fluctuations in side chain placement may have significant effects on the properties of the model. In general, structures are well conserved in the absence of high sequence homology [55]. In these cases, it is vital that structure-based alignments are used for generating the modeling input, and that as many templates as possible are included [56].

8. Conclusions

Overall we find that previous hypotheses describing the complementary roles of electrostatic and hydrophobic interactions involved in binding to the interface are well documented. It appears that initial approach and orientation of the enzyme is guided largely by electrostatic effects, particularly in the case of hGIIA. Other, more complex interactions may also play a vital role, including binding to heparin sulfate proteoglycans or other receptors [57]. The precise nature and extent of these interactions is under investigation [58]. Upon close contact with the interface, desolvation and aromatic residue insertion exert more influence. This allows for the tight association of membrane and interface which permits the substrate to diffuse into the active site.

One of the more intriguing aspects of the PLA2 family is that in spite of a highly conserved tertiary fold, there is a fair amount of sequence flexibility and very divergent roles in vivo. All of these enzymes catalyze the hydrolysis of the sn-2 ester bond of glycerophospholipids, and it is known that family members can often act on multiple species of lipid with similar activity. The apparent rate and in vivo activity, however, are very much regulated by the affinity of the enzyme for the interface. Based on the conservation seen in the active site residues, it seems that selective inhibitors for PLA2s will need to interact specifically with the substrate channel or the i-face of the protein. This approach has recently lead to a major breakthrough with the design of a selective and tight binding inhibitor of hGX [59].

An examination of Table 2 shows that while sequence identities among PLA2s is modest (33–56%), the sequence similarities and mainchain conformations are quite conserved. The least identical pair (hGX and sGIA, 33%) show 88% similarity and a 1.14 Å backbone RMSD. These characteristics indicate that overall fold and general chemical properties are necessary for proper function, and that differences in interfacial affinity and activity are controlled by protein-specific sidechain variability, interactions, and properties. As shown in Fig. 4, many of the interfacial residues (indicated by asterisks) are in structurally conserved regions (boxes in figure), but are not highly conserved in sequence across the family.

There are several factors that hinder a fuller understanding of interfacial interactions. Namely these are obtaining structural analogs of proteins associated to interfaces and characterizing the complicated electrostatic environments of both the protein and bilayer. The mutagenesis studies reviewed here indicate that for many PLA2s, no one residue is the linchpin for interfacial recognition, although aromatic residues do impart affinity for zwitterionic surfaces. Instead, it seems that the overall properties of both enzyme and interface are the driving force behind association, and ultimately in control of PLA2 function.

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References


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