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Review Article

Computational and theoretical approaches for studies of a lipid recognition protein on biological membranes

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Many cellular functions, including cell signaling and related events, are regulated by the association of peripheral membrane proteins (PMPs) with biological membranes containing anionic lipids, e.g., phosphatidylinositol phosphate (PIP). This association is often mediated by lipid recognition modules present in many PMPs. Here, I summarize computational and theoretical approaches to investigate the molecular details of the interactions and dynamics of a lipid recognition module, the pleckstrin homology (PH) domain, on biological membranes. Multiscale molecular dynamics simulations using combinations of atomistic and coarse-grained models yielded results comparable to those of actual experiments and could be used to elucidate the molecular mechanisms of the formation of protein/lipid complexes on membrane surfaces, which are often difficult to obtain using experimental techniques. Simulations revealed some modes of membrane localization and interactions of PH domains with membranes in addition to the canonical binding mode. In the last part of this review, I address the dynamics of PH domains on the membrane surface. Local PIP clusters formed around the proteins exhibit anomalous fluctuations. This dynamic change in protein-lipid interactions cause temporally fluctuating diffusivity of proteins, i.e., the short-term diffusivity of the bound protein changes substantially with time, and may in turn contribute to the formation/dissolution of protein complexes in membranes.

Key words: Peripheral membrane protein, pleckstrin homology domain, phosphatidylinositol phosphate, protein-lipid interaction, molecular dynamics simulation

Many trafficking and signaling events within cells are triggered by the association of peripheral membrane proteins (PMPs) with biological membranes [1,2]. During this process, both protein-lipid and protein-protein interactions induce spatiotemporal macromolecular crowding and complexity and thus influence the diffusion and interaction of biomolecules in the membranes [3,4]. A variety of proteins transiently assemble to specific locations and then exert their functions via colocalization with partners [5,6].

The association of PMPs on membrane surfaces often requires lipid-binding modules, e.g., the pleckstrin homology (PH) domain [1,2]. PH domains are an extensively studied, structurally conserved family and an important class of membrane recognition domains [7] that bind to specific lipids, i.e., phosphatidylinositol phosphates (PIPs) [6,8], in biological membranes. PH domains consist of 120 residues with an antiparallel β sheet architecture followed by one or two amphipathic α helices [5]. The majority of PH domains have a KXn(K/R)XR motif in the loop connecting strands β1 and β2. This positively charged sequence has been shown to reg-

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◀ *Significance* ▶

Association of peripheral membrane proteins (PMPs) on cell membranes are crucial for maintaining cellular functions. This association is triggered by the lipid recognition modules, e.g. pleckstrin homology (PH) domains, within the PMPs. Here I review recent progress of computational and theoretical approaches for investigating the molecular details of interaction and dynamics of PH domains on biological membranes. These approaches can be applicable for other classes of PMPs that interact with specific lipids.

ulate the contacts of the PH domains with different types of PIPs. To date, the structures of many PH domains have been determined (~150 structures), and these structures often do not contain bound lipid molecules. Even when these structures have a bound lipid molecule, it is only the head group of bound inositolphosphates (InsPs, i.e., the PIP headgroup). Moreover, although the structures and membrane interactions of different PH domains have been studied [9], these studies have not provided direct indications of their exact position and orientation on the membrane surface. Additionally, binding of PH domains to membranes may be also coupled with conformational changes within the protein and partial penetration of the protein into the membranes. Thus, it remains challenging to elucidate the molecular details of their interactions with lipid molecules and their dynamics on membranes by experimental approaches.

Molecular dynamics (MD) simulations can be useful as a computational microscope [10], enabling the user to switch from "zooming in" to atomic resolution for examination of detailed molecular interactions to "zooming out" to lower resolution to address the dynamic phenomena for large-scale systems with longer timescales using coarse-grained (CG) models [11,12]. Here, I summarize recent progress in computational and theoretical approaches for studying the interactions and dynamics of PH domains on biological membranes, which can be extended to other classes of PMPs. I first discuss multiscale MD simulations for determining the mode of interaction of PH domains with PIP-containing lipid membranes. I then evaluate the dynamic behaviors of PH domains on the membrane surface, explaining how the diffusivity of the protein changes with respect to time, dependent on protein-lipid interactions.

Association of PH domains with membranes

Atomistic MD (AT-MD) simulations have been used to study the association of key lipid recognition domains, e.g., BAR [13], C1 [14], C2 [15], FYVE [16], PX [16], and PH domains [17,18], with lipid membranes and the GLA domain with membrane mimetic models [19]. Detailed analyses can be conducted for the proteins, e.g., penetration into the membranes, conformational fluctuation, and specific molecular interactions. However, for the AT-MD simulations, it is rather difficult to investigate the complete description of the interaction of PMPs with biological membranes due to the underlying slow dynamics of proteins, e.g., diffusion, rotation, and conformational changes. The longer time scale of the position exchange of lipids even in the pure lipid membranes [20] also causes difficulties with regard to sufficient sampling of protein-lipid interactions. In addition, these approaches require a degree of prior knowledge of the nature of the lipid recognition region of the protein and/or of the orientation and position of the protein relative to the membrane surface.

To overcome these limitations of AT-MD simulations, the use of coarse-grained (CG) models is a powerful and flexible approach for a broad range of biological systems [12,21], e.g., lipid self-assembly and deformation, diffusion of biomolecules, and protein-lipid and protein-protein interactions. In CG models, to reduce computational costs rather than accuracy at the atomistic level, an average of four heavy atoms plus associated hydrogens are represented by a single CG particle. Large integration time steps can be used because certain degrees of freedom of light mass atoms are not explicitly considered. This simplification smooths the underlying free energy landscape. Currently, using CG models can achieve observable time scales of micro- to milliseconds depending on the system size [10,22], whereas AT-MD simulations enable simulations of the nano- to microsecond time scale for the system with the same length scale. To date, CG-MD simulations have been applied for lipid binding proteins, e.g., BAR [23], talin [24], and phosphatase and tensin homolog (PTEN) [25], and provide good results compared with experimental observations. Thus, the combined use of AT-MD and CG-MD simulations depending on the time and length scales can be used to elucidate the detailed interactions of PMPs on the membrane surface. With extended AT-MD simulations using typical configurations from CG-MDs as initial configurations, detailed analyses and verification of the accuracy of CG-MDs can be performed. Note that a higher level of coarse-graining is needed to study the mesoscopic features of membrane deformation and tubulation by PMPs [26,27].

Recently, high-throughput multiscale MD simulation was conducted for 13 different PH domains which have bound InsPs within the structures [28] (see Fig. 1); GRP1 [29], ARNO [30], PLC-δ1 [31], DAPP1 [32], PDK1 [33], PEPP1, PKB/Akt [34], C-PH [35], Kindlin-2 [36], and Btk [37] PH domains that do have the KXn(K/R)XR motif (canonical PIP-binding site); ArhGAP9 [38] and β-spectrin [39] PH domains that do not have the KXn(K/R)XR motif (noncanonical PIP-binding site); and the ASAP1 PH domain [40] that is proposed to have both canonical and non-canonical PIP-binding sites. The results of the CG-MD simulations for the PH/PIP interactions agree well with both experimental measurements and more detailed AT-MD simulations. Interestingly, in 83% of the final membrane complexes obtained by the CG-MD simulations (13 PH domains), a PIP molecule binds to the same binding site suggested by the PH/InsP complexes obtained by nuclear magnetic resonance (NMR) or X-ray crystallography [28] (see Fig. 1D). The preferred orientation of the PH domains relative to the membranes was determined by calculation of the density landscapes (see Fig. 1B). Analysis of the contacts between the PH domains and the PIPs suggests that all PH domains associate with the membrane via the positively charged loop connecting the β1 and β2 strands (see Fig. 1C). This is consistent with the elimination of their interactions with PIP molecules by mutations in the β1/β2 loop residues. In CG-MD simulations, the association of PH domains with PIPs decreases with a single
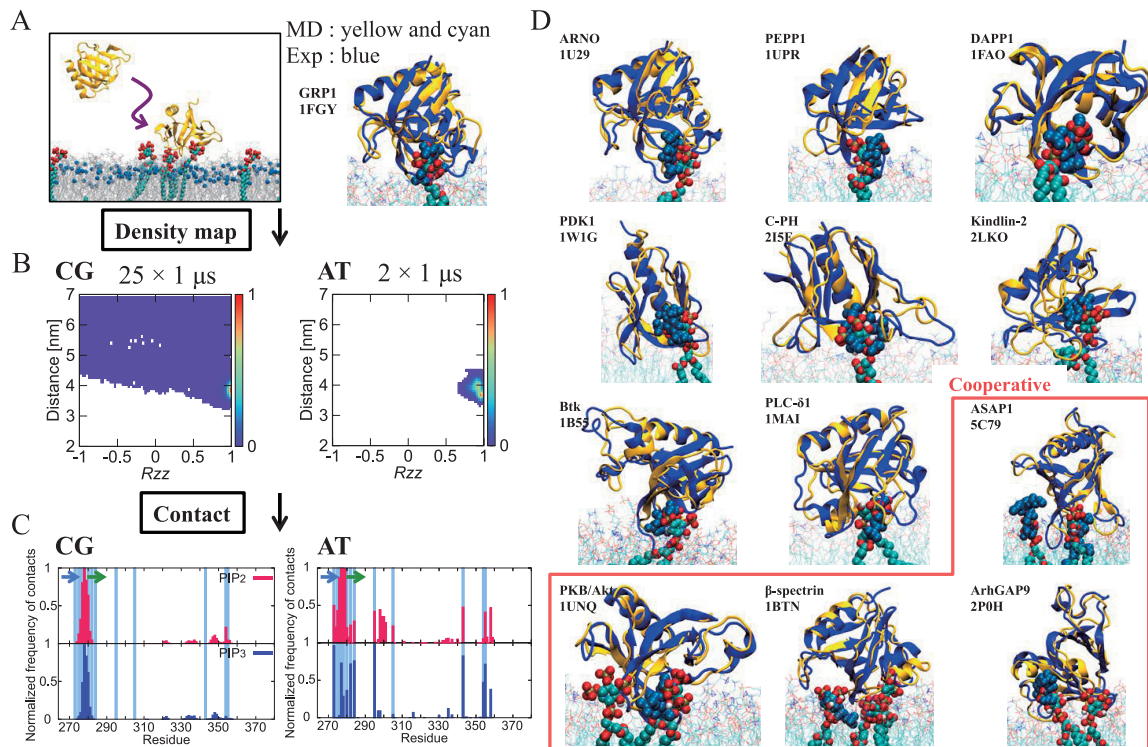
Figure 1 The PH domain/membrane simulation pipeline for a variety of PH domains [28]. (A) Snapshot of a selected simulation demonstrating the localization of the general receptor of phosphoinositides 1 (GRP1) PH domain to the lipid bilayer. Alignment of the PH/PIP complexes derived from the simulation approach (with PH domains in yellow and PIP molecules in cyan/red/bronze/silver) with the corresponding crystal structures (PH domains and PIP both in blue). These complexes were obtained from the maxima in the density maps shown in (B). (B) Normalized density map of the GRP1 PH domain (*zz* component of rotational matrix versus distance between a PH domain and the lipid membrane). The ensemble used for the calculation are 25 1 µs for CG-MD and 2 1 µs for AT-MD. (C) Normalized average number of contacts between the GRP1 PH domain and PIPs. The light blue colors represent the experimental contacts observed in the crystal structure. (D) PH/PIP complexes derived from simulation.

mutation within the protein [28,41]. Note that still unclear whether the full-length proteins may alter the associations of the PH domains.

In addition to either canonical or noncanonical lipid-binding sites, the simulation approach enables researchers to identify secondary lipid-binding sites on PH domains. Identification of cooperatively bound PIP on the ASAP1 PH domain is in good agreement with a previous crystallographic study [40] (see Fig. 1D). Two PIP-binding sites have also been identified for PKB/Akt, β-spectrirn, ArhGap9, and Dok7 PH domains, as expected from the structural data [28,42]. This is due to the fact that these PH domains lack a KXn(K/R)XR motif which is found in other PH domains [38,43]. Dissociation of the PIP lipid from the noncanonical site results in reorientation of the protein [28]. Thus, such interactions may regulate the orientation of the PMPs on the membrane surfaces. Multiple lipid interactions are crucial for the binding of PH domains to membranes.

However, approximations implied in the CG models make it difficult to analyze the specificity of the PH domains for different PIP species [44–47]. Notably, during extended AT-MD simulations of a few microseconds, PIP lipid interactions with the PH domains were generally retained. This suggests that the AT-MD simulations remain too short for direct analysis of the specificity for different species of PIPs.

Calculation of potential of mean force (PMF) allows us to study the affinity of PIP molecules with PH domains [41,48]; however, the umbrella sampling method for PMF calculations depends on the initial configuration and does not sample all states on the membrane surface. Alternatively, the replica-exchange umbrella sampling method may be feasible for assessing the convergence of the simulation toward representative equilibrium sampling [49].

PIP cluster and diffusive dynamics of PH domains on membrane surfaces

Investigation of the dynamic behavior of the PH domain on the membrane surfaces can be addressed using CG-MDs. In addition to a bound PIP in the binding site, PIPs form cluster around PH domains [50,51] (see Fig. 2). The fluctuation in the cluster size exhibits an enigmatic $1/f$ noise in the power spectra, $S(f) \propto f^{-\beta}$ with a power-law exponent $\beta = 1.46$. The $1/f$ noise reflects the underlying long-term correlated motion of protein-lipid interactions, in which lipids exhibit transient subdiffusion originating from the viscoelasticity
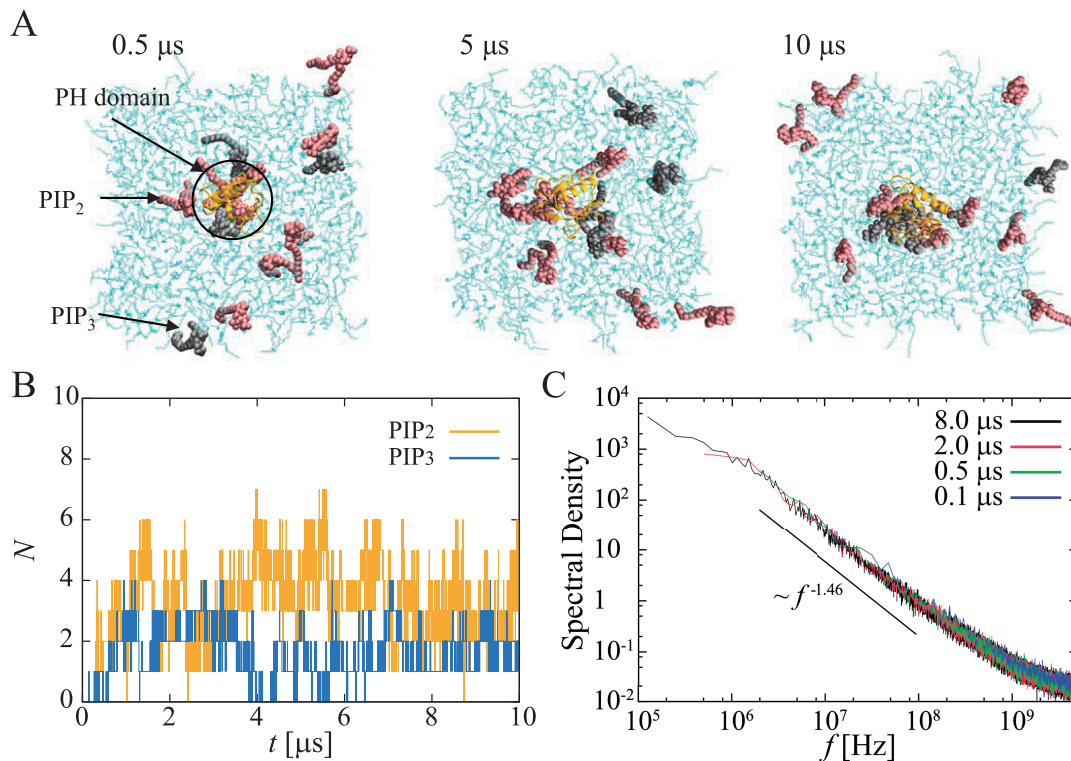
Figure 2 Cluster of PIP lipids around PH domains [51]. (A) Snapshots of the membrane-bound DAPP1 PH domain. The leaflet (cyan) where the PH domain binds is shown. PIP_2 and PIP_3 lipids are shown as pink and gray, respectively. (B) Time series of the number of PIP_2 and PIP_3 molecules around the PH domain. (C) Ensemble-averaged PSDs of the number of PIP_3 molecules around the PH domain. The different colored lines represent different measurement times, and their power spectra coincide without fitting.

[52–55]. To determine the biological significance of the observed $1/f$ noise, a simple theoretical model was proposed in which the dynamic processes reflected in $1/f$ noise may help to stabilize the binding of PH domains by increasing the residence times of the protein on the membrane surfaces [51].

The clustering of PIPs also affects the diffusivity of PH domains on the membrane surfaces. From the trajectory $\vec{r}(t)$ of a single molecule, the diffusivity is often characterized by the time-averaged mean square displacement (TAMSD),

$$\overline{\delta^2(\Delta; t)} = \frac{1}{t-\Delta} \int_0^{t-\Delta} [\vec{r}(t'+\Delta) - \vec{r}(t')]^2 dt', \quad (1)$$

where $\Delta(\ll t)$ is the lag time. In simple diffusion processes, the TAMSD increases linearly, $\overline{\delta^2(\Delta; t)} \sim 2dD\Delta$, where d is the dimension and D is the diffusion coefficient. Using the single particle tracking technique, diffusion constants of PMPs on various lipid membrane surfaces have been measured [56–59]. Tandem domain formations of proteins, e.g., PH domains [57] and C2 domains [60], slow down the diffusivity of the proteins. In MD simulations, the TAMSDs of PH domains on a PIP-containing membrane surface exhibit transient subdiffusion, i.e., $\overline{\delta^2(\Delta; t)} \propto \Delta^{\alpha}$ with an exponent $\alpha \approx 0.7$ for shorter lag times, switching to $\overline{\delta^2(\Delta; t)} \propto \Delta$ for longer lag times (see Fig. 3) [61]. Although some TAMSDs

exhibit subdiffusion after the transition time due to the specificity of trajectories by finite time measurement (crossover will appear if the measurement time is increased), the crossover in the mean TAMSD is clear around 10 ns. The crossover point of the exponent α around 10 ns corresponds to the crossover point of the transient subdiffusion of lipids within pure lipid bilayers [52–55]. Subdiffusive motions have also been observed both in experiments and simulations, e.g., diffusion of transmembrane proteins within membranes [62–64] and of water molecules on membrane surfaces [65].

The PH domains on the PIP-containing membrane show two diffusive properties [61]. One is correlated motions relevant to fractional Brownian motion (FBM) [66,67], attributed to the motion of the lipids with which the PH domain interacts [52–55]. The other is temporally fluctuating diffusivity, i.e., the short-term diffusivity of the protein changes substantially with time. This is completely different from the ergodic diffusion process, e.g., Brownian motion, in which there would be no intrinsic differences between diffusivities for short- and long-term measurements. This substantial fluctuation of the diffusivity originates from protein-lipid interactions. The diffusivity of the protein when more PIPs are bound is lower than that when fewer PIPs are bound (see Fig. 3). Moreover, the diffusion process is well described by a fluctuating diffusivity model, called the
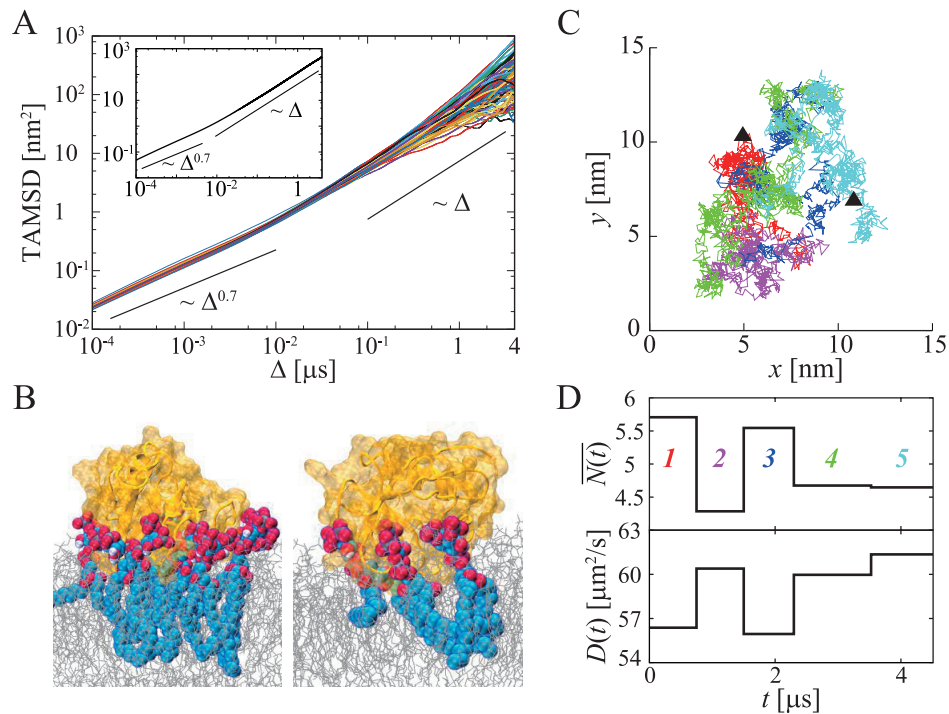
Figure 3 Diffusion process of the DAPP1 PH domain [61]. (A) The TAMSDs of 97 trajectories of the PH domain on the membrane surface. The measurement time for each trajectory t is 8 µs. The inset shows the mean of TAMSDs. (B) Snapshots of the PH domain in the many PIP bound state (left) and few PIP bound state (right). The PH domain, lipid bilayer, and bound PIP are colored yellow, silver, and cyan/red, respectively. (C) Lateral trajectory of PH domain on the membrane surface. Colors of the trajectory correspond to each state in (D). The black triangles indicate the start and end points. (D) Time series of the short-time diffusivity and the time-averaged number of bound PIPs in each diffusive state.

Langevin equation with fluctuating diffusivity (LEFD) model [68–70]. These results suggest that lipid molecules not only act as anchors for lipid recognition proteins, but may also regulate their diffusivity and thus potentially modulate their interactions with other proteins and receptors. Such heterogeneous diffusion induced by protein-lipid interactions may be a universal property for other classes of PMPs that interact with specific lipids.

Conclusion

Here, I have provided a review of the recent computational and theoretical understandings of the association of PH domains with biological membranes. Combination of CG-MD and AT-MD simulations can provide insights into the specific molecular mechanisms of the formation of protein/bilayer complexes and diffusion of proteins on membrane surfaces, which are often difficult to obtain using experimental techniques. Given the progress in computational approaches that show good agreement with experiments, it is anticipated that these approaches will be extended systematically to the studies of other family of PMPs with biological membranes. Moreover, it is important to reveal the molecular interactions and diffusion in more biologically realistic systems, including various lipids, transmembrane proteins, and PMPs.

Cell membranes are spatiotemporally inhomogeneous environments as a consequence of the formation of lipid domains, the crowding of a variety of lipid and protein species, and interactions with cytoskeletal components of the cell [71]. The electrostatic protein-lipid interactions and effects of ions reorganize the local lipid environment creating PIP-enriched microdomains [72–74]. Such heterogeneity determines the diffusive nature of PMPs on membrane surfaces. The diffusion of PMPs and changes in their local lipid environment may then contribute to the dynamics of the formation/dissolution of signaling complexes and/or the recruitment/detachment of other PMPs and integral membrane proteins to the specific locations. In addition, dissociation from the membrane allows PMPs to explore large areas in a short time [75]. These results may contribute to an enhanced probability of encountering target complexes on cell membrane surfaces, as theoretically shown to be intermittent search strategies observed in a variety of biological processes [76,77].

Acknowledgements

Conflict of Interest

The author declares no conflicts of interest.

Author Contributions

E. Y. wrote the manuscript.

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