BIOPHYSICS

Dynamic interactions between a membrane binding protein and lipids induce fluctuating diffusivity

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Pleckstrin homology (PH) domains are membrane-binding lipid recognition proteins that interact with phosphatidylinositol phosphate (PIP) molecules in eukaryotic cell membranes. Diffusion of PH domains plays a critical role in biological reactions on membrane surfaces. Although diffusivity can be estimated by long-time measurements, it lacks information on the short-time diffusive nature. We reveal two diffusive properties of a PH domain bound to the surface of a PIP-containing membrane using molecular dynamics simulations. One is fractional Brownian motion, attributed to the motion of the lipids with which the PH domain interacts. The other is temporally fluctuating diffusivity: that is, the short-time diffusivity of the bound protein changes substantially with time. Moreover, the diffusivity for short-time measurements is intrinsically different from that for long-time measurements. This fluctuating diffusivity results from dynamic changes in interactions between the PH domain and PIP molecules. Our results provide evidence that the complexity of protein-lipid interactions plays a crucial role in the diffusion of proteins on biological membrane surfaces. Changes in the diffusivity of PH domains and related membrane-bound proteins may in turn contribute to the formation/dissolution of protein complexes in membranes.

INTRODUCTION

Cell membranes provide a unique and complex environment for biological reactions, in which both protein-lipid and protein-protein interactions within the membranes play a key role (1–3). Diffusion of biomolecules within membranes is crucial for regulating many aspects of cell function. Macromolecular complexity and crowding cause spatiotemporal heterogeneity and thus influence the diffusion process in cell membrane environments.

Peripheral membrane proteins are present within the cytoplasm of cells and associate with cell membrane surfaces in a lipid-dependent fashion. They play key roles in many trafficking and signaling events within cells. Association of peripheral proteins on membrane surfaces is determined by lipid-binding modules, of which the pleckstrin homology (PH) domains are a well-studied family. PH domains are a structurally conserved family of proteins that bind to specific lipids [phosphatidylinositol phosphates (PIPs)] that are present in cell membranes (4, 5). Although structures and membrane interactions have been studied for different PH domains (6–8), understanding the diffusive behavior of PH domains bound to a cell membrane surface remains challenging (9–11). Recently, a number of studies have suggested that PIP molecules cluster around membrane-bound peripheral proteins (12, 13). This clustering may affect the diffusivity of peripheral proteins on the membrane surfaces and is thus likely to play a role in regulating their function.

Using single-particle tracking techniques, one can obtain the trajectories of biomolecules. Diffusion is often characterized by the time-averaged mean square displacements (TAMSDs)

\[
\overline{\delta^2(\Delta; t)} = \frac{1}{t - \Delta} \int_0^{t-\Delta} \left[ \bar{r}(t' + \Delta) - \bar{r}(t') \right]^2 dt'
\]

where \(\bar{r}(t)\) and \(\Delta\) are the position of the tracked particle and the lag time, respectively. In simple diffusion processes, diffusivity is characterized by the slope of the TAMSD for long-time measurements, that is, \(\delta^2(\Delta; t) \sim 2d\Delta\), where \(d\) is the dimension and \(D\) is the diffusion coefficient. In this case, the diffusion coefficient is uniquely determined depending on the viscosity of the medium and/or the shape of the Brownian particle. However, in living cells, proteins can change their shapes, and properties of the surrounding environments change with time. Therefore, the diffusivity obtained by long-time measurements fails to capture the short-time diffusivity, defined as the diffusivity obtained by short-time measurements, which is considered to be intrinsically fluctuating under diffusion processes. Moreover, anomalous diffusion, seen as a sublinear time dependence of TAMSDs, is not unusual but rather is ubiquitously observed for both proteins in cell membranes (14, 15) and, for example, mRNA (16), chromosomal loci (17), lipid granules (18), and insulin granules (19) within cells. Moreover, using molecular dynamics (MD) simulations, subdiffusive motions have also been observed in the diffusion of lipids (20–23), of transmembrane proteins (24), and of water molecules at the surface of membranes (25).

Various stochastic models of anomalous diffusion have been proposed to interpret the physical origin of the diffusion process on the assumption that the environment is homogeneous (26, 27). However, this assumption is unlikely to be valid in a cell membrane that presents a heterogeneous environment (3). Furthermore, the quenched trap model, that is, diffusion on a random energy landscape, provides a rich behavior that is different from that of a homogeneous environment (28, 29). Revealing the origins of diffusion is important because it will allow us to understand the physical properties of a range of processes, for example, viscoelasticity with crowding of macromolecules (16, 18, 20–23, 25), transient immobilization in the presence of obstacles with heavy-tailed trapping (14, 19, 25), and jamming in the presence of obstacles (14, 19). Diffusivity may also change temporally because of changes in the surrounding environment or because of dynamically fluctuating shapes of biomolecules (30, 31). Stochastic models for heterogeneous diffusion processes, in which diffusivity is a spatiotemporally random quantity, have been developed recently in order to interpret the anomalous diffusion of biomolecules in heterogenous
crowding environments [for example, spatial heterogeneity (32) and temporal heterogeneity (33–38)].

Here, using MD simulations, we investigate the diffusive behavior of the DAPP1 PH domain on a lipid membrane surface, exploring how the diffusivity of the protein changes with respect to time. Moreover, we show that the fluctuating diffusivity of the bound protein arises from the underlying protein-lipid interactions, which, in turn, dynamically change in time, and that the process is ergodic.

RESULTS
Simulations of PH domain interactions with a membrane
To investigate the diffusion process of a peripheral protein on a membrane surface, we performed coarse-grained MD (CG-MD) simulations (39) of the DAPP1 PH domain interacting with a PIP-containing lipid bilayer membrane. In the initial configuration of each simulation, the PH domain was displaced ca. 9 nm away from the lipid bilayer surface. One hundred simulations were run, each for 10 μs from different initial orientations of the PH domain relative to the bilayer, thus yielding a total simulation time of 1 ms. We tracked the protein on the membrane surface and analyzed PH domain diffusion for the last 8 μs of each trajectory for which the protein was bound to the membrane (see fig. S1). Note that 3 trajectories were removed from the initial ensemble of 100 as the protein molecule, having bound, subsequently dissociated from the bilayer. Additionally, we subtracted the center of mass (COM) of the associated bilayer leaflet from the trajectories of the protein to remove effects of COM motion of the membrane as a whole (20, 22, 24, 25). The TAMSD of the PH domain on the lipid membrane surface exhibits transient subdiffusion, that is, \( \delta^2(\Delta; t) \propto \Delta^a \) for shorter lag times, switching to \( \delta^2(\Delta; t) \propto \Delta \) for longer lag times (see Fig. 1). The power-law exponent \( a \) changes from 0.7 to 1.0 at a crossover point around 10 ns, which corresponds to the crossover point for anomalous diffusion of lipids within pure lipid bilayers (20–24). Note that the diffusion coefficient is of the same order of magnitude as the experimentally measured diffusion coefficient of the GRP1 PH domain (9, 10, 13). Similar transient subdiffusion is also observed for other PH domains (13). Moreover, we confirmed that there is no aging of TAMSDs, that is, \( \langle \delta^2(\Delta; t) \rangle = \text{const.} \), and that there is ergodicity of the diffusion process, that is, the ensemble-averaged MSD is consistent with the TAMSD (see fig. S2).

Anticorrelated motion of the PH domain over shorter time scales
The diffusive properties of lipids are known to show correlated motions relevant to fractional Brownian motion (FBM) (20–23). The correlated motions of lipids were also shown to affect the dynamics of interfacial water molecules on the membrane surface (25, 40). To investigate the impact of the FBM of the lipid molecules on the PH domain, we calculated the displacement autocorrelation function (DAF) of the protein

\[
C_A(t) = \langle (x(t + \Delta) - x(t))(x(\Delta) - x(0)) \rangle / \Delta^2
\]

Figure 2A shows the normalized DAF \( C_A(t)/C_A(0) \) for \( \Delta = 0.1 \) and 2 ns. The DAF of free FBM decays from negative values to zero via a power law (41), \( C_A(t)/C_A(0) \sim - (\alpha - \alpha^2)(\Delta/t)^2 - \alpha^2/2 \). The normalized DAF for \( \Delta = 0.1 \) ns agrees well with the theoretical behavior of free FBM with \( \alpha = 0.7 \) (41). Although the normalized DAF for \( \Delta = 2 \) ns is not consistent with FBM, we can clearly see a cutoff around 10 ns, which implies that TAMSDs correspond to normal diffusion for \( \Delta > 10 \) ns. The behavior of the protein exhibits anticorrelation comparable to the anticorrelated motions of lipids (20–23). Thus, the diffusion process of the peripheral PH protein on the membrane surface at shorter time regions is affected by the FBM of lipids with which it interacts.

To confirm whether the diffusive features are well described by the FBM, we examined the Gaussianity of the displacement. In particular, we calculated the propagator, that is, the probability that a particle is...
found in \((x, x + dx)\) at the lag time \(\Delta\). However, the normalized propagator \(P(x, \Delta)\) has a non-Gaussian shape (see Fig. 2B). Therefore, the anomalous diffusion of the PH domain may be accounted for by the coexistence with other diffusive properties.

**Fluctuating diffusivity of the PH domain**

Interactions between the protein and PIP molecules in the bilayer are crucial for the localization of the PH domain on the membrane surface. As shown in Fig. 3A, the number of PIP molecules interacting with a bound PH domain changes with time. These dynamic interactions are expected to affect the diffusivity of the protein because the diffusivity crucially depends on the properties of the PH/PIP complex, including the number of PIPs present in this complex. In other words, the short-time diffusivity (corresponding to the short-term, that is, sub-nanosecond, subdiffusion regime) may change with time as the number of bound PIPs changes. To better characterize the diffusive behavior of the PH domain, we propose a new method to estimate the short-time diffusivity. To the best of our knowledge, there is currently no method to estimate the short-time diffusivity without knowing the times at which diffusivity changes substantially. Using our estimation method (see Materials and Methods for more details), we can detect variations of the short-time diffusivity as a function of time in a trajectory (see Fig. 3B). Here, we obtained five different diffusive states over the duration of the simulation. Thus, we have successfully detected the fluctuating diffusivity of the PH domain. To investigate the effect of clustering of PIPs about the PH domain on the diffusivity of the protein, we calculated the number of PIPs bound by the PH domain in each diffusive state (see Fig. 3B). We then examined the correlation between the time-dependent diffusivity \([D(t)]\) and the number of bound PIPs \([N(t)]\) across the whole ensemble of 97 simulations analyzed [see Fig. 3C; the probability density functions (PDFs) of \(D(t)\) and \(N(t)\) are shown in fig. S3]. It is evident that the diffusivity of the protein is lower when more PIPs are bound than when fewer PIPs are bound. There is a negative correlation (correlation coefficient = −0.42) between the short-time diffusivity and the number of bound PIP molecules. In Fig. 3D, we show the typical trajectory of the PH domain bound to the membrane surface corresponding to Fig. 3B. Although the PH domain diffuses in the same place, the short-time diffusivity differs. This means that the short-time diffusivity of the PH domain undergoes a temporal fluctuation depending not on spatial heterogeneity but instead on temporal changes in the number of bound PIP molecules.

**Heterogeneous diffusion with fluctuating diffusivity**

If a system is non-ergodic and/or the time average is not taken for a sufficiently long time period, the TAMSD does not coincide with the MSD. In this case, the TAMSD exhibits broad scattering. The magnitude of the fluctuations of the TAMSD can be quantified by the relative standard deviation (RSD) \((36–38, 42–44)\)

\[
R(t;\Delta) = \sqrt{\frac{\langle \delta^2(\Delta; t) \rangle^2}{\langle \delta^2(\Delta; t) \rangle^2}}
\]

In the case of non-ergodic diffusion processes, for example, the continuous-time random walk (42–44) and annealed transit time models (35), the RSD of TAMSDs converges to a nonzero value for all \(\Delta \ll t\) as \(t \to \infty\). This is totally different from ergodic diffusion processes, for example, Brownian motion and FBM in the subdiffusion case (45), for which the RSD converges to 0 with a power-law form \(t^{-0.5}\). In this case, there would be no intrinsic differences between diffusivities for short-time measurements and for long-time measurements. In other words, fluctuations of the TAMSD come from the finite measurement times. However, the difference from the scaling \(t^{-0.5}\) will imply a possibility that the short-time diffusivity is intrinsically fluctuating. Figure 4 shows the RSD of TAMSDs of the membrane-bound PH domain. The convergence of the RSD to 0 is very slow, that is, the power-law exponent is below −0.5, although the diffusion process is ergodic, as shown by the agreement between the time-averaged and ensemble-averaged MSDs (see fig. S2).

To interpret this, we consider a Langevin equation with fluctuating diffusivity (LEFD) model (36–38) as a temporally heterogeneous diffusion process, \(dx(t)/dt = \sqrt{2D(t)}\omega(t)\), where \(\omega(t)\) is the white Gaussian noise with \(\langle \omega(t) \rangle = 0\) and \(\langle \omega(t)\omega(t') \rangle = \delta(t - t')\). The LEFD model is reasonable because the PH domain has been shown to exhibit fast and slow diffusivities depending on the number of bound PIPs (see above), and the diffusion process of this model is ergodic. To capture the essential features of the observed heterogeneous diffusion, we consider the diffusivity \(D(t)\) to vary dichotomously, that is, \(D_s\) for a slow state and \(D_f\) for a fast state \((D_s < D_f)\). From the PDF of the diffusion coefficient, \(D = \delta^2(\Delta; t)/2d\Delta\) for \(\Delta = 0.1\) \(\mu s\) and \(t = 1\) \(\mu s\), we used \(D_f = 17\) and \(D_s = 14\) \(\mu m^2/\mu s\) (see fig. S4). In the LEFD with a two-state model, sojourn time for each state is a random variable, and we assume that these distributions follow power-law
**DISCUSSION**

In summary, we have used extensive (1 ms) MD simulations to investigate the diffusive properties of the DAPP1 PH domain bound to the surface of a model cell membrane. Although the underlying diffusion process is ergodic, the diffusivity of the protein fluctuates anomalously, which may be attributed to the dynamic interaction between the PH domain and the PIP molecules to which it is bound.

Cell membranes are spatially and temporally inhomogeneous environments as a consequence of the formation of lipid (nano)domains, the crowding of a variety of lipid and protein species, and interactions with cytoskeletal components of the cell (3). These components of the membrane environment are temporally and spatially regulated. Dynamic interactions of membrane proteins with lipids add a further level of complexity that is therefore expected to make the diffusion process of membrane proteins even more heterogeneous. In our study, we have shown that this heterogeneity determines the diffusive nature of key peripheral proteins on membrane surfaces. In particular, the number of PIP molecules that are bound to a PH domain alters its diffusivity. The stages of our method were the following: First, we calculated the TAMSD restricted to the time window $[t, t + T - \Delta]$, and thus, we obtained the temporal diffusion coefficients as a function of $t$.

**Estimation of short-time diffusivity**

Here, we introduced an estimation method to detect the short-time diffusivity. The stages of our method were the following: First, we calculated the TAMSD restricted to the time window $[t, t + T - \Delta]$, and thus, we obtained the temporal diffusion coefficients as a function of $t$.

$$D(t; \Delta, T) = \frac{1}{2d\Delta(T - \Delta)} \int_{t}^{t+T-\Delta} \left[ \vec{r}(t') + \Delta - \vec{r}(t') \right] dt'$$  \hspace{1cm} (4)
calculating a diffusion coefficient in fig. S3. However, this failed to capture the short-time diffusivity because some time window \([t_r, t + T - \Delta]\) contained the point at which the short-time diffusivity changed substantially. For this reason, we introduced the renewal time at which the diffusivity changed. The renewal time was defined as the temporal diffusivity coefficients crossed its mean. We divided the obtained trajectory of \(D(t, \Delta, T)\) into two states, a fast state \(F\) and a slow state \(S\), using the average calculated by the whole trajectory. The transition point \(t_i\) of each state was estimated by \(S \rightarrow F [\rho_S(t_i) < \rho_F(t_i)]\) or \(F \rightarrow S [\rho_F(t_i) < \rho_S(t_i)]\), where
\[
\rho_F(t_i) = \frac{1}{T_c} \int_{t_i}^{t_i + T_c} 1(D(t'; \Delta, T) > D_{\Delta}) dt'
\]
\[
\rho_S(t_i) = \frac{1}{T_c} \int_{t_i}^{t_i + T_c} 1(D(t'; \Delta, T) < D_{\Delta}) dt'
\]
using the average \(D_{\Delta}\), and we used \(T_c = 10\) ns. The renewal time detected the transition point from a fast to a slow diffusive state or vice versa. Because we knew the renewal times \(t_1, \ldots, t_n\), we calculated the short-time diffusion coefficient in the time window \(\left[t_i, t_{i+1}\right]\)
\[
D(t) = \frac{1}{2d\Delta(t_{i+1} - t_i - \Delta)} \int_{t_i}^{t_{i+1} - \Delta} \left[ \bar{r}(t' + \Delta) - \bar{r}(t') \right]^2 dt'
\]
where \(t_i\) is the \(i\)th renewal time, and we used \(\Delta = 0.1\) ns.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/3/1/e1601871/DC1

**REFERENCES AND NOTES**


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