Diffusion of Integral Membrane Proteins in Protein-Rich Membranes

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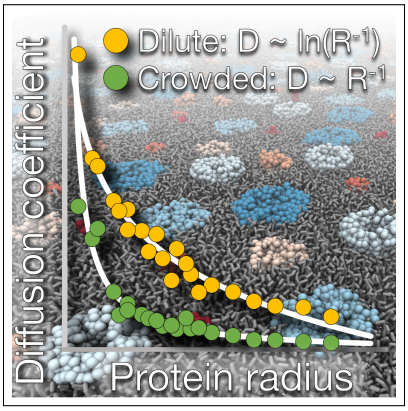
Abstract

The lateral diffusion of embedded proteins along lipid membranes in protein-poor conditions has been successfully described in terms of the Saffman–Delbrück (SD) model, which predicts that the protein diffusion coefficient D is weakly dependent on its radius R as $D \propto \ln(1/R)$. However, instead of being protein-poor, native cell membranes are extremely crowded with proteins. Based on extensive molecular simulations we here demonstrate that protein crowding of the membrane at physiological levels leads to deviations from the SD relation and to the emergence of a stronger Stokes-like dependence $D \propto 1/R$. We propose that this $1/R$ law mainly arises due to geometrical factors: smaller proteins are able to avoid confinement effects much better than their larger counterparts. The results highlight that the lateral dynamics in the crowded setting found in native membranes is radically different from protein-poor conditions and plays a significant role in formation of functional multi-protein complexes.

Graphical TOC Entry



In living biological cells, the plasma membrane hosts numerous integral proteins. Together with membrane lipids and other macromolecules, they are thermally driven to diffuse[1,2] along the plasma membrane to form functional protein oligomers[3,4] and lipid-protein assemblies[5,6] involved in, *e.g.*, metabolism, recognition, and signaling.

In this context, the major challenge is to understand protein diffusion under the crowding of proteins. Membranes of living biological cells are highly heterogeneous, partitioned, and extremely rich in proteins,[7–10] with typical lipid/protein ratios ranging between 50 and 200.[11,12] The average in-plane distance between membrane proteins is just a few nanometers, implying that the proteins are in constant interplay colliding with one another. However, how this dynamical interplay induced by protein crowding influences membrane protein diffusion remains poorly understood.

It is known that crowding, together with other phenomena occurring in the membranes of living cells, leads to complex effects[13,14] but their characterization in the nanoscale has turned out to be difficult. Molecular simulations would be an excellent approach or even the method of choice to explore this challenging topic but, surprisingly, quite little has been done until now. Simulations[15,16] have revealed the emergence of crowding-induced anomalous diffusion[13,14] in lipid membranes, complementing earlier Monte Carlo simulations with immobile objects[17] and clarifying the interpretation of fluorescence correlation spectroscopy experiments[18] that originally confirmed anomalous diffusion to take place in lipid bilayer systems. Besides this, simulations have largely just supported the experimental observations[19] that diffusion slows down for increasing crowding.[15,20,21] As to recent progress on the experimental side, modern atomic force microscopy, single-particle tracking, and the variety of super-resolution microscopy techniques are able to detect, *e.g.*, heterogeneity, anomalous diffusion, and nonergodicity in the motion of membrane proteins.[22–24] These techniques have potential to generate breakthrough insight on how functional protein complexes form in native membranes through diffusion and oligomerization. Planning and interpretation of these experiments is difficult, though, given the lack of theoretical understanding of how membrane

protein diffusion takes place under protein-rich conditions.

Meanwhile, in the protein-poor limit the diffusion of proteins is fairly well understood.[1,19] Experimental data have provided compelling evidence that for a membrane protein of lateral radius R, the protein diffusion coefficient D scales logarithmically as $D \propto \ln(1/R)$. This logarithmic dependence has been observed for a variety of membrane proteins[1,25–27] and aggregated peptides.[28] Computer simulations of proteins in protein-poor conditions also support this relation,[20,29] even in the presence of hydrophobic mismatch.[30] While deviations from this relation have also been reported,[31–33] they have been suggested to result from local membrane deformations[34] or experimental setups.[25]

Theoretically, the logarithmic dependence of D on R is predicted by the Saffman–Delbrück (SD) model derived for protein-poor conditions. The SD model links the diffusion coefficient of a protein with the physical properties of the lipid membrane and the surrounding solvent,[35,36]

$$D_{\mathrm{SD}} = \frac{k_{\mathrm{B}}T}{4\pi\mu_{\mathrm{m}}h} \times \left[\ln\left(\frac{h\mu_{\mathrm{m}}}{\mu_{\mathrm{f}}R}\right) - \gamma\right], \tag{1}$$

where μ_{m} and μ_{f} are the viscosities of the membrane and the surrounding solvent (typically water), h is the hydrophobic thickness of the membrane, and $\gamma \approx 0.5772$ is the Euler–Mascheroni constant. The SD model treats the lipid bilayer as a continuum liquid, thus failing to describe the diffusion of membrane lipids[37] unless the interleaflet friction is considered.[38] The SD model should be valid for membrane inclusions smaller than the SD length $L_{\mathrm{SD}} = h\mu_{\mathrm{m}}/(2\mu_{\mathrm{f}})$ with a typical value of ∼100 nm.[39] This holds for membrane proteins, but for diffusing objects larger than L_{SD}, the SD model was extended and a Stokes-like dependence $D \propto 1/R$ was found.[39,40] This relation successfully describes domain diffusion in monolayer experiments,[41,42] computer simulations on large inclusions,[29] and diffusion in viscous membranes.[43] However, the interpretation of the parameters, such as R, in the SD model is not obvious,[44] and fits to data measured in living cells provide nonphysical val-

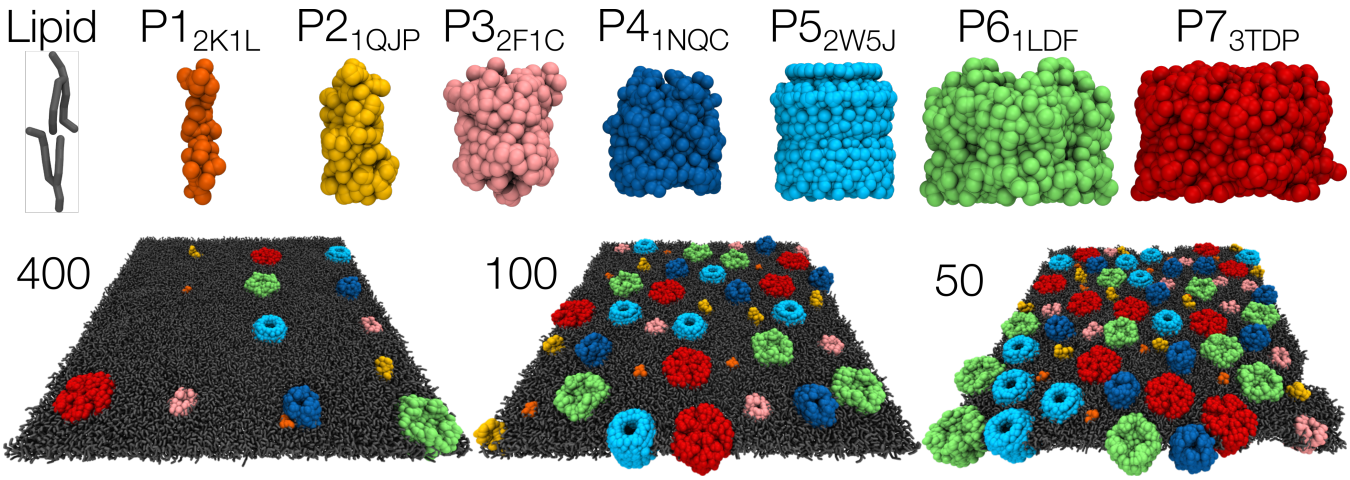


Figure 1: Top: Transmembrane proteins in the polydisperse coarse-grained simulations. labeled P1 to P7; the subscript is the PDB identifier (Tables S1 and S2 in the Supplemental Information). Two DPPC lipids, making up the bilayer, are also shown to scale. Bottom: Examples of the simulated polydisperse CG membranes with a varying lipid/protein ratio of 400, 100, and 50 lipids per protein per leaflet (left to right). The rightmost system has also appeared in Ref. 13.

ues for them.[27] Still, the SD model is consistent with extensive experimental data for the logarithmic dependence on R and hence widely accepted under dilute conditions.

However, given the assumptions made in deriving the SD model for infinitely dilute protein concentrations, can one assume its applicability to biologically relevant membrane systems characterised by protein crowding? If not, then the central questions are, what is the size dependence $D(R)$ replacing the SD law, and for what physical reasons?

In this work, we tackle these outstanding questions by molecular dynamics (MD) simulations. Previous MD studies have addressed the effects of crowding and highlighted decreased diffusivities,[15,20,21] an extended subdiffusion regime,[15,16,20] and deviations from Gaussian statistics.[16] Despite all the experimental and computational efforts, however, no studies have attempted to probe the validity of the SD model in crowded membranes, or found rigorous descriptions for $D(R)$ in the protein-rich limit.

Based on the present work on membranes hosting a polydisperse set of proteins, we find that in dilute conditions we reproduce the SD-like weak $D \propto \ln(1/R)$ dependence. However,

protein crowding at physiological levels is here shown to result in a crossover to a significantly more pronounced Stokes-like $D \propto 1/R$ relation. We argue that this $1/R$ law mainly arises due to geometrical factors: smaller proteins are able to avoid confinement effects much better than their larger counterparts. This claim is in full agreement with our additional simulations of crowded membranes with a monodisperse protein population, and with simulations of a 2D Lennard-Jones liquid.

To elucidate the effect of protein crowding on the $D(R)$ relationship, we performed MD simulations using two very different models. First, we simulated extensive membranes in the coarse-grained (CG) scheme[45–47] using the Martini model. These membranes contained a polydisperse mixture of seven transmembrane proteins with effective radii between 1 and 4.5 nm, thus mimicking realistic conditions to a satisfactory extent. In each of the simulated five membranes, with lipid/protein ratios equal to 50, 75, 100, 200, and 400 per leaflet, the relative concentrations of the seven protein types were equal. These proteins, chosen to have minimal extramembrane domains and a cylindrical shape, are shown in Fig. 1, along with examples of simulated membrane systems. The protein–protein interactions were slightly reduced, resulting in realistic transient oligomerization. Additionally, we used this Martini model to simulate crowded membranes with a monodisperse set of proteins and a lipid/protein ratio of 50 per leaflet. Here, in addition to the proteins shown in Fig. 1, the simulations also included two other small proteins (see Section S1.4). Second, we simulated 2D Lennard-Jones (2DLJ) fluids with 15 circular inclusions of different radii. The free LJ particle/inclusion ratio varied between 300 and 1000, which provides similar protein/inclusion area coverages than the CG Martini systems. Details on the CG and 2DLJ models and simulations are provided in Sections S1.1–S1.4.

The simulations of polydisperse CG models were run for 100 μs each, which allowed the reliable extraction of D in the microsecond regime, where diffusion is no longer anomalous but normal[15,16] (Section S2.2). The extracted lipid and protein D are shown as a function of the lipid/protein ratio in the top panel of Fig. 2. The effect of crowding is somewhat

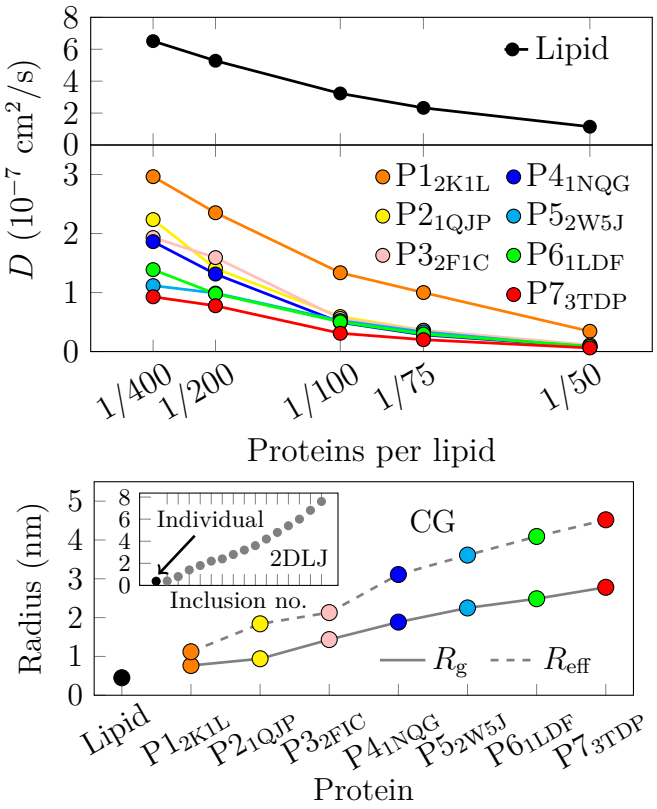


Figure 2: Top: Lipid and protein diffusivities from polydisperse CG simulations. Note that the x axis is linear in protein/lipid values.[15] Bottom: Gyration radii and effective radii of the proteins extracted from single-protein simulations. Inset: size distribution in the 2DLJ system. Coloring as in Fig. 1.

nonlinear, in agreement with earlier works.[15,48] In particular, the effect of crowding on the Ds of the smallest proteins and the lipids is most severe. The diffusion coefficients measured for each individual protein reveal fairly small scatter around the mean, indicating that no proteins are immobilized by, *e.g.*, extensive aggregation or confinement (Section S2.6), except for the $\mathrm{P2_{1QJP}}$, see below.

It is established that the radius of a diffusing protein is not simply that of the bare protein alone but includes a shell of lipids moving together with the protein.[44,49] We estimated these effective radii R_{eff} from average lipid displacements in the vicinity of the protein in single-protein simulations (Sections S1.2 and S2.1). Protein R_{eff} as well as their bare gyration radii R_g are shown in the bottom panel of Fig. 2. R_{eff} values exhibit a systematic increase as

a function of R_g. Superimposing the lipid displacement profiles reveals that the thickness of the lipid shell is approximately 1.5 lipid layers thick and independent of the size of the protein as well as of the lipid/protein ratio (see Section S2.1).

Fig. 3 depicts the protein diffusion coefficient as a function of R_{eff}. Fits of the logarithmic SD-like (1) and the Stokes-like behavior ($D = k_{\text{B}}T\lambda\,(4\pi\mu_{\text{m}}hR)^{-1} + c$ with characteristic length λ) are shown in Figs. 3 and S9. The value of constant c was insignificant. It is evident that at low protein concentrations the data follow $D \propto \ln(1/R)$, in line with previous studies. However, at higher protein concentrations $D(R)$ becomes increasingly Stokes-like. This observation is independent of the definition of the protein radius (Section S2.3), even though R_{eff} provides slightly better fits. The change from the SD-like to the Stokes-like behavior upon crowding is evident in the double logarithmic scale (insets of Figs. 3 and S9). Importantly, these conclusions for the crossover from the logarithmic SD-like to the Stokes-like behavior hold for the polydisperse CG Martini model, and also for the 2DLJ model. Further, under protein crowding, the Stokes-like behavior is observed also in monodisperse CG systems (see Section S2.9). As an exception to the general trend, the diffusion coefficients calculated for the second smallest protein (P2_{1QJP}) do not follow the observed tendencies in most of the systems due to its tendency to aggregate with the largest protein (P7_{3TDP}) (see Section S2.4), which serves as a reminder that simplified theoretical models cannot account for specific interactions in biological systems. Yet, in monodisperse crowded CG systems (Section S2.9) P2_{1QJP} diffuses freely in the absence of P7_{3TDP} and hence follows the $D \propto 1/R$ behavior. Overall, the observation of the Stokes-like behavior for crowded membranes is our first main result.

Setting the hydrophobic thickness of the membrane $h = 4$ nm[50] and the water viscosity $\mu_{\text{f}} = 0.7$ mPa s,[51] and using R_{eff}, we extract 2.7 mPa s for the membrane viscosity μ_{m} from fits to the SD model (1) for the most dilute system. This agrees favorably with 3 mPa s measured for a pure DPPC bilayer,[50] suggesting that the $D \propto \ln(1/R)$ dependence arises from an underlying SD-like relationship.

Our extensive CG simulations already provide compelling evidence that at high protein crowding, corresponding to physiological conditions (Section S2.8), the $D \propto \ln(1/R)$ relation gets replaced by the $D \propto 1/R$ one. We also considered a 2DLJ model that contains no coupling between the diffusion plane and the surrounding solvent. This model successfully reproduced the key features of membrane dynamics in our earlier work.[16,52] Remarkably, the change in the dependence of $D(R)$ from SD to Stokes-like behavior is also convincingly reproduced by the 2DLJ systems, see Fig. 3 (and Section S2.5).

The extracted μ_{m} from the dilute CG simulations gives $L_{\mathrm{SD}} \approx 7.7$ nm. The SD model assumes that the diffusing proteins have radii smaller and inter-protein distances larger than L_{SD}, and that the host membrane and the surrounding solvent are infinite. Otherwise, the finite size of the simulation system might affect its dynamic properties.[38] The observation that $D \propto \ln(1/R)$ is observed in our state-of-the-art simulations, which only partly fulfil these requirements,[39] suggests that the regime where the relation holds is actually broader than what is expected based on the SD model. Alternatively, the $D \propto \ln(1/R)$ law might result from another underlying and unresolved mechanism, supported by the similar behavior of the 2DLJ simulations without membrane–solvent coupling. The observation of the $D \propto \ln(1/R)$ relation in conditions where SD is not expected to hold is our second main result.

Fig. 3 combines data from the polydisperse CG (colored dots) and the 2DLJ simulations (black dots) for the dilute (top, 400 lipids per protein, 1000 free LJ particles per inclusion) and crowded (bottom, 50 lipids per protein, 300 free LJ particles per inclusion) cases using dimensionless units (both diffusivities and radii are divided by the corresponding values of a lipid or a single LJ particle (see Section S1.5)). While the compared systems do not have exactly the same protein/lipid or inclusion/free LJ particle ratios nor the same surface coverage of proteins/inclusions, this normalization reveals the striking similarity of the functional relationship between diffusion coefficients and radii in these two fundamentally different systems. The data for the dilute systems are well fitted by the $D \propto \ln(1/R)$ model. The crowded case does not follow this SD-like dependence, but is instead described accurately

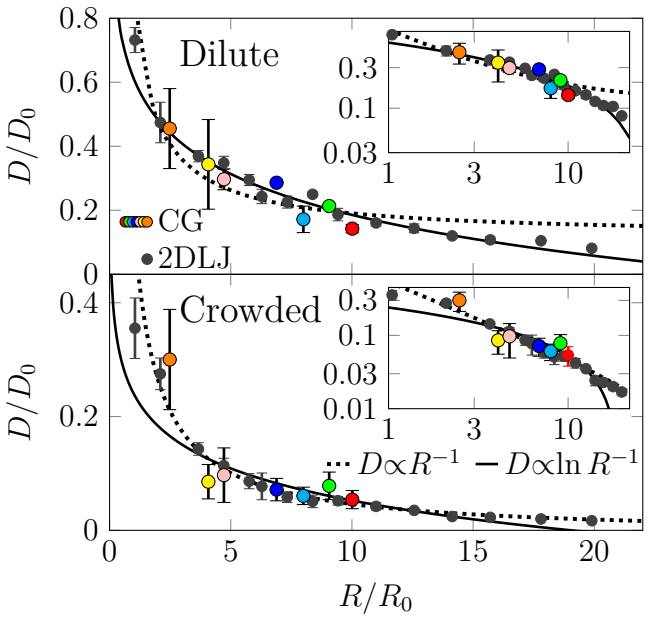


Figure 3: Diffusion coefficients versus protein/inclusion size of the polydisperse CG membrane and 2DLJ models shown in dimensionless units. Unscaled data are shown in Sections S2.3 and S2.5. In the dilute case the data fully agree with the SD model, whereas in the crowded case the $1/R$ dependence of the diffusivity is evident. Coloring as in Fig. 1.

by the Stokes-like $1/R$ law. This full *quantitative* consistency of the $D(R)$ behavior between the CG and the 2DLJ models is our third main result.

What is the physical origin of the observed change in the functional form of $D(R)$? Protein crowding increases membrane viscosity, which results in an increase of the SD length and hence should expand the validity of the SD model. However, we instead find that the SD model no longer describes diffusion in the crowded regime. The presence of other proteins violates the basic assumption in the SD model of a single protein embedded in an isotropic continuum liquid. Could inter-protein interactions and aggregation lead to smaller effective protein mobilities and thus effect deviations from the SD law? This effect is excluded as due to the specifics of our model setup (Sections S1.1 and S1.3) no significant aggregation between proteins or inclusions was observed, except for the aforementioned $\mathrm{P2}_{\mathrm{1QJP}}$–$\mathrm{P7}_{\mathrm{3TDP}}$ interaction (see Section S2.4). Crowding may also alter the protein and lipid dynamics due to confinement, and proteins mutually act as moving obstacles. It was recently found that

proteins need to escape their confinement to properly sample the membrane plane and reach the normal diffusion regime.[15] This escape probability is directly proportional to the protein cross section and hence radius. In the crowded systems the smallest proteins are able to travel longer distances by slipping through small openings between proteins that are impenetrable for larger proteins. This is visualized in Section S2.7 showing 35 μs long trajectories of each of the protein types in the most crowded system. The trajectory of the smallest protein shows both localized rattling motion and rapid movement across longer distances. These spurts correspond to events where the small protein slips through an opening between the larger proteins.

The hypothesis that geometric confinement is responsible for the crossover from the $D \propto \ln(1/R)$ behavior to the $1/R$ law is further supported by the 2DLJ simulations, in which the diffusion of disc-shaped inclusions of different radii undergoes an identical transition, as demonstrated *quantitatively* in Fig. 3. Also, given that the 2DLJ system is void of any hydrodynamic interactions with the solvent, it is obvious that the change in $D(R)$ must arise from geometric factors: The embedded discs act as obstacles and give rise to confinement effects. Since hydrodynamics is not predominant for this system, the dynamics are likely mostly affected by collisions, which can effectively be modeled by the 2D Enskog theory.[53] While this theory was derived for fairly dilute conditions, it interestingly suggests a $1/R$ dependence of the collision rates on the particle radius.

Does the observed change in the $D(R)$ dependence have any biological relevance? Proteins occupy at least 20% of the surface area of a red blood cell membrane.[11] This corresponds to between 100 and 200 lipids per protein. Based on data for lateral protein concentration in the rod outer segment and in the baby hamster kidney cell membranes, an estimate of 50 lipids per protein has been made.[12] In the present work, we found that the crossover from the SD law to the Stokes-like regime takes place at about 200–300 lipids per protein (Section S2.8). Therefore, in cell membranes exceptionally crowded with proteins the diffusion takes place in a regime characterized by Stokes-like scaling $D \propto 1/R$.

Hence, in the crowded case there is an order of magnitude difference between the diffusion coefficients of the smallest proteins and protein complexes that can be as large as 10 nm, such as the nuclear pore complex. Under crowding, large protein complexes are essentially immobile but interact with small proteins that diffuse rapidly and aggregate with the complex to render them functional. The dynamics in the crowded setting is therefore radically different from protein-poor conditions, where the diffusion of proteins is largely independent of their size as highlighted by the SD law.

We demonstrated that the $D \propto \ln(1/R)$ relation akin to the celebrated SD model fails to describe protein diffusion under protein crowding. Such conditions favor the diffusion of smaller proteins that are able to escape geometric confinement. Remarkably, this behavior is reproduced in a simple 2DLJ fluid, providing compelling support for the hypothesis that the observed crossover from $D \propto \ln(1/R)$ to $D \propto 1/R$ in crowded membranes is mainly effected by geometric constraints and thus the ability of a given protein to escape confinement. Importantly, the change from the SD-like to the Stokes-like behavior occurs at protein concentrations that are relevant for cellular membranes.[11,12] This suggests that while the SD model has been successful in describing diffusion coefficients in dilute model systems, it should be applied to more realistic biological membranes with extreme care.

Acknowledgement

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Supporting Information Available

Details on the employed models, the setup of the simulations, and additional results.

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