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COMMUNICATION

Translational and rotational diffusion of micrometer-sized solid domains in lipid membranes

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We use simultaneous observation of translational and rotational Brownian motion of domains in lipid membranes to test the hydrodynamics-based theory for the viscous drag on the membrane inclusion. We find that translational and rotational diffusion coefficients of micrometer-sized solid (gel-phase) domains in giant unilamellar vesicles showing fluid–gel phase coexistence are in excellent agreement with the theoretical predictions.

Brownian motion in lipid membranes is characterized by a peculiar feature, which sets it apart from diffusion in homogeneous bulk media. As first realized by Saffman and Delbrück (SD),^{1,2} the presence of hydrodynamic interactions mediated *via* the fluid media surrounding the membrane leads to appearance of an additional hydrodynamic length scale known as the Saffman–Delbrück (SD) length. The SD length l_{SD} is determined by the surface viscosity of the membrane η and the bulk viscosities of the surrounding media, μ_1 and μ_2 : $l_{SD} = \eta/(\mu_1 + \mu_2)$. Translational and rotational drag on a membrane inclusion with the characteristic size a then exhibits different behavior depending on the ratio a/l_{SD} : when the reduced inclusion size $\varepsilon = a/l_{SD}$ is small, $\varepsilon \ll 1$, the 2D membrane dynamics dominates the motion of the inclusion; on the other hand, for very large membrane inclusions, for which $\varepsilon \gg 1$, the motion of the inclusion is essentially controlled by the 3D dynamics of the fluid media surrounding the membrane.

Simple analytical expressions for the translational and rotational diffusion coefficients have been derived in the limits of very small (SD approximation)^{1,2} and very large membrane inclusions.³ The exact solution which is valid for arbitrary combinations of the inclusion radius and the viscosities of the membrane and surrounding media has been derived for a solid circular inclusion by Hughes, Pailthorpe, and White (HPW).³ Unfortunately, the very unwieldy form of this solution has prevented its practical use for a long time.

To overcome these difficulties and provide a practical tool for analysis of membrane diffusion data, we have previously developed a simple high-accuracy analytical approximation to the exact HPW solution for the translational diffusion coefficient of a membrane inclusion.⁴

$$\hat{D}_T(\varepsilon) = k_B T / (4\pi\eta) \times [\ln(2/\varepsilon) - \gamma + 4\varepsilon/\pi - (\varepsilon^2/2)\ln(2/\varepsilon)] \times [1 - (\varepsilon^3/\pi)\ln(2/\varepsilon) + \beta(\varepsilon, b_{T1}, b_{T2}, c_{T1}, c_{T2})]^{-1}, \quad (1)$$

where $\gamma = 0.577215$ is the Euler constant,

$$\beta(\varepsilon, p, q, v, w) = v\varepsilon^p / (1 + w\varepsilon^q) \quad (2)$$

is a bridging function, and $b_{T1} = 2.74819$, $b_{T2} = 0.51465$, $c_{T1} = 0.73761$, and $c_{T2} = 0.52119$.[†] This approximation allows one to easily predict the translational diffusion coefficient of membrane inclusions for arbitrary combinations of the inclusion size, membrane surface viscosity, and viscosities of the media surrounding the membrane. As a result, it has become a versatile tool in the analysis of experimental data^{4–6} and results of numerical simulations,^{7,8} as well as in making theoretical predictions on the critical dynamics of lipid membranes.^{9,10}

In this Communication, we develop a simple and accurate approximation for the rotational diffusion coefficient of a membrane inclusion. We use it, along with our previous results⁴ for the translational diffusion coefficient eqn (1), to analyze experimental data on rotational and translational diffusion of micrometer-sized solid domains in lipid membranes in the cross-over region from the 2D membrane-dominated to the 3D bulk fluid-dominated dynamics. By this means, we experimentally test the hydrodynamic theory^{1–3} for the viscous drag on membrane inclusions.

Using a similar approach as previously,⁴ we present the rotational diffusion coefficient in the following form: $D_R(\varepsilon) = D_{R0}\Delta_R(\varepsilon)$. Here $D_{R0} = k_B T (\mu_1 + \mu_2)^2 / (4\pi\eta^3)$ is a coefficient which is independent of the membrane inclusion size and characterizes the scale of the rotational diffusion coefficient in the membrane of a given surface viscosity η which is surrounded by two fluid media with the bulk viscosities $\mu_{1,2}$. The quantity $\Delta_R(\varepsilon)$ is the reduced rotational mobility, for which the exact solution is known,³ but can only be computed numerically. One of the goals of the present work is to develop a simple, yet high-accuracy, approximation $\hat{\Delta}_R(\varepsilon)$ to this quantity.

Finding the exact HPW solution for the reduced rotational mobility $\Delta_R(\varepsilon)$ is equivalent to solving the infinite system of linear equations, eqn (5.26), of the HPW work.³ In practice, we found that truncating the system to 100 equations provides stable and accurate results within the whole range of $\varepsilon = 10^{-4}$ to 10^6 . Integrals involving products of Bessel functions were evaluated numerically using the approach described in our previous publication.⁴

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The asymptotic behavior of $\Delta_R(\epsilon)$ is known in the limits of very small and very large inclusion sizes: when $\epsilon \rightarrow 0$, the SD approximation¹⁻³ gives $\hat{\Delta}_{R,SD}(\epsilon) = 1/\epsilon^2$; for $\epsilon \rightarrow \infty$, the HPW asymptotic expression (3) gives $\hat{\Delta}_{R,HPW}(\epsilon) = 3\pi/(4\epsilon^3)$. Based on the comparison with the exact HPW solution, we conclude that the asymptotic expressions for small-size and large-size inclusions hold only for $\epsilon < \sim 0.1$ and $\epsilon > \sim 50$, respectively (Fig. 1).

We develop our approximation $\hat{\Delta}_R(\epsilon)$ using analytical asymptotic matching to $\Delta_R(\epsilon)$ and choosing an appropriate bridging function which would provide a correct cross-over behavior and high overall accuracy of the approximation. We find that the following expression $\hat{\Delta}_R(\epsilon) = [\epsilon^2 + 4\epsilon^3/(3\pi) + \beta(\epsilon, b_{R1}, b_{R2}, c_{R1}, c_{R2})]^{-1}$ with the bridging function eqn (2), and parameters[†] $b_{R1} = 2.91587$, $b_{R2} = 0.68319$, $c_{R1} = 0.31943$, and $c_{R2} = 0.60737$, provides a good trade-off between the simplicity of the expression and quality of approximation: With this choice, the relative accuracy of our approximation is better than 0.07% within the whole range of ϵ , i.e. for arbitrary combinations of the inclusion radius and viscosities of the membrane and the surrounding fluid media (Fig. 1).

Thus, the closed-form high-accuracy approximation for the rotational diffusion coefficient of a circular solid inclusion in a lipid membrane reads as follows:

$$\hat{D}_R(\epsilon) = k_B T (\mu_1 + \mu_2)^2 / (4\pi\eta^3) \times [\epsilon^2 + 4\epsilon^3/(3\pi) + \beta(\epsilon, b_{R1}, b_{R2}, c_{R1}, c_{R2})]^{-1}. \quad (3)$$

As an experimental system, we chose giant unilamellar vesicles (GUVs) consisting of the equimolar mixture of two saturated phospholipids, DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) and DPhPC (1,2-diphytanoyl-*sn*-glycero-3-phosphocholine) (Avanti Polar Lipids, Alabaster, AL). GUVs were produced by electroformation on a platinum wire¹¹ in a 300 mM solution of sucrose (Sigma-Aldrich) in deionized degassed water at 60 °C, and were transferred for observation into chambers containing an iso-osmolar solution of glucose (Sigma-Aldrich) in deionized degassed water. As a result, the sample consisted of GUVs filled with 300 mM aqueous solution of sucrose which were surrounded by an aqueous solution containing 225 mM glucose and 75 mM sucrose. To facilitate fluorescence videomicroscopy imaging, the fluorescent lipid marker Texas Red-DPPE (Invitrogen, Eugene, OR) was added to the lipid

mixture at a concentration of 0.1 mol%. The temperature of the sample was maintained at 23.5 ± 1 °C.

Fluorescence video-microscopy measurements were carried out using an IX71 inverted microscope equipped with an UPlanApo 60×/1.20w water immersion objective (both Olympus, Hamburg, Germany). A conventional 100 W HBO mercury arc lamp in combination with a suitable filter was used to excite fluorescence. Movies were recorded at a resolution of 0.108 μm per pixel at a rate of 33.3 frames per s (0.03 s per frame) using a Neo sCMOS camera (Andor Technology, Belfast, Northern Ireland). Observations of translational and rotational Brownian motion of solid (gel-phase) domains in the lipid membrane were carried out on upper poles of vesicles with radii $R_{GUV} \geq 20 \mu\text{m}$ which firmly adhered to the bottom of the observation chamber and did not show any detectable motion on the time scale of ~ 10 s. To avoid effects of vesicle curvature on the results of diffusion coefficient measurements, the domains were tracked within the upper pole region of a vesicle at projected distances from its pole not exceeding $R_{GUV}/3$. The effective radius a of a domain was calculated from its area S as $a = (S/\pi)^{1/2}$. Viscosities of the aqueous sucrose and sucrose-glucose solutions were measured using a Physica MCR 301 rheometer (Anton Paar, Ostfildern, Germany) at 23.50 ± 0.03 °C and were found to be 1.20 ± 0.03 mPa s and 1.09 ± 0.03 mPa s, respectively.

According to the previously published phase diagram of DPhPC/DPPC membranes,¹² bilayers made of the equimolar DPhPC/DPPC lipid mixture exhibit fluid-gel coexistence at room temperature.

In our experiments, we frequently observed that gel domains formed in these membranes are diamond-shaped (Fig. 2). When GUVs were not osmotically tensed (the condition used in the domain tracking experiments described here) the diamond-shaped domains had the semiaxis ratio of 1.42 ± 0.05 , as one can see in Fig. 2. (We note here parenthetically that we found that the aspect ratio of the diamond-shaped domains depends on the membrane tension: we observed that an increase in the osmolarity of the medium surrounding the vesicles leads to formation of more elongated diamond-shaped domains with a higher semiaxis ratio.) This well defined geometric shape should reflect the molecular organization of the lipids in the gel phase, as it has been argued previously.¹³

The diamond shape of the solid membrane domains facilitates simultaneous tracking of their positions and orientations and thus allows one to extract the information on their translational and rotational diffusion coefficients. At the same time, the domains are not too strongly elongated, so that the expressions for the translational and rotational diffusion coefficients eqn (1) and (3), both based on the theory developed for a circular-shaped membrane inclusion, should still be applicable in this case.

To check whether the latter assumption is justified, we calculated viscous drag coefficients for in-plane translational and rotational motion of an elliptic disk¹⁴ which has the same semiaxis ratio $a_1/a_2 = 1.42$ as our diamond-shaped domains, and compared the results with those of the equivalent circular disk with the radius $a = (a_1 a_2)^{1/2}$. This should give an idea of how strongly the shape anisotropy will manifest itself in the Brownian motion of very large domains with $a \gg l_{SD}$ (for smaller domain sizes, the effect of the shape anisotropy will be progressively less pronounced – see, e.g., ref. 15). We found that the anisotropy of the drag coefficient (and therefore, diffusion coefficient) of the elliptic disk is $\sim 6\%$, which is of the order of the experimental error of the present study and thus justifies our assumption. Moreover, the mean in-plane diffusion

Fig. 1 Reduced rotational mobility of a membrane inclusion: the exact HPW result $\Delta_R(\epsilon)$ (circles), SD approximation $\hat{\Delta}_{R,SD}(\epsilon)$ (dashed line), HPW large- ϵ asymptotics $\hat{\Delta}_{R,HPW}(\epsilon)$ (dotted line), and the proposed empirical approximation $\hat{\Delta}_R(\epsilon)$ (solid curve). The upper panel shows the relative errors of the corresponding approximations.

Fig. 2 Fluorescence microscopy images of the upper pole area of a giant unilamellar vesicle with diamond-shape gel-phase domains (dark) recorded consecutively every 10 s. Vesicle radius $R_{\text{GUV}} = 20 \mu\text{m}$. Membrane composition: DPhPC/DPPC 50 : 50. Temperature: $23.5 \pm 1^\circ\text{C}$. Scale bar: $5 \mu\text{m}$.

coefficient of such an elliptic disk is just $\sim 1\%$ lower than that of the equivalent circular disk. The in-plane rotational diffusion of the elliptic disk at the above semiaxis ratio is $\sim 6\%$ lower than that of the equivalent circular one, which is again of the order of our experimental accuracy. By using the analogy with the results for translational and rotational diffusion coefficients of a deformed sphere,¹⁶ we conclude that the rotational diffusion coefficient should generally be more sensitive to deviations of the domain shape from the circular one, which means that in this case the use of the model implying the circular inclusion shape may result in somewhat higher membrane viscosity values recovered from the rotational diffusion data.

Translational and rotational mean-square displacements, $\text{MSD}_{\text{T}}(t)$ and $\text{MSD}_{\text{R}}(t)$, of individual diamond-shaped gel domains were calculated from their time-dependent positions and orientations. The translational and rotational diffusion coefficients D_{T} and D_{R} were determined by fitting the dependences $\text{MSD}_{\text{T}}(t) = 4D_{\text{T}}t + \delta_{\text{T}}$ and $\text{MSD}_{\text{R}}(t) = 2D_{\text{R}}t + \delta_{\text{R}}$ to the mean-square displacement data using weighted linear least squares, as suggested elsewhere.¹⁷ The free offset terms $\delta_{\text{T,R}}$ were used to account for the experimental errors of single particle tracking.¹⁸ The experimental values of D_{T} and D_{R} along with the corresponding error estimates¹⁷ are shown in Fig. 3 versus the effective radii of domains.

With the known viscosities of the media surrounding the membrane, the analysis of the diffusion data allows one to estimate the viscosity of the liquid disordered phase of the membrane. The

weighted least-squares analysis of the translational and rotational diffusion datasets using eqn (1) and (3) yields very close values for the viscosity of the fluid membrane phase η : $(2.1 \pm 0.1) \times 10^{-9}$ and $(2.3 \pm 0.1) \times 10^{-9} \text{ Pa s m}$, respectively, whereas the global fit of these two datasets gives $\eta = (2.2 \pm 0.1) \times 10^{-9} \text{ Pa s m}$ (Fig. 3). These viscosity values correspond to the Saffman–Delbrück length $l_{\text{SD}} \approx 1 \mu\text{m}$. Thus, our experimental data cover the cross-over region from the 2D membrane-dominated to the 3D bulk fluid-dominated dynamics, which is illustrated in Fig. 3 by the corresponding asymptotic dependences.

As it is clear from the DPhPC/DPPC phase diagram,¹² the fluid phase in the equimolar DPhPC/DPPC mixture is strongly enriched with the low-melting DPhPC lipid. Unfortunately, we could not find published experimental data on the surface viscosity of either the fluid phase in the equimolar DPhPC/DPPC mixture or the pure DPhPC. We, however, can compare our results with those obtained for a DPhPC/cholesterol/DPPC mixture.¹⁹ There, the mean of the viscosities of the DPhPC-enriched liquid disordered and more viscous DPPC- and cholesterol-enriched liquid ordered phase was determined and found to be $(4 \pm 1) \times 10^{-9} \text{ Pa s m}$. This value, which can serve as an upper estimate of the surface viscosity of DPhPC-enriched fluid phase, is in a very reasonable agreement with the values obtained in the present work.

Thus, our experimental results unambiguously show that both the translational and rotational diffusion of solid membrane domains are consistently described by the hydrodynamic theory originally

Fig. 3 Translational (D_{T}) (A) and rotational (D_{R}) (B) diffusion coefficients of diamond-shaped gel-phase domains in DPhPC/DPPC giant unilamellar vesicles and their ratio $D_{\text{T}}/D_{\text{R}}$ (C). Solid lines in panels (A) and (B) show the global weighted least-squares fit of translational and rotational data using eqn (1) and (3). The dashed and dotted lines show the small-size (SD approximation) and large-size asymptotic dependences. Lines in panel (C) represent ratios of the corresponding model predictions shown in panels (A) and (B). Because of a limited time resolution, rotational diffusion coefficients could be determined only for domains with the effective radius $a > 0.7 \mu\text{m}$. Membrane composition: DPhPC/DPPC 50 : 50. Temperature: $23.5 \pm 1^\circ\text{C}$.

developed by Saffman and Delbrück^{1,2} and further extended by Hughes, Pailthorpe, and White.³

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Notes and references

† While preparing the present work for publication, we discovered an unfortunate typo in the first significant digit of the coefficient b_{T2} in ref. 4. The correct value of b_{T2} is given in the present paper. Note that all calculations reported in our previous publications and the present work were carried out using the correct value of b_{T2} . Whereas the use of the correct value of b_{T2} guarantees that the maximum relative error in the approximation of D_T does not exceed 0.015% within the whole range of ε ,⁴ the use of the expression with a typo in b_{T2} increases this error up to ~3.8%, which is fortunately still low enough to ensure that the results and conclusions of the works by other groups based on application of our expression with a typo in b_{T2} are not affected by this misprint.

‡ The values of the parameters b_{R1} , b_{R2} , c_{R1} and c_{R2} were determined by numerical minimization of the sum of squared residuals $\sum_i (\ln \Delta_R(\varepsilon_i) - \ln \hat{\Delta}_R(\varepsilon_i))^2$ on a grid of 893 uniformly log-spaced ε values covering the range of $\varepsilon = 10^{-4}$ to 10^5 .

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