

Mismatch of bulk viscosity reduces interfacial diffusivity at an aqueous-oil system

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A significant decrease in the diffusivity of phospholipid molecules at an aqueous-oil monolayer was observed experimentally when the bathing solutions had different viscosities $\eta_1 \neq \eta_2$. Near the point of isoviscosity $\eta_1 \approx \eta_2$, the diffusion constant at the monolayer can be drastically affected by a slight change in the viscosity mismatch. We interpreted this phenomenon by considering the effect of vertical fluctuations which affect the long-term dynamics of two-dimensional lateral diffusion at the monolayer.

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The Brownian motion of phospholipid molecules and proteins in a two-dimensional biological membrane plays a key role in many biological processes [1,2]. According to the fluctuation-dissipation theorem, the diffusion coefficient can be written as

$$D = k_B T / \Gamma, \quad (1)$$

where D denotes the diffusion constant, k_B denotes the Boltzmann constant, T denotes the absolute temperature, and Γ denotes the friction coefficient. In principle, Eq. (1) should be valid not only for a three-dimensional isotropic system but also for a two-dimensional system. However, to solve hydrodynamic creeping-flow equations for the diffusion of a cylindrical molecule, such as a phospholipid or a protein, in a two-dimensional, continuous, viscous, and incompressible fluid, we must face the Stokes' paradox, where it becomes impossible to fulfill all of the boundary conditions [3].

A basic theory to avoid Stokes' paradox has been proposed by Saffman and Delbrück [4,5]. They treated a membrane as a two-dimensional continuous sheet with very small thickness h and viscosity η , bounded on both sides by three-dimensional fluids with viscosities η_1 and η_2 ($\approx \eta_1$). However, they ignored the concreteness of the lipid molecular structure. The friction coefficient Γ in the Saffman-Delbrück model can be written as

$$\Gamma = 4\pi\eta h \left(\ln \frac{\eta h}{\eta_1 R} - \gamma \right)^{-1}, \quad (2)$$

where R denotes the radius of a cylindrical molecule and $\gamma=0.5772$, Euler's constant. Hughes *et al.* extended the model of Saffman and Delbrück toward the parameter area $\epsilon \leq 1$ [6,7],

$$\Gamma = 4\pi\eta h \left(\ln \frac{2}{\epsilon} - \gamma + \frac{4}{\epsilon} - \frac{\epsilon^2}{2} \ln \frac{2}{\epsilon} \right)^{-1}, \quad (3)$$

where $\epsilon = (\eta_1 + \eta_2)R / \eta h$. The condition $(\eta_1 + \eta_2) \ll \eta$ is required for Eqs. (2) and (3). Equations (2) and (3) indicate that the mobility at a two-dimensional liquid should be proportional to $\eta_1 + \eta_2$.

There have been intensive studies toward the extension and improvement of the Brownian motion at an interface [8–10]. For example, Fischer *et al.* considered three-dimensional objects that are, while immersed in a monolayer or membrane, protruding into the surrounding three-dimensional fluid and calculated the friction coefficient of the objects as a function of the vertical position around an interface [9]. Naji *et al.* proposed that the mobility changes according to the $1/\epsilon$, where $\epsilon \gg 1$ [10]. Vaz *et al.* indicated that the Saffman-Delbrück model is not applicable to the case of lipid diffusion in a bilayer membrane at an aqueous-aqueous interface and asserted that a free-volume area model is more suitable [11]. However, some experimental reports are not fully consistent with these theories that include the Saffman-Delbrück model [12,13]. In particular, experiments at an oil-water monolayer interface seem to reveal intriguing diffusion behaviors. Although the interaction of oil with the tail groups of phospholipid molecules might be responsible for the low mobility at an aqueous-oil monolayer [13,14], a more systematic investigation is clearly needed.

In this study, we focused on the impact of a mismatch of the viscosities of the upper and lower bathing fluids, which may provide important insights into the diffusion dynamics at the interface. We experimentally investigated the lateral mobility of phospholipid molecules at an aqueous-oil interface with changes in the viscosities of both the aqueous solution η_{aq} and oil phase η_{oil} over a broad range of viscosity ratios: $\eta_{aq}/\eta_{oil} \leq 1$ to $\eta_{aq}/\eta_{oil} \gg 1$. Micrometer-scale Phospholipid-coated Droplets (MPDs) [12] have been adapted. In MPDs, the phospholipid molecules are arranged spontaneously at the oil-water interface with the minimization of the interfacial energy. We found that, in addition to the average viscosity $(\eta_{aq} + \eta_{oil})/2$, the difference in viscosity $|\eta_{aq} - \eta_{oil}|$ has a pronounced effect on the lateral diffusion at the interface, i.e., the diffusivity reaches a maximum value at the point of isoviscosity $\eta_{aq} = \eta_{oil}$, and deviation from this point results in a dramatic decrease in the diffusion coefficient, which cannot be predicted by the existing theoretical models.

The viscosity of the aqueous phase was adjusted as desired through the addition of 50, 250, 300, 335, 575, or 807 mg/ml polyethylene glycol (PEG, molecular weight = 15,000, radius of gyration $R_g = 4.2$ nm, and critical concentration $c^* = 107$ mg/ml) in a phosphate buffer saline (10 mM

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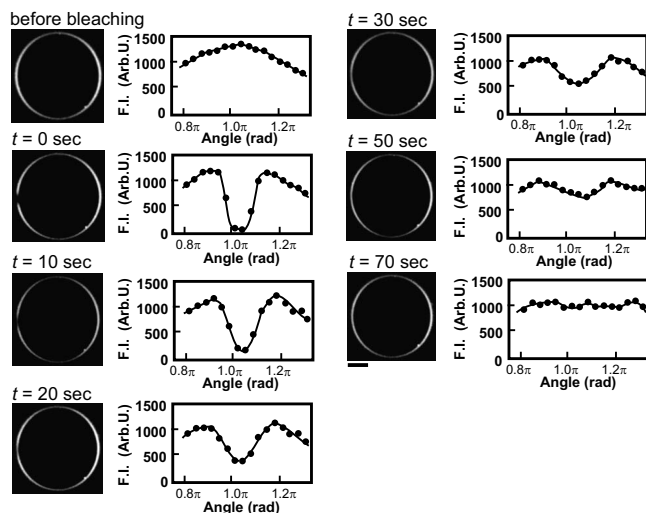


FIG. 1. Typical example of a FRAP measurement on an MPD. Time-sequence confocal images and bleaching profiles of an MPD, where the oil phase is mineral oil and the aqueous phase is 385.0 mg/l PEG solution (viscosity=75.2 cP at 20 °C). F.I.=fluorescence intensity and Arb.U.=arbitrary unit. Scale bar is 50 μm .

sodium phosphate including 0.9% NaCl, pH of 7.4). The viscosities of the PEG solution at 20 °C are 3.6, 28.5, 44.2, 75.2, 148, and 421 cP, respectively. The viscosity of the oil phase was changed by using different organic solvents: mineral oil (viscosity at 20 °C=22.2 cP), linseed oil (43.0 cP at 20 °C), or rapeseed oil (77.8 cP at 20 °C). To observe the two-dimensional diffusivity of liquid molecules, we used droplets with a size of several hundreds of micrometers which are covered with a phospholipid membrane (MPDs) [12]. At the aqueous-oil interface of an MPD, a 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) layer is formed which includes 0.5 mol % of the fluorescent molecule 1-oleoyl-2-[1,2-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino] dodecanoyl]-*sn*-glycero-3-phosphoethanolamine (NBD-PE). MPDs were prepared as follows: 0.25 mM DOPE including 0.5 mol % NBD-PE was dissolved in oil under ultrasonication for 60 min at 50 °C. After the oil cooled completely from 50 to 20 °C, 2 μl of PEG solution was added to 50 μl of oil containing lipids and mechanically agitated by repeated pipetting for ~ 60 s.

The lateral mobility of phospholipid molecules was measured by fluorescence recovery after photobleaching (FRAP), which is a useful method for measuring the Brownian motion of particles in a lateral membrane [15,16]. FRAP measurements and data analyses were performed as described in Ref. [12]. To perform measurements in the steady state, the adsorption or desorption of lipid molecules at an interface is a considerable problem [17]. In our case, the adsorption or desorption of DOPE through an interface (from oil to aqueous phase) is almost negligible because of the very low solubility of DOPE in aqueous solution. Therefore, the adsorption or desorption between the oil phase and the interface of a MPD should be considered. To reach an equilibrium condition for the density of DOPE at the interface, MPDs were stood still for 1 h at 20 °C before the measurements. Under

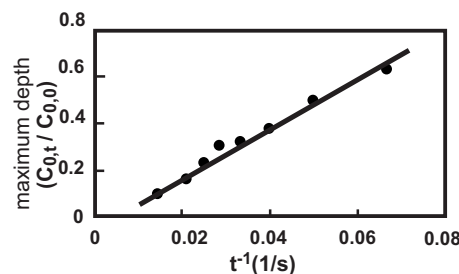


FIG. 2. Maximum bleach depth versus inverse of time after photobleaching for the MPD depicted in Fig. 1. From the slope, the diffusion constant is $D=6.23 \times 10^{-9}$ cm^2/s .

this procedure, we obtained data with good reproducibility.

Figure 1 exemplifies the results of FRAP measurement for an MPD in mineral oil (left, confocal fluorescence images; right, fluorescence intensity profiles). Before bleaching, the white ring of an MPD was uniform. Just after photoirradiation, a bleached spot appeared clearly on the white ring in confocal images at $t=0$ s, and gradually recovered. At $t=70$ s after photoirradiation, the bleached region had recovered almost completely. This time evolution indicates that the unbleached NBD-PE species diffused into the bleached spot. Figure 2 shows the time dependence of the maximum bleach depth for the results given in Fig. 1. For the vertical axis in Fig. 2, the maximum bleach depth at each time $C_{0,t}$ is given relative to the depth before bleaching $C_{0,0}$. The data were analyzed by a linear least-squares fitting equation, and the diffusion coefficient of DOPE at an aqueous-oil interface was calculated. A recovery behavior similar to that shown in Figs. 1 and 2 was also observed in the other samples.

Figure 3 shows the diffusion coefficients of DOPE at an aqueous-oil interface as a function of η_{aq} . The vertical dashed lines indicate the viscosity of the oil phase η_{oil} . In every case we examined, the highest diffusion coefficient was observed when the viscosity of the oil phase η_{oil} was nearly equal to that of the aqueous solution ($\eta_{oil} \approx \eta_{aq}$). For both $\eta_{oil} > \eta_{aq}$ (region to the left of the vertical dashed line in each plot in Fig. 3) and $\eta_{oil} < \eta_{aq}$ (region to the right of the vertical dashed line), the mobility was lower than that for $\eta_{oil} \approx \eta_{aq}$.

There seems to be no appropriate theory to address the

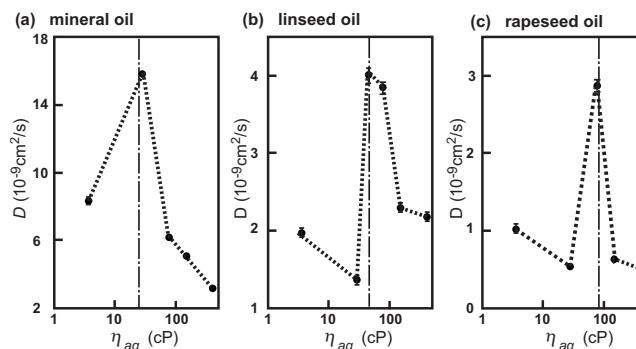


FIG. 3. Plots of the diffusion coefficient D versus the viscosity of the PEG solution (aqueous phase). The vertical dashed lines indicate the viscosity of the oil phase. The oil phases are (a) mineral, (b) linseed, and (c) rapeseed oils.

present observation. Nevertheless, several possibilities can be proposed. For example, by changes in the concentration of PEG in the aqueous phase, the interfacial energy between the lipid head group and the aqueous phase may be modified. Similar effect may be expected for the oil and lipid tail pair. In addition, due to the mismatch of the bulk viscosity ($\eta_1 \neq \eta_2$), each side of a lipid molecule experiences a different amplitude of drag, which causes a change in the morphology of the lipid. Although these factors may be important, they may be difficult to identify to establish a meaningful comparison with the experiments. Here, as an alternative, we propose a simple model based on a Brownian motion by smearing the details for particular systems. This approach leads to a generic scenario that arises purely from the dynamic property of a Brownian particle fluctuating around the interface.

Let us consider the motion of a Brownian particle at an interface (x - y plane) at $z \approx 0$, which is surrounded by viscous three-dimensional fluids. While a Brownian particle diffuses laterally in a plane, it also exhibits vertical fluctuations around the interface. When there is a viscosity mismatch $\eta_1 \neq \eta_2$, the vertical fluctuation produces a temporal modification of the drag friction for lateral motion. Thus, lateral diffusive motion is coupled with the vertical fluctuation mode, which may affect the long-term diffusion behavior in the lateral direction, which leads to the renormalization of the diffusion coefficient. The essential features of this effect can be captured by the following dynamic equation:

$$\Gamma_x(z) \frac{dx}{dt} = \xi_x(t), \quad (4)$$

$$\Gamma_z \frac{dz}{dt} = -kz + \xi_z(t), \quad (5)$$

where the random forces ξ_x , ξ_y are Gaussian white noises with a mean of zero and $\langle \xi_\alpha(t) \xi_\beta(t') \rangle = 2\Gamma_\alpha k_B T \delta(t-t') \delta_{\alpha\beta}$ ($\langle \dots \rangle$ represents a statistical average). Equation (5) includes a restoring force with the spring constant k , which ensures that the particle is trapped around the interface region with variance $\sim \sqrt{k_B T/k}$. Note that the friction coefficient Γ_z in the vertical direction should also be position dependent, but we adopt an approximation of position independence for simplicity. The equation of motion in the y direction is given by the same formulas as for the x direction. With the bulk friction coefficients Γ_1 and Γ_2 in the respective phases, the functional form of $\Gamma_x(z)$ may be simply assumed to be

$$\Gamma_x(z) = \bar{\Gamma} + \frac{\Delta\Gamma}{2} \tanh\left(\frac{z}{\omega}\right), \quad (6)$$

where $\bar{\Gamma} = (\Gamma_1 + \Gamma_2)/2$, $\Delta\Gamma = |\Gamma_1 - \Gamma_2|$, and ω characterizes the interfacial width [18]. In the crude approximation, the upper and lower bounds for Γ_x are proportional to $\max\{\eta_1, \eta_2\}$ and $\min\{\eta_1, \eta_2\}$, respectively.

When the amplitude of the vertical fluctuation is small (large k), we can only retain a linear term $\Gamma_x(z) \approx \bar{\Gamma} + \frac{\Delta\Gamma}{2} \left(\frac{z}{\omega}\right)$

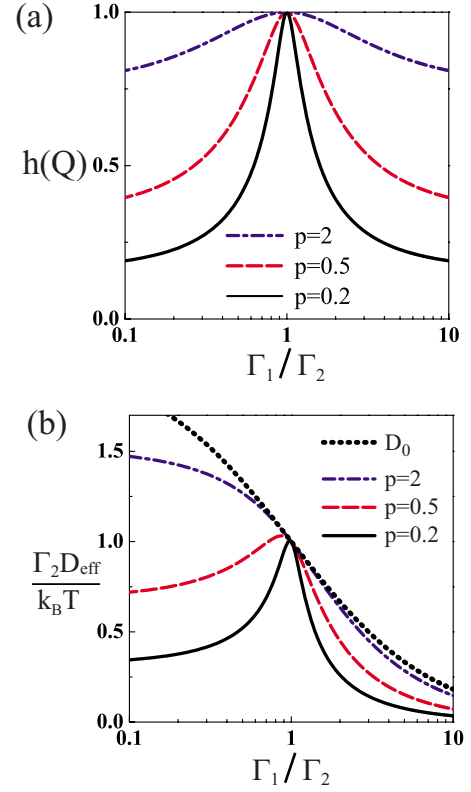


FIG. 4. (Color online) (a) Suppression factor $h(Q)$ and (b) diffusion coefficient D_{eff} as functions Γ_1/Γ_2 [in (b), Γ_2 is fixed].

in the friction fluctuation. By substituting the solution of Eq. (5) into Eq. (4), we can calculate the lateral mean-square displacement as

$$\langle [\Delta x(t)]^2 + [\Delta y(t)]^2 \rangle = 4D_{eff}t \quad (t \gg \Gamma_z/k), \quad (7)$$

from which the effective diffusion coefficient is identified as

$$D_{eff} = D_0 h(Q). \quad (8)$$

In addition to the normal term $D_0 = k_B T / \bar{\Gamma}$, there is a factor

$$h(Q) = 2Q \exp(Q^2) \text{erfc}(Q), \quad (9)$$

where $\text{erfc}(x) \equiv \int_x^\infty dt \exp(-t^2)$ is an error function. Equation (9) manifests a nontrivial effect associated with the viscosity mismatch as a function of the dimensionless parameter

$$Q = \frac{\bar{\Gamma}}{\Delta\Gamma} p \approx \frac{\bar{\Gamma}}{\Delta\Gamma}, \quad (10)$$

where $p = \sqrt{2}\omega / \sqrt{\langle z^2 \rangle}$ is a ratio of the interfacial width with respect to the mean-square fluctuation of the particle in the vertical direction. While this quantity is expected to be on the order of unity, its precise value would change to reflect the details in the individual models under investigation. In Fig. 4(a), the factor $h(Q)$ is plotted against Γ_1/Γ_2 . The equality $h(Q)=1$ holds only when $\Gamma_1=\Gamma_2$, and a viscosity mismatch introduces a suppression $h(Q) < 1$. As a consequence, the diffusion coefficient D_{eff} becomes smaller than the expected value D_0 [Fig. 4(b)] and tends to exhibit a maximum at $\Gamma_1=\Gamma_2$. The degree of the suppression factor $h(Q)$ at a

given viscosity mismatch $\Delta\Gamma$ depends on the value of p rather sensitively. Physically, $p \geq 1$ corresponds to a strongly bound particle (with small vertical fluctuation) and $p \lesssim 1$ indicates the opposite. As the value of p decreases, the suppressive tendency increases, and the calculated curve at $p \approx 0.2$ is quite similar to the experimental results. However, for such a small p value, the linearization of $\Gamma_x(z)$ might not be a good approximation. Furthermore, the calculated self-diffusion coefficient is expected to show a trend that is qualitatively similar to the experimentally measured cooperative-diffusion coefficient with regard to the viscosity-mismatch effect, although these two diffusion coefficients are not identical to each other unless the system is sufficiently dilute. These points should be carefully checked along with the effect of the z dependence of $\Gamma_z(z)$ in a future investigation.

In summary, we have found that a viscosity mismatch in the surrounding three-dimensional fluids $\eta_1 \neq \eta_2$ suppresses the Brownian motion of phospholipid molecules at an

aqueous-oil interface. The analysis of a simplified model suggests that the out-of-plane fluctuation mode may be an important factor that is responsible for this observation, although a more detailed theoretical analysis of the dissipation mechanisms is necessary. Other factors, such as the microscopic lipid structure and the relative interfacial tension in three-component systems (oil-water-lipid), may also play some role, which might be dominant in certain situations. Further studies may be able to reveal the impact of such additional contributions, which will be necessary to make quantitative predictions.

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