Microscopic versus macroscopic diffusion in one-component fluid phase lipid bilayer membranes

Dear Sir:

The lateral diffusion of membrane lipids in one-component Lα phase (fluid phase) lipid bilayer membranes has been studied over the last twenty years by techniques that measure: (*a*) extreme short range diffusion over distances on the order of about two lipid diameters using methods such as quasielastic neutron scattering (1, 2); (*b*) intermediate range diffusion over several lipid diameters using methods involving bimolecular reactions (3, 4); and (*c*) long range diffusion over several micrometers using methods such as fluorescence recovery after photobleaching (5) and magnetic resonance techniques (6–8). The general conclusion seems to be that there is a discrepancy of about two orders of magnitude between the translational diffusion coefficients (D_t) measured by the first group of techniques ($D_t \approx 10^{-6}$ cm^2 s^{-1}) and the last group ($D_t \approx 10^{-8}$ cm^2 s^{-1}). The second group of techniques report values of D_t that lie between these extremes. For simple liquids it is difficult to understand why the self diffusion rates for short and long ranges should be different.

We attempt here to reconcile the differences observed between the different methods. For the sake of simplicity, we only consider the extreme cases of diffusion measured by quasielastic neutron scattering ($D_t \approx 10^{-6}$ cm^2 s^{-1}) and fluorescence recovery after photobleaching ($D_t \approx 10^{-8}$ cm^2 s^{-1}). In the free-volume model for diffusion in a lipid bilayer (see reference 5 and literature cited therein) translational diffusion of a lipid molecule occurs when a free volume (area) of a critical size appears in the immediate vicinity of the test particle. The occurrence of the free volume is a result of random density fluctuations in the lipid bilayer. The situation is pictorially depicted in Fig. 1. The test particle is now free to move into the free space leaving the space it originally occupied free. Now, one of two things can happen: the test particle may move back into its original position, or another lipid molecule may move into the position originally occupied by the test particle. Only in the second case will there be an "effective displacement" of the test particle. Fluorescence recovery after photobleaching and other methods that study the translational diffusion over very long ranges (5–8) measure the sum of a very large number of such "effective displacement" steps. On the other hand, quasielastic neutron scattering measures displacements over very short time periods ($< 10^{-9}$ s), which, for the case of lipid bilayers, means displacements on the order of ~ 10 Å, that are about the size of two lipid diameters or the diameter of our test particle and the neighboring free volume. Thus, this technique simply measures the "rattling-about" of the lipid particle in a vacant space and probably not the "effective displacement" defined above.

If these "effective displacements" are viewed as steps in a random walk on a lattice where the distance between two lattice points is the average diameter of a lipid molecule, a mean hopping frequency for the test particle or, what is the same thing, for the free volume can be simply calculated (4). For the case of $D_t \approx 10^{-8}$ cm^2 s^{-1} measured by fluorescence recovery after photobleaching (5), the hopping frequency is 6.3×10^6 s^{-1}. The reciprocal of this value (1.6×10^{-7} s) is the lifetime of the free volume in a given lattice position.

Viewed from the standpoint of continuum fluid hydrodynamic models for diffusion in thin quasi-two-dimensional viscous fluid sheets, such as membranes that are surrounded by a three-dimensional bounding fluid (see reference 9 and references therein), the mean squared displacement varies linearly with time regardless of distance. The fact that D_t measured for very short range displacements by quasielastic neutron scattering is about two orders of magnitude larger than D_t, obtained by methods that measure very large scale displacements, suggests that lipid particle displacement in a fluid lipid bilayer is not adequately described by the assumption that the bilayer is a continuum fluid. Rather, lipid diffusion should be viewed as occurring by discrete jumps whose lengths are about the same as the diameter of a lipid molecule.

It is somewhat more difficult to understand the diffusion coefficients measured for intermediate ranges cited earlier. One aspect that must be considered is that all of the methods used in the measurement of this value involve bimolecular reactions between probes that are characteristically incorporated at molar fractions of $> 1\%$ in the lipid bilayer. At these concentrations the probe molecules cannot be simply considered to be reporter groups but should actually be considered to be chemical components of the system. Under these conditions, possible phase separations resulting in domains which are relatively rich in the probe become a real possibility. The consequence of the existence of such domains is an overestimation of the mean probe–probe separation distance, a parameter that is critical for the derivation of the diffusion coefficient from these measurements, and may lead to an overestimation thereof. Another aspect of these measurements is that the value of the diffusion coefficient derived from the measured

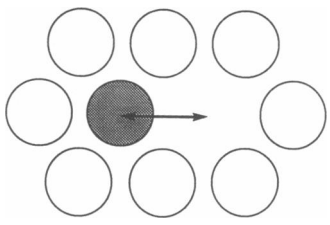

FIGURE 1 Pictorial depiction of displacement in terms of the free-volume model for diffusion in a lipid bilayer (5). The lipid molecules are shown as circles in a hexagonal lattice. The unoccupied lattice space represents the free volume and the shaded lipid molecule the test particle. The double-headed arrow is used to indicate the possible motions of the test particle during the lifetime of the free volume in the lattice space shown.

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bimolecular reaction rate constant is dependent upon the model used for interpretation of the data.

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