Lateral Lipid Diffusion Dominates NOESY Cross-Relaxation in Membranes

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Recently, we compared experimentally determined rates of NOESY cross-relaxation in liquid-crystalline phospholipid bilayers with theoretical values calculated from a 10 ns molecular dynamics (MD) simulation. The MD simulation provided autocorrelation functions, and consequently, spectral density functions of magnetic dipole−dipole interactions in a membrane. Correlation functions decayed as a result of lipid motion, including bond vibrations, gauche/trans isomerization of hydrocarbon chains and headgroups, molecular rotation and wobble of the entire lipid, and lateral diffusion of lipid molecules.[1] We calculated intensity factors and correlation times for the different motions to quantitate their contribution to the correlation functions. The analysis showed unambiguously that motions with correlation times that significantly exceeded the length of the simulation made the largest contribution to cross-relaxation. Excellent agreement was achieved, assuming a correlation time of 170 ns for the slowest motion[1]—a value that is suggestive for lateral lipid diffusion.[2] In this contribution, we provide experimental evidence that the rate of lateral diffusion is indeed a universal scaling factor for rates of cross-relaxation in lipid membranes.

First, let us explain how lateral diffusion influences cross-relaxation. The cross-relaxation rates, Γ_{ij}, depend on the spectral densities $J_{ij}(\omega)$

$$\Gamma_{ij} = \zeta[3J_{ij}(2\omega_0) - 1/2J_{ij}(0)]$$

where ω_0 is the proton Larmor frequency and ζ is a factor that combines the constants. Because all measured cross-relaxation rates at ambient temperature are negative, we concluded that the spectral densities at zero frequency, $J_{ij}(0)$, are dominant. The spectral density functions in lipid bilayers can be approximated by a sum of terms that are linked to the various motions listed above

$$J_{ij}(\omega) = \sum_n 2a_n\tau_n/(1 + \omega^2\tau_n^2)$$

where τ_n are correlation times and a_n the corresponding intensity factors.[1] Spectral density at frequency zero is proportional to the sum of products $a_n\tau_n$. Typical intensities of the motions with the longest correlation time are of the order of five percent of total intensity.[1] Despite their small amplitude, the large value of the corresponding correlation time, $\tau_n = 170$ ns, makes the slowest motion, presumably lateral diffusion, the dominant contribution to spectral density. Consequently, all rates of lipid cross-relaxation are predicted to scale with a single correlation time that depends on the rate of lateral diffusion and has the same temperature dependence as lateral diffusion. This prediction was tested experimentally.

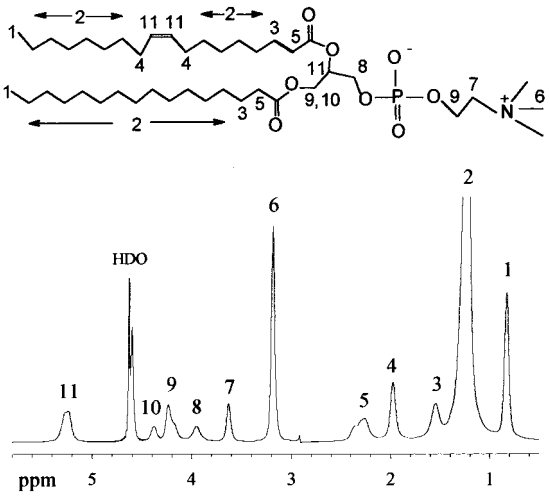

Figure 1. 500 MHz magic-angle spinning ^{1}H NMR spectrum of POPC in 50 wt % D_2O with peak assignment, recorded at a spinning speed of 10 kHz.

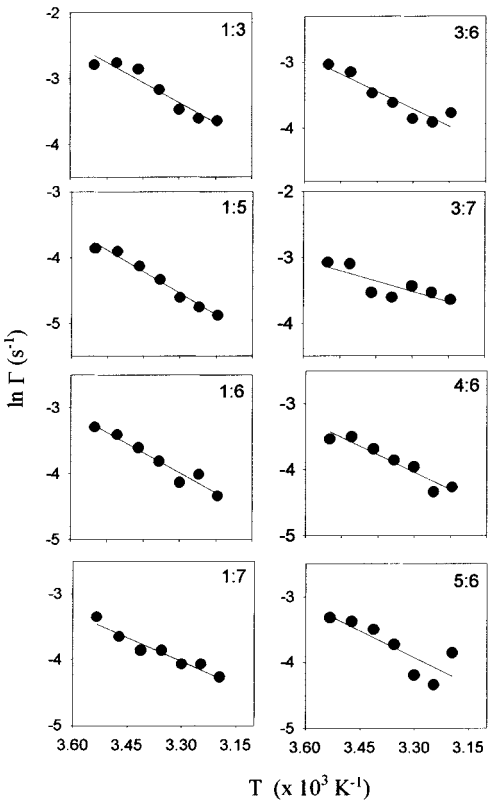

Figure 2. Natural logarithm of cross-relaxation rates between lipid resonances vs inverse absolute temperature. Two-dimensional proton NOESY experiments were conducted at mixing times of 5 and 250 ms. Cross-peak intensities were determined by two-dimensional integration. The cross-relaxation rates were computed by solving the relaxation rate matrix as described previously.[3] Every panel in the graph represents the temperature dependence of one cross-relaxation rate, e.g., panel 1:3 shows the cross-relaxation rate between lipid resonances 1 and 3 (resonance assignments given in Figure 1). The lines are a linear, least-squares fit to the experimental rates. Within experimental error all curves have the same slope corresponding to an activation energy of 4.9 ± 0.6 kcal/mol.

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The lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was hydrated with 50 wt % D_2O and investigated in its liquid-crystalline lamellar phase (Figure 1). POPC was chosen for its low main-phase transition temperature of −2 °C that enables measurements in the biologically relevant temperature range of 10−40 °C. Two-dimensional NOESY ^{1}H NMR experiments were conducted on a Bruker DMX500 widebore spectrometer equipped with a Bruker double gas bearing MAS probe for 4 mm rotors spinning at 10 kHz. Data were acquired and analyzed as described previously.[3,4]

For quantitative analysis of cross-relaxation rates as a function of temperature, we selected cross-relaxation rates that are solely caused by intermolecular lipid−lipid interactions,[3,4] like the cross-peaks between the terminal methyl and headgroup protons, the terminal methyl and the upper hydrocarbon chain protons, and the upper hydrocarbon chain and headgroup protons. The temperature dependence of these rates as an Arrhenius plot is shown in Figure 2. All dependencies were well approximated by linear curves. Their slopes are identical within experimental error, confirming that the same relaxation mechanism is responsible for all rates. The activation energy of this relaxation mechanism, 4.9 ± 0.6 kcal/mol, is in excellent agreement with the activation energy of POPC lateral diffusion, 5.4 kcal/mol, that we calculated from data of Köchy and Bayerl.[5]

The result strongly supports our model that lateral lipid diffusion is the major contributor to cross-relaxation in lipids. Strictly speaking, this is correct for intermolecular cross-relaxation only. However, our experiments on protonated lipid in a deuterated matrix have shown that the majority of cross-relaxation rates is dominated by intermolecular contributions.[3,4] Furthermore, cross-relaxation between lipids and membrane-incorporated molecules, for example, alcohol[6] and indole analogues,[7] should also be dominated by correlation times from the diffusion of incorporated molecules. Measurement of NOESY cross-relaxation rates is an alternative method for obtaining diffusion rates in membranes. The experiments require only milligram-size samples and do not employ membrane-perturbing labels. Even experiments on diffusion in complex biological membranes that contain a variety of lipids and proteins appear to be feasible.

Supporting Information Available: NOESY contour plot of POPC and tables of relaxation matrices of POPC as a function of temperature (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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