Length dependent effect of added alkane on fluidity and inter-leaflet coupling of lipid membranes

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[Background] In biomembranes, various kinds of small organic molecules, such as sterols and fatty acids, regulate the physical properties of lipid bilayers to maintain cell functions. One of the typical effects of these organic molecules is on membrane fluidity, which has long been an important topic in biomembranes. Studies in biologically relevant systems have suggested that membrane fluidity affects various cell functions, such as enzyme activity, transport process, hormone action, and immune response. [1] Not only the bilayer's fluidity itself but also the dynamic coupling/decoupling of the outer and inner leaflets of bilayers is possibly controlled by such small organic molecules. However, the effects of small organic molecules on the bilayer fluidity and the inter-leaflet coupling have not been fully understood.

The inter-leaflet coupling is known to connect the elastic bending modulus, κ, and the area compressibility modulus, K_A, in a thin elastic sheet theory. [2] As explained more in detail in the following section, recent neutron spin echo (NSE) studies start to measure both κ and K_A independently by measuring both bending and thickness fluctuations in a lipid bilayer. [3,4]

In this experiment, we investigated the effect of small organic molecules on the membrane fluidity utilizing NSE. We used synthetic lipid bilayers with *n*-alkanes. The effects of *n*-alkanes on the phase behavior, the bilayer structure, and its elasticity, have been extensively studied by us. [5,6] Change in the phase behavior of the lipid bilayers strongly depends on the alkane length.[5] We speculated that the membrane fluidity also relates to the intermolecular force in the membrane. For the systematic understanding of the effects of various organic molecules on the membrane properties, the alkane length dependence of the membrane fluidity will be an ideal system to explore.

[Methods] The NSE technique has been traditionally used to determine membrane's elastic bending modulus, κ. However, the recent development of membrane theories and experimental techniques started to shed light on more detailed membrane properties. [7,8]

Recently, thickness fluctuations in lipid membranes have been successfully measured using NSE, [3,4,9] and this technique was shown to be potential means to access β, which characterizes the inter-leaflet coupling. [4] The bending fluctuations have been modeled by Zilman and Granek [10] as the intermediate scattering function decays following a stretched exponential function with a stretching exponent of 2/3. The decay rate $\Gamma(q)$, where q is the momentum transfer, follows q^3 for bending fluctuations, while the thickness fluctuations are seen as a peak in $\Gamma(q)/q^3$ with an underlying q^3 dependence as follows: [4]

$$\frac{\Gamma}{q^3} = 0.0069\sqrt{\frac{k_B T}{\kappa}}\frac{k_B T}{\eta} + \frac{(\tau_{TF} q_0^3)^{-1}}{1+(q-q_0)^2\xi^2} \quad (1)$$

where the first term indicates the contribution from the bending fluctuations and the second term represents the thickness fluctuation contributions. η is the solvent viscosity, τ_{TF} represents the relaxation time for the thickness fluctuations, q_0 denotes the peak location in Γ/q^3 representation which is identical to the dip location of the bilayer form factor measured by SANS, and ξ indicates the half width at half maximum (HWHM) of the Lorentz function. The fractional change in the thickness, σ_h, is ex-

pressed as $\sigma_h = \Delta h/h = 2(q_0\xi)^{-1}$, where h represents the bilayer thickness. Neglecting changes of the molecular volume, σ_h is compensated for by the fractional change in the area, σ_A, as $\sigma_h^2 = \sigma_A^2$, and a simple statistical mechanical relation connects area compressibility modulus K_A and σ_h as $K_A = k_B T/\sigma_h^2 A_0$. [4,11] Therefore, the measurement of the thickness fluctuation amplitude yields to estimate K_A. On the other hand, in a thin elastic sheet theory, a relation between κ and K_A is formulated as $K_A = \beta\kappa/d_t^2$, where d_t is the thickness of the hydrocarbon region of the membrane. [2] These two independent measure of κ and K_A by the bending and thickness fluctuations, respectively, allows one to estimate a change in β if any.

[Results] We have performed an NSE experiment on the NGA-NSE and SANS experiment on the NG7, NIST, using dipalmytoylphosphatidylcholine (DPPC) bilayers with and without alkanes. We measured the cases for *n*-octane (C8), *n*-decane (C10), *n*-dodecane (C12) and *n*-tetradecane (C14). We independently measured the bending and thickness fluctuations by using protiated and alkanes in D_2O for the bending fluctuations, while the thickness fluctuation measurements were performed by employing tail-deuterated DPPC, deuterated *n*-alkanes and D_2O.

As the alkane length increased, κ became lower, which means that the membrane became more flexible. This trend is very interesting considering SANS results indicating that membrane thickness increases with increasing the alkane length. The thickness change is also supported by the results of thickness fluctuations (Fig. **??**). The peak derived from thickness fluctuation shifted to lower-q with increasing alkane length. κ should be larger as membrane thickness becomes larger if the membrane viscosity and molecular volume are not changed by the incorporation. Therefore, the decrease in κ suggests the possibility of changes in them. As peak width ξ is inversely proportional to thickness fluctuation amplitude ($\sigma_h = 2(q_0\xi)^{-1}$), the observed increase in ξ indicates that the thickness fluctuation is suppressed by *n*-alkanes. The results also show the alkane length dependence of ξ. It is possible that shorter alkane changes the membrane structure or viscosity more largely, resulting in the larger suppress.

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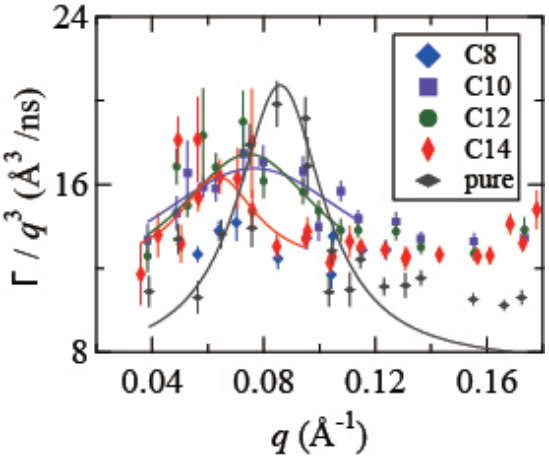

Fig. 1. $\Gamma(q)/q^3$ for DPPC with and without alkanes (C8 C10, C12 and C14). Red diamond: our unpublished previous result. Black diamond: result from [4].