

# Evaluation of Membrane Lipid Dynamics by Time-Resolved Small-Angle Neutron Scattering

M. Nakano(A), M. Fukuda(A), T. Kudo(A), H. Endo(B)

(A)Graduate School of Pharmaceutical Sciences, Kyoto Univ., (B)ISSP-NSL, Univ. of Tokyo

In biological plasma membranes interbilayer transport and transbilayer movement of phospholipids are controlled by phospholipid transfer proteins and translocase enzymes [1]. Evaluation of these lipid dynamics is, however, limited due to lack of its methodology.

Richter and co-workers [2] have reported that the unimer exchange of polymer micelles in dimethylformamide can be detected by small-angle neutron scattering (SANS) technique using hydrogenated and deuterated polymers. This technique can be applied to phospholipids since the transfer of the lipids is known to be a slow process with a half-life of hours, i.e., detectable timescale by SANS. In this study, large unilamellar vesicles (LUVs) of dimyristoylphosphatidylcholine (DMPC) were prepared and used for time-resolved SANS (TR-SANS) measurements to evaluate the rate of interbilayer and transbilayer transfers of the lipid.

DMPC and d54-DMPC were obtained from Avanti Polar Lipids Inc. (Alabaster, AL). LUVs consisting of d54-DMPC (D-LUV) or DMPC (H-LUV) with a diameter of ca. 100 nm were prepared by extrusion method using Tris-buffered saline containing equivalent volume of D<sub>2</sub>O and H<sub>2</sub>O. LUVs consisting of 1:1 mixture of both lipids (D/H-LUV) were also prepared by mixing these lipids before hydration. Phospholipid concentration of each LUV preparation was set to 20 mM. SANS measurements were performed using SANS-U with 7° of incident neutron beam. Sample-to-detector distance was set to 4 m.

D-LUV and H-LUV showed almost identical scattering profile, while D/H-LUV exhibited little scattering, suggesting that the scattering length density of D/H-LUV accords with that of solvent, i.e., contrast

matching condition.

When equimolar amounts of D-LUV and H-LUV are mixed, scattering intensity should be equal to the average of each LUV preparation if there is no intervesicular lipid exchange. On the other hand, the lipid exchange between D- and H-LUV reduces the difference in the scattering length density of LUVs from solvent (i.e., contrast) with time. In this case, decay of the contrast depends on both the lipid exchange and flip-flop, and can be described by a double-exponential decay function with rate constants of the exchange and flip-flop.

TR-SANS experiments were carried out at four different temperatures. Each measurement was started immediately after mixing equivalent volume of D-LUV and H-LUV. The data were collected every 3 min. Time-course of the normalized contrast was calculated from the scattering intensity. The contrast decayed more steeply with an increase in temperature. In addition, the normalized contrasts reached below 0.5, suggesting an involvement of flip-flop. The obtained contrast decays were well reproduced by the double-exponential function, which are represented by solid curves in Figure 1. Arrhenius plots of the obtained parameters exhibited good linear relationship, suggesting high accuracy of the data. The half-lives of the lipid exchange and flip-flop at 37 °C were estimated at about 150 and 510 min, respectively. The results presented here demonstrate that TR-SANS is a powerful method to determine the membrane lipid dynamics.

## References

- [1] J. C. M. Holthuis and T. P. Levine, Nat. Rev. Mol. Cell Biol. 6 (2005) 209.
- [2] L. Willner et al., Europhys. Let. 55 (2001) 667.

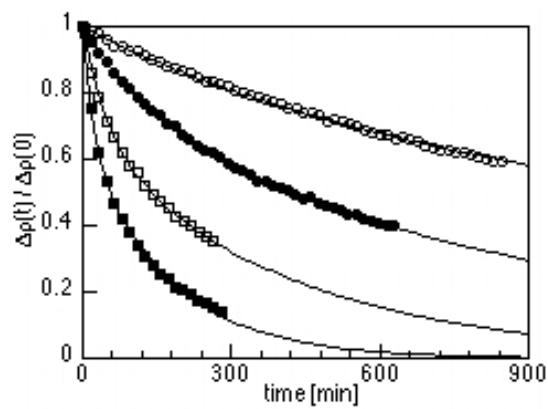


Fig. 1. Contrast decays of LUVs after mixing D- and H-LUV at four different temperatures. Solid curves are fitting curves according to the double-exponential function.