（ ※本報告書は英語で記述してください。ただし、産業利用課題として採択されている方は日本語で記述していただいても結構です。 ）

TOKAI CROSS **Experimental Report** J-PARC

承認日Date of Approval 2013/11/23
承認者Approver Jun-ichi SUZUKI
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課題番号 Project No. 2012A0127

実験課題名 Title of experiment

Time-resolved small-angle neutron scattering studies on the lipid transfer among mixed disk-like lipid bicelles

実験責任者名 Name of principal investigator

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所属 Affiliation

National Tsing Hua University (TAIWAN)

装置責任者 Name of responsible person: Dr. Jun-ichi Suzuki

装置名 Name of Instrument/（BL No.）

BL-15 (TAIKAN)

実施日時 Date and time of Experiment

December 4, 2012, 9:00 AM – December 8, 2013, 9:00 AM

試料、実験方法、利用の結果得られた主なデータ、考察、結論等を、記述して下さい。（適宜、図表添付のこと）

Please report your samples, experimental method and results, discussion and conclusions. Please add figures and tables for better explanation.

1. 試料 Name of sample(s) and chemical formula, or compositions including physical form.

1. DPPC: 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, $C_{40}H_{80}NO_8P$
2. d62-DPPC: 1,2-dipalmitoyl-sn-glycero-3-phosphocholine-d62, $C_{40}H_{18}D_{62}NO_8P$
3. diC_7PC: 1,2-diheptanoyl-sn-glycero-3-phosphocholine, $C_{22}H_{44}NO_8P$

2. 実験方法及び結果（実験がうまくいかなかった場合、その理由を記述してください。）

Experimental method and results. If you failed to conduct experiment as planned, please describe reasons.

The biological membranes consist of several kinds of lipids, cholesterols and membrane proteins. Signal transferring among cells highly depends on the membrane functions. Lipids distribution in the membrane is critical to determine the local functions of the membrane, such as to form raft domains. Lipid transport occurs by in-plane diffusion, flip flop, exchange (trans bilayer) among membranes. It is desirable to determine the fundamental lipid transport processes, such as flip-flop and lipid exchange. There were several successful studies using time-resolved small-angle neutron scattering (TR-SANS) to study such a problem by mixing deuterated vesicles with protonated vesicles. Due to the relatively small size (about 100 nm), there is a high curvature for such model systems, which would affect significantly the lipid transport rates. By mixing suitable amounts of long-chain lipids with short–chain lipids, planar nanodisc bicelles could be formed with the center region dominated by the bilayer of long-chain lipids and the short-chain lipids forming the rim. The typical size of the disk bicelles is around 30 nm. Using such a planar bilayer system, the effect of curvature or asymmetry can be completely eliminated. The results can be compared with the results from vesicles to identify the difference due to curvature effect.

2. 実験方法及び結果（つづき） Experimental method and results (continued)

The time-resolved SANS (TR-SANS) measurements were conducted at the BL-15 Taikan of J-PARC at Tokai, Japan. Both deuterated and protonated DPPC were mixed with suitable amounts of diC7PC to form bicelles of different sizes. The bicelle size could also be an important factor in determining the lipid exchange rate. The effect due to bicelle concentration was also checked. Typically, the deuterated bicelles were mixed with protonated bicelles right before the start of the TR-SANS measurements. Each mixture took about 5~8 hours to collect the scattering spectrum. Due to the time-of-flight nature of the instrument, each recorded neutron possesses position and time information that facilitates time slicing of the scattering data at desired time step. Due to problems with the data reduction software, the complete data set was still not available for the time-resolved measurements at this moment. The results of static measurements of the protonated biclelles as well as the bicelles with d62-DPPC are shown in Fig. 1 for different mixing ratios of DPPC to diC_7PC. The amounts of the DPPC or d62-DPPC were kept at the same concentration of 10 mM in each sample, while the concentration of the diC_7PC was varied to change the size of the disc bicelles. For the d62-DPPC/ diC_7PC bicelles in D_2O solution, the main scattering contrast will be from the ring (diC_7PC) of the disc bicelle and the scattering profiles are different from the protonated bicelles.

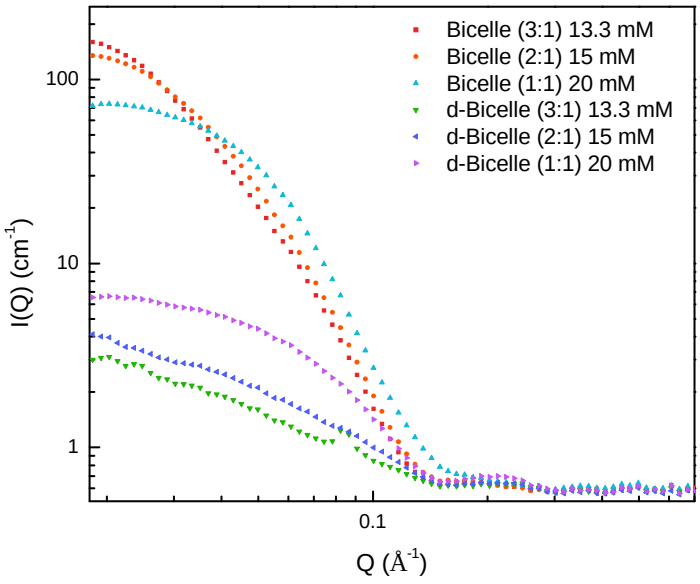

Fig. 1 The measured SANS profiles of the bicelles consist of mixing protonated DPPC with diC7PC, and the SANS profiles of the bicelles consist of mixing d62-DPPC with diC7PC.