（ ※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。 ）

CROSS TOKAI **Experimental Report** J-PARC	承認日 Date of Approval 2017/9/19 承認者 Approver Juni-chi Suzuki 提出日 Date of Report 2017/6/28
課題番号 Project No. 2017A0084 実験課題名 Title of experiment Determination of peptide-induced phospholipid flip-flop by time-resolved measurements 実験責任者名 Name of principal investigator Minoru Nakano 所属 Affiliation Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama	装置責任者 Name of Instrument scientist Kazuki Ohishi 装置名 Name of Instrument/(BL No.) TAIKAN (BL15) 実施日 Date of Experiment 2017/05/21-2017/05/26

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.

Large unilamellar vesicle of 1-palmitoyl-2-oleoylphosphatidylcholine (H-LUV) and large unilamellar vesicle of 1-palmitoyl-d_{31}-2-oleoylphosphatidylcholine (D-LUV) were prepared by extrusion method using Tris buffer containing 30% D_2O. Methyl-β-cyclodextrin (MβCD) was dissolved in Tris buffer containing 30% D_2O. Transmembrane peptide (Q-peptide, Acetyl-ALALALALAQWLALALALAKKKK-amide) and its negative-control peptide (L-peptide, Acetyl-ALALALALALWLALALALAKKKK-amide) were synthesized using Fmoc-based chemistry and purified by reverse-phase HPLC equipped with a C18-column. The peptides obtained were dissolved in ethanol.

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.

We have previously demonstrated by time-resolved SANS experiment that transmembrane model peptides with a hydrophilic residue located within the center of a hydrophobic Leu-Ala repeat sequence enhanced the flip-flop of 1-palmitoyl-2-oleoylphosphatidylcholine (Kaihara et al., Chem Phys 2013, 419, 78). An NBD fluorescence-quenching assay has also revealed that the membrane-spanning sequence of an ER-resident membrane protein, EDEM1, which contains hydrophilic Arg/His residues, robustly promoted flip-flop in artificial membranes (Nakao et al., Biophys J 2016, 110, 2689). Based on these findings, we hypothesized that the flip-flop of phospholipid membranes could be controlled artificially by just adding peptides. In this study we designed peptides that consist mainly of the hydrophobic Leu-Ala repeat sequence and of four lysine residues at C-terminus. Hence, it is considered that the peptides are inserted into vesicles from their N-terminal side immediately on addition, and form a membrane-spanning configuration.

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

Time resolved SANS experiments were performed at J-PARC BL15 TAIKAN for mixtures of D-LUV (15 mM) and H-LUV (15 mM) in Tris buffer containing 30% D_2O at 37 °C. The D_2O volume fraction of 30% was settled on so that the solvent has the intermediate value of the scattering length density between that of D-LUV and H-LUV. Scattering data collected for every one minute were processed including conversion to I-Q data, subtraction for the solvent scattering, and integration of I(Q) between $0.007 < Q < 0.011$ Å^{-1}. After adding 1 mM MβCD, the scattering intensity from the mixtures of vesicles was decreased due to MβCD-mediated phospholipid exchange between D- and H-LUVs, and the intensity decay was reached plateau within 3 h. In this context, both D- and H-LUVs would contain equal amounts of D- and H-lipids (and hence contrast matching) in their outer leaflet, whereas inner leaflet of D- or H-LUVs consists exclusively of either D- or H-lipid, respectively, since the flip-flop of the phospholipids used in this study has been shown not to occur (Nakano et al., J Phys Chem B 2009, 113, 6745).

Afterward, an ethanol solution of the peptide was added. As shown in Figure 1, the scattering intensity was decreased by the addition of Q-peptide, which contains a hydrophilic Glu residue in the center of its transmembrane sequence. The intensity of the sample finally reached down to that of the solvent, suggesting that Q-peptide entirely scrambled lipids between inner and outer leaflets. On the other hand, the addition of L-peptide, whose transmembrane domain consists merely hydrophobic residues and no hydrophilic residue, reduced the scattering intensity only slightly, and the extent of the intensity reduction was similar to that when ethanol was solely added without peptides. This indicates that L-peptide has no activity to scramble the bilayer. Since the Q- and L-peptides differ in that the former substitutes Glu for central Leu residue in the latter, the Glu residue localized in the middle of bilayers could increase the local polarity around the residue, opportunity for head group penetration of lipids, and eventually the frequency of the flip-flop. The time-resolved data clearly detected presence or absence of the peptides' ability to induce phospholipid flip-flop.

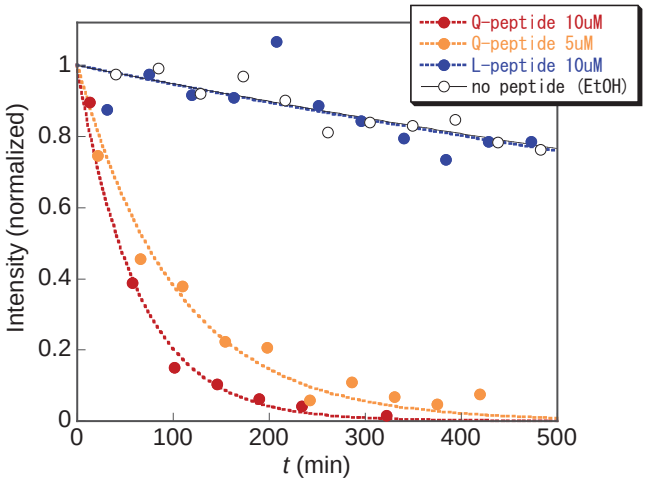

Figure 1. SANS intensity decays after injection of transmembrane peptide (Q- or L-peptide in EtOH) into the mixture of D- and H-LUVs at $t = 0$. MßCD was mixed 180 min before the peptide solution was injected.