（ ※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。 ）

| MLF Experimental Report | 提出日 Date of Report<br>July 31, 2013 |
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| 課題番号 Project No. 2013A0088<br>実験課題名 Title of experiment<br>In-Situ Neutron Reflectivity Studies on the Adsorption of DNA by Mixed Diblockcopolymer and Cationic Lipid Monolayers during Compression of the Complex Monolayer and the Effects of Brush Size, Brush Density and Divalent Ions on DNA Condensation<br>実験責任者名 Name of principal investigator<br>Tsang-Lang Lin<br>所属 Affiliation<br>National Tsing Hua University (TAIWAN) | 装置責任者 Name of responsible person<br>Dr. N. Yamada<br>装置名 Name of Instrument/（BL No.）<br>BL-16 (ARISA II)<br>実施日 Date of Experiment<br>May 1, 2013, 9:00 AM – May 4, 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. diblockcopolymer poly(styrene-b-N-methyl-4-vinyl pyridinium iodide (PS-P4VPQ), (CH2CHC6H5)n-(CH2CHC5H4NCH3I)m
2. DNA, C232 N92O139P22
3. DC-Cholesterol: 3ß-[N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterol hydrochloride, $C_{32}H_{57}N_2O_2Cl$

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.

Recently, there were many studies on the cationic lipid-DNA interactions in order to develop efficient delivery systems for nonviral gene therapy. These studies focused on the structure and interaction of cationic lipid-DNA complex in solution. On the other hand, the interaction of DNA with the monolayer at the air-liquid interface or at solid surfaces is fundamentally different from the interaction in bulk solutions. Other than using cationic lipids, some amphiphilic diblockcopolymers can form highly stable vesicles or micelles in solutions that can form complex with DNA. For the cationic diblockcopolymer monolayer suspended at the air-water interface, the cationic brushes can extend into the water phase and form a thick charged brush layer that could adsorb the DNA to form a three-dimensional brush-DNA complex layer. Our previous neutron reflectivity studies of the DNA adsorption by cationic diblockcopolymer PS-P4VPQ monolayer at the air-water interface showed that high amounts of DNA readily adsorbed by the cationic brushes and the adsorption can be further enhanced by the addition of divalent cations.

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

The surface charge density is an important factor in the DNA adsorption and it can be varied by mixing the diblockcopolymers with cationic lipids. The effect of the existence of some planar surface charge domains formed by DC-Cholesterol among the thee-dimensional cationic brusher layer domains (PS-P4VPQ) on the DNA adsorption will be examined. Three-day beamtime was awarded by the J-PARC to conduct neutron reflectivity measurements at the BL16 SOFIA of MLF. A newly installed Langmuir trough was used to prepare the mixed monolayer on $D_2O$/DNA solution. We were the first official user group to use a LB trough for in-situ neutron reflectivity measurements at BL-16, the ARISA II), J-PARC (as shown in Fig. 1). For each sample, three incident angles were used: 0.4, 1.0 and 2.2 degrees to cover a *Q*-range up to about 0.2 $Å^{-1}$. It took about one hour to four hour measurement time for each sample depending on the intensity of the reflected beam. The neutron reflectivity measurements were helped by Dr. N. Yamada of KEK. Fig. 2 shows the measured neutron reflectivity profiles from a mixture of PS-P4VPQ and DC-cholesterol monolayer at a molecular ratio of 1:20. In the presence of DNA, an interference fringe develops at around $Q = 0.07$ $Å^{-1}$. The addition of the divalent ions, $Ca^{+2}$, further enhances the intensity of the interference peak, which indicates an increase of the adsorbed DNA due to the DNA condensation induced by divalent ions. Further detailed analysis can reveal the exact amounts of the adsorbed DNA and the distribution (depth neutron scattering length density profiles).

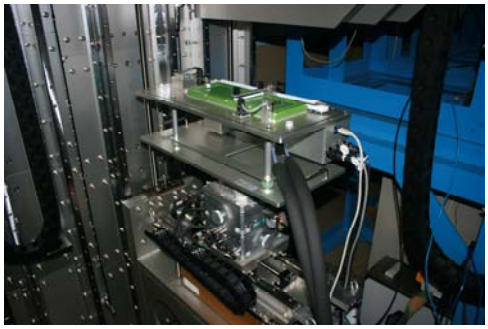

Fig. 1 The Langmuir-Blodgett trough used in the in-situ neutron reflectivity study to prepare monolayer at the air-$D_2O$ interface.

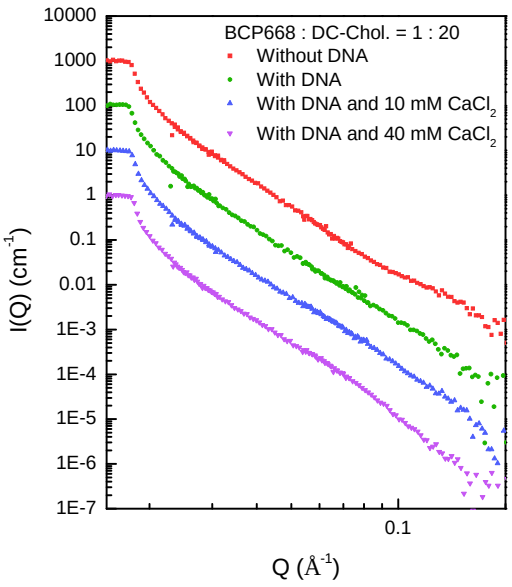


Fig. 2 The measured neutron reflectivity profiles from a mixture of PS-P4VPQ and DC-cholesterol monolayer at a molecular ratio of 1:20. The DNA concentration in the subphase is 1 micro molar.