

Neutron reflection measurement on the absorption of DNA onto the lipid monolayer and cholesterol at the air-water interface

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For the first time, we used the AMOR neutron reflectometer at Swiss Spallation Neutron Source (SINQ) for air-liquid interface measurements. A D₂O water surface was used to calibrate the instrument. We then measured phospholipid monolayers on the air-water interface. With the Langmuir trough, we could control the surface pressure of the lipid monolayers. We also measured the neutron reflection from the lipid monolayers spread and compressed to different surface pressures on the D₂O subphase of 1 μM of DNA from herring tests. The interest lies in the absorption effect of the negatively charged DNA onto the zwitterionic heads of the phospholipid monolayer and positively charged cholesterol monolayer at the air-water interface. Following is the description of measurement details and results.

1. D₂O test measurement

For liquid surfaces the reflected surface is being inherently horizontally oriented. We used a supermirror to bend down the beam for neutron reflection (NR) measurements using the time-of-flight mode. Since the available neutron spectrum for AMOR is between 3-9 Å in principle we could only cover a dynamic Q-range of 3 orders of magnitude for each incident angle. To obtain a decent dynamic Q-range, we used two angles of the reflecting supermirror of 0.5° and 1.5° for covering a Q-range of 0.012-0.1 Å⁻¹ for each system. For higher incident angle, the incident beam intensity reflected from the supermirror decayed severely and failed to give measurable reflection intensity. The beam size used for the 0.5° and 1.5° were 1mm and 3 mm, respectively, for a balance between intensity and Q-resolution.

In Fig. 1 we show the reflectivity measured for the D₂O surface using 0.5° incident angle. The data can be fitted with the D₂O Fresnel curve of a scattering-length-density of $6.33 \times 10^{-6} \text{ Å}^{-2}$, corresponding to a critical Q edge of 0.017 Å^{-1} for total reflection, with a surface roughness of 3 Å (dashed curve). The data in the higher Q region deviate gradually from the fitted curve (after $Q \sim 0.04 \text{ Å}^{-1}$) revealing an increasingly large effect of the fast-neutron-background effect. For a comparison, we also show the D₂O data measured using the NG 7 neutron reflectometer at the National Institute of Standards and Technology (NIST), USA. To reduce the background scattering by shifting the detector from the specular position by 4 mm in the following measurements. The Langmuir-trough in the experiments at AMOR was used by courtesy of R. Steitz, HMI, Berlin, Germany.

2. Absorption of DNA onto phospholipid monolayer at the air-water interface

We are interested in the absorption effect of DNA onto the phospholipid monolayer at the air-water interface. The absorption mechanism is expected to be of electrostatic interactions between the negatively charged DNA and the electric dipole of the zwitterionic heads of phospholipids, of electric dipoles pointing down into water to attract DNA molecules. The adsorption of DNA may induce structural changes to the lipid monolayer at the air-water interface, for instances, the re-orientation of the lipid head group conformation, as a consequence, a reduction of the tilt angle of the lipid chains. These structural changes may be perceived

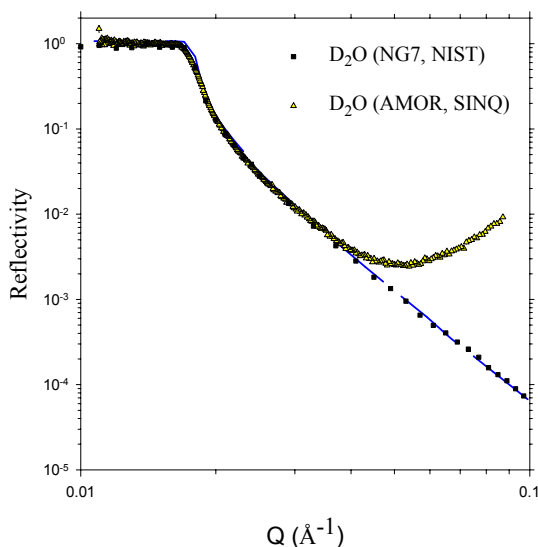


Figure 1: Neutron reflection data for D₂O measured at the AMOR neutron reflectometer (triangles). Also shown were the D₂O data (squares) measured at the NG7 neutron reflectometer at NIST, fitted with the Fresnel curve of a 3 Å surface roughness.

by the neutron reflection measurement for the change of layer thickness of the lipid monolayer.

Our surface pressure-area (π -A) isotherm results (Fig. 2) indicates that the DNA (1 μM) in water solution adsorbs to the surface DPPC monolayer and changes the isotherm significantly. The earlier rising surface pressure for the DPPC on DNA solution, compared to that on pure water, is attributed to the additional charge interactions between the DNA adsorbing underneath the surface monolayer of DPPC.

We spread 20 μl of a phospholipid (DPPC) solution in chloroform (3.5 mg/ml) on a D₂O solution of 1.0 μM DNA. The DNA was purified from the herring tests sodium salt of M_w of 700 b_p, where $b_p = 649 \text{ a.m.u.}$. To increase the neutron sensitivity, we used a deuterated phospholipid, dipalmitoyl-phosphatidylcholine, with the two aliphatic chains deuterated (d₆₂-DPPC, $M_w = 796$). With the Langmuir trough setup on the AMOR beamline, we could compress the surface slightly for a non-zero surface pressure $\pi = 1.2 \text{ mN/m}$,

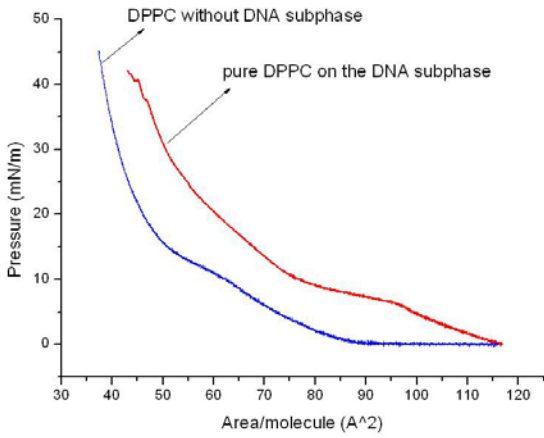


Figure 2: Surface pressure-area isotherms for DPPC on pure water and water subphase containing 1 μM DNA, respectively.

to assure the existence of the DPPC molecules on the water surface. We then waited for two hours for equilibrium.

At the end, the pressure stabled at 3.2 mN/m. Presumably, DNA molecules in the solution absorbed to the surface monolayer and thus increased the surface pressure. Neutron reflection measurement was then conducted at this moment. Further, we compressed the surface monolayer to 23 mN/m ($A = 65 \text{ \AA}^2/\text{mol.}$) and measured the reflection again. The result is shown in Fig. 3.

In Fig. 3, the reflectivity data measured at low π for the lipid/DNA system resembles that for pure D_2O (dashed curve). In the Q -region near 0.1 \AA^{-1} , the data subject large uncertainty due to the difficulty in subtracting the true background. On the other hand, the data measured at higher π of 23 mN/m show a significant change of the reflectivity profile from the D_2O curve. At this pressure, the DPPC monolayer was assumed to form a compact monolayer, based on the surface isotherm measured. The oscillation of the reflectivity data profile reflects the layer structure of the compact surface monolayer. Nevertheless, the effect is complex by the absorption of the DNA to the lipid monolayer. This should become clear when the NR measurement for the pure DPPC system on D_2O subphase containing no DNA at similar surface conditions is performed, which was not completed due to the limited number of measurements within the beam time available.

On the other hand, since the DPPC monolayer thickness is estimated to be 26 \AA , we would need data covering a Q -range up to $\sim 0.2 \text{ \AA}^{-1}$ to distinguish the Kiessig fringe from the reflectivity decay due to surface roughness. Unfortunately, the current AMOR setup cannot provide such a desired Q -range for liquid surfaces. From the current data, we would have difficulties to separate out the DNA absorption effect on the lipid monolayer, even if we measure the NR data for the pure DPPC monolayer system.

We would recommend to see two serious supermirrors for larger incident angles, therefore larger Q values. Each of the two mirrors can share half of the incident angle, thus increasing the reflection from each mirror and the incident beam intensity for reflection measurement.

3. Absorption of DNA onto cholesterol monolayer at the air-water interface

For even stronger charge interactions between the surface

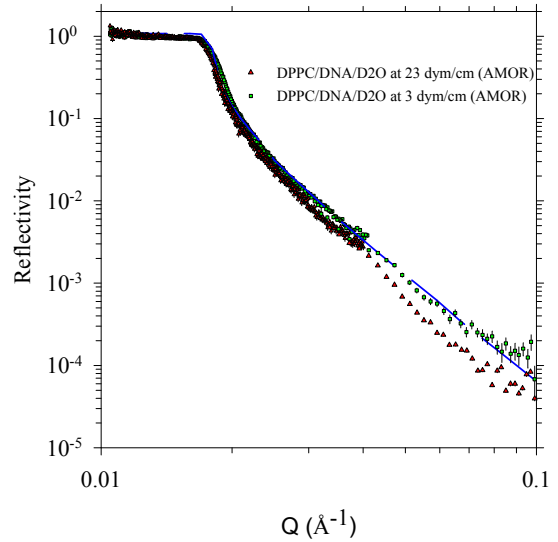


Figure 3: Neutron reflection data for the d_{62} -DPPC/ D_2O (DNA) system at surface pressures of 3 mN/m (squares) and 23 mN/m (triangles), respectively. The dashed curve is the D_2O Fresnel curve of 3 \AA surface roughness.

monolayer and the DNA in solutions, we replaced DPPC by positively charged cholesterol, β -N-(dimethyl-aminoethyl) carbamate (DC-Chol) of $M_w = 537.3$. According to the π -A isotherms and X-ray reflectivity of the LB film, DC-Chol can form a monolayer on the air-water interface. The attraction of the positively charged DC-Chol monolayer to the negatively charged DNA is even stronger, compared to that between DPPC and DNA, as indicated by the strongly modified π -A isotherm of the DC-Chol when compressed on the water solution containing $0.8 \mu\text{M}$ DNA (Fig. 4).

We spread $22 \mu\text{l}$ of DC-Chol solution in chloroform (2.5 mg/ml) on D_2O and compressed to $A = 65 \text{ \AA}^2/\text{mol.}$ ($\pi = 1.6 \text{ dyn/cm}$), where the neutron reflection data were collected.

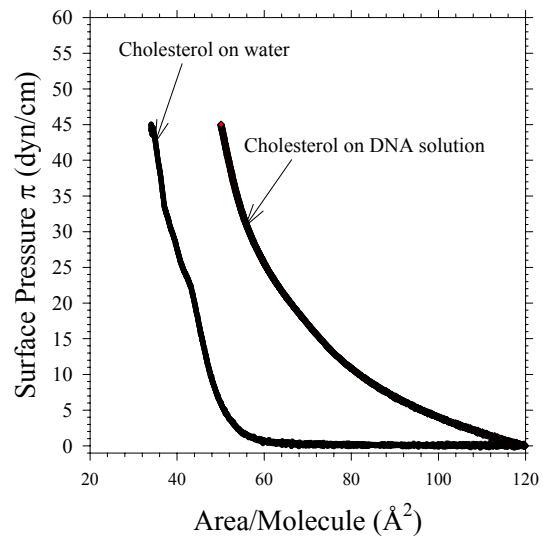


Figure 4: Surface pressure-area isotherms for DC-Chol on pure water and DNA water solution, respectively.

We also measured for DC-Chol spread on a D₂O solution of 0.8 μ M DNA, with A compressed to the same value ($\pi = 13$ dyn/cm). The result is shown in Fig. 5. The higher surface pressure for the cholesterol/DNA system reflects the adsorption of DNA.

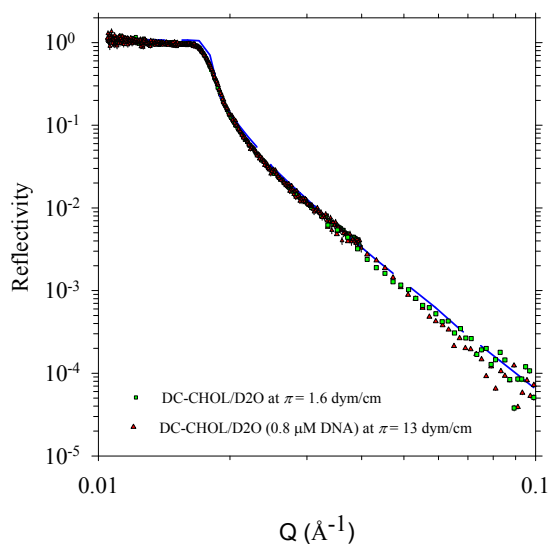


Figure 5: Neutron reflection data for DC-Chol on pure D₂O subphase (squares) and on the D₂O subphase of 0.8 μ M DNA (triangles). The surface excess $A = 65 \text{ \AA}^2/\text{mol}$. is the same for both measurements. The dashed curve is the D₂O Fresnel curve with 3 \AA surface roughness.

In Fig. 5, the reflectivity profiles measured for the DC-Chol monolayer on pure water surface and on the water subphase containing DNA are similar in the low-Q region. Nevertheless, the latter decreases slightly more as Q increases, reflecting a larger roughness effect or larger thickness (shorter oscillation period in Q) due to the adsorption of DNA onto the cholesterol monolayer at the air-water interface. As also mentioned in the previous case for the DPPC/DNA system, within the limited Q-range, the small difference in reflectivity profiles measured is difficult to trace back to the corresponding structural differences (several to 10 \AA) of the cholesterol monolayer changed by the DNA adsorption.