

Supported Biomembrane Films from Proteins and Lipids

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Lipid/protein complex films have attracted intensive interest as a model system to investigate membrane structure, membrane permeability and the interaction of lipids and proteins. In this work, lipid and protein multilayer films were studied by neutron reflectivity. Negative charged lipid DMPA was adsorbed onto a positive charged polymer cushion. DMPA did not form a simple bilayer structure on the polymer surface. A bilayer structure was formed on a HSA surface after deposition of a HSA layer onto the initial DMPA covered surface.

Lipid bilayers are suitable model systems for studying membrane recognition and signal transduction processes.¹ Especially the combination of proteins with lipid layers attracts a widely attention since the later provides a natural environment for the immobilization of receptors and antibodies.² Recently, L- α -dimyristoylphosphatidic acid (DMPA)/human serum albumin (HSA) hollow capsules and its application as controlled drug release system have been investigated.^{3,4} In order to understand the interaction between the lipid and the protein in the complex film, DMPA/HSA multilayer films were fabricated by Layer-by-Layer (LbL) technique. Using neutron reflectivity (NR) measurements we wanted to study in-situ the formation of DMPA/HSA “sandwich” like structures and gain information about the partial densities of the individual components by use of contrast matching.

The experiments were performed in a homemade liquid/solid experimental cell in ToF mode at the reflectometer AMOR at three angles of incidence. This way the whole necessary Q range was accessible. The first NR experiment was successfully carried out on the polyelectrolyte cushion that was prepared in advance by LbL deposition of poly (sodium 4-styrenesulfonate) PSS and poly (allylamine hydrochloride) PAH on a Si block. Then lipid was introduced into the experimental cell and left for adsorption for 1h. The lipid dispersion adsorbed to the polyelectrolyte support had an initial concentration of 0,5 mg/mL and the size of the vesicles prepared by extrusion was 50 nm. The lipid adsorption and NR measurements were performed at a temperature above the main phase transition temperature of the phospholipid. After the adsorption remaining free lipid was washed away by D₂O. The NR experiment was performed against D₂O. We found that DMPA formed a complicated layer structure rather than a simple bilayer structure (Figure 1). The reflectivity curve could not be fitted with a single box model. The experiment was continued with in-situ adsorption. The NR curve shows that HSA formed a monolayer on the DMPA covered surface. Next DMPA was introduced again. The neutron reflectivity curve showed further deposition of a DMPA bilayer on the HSA surface. Probably the charge density of these two different surfaces and the interaction between DMPA/polymer and DMPA/HSA should be considered in order to understand why DMPA formed different structure on polymer cushion and HSA surface.

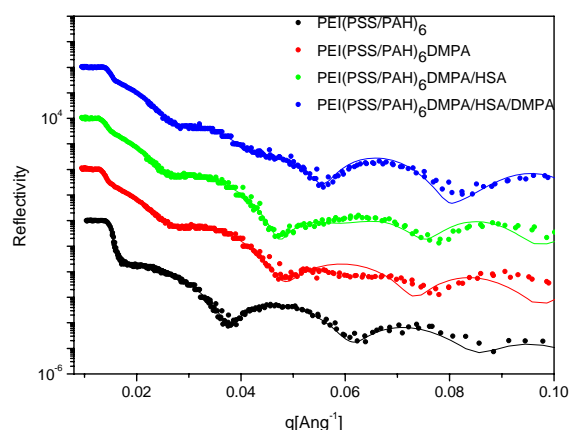


Figure 1: Neutron reflectivity profiles of (a) PEI(PSS/PAH)₆, (b) PEI(PSS/PAH)₆/DMPA, (c) PEI(PSS/PAH)₆/DMPA/HSA and (d) PEI(PSS/PAH)₆/DMPA/HSA/DMPA multilayer film.

Table 1: Fitting parameters that give best fit to the experimental reflectivity curves for supported DMPA/HSA layer on polymer cushion.

	Layer	Thickness (nm)	SLD(10 ⁻⁶ × Å ⁻²)	Roughness (sigma)
a	PEI(PSS/PAH) ₆	25.8 ± 0.2	3.913 ± 0.06	15.523 ± 3
b	PEI(PSS/PAH) ₆ /DMPA	10.1 ± 0.7	0.794 ± 1	5 ± 2
c	PEI(PSS/PAH) ₆ /DMPA/HSA	4.1 ± 0.1	2.384 ± 0.07	5 ± 3
d	PEI(PSS/PAH) ₆ /DMPA/HSA/DMPA	4.9 ± 0.5	4.93 ± 0.1	5 ± 7

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