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Experimental report

The effect of cholesterol and/or melatonin on the amyloid-β peptides loaded model membranes – Neutron Reflectometry study

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Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative disease characterized by dementia and memory loss for which no cure or effective prevention is currently available. One of the hallmarks of AD is the formation of senile plaques, primarily consisting of amyloid-β (Aβ) peptides. The crucial role in this process is thought to be imparted by peptide-membrane interactions, modulated by membrane composition. In particular, cholesterol was found to segregate in the immiscible plaques when present in elevated concentrations, and even further intensification was observed in the anionic membranes by the presence of Aβ peptides[1]. However, the changes to the structural properties of membrane are known to be accompanied also by the changes in membrane physico-chemical properties. The cholesterol increases the order of lipid hydrocarbon chains and increases the stiffness of membrane. On the other hand, melatonin was found to increase the fluidity of membrane and counteracted the effect of cholesterol (see Figure 1). Interestingly, decreasing levels of melatonin in the brain tissue were correlated with the aging, suggesting the melatonin's potential role in preventing the development of AD whose probability also increases with age.[2] We have extended our previous studies investigating the effect of the addition of cholesterol and/or melatonin on the structural properties of model phospholipid membrane,[3] those looking at the interactions with Aβ peptide. We decided to utilize neutron reflectometry, because this method is rather precise in the determination of thin layer structures and its changes.

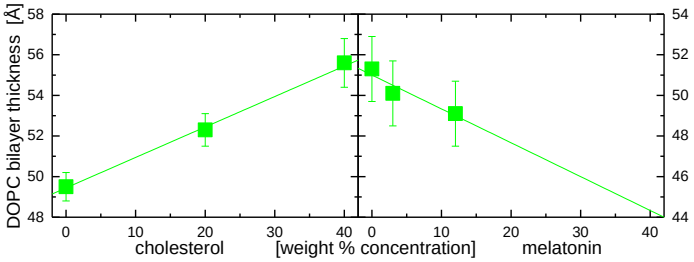

Figure 1: The interactions of cholesterol and/or melatonin with DOPC model membrane. The graph shows an increase of bilayer thickness with the increasing cholesterol, while opposing effect is caused by the addition of melatonin.

Experiment

The solutions of increasing complexity consisting of DOPC, 29 mol% cholesterol, 29 mol% melatonin, and 3 mol% of transmembrane Aβ segment A$\beta_{25\text{-}35}$ were prepared. DOPC was purchased from Avanti Polar Lipids (Alabaster, USA), the rest of compounds from Sigma (St. Luis, USA). The solutions were spread on the surface of silicon wafer substrate 8.5x6 cm^2, covered by another piece of identical crystal and merged under the water. Mutual sliding of crystals results in the creation of planar bilayer on the surface of the crystals, while aqueous ambient ensures the rinsing of extra lipid. The surface of the crystals was treated before the solution deposition in order to get rid of all impurities and

to obtain perfect hydrophilic properties employing standard method as follows. The crystals were merged in the solution of H_2O:NH_4OH (27 %) with volume ration 5:1 and heated up to 70°C. Afterwards, one volume unit of H_2O_2 (30 %) was added and the crystals were kept in these conditions for 15 minutes. The final experimental sample is mounted so that the bilayer normal is horizontal, and a hydrating water chamber is formed by the Si wafer substrate, a rubber o-ring, and aluminium chamber cover. The experimental data were collected at multifunctional neutron reflectometer with horizontal sample plane during acquisition time ~ ½ day/sample.

Results and discussions

Unfortunately, due to instrument technical issues, the experiment failed. Despite the problems preventing us from utilizing the data in a proper analysis, the measurements revealed clearly a successful sample preparation. Figure 2 suggests a single layer of lipid membrane deposited via the vesicle deposition method on top of the Silicon wafer.

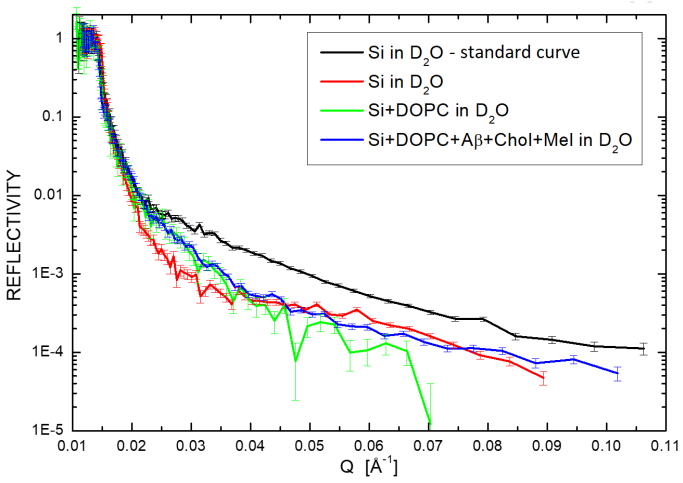

Figure 2: Reflectivity data taken at the GRAINS instrument in March 2017. The distortion of neat Si wafer data reveals the technical issues. Nevertheless, the differences of further data suggest a potentially successful preparation of samples.

References:

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