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E x p e r i m e n t a l r e p o r t

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

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Introduction

Cell membrane, being an interface between inner cellular organs and cell ambient, is a crucial feature facilitating not only transport of matter, but it is also found to be the place of acting for numerous diseases. Alzheimer's disease (AD) is considered to be one of them. The suggestion is built around the fact, that plaques, primarily consisting of amyloid-*β* (A*β*) peptides, are frequently observed in the brain tissue of people suffering from the AD. In order to investigate the A*β* plaques formation on the surface of the neural cells and A*β* peptides interaction with biological membranes, detailed studies carried out on model bilayer systems are inevitable. Due to this, we performed initial experiments employing neutron reflectometry, where we attempted to scrutinize several model bilayer systems (comprising fractions of DOPC, melatonin and cholesterol). In the case of obtaining promising results regarding the observation of some differences in mutual membranes thickness and/or neutron scattering length density profiles, further experiments with A*β* peptides introduced in the systems will be proposed.

Experiment

The solutions of increasing complexity consisting of DOPC, 29 mol% cholesterol and 29 mol% melatonin 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 modified surface ($Si/Ni/Si/SiO_2$) 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 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. Since the examination of this kind of systems at GRAINS facility has been done for the first time, measurement of each system was repeated. From the samples data comparison, information on quality of sample preparation can be inferred.

Results and discussions

Dependences of reflectivity *R* on momentum transfer change *q* of all the samples are displayed in Fig. 1. Owing to new special $Si/Ni/Si/SiO_2$ layer on the surface of the crystal, enhanced contrast and resolution was achieved when compared to previous measurements performed utilizing crystal with untreated Si/SiO_2 surface. One can notice clear difference between *R(q)* of pure substrate (red squares) and the rest of the data. Shift of the peaks' maxima to the lower *q* with respect to pure substrate points to the surface layer thickness increment. This is the evidence of single lipid bilayer deposition on the crystal surface.

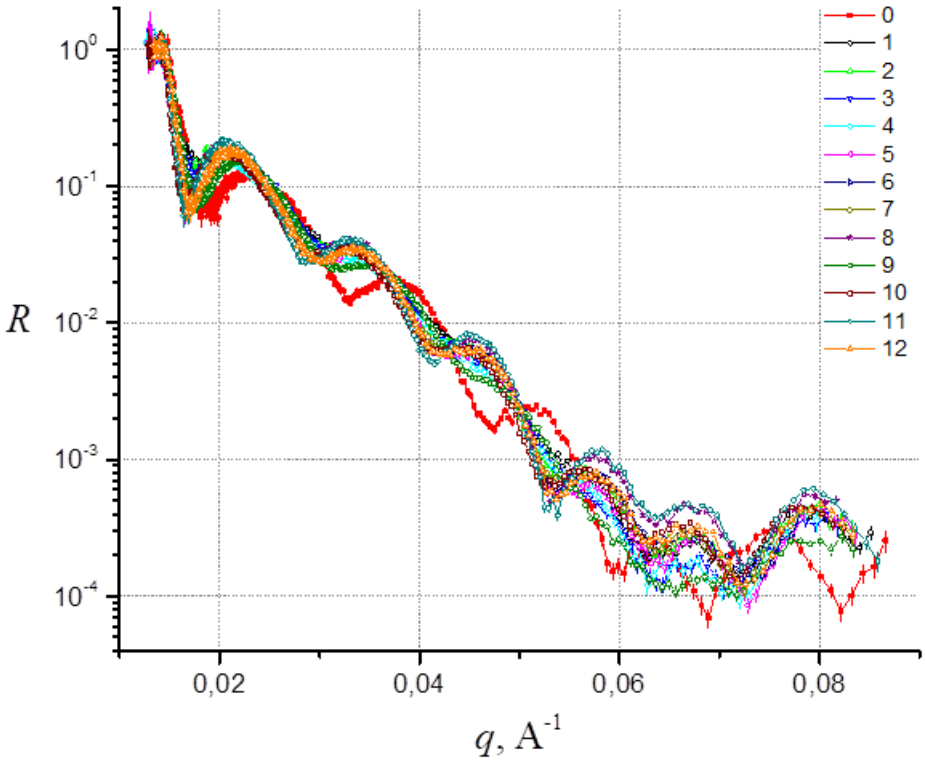


Figure 1: Reflectivity data taken at the GRAINS instrument in February 2019. Difference between pure substrate (red squeres) and substrate + corresponding bilayer is evident. 1. DOPC; 2. DOPC + Cholesterol; 3. DOPC + Melatonin; 4. DOPC + Cholesterol + Melatonin; 5. DOPC; 6. DOPC + Cholesterol D7; 7. DOPC + Cholesterol D7 + Melatonin; 8. DOPC + Cholesterol; 9. DOPC + Melatonin; 10. DOPC + Cholesterol + Melatonin; 11. DOPC + Cholesterol D7; 12. DOPC + Cholesterol D7 + Melatonin

From mutual comparison of the data obtained for the same systems from repeated measurements (Fig. 2) we can conclude that, in general, the shape of the corresponding curves can be regarded as similar. Importantly, rather good accordance of local extremes' (maxima and minima) positions is apparent. Progressive difference between the two curves can be seen particularly for higher q values with exception of system DOPC + Cholesterol D7 + Melatonin, where the best mutual data agreement was observed. Since the data quality of repeated measurements appears higher (considering the accuracy of minima identification), they will be assumed in the further analysis (Fig. 3).

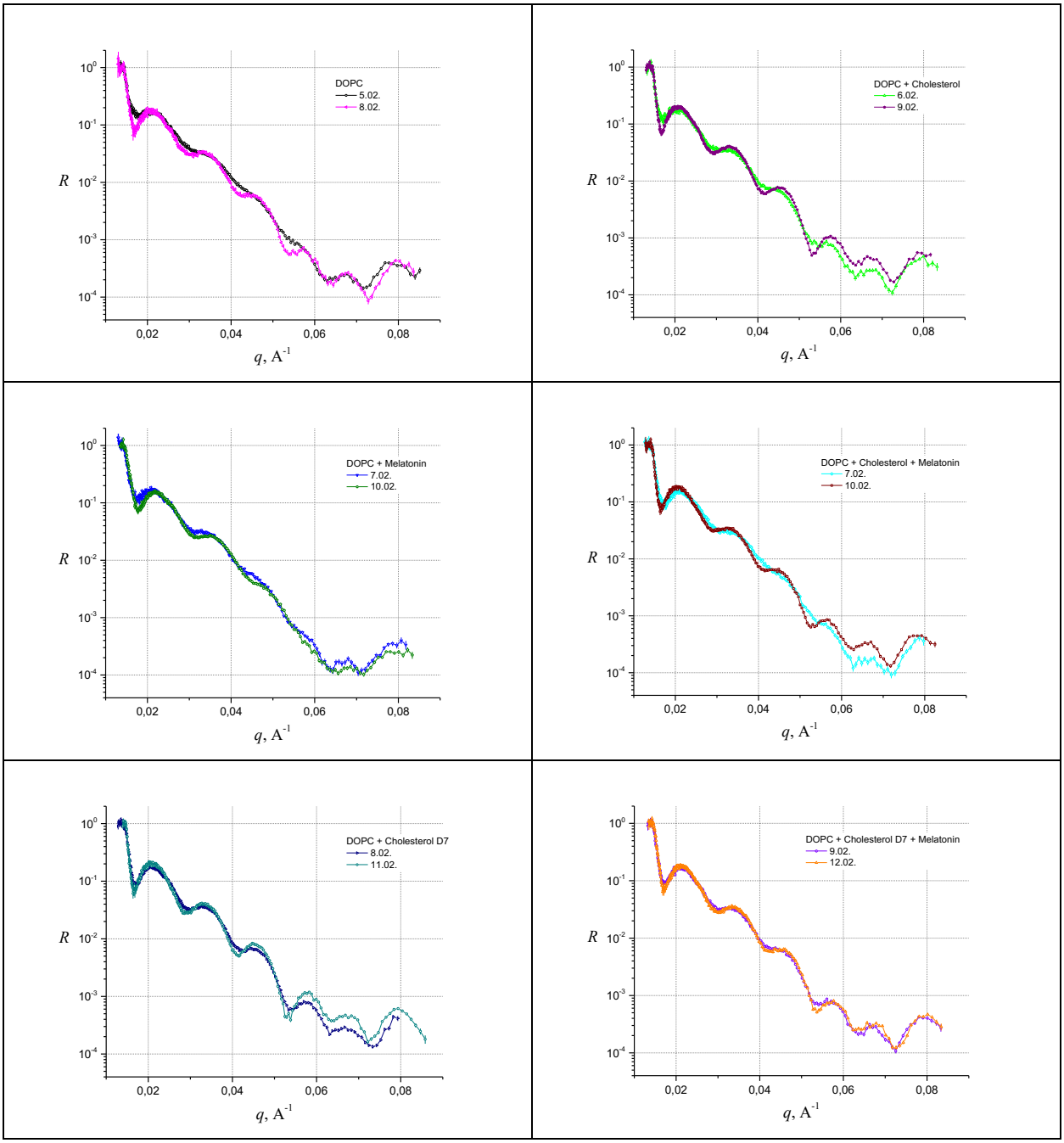


Figure 2: Mutual comparison of reflectivity data obtained for all investigated lipid bilayer systems in two different measurements. The date of data collection (February 2019) is indicated in the legend.

Without profound analyzing or modeling, one can recognize slight shifts (Fig. 3) of maxima positions corresponding to DOPC bilayers modified by cholesterol (violet) and melatonin (green) to the left and right, respectively, when compared to pure DOPC membrane (magenta). The shifts correspond to bilayer thickness changes and have been found in qualitative agreement with the evidence of cholesterol and melatonin counteracting on lipid membrane reported earlier[3].

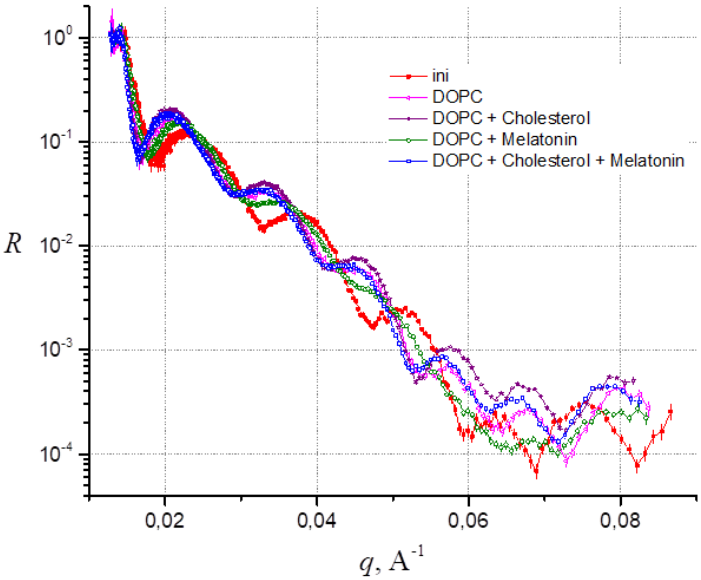
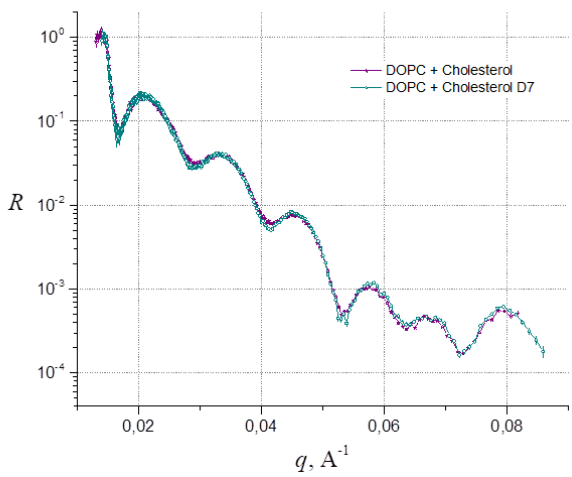


Figure 3: Reflectivity data of higher quality collected from repeated measurements of all the systems.

According to Fig. 4 and Fig. 5, the cholesterol deuteration affected the reflectivity of neutrons by the systems insignificantly. Nevertheless, some discrepancies have been found in the case of DOPC + Cholesterol + Melatonin.



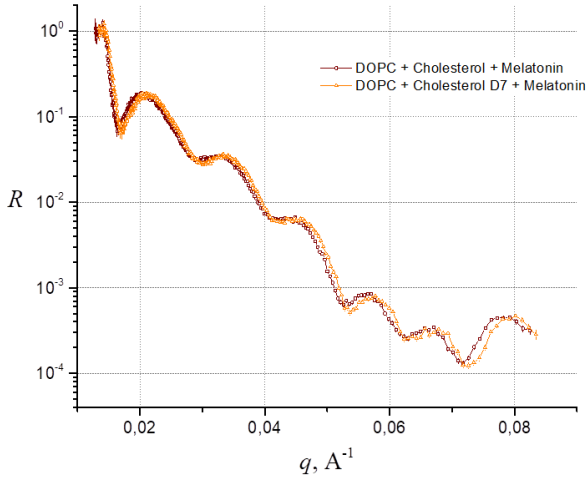


Figure 4, Figure 5: The effect of deuterated Cholesterol D7 on reflectivity of model bilayers.

Conclusions

Investigation of model DOPC bilayers doped by cholesterol and melatonin by means of neutron reflectometry led to several crucial conclusions. First, sufficient contrast inevitable for proper data analysis and different system identification has been achieved. Second, the process of single bilayer preparation and deposition on the crystal surface has been handled. Third, the possible utilization of this method for further extended experiments involving Aβ peptides has been approved. Profound analysis and evaluation of experimental data gathered in the study are on the way.

References:

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