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Keynote 6 - Molecular transport in lipid membranes investigated by neutron scattering

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The phospholipid bilayer is the basic structural motif of most biological membranes. As such, many biological processes occur within or in the proximity of the cell membrane, and therefore, interest in the properties and behavior of lipids in membranes is considerable. For example, it is found that in nature the lipid distribution across the inner and outer leaflet of cell membranes is asymmetric and this asymmetry plays a prominent role in processes like cell fusion, activation of the coagulation processes and the recognition and removal of apoptotic cells by macrophages. Therefore, there is great interest in studying the factors determining lipid movement across membranes as well as the resulting lipid mapping in the membrane, both of which are far from being understood and characterized.

In the literature, big discrepancies in the timescale of the occurrence of lipid flip-flop in model bilayer systems are found, partly due to the fact that these measurements were based on the indirect observation of the process and hampered by artifacts emerging from these different methodologies. Combining time resolved small angle neutron scattering and neutron reflectivity, we show that it is possible to capture inter and intra vesicular exchange as well as lipid composition differences in the leaflets of a model bilayer with the sub-nanometer spatial resolution and for times scales as short as a few minutes. Starting from these results we extensively studied, temperature and time dependence of the structure of lipid bilayers looking for traces of structural asymmetry and consequent relaxation towards an equilibrated symmetric bilayer.

By in situ monitoring the structure of a) a solid supported lipid bilayer exposed to a solution of isotopically labeled vesicles and b) a bulk mixture of hydrogenated and deuterated vesicles, we can provide new insight on the characteristics of inter- and intra-bilayer rearrangement processes. I will report on the rates and energetics of pure lipids as well as cholesterol transfer in different lipids environments. Particularly, we markedly found that cholesterol moves very slowly (tens to hundreds of minutes) across a single bilayer (flip-flop), over a large energy barrier which was not significantly different from the time or energy that it took cholesterol to move between vesicles (exchange). These results will be discussed with respect to MD predictions and to the presence of defects that can render the lipid intra-bilayer movement (flip-flop) the time limiting process or not.

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