Experimental report

21/04/2016

Proposal: 9-13-598 **Council:** 4/2015

Title: QENS study of the influence of transmembrane proteins on the dynamics of phospholipid vesicles

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Lisa LAUTNER

Experimental team: Lisa LAUTNER
Torben SCHINDLER

Local contacts: Tilo SEYDEL

Samples: DMPC

Instrument	Requested days	Allocated days	From	To
IN16B	4	3	02/12/2015	05/12/2015

Abstract:

Biological membranes are involved in a variety of cell functions as i.a. the regulation of signal and molecular transport into and out of cells. The interactions between the different lipids and proteins which are the main components of the membrane influence the regulation of these cell processes and are thereby strongly coupled to the membrane structure, composition and dynamics.
Hence, we intend to study the molecular mobility of fully hydrated 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) phospholipid vesicles in absence and presence of different amounts of the transmembrane protein transferrin receptor (TFRC) as well as the protein dynamics itself. In order to obtain a detailed understanding of the molecular dynamics of biological membranes on an atomistic scale, with high spatial as well as temporal resolution the combination of the complementary methods Quasielastic Neutron Scattering (QENS) and Molecular Dynamics (MD) simulations will be used. MD simulations of DMPC bilayers with the embedded protein TFRC are currently in preparation and will be compared to the QENS experiments. Therefore, we apply for 4 days of beamtime at the backscattering spectrometer IN16B.

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EXPERIMENTAL REPORT

Experiment Title

QENS study of the influence of transmembrane proteins on the dynamics of phospholipid vesicles

Proposal number	**9-13-598**
Instrument	**IN16B**
Date of Experiment	**02.12.15-05.12.15**
Local Contact	**Tilo Seydel**

Experimental Team

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Proteins and lipids constitute a major part of biological cell membranes. The lipid-protein interactions effect a variety of important cell function as i.a. the regulation of molecular and signal transport which are strongly coupled to structure, composition and dynamics of the membrane. The transmembrane protein transferrin receptor (TFRC) plays a crucial role in the transport of iron into the cell as well as in the regulation of the cellular iron concentration [1]. In order to perform his functions the transmembrane protein needs to be mobile in the membrane. The two-dimensional movement of such membrane receptors provide a mechanism for interactions between receptors in the plane of the membrane. The lateral diffusion of the receptor proteins can lead to formation of clusters which may also be mobile [2]. The understanding of the lateral mobility of membrane proteins and protein cluster formation is a first step to understand the dynamics of cell surface processes [3].

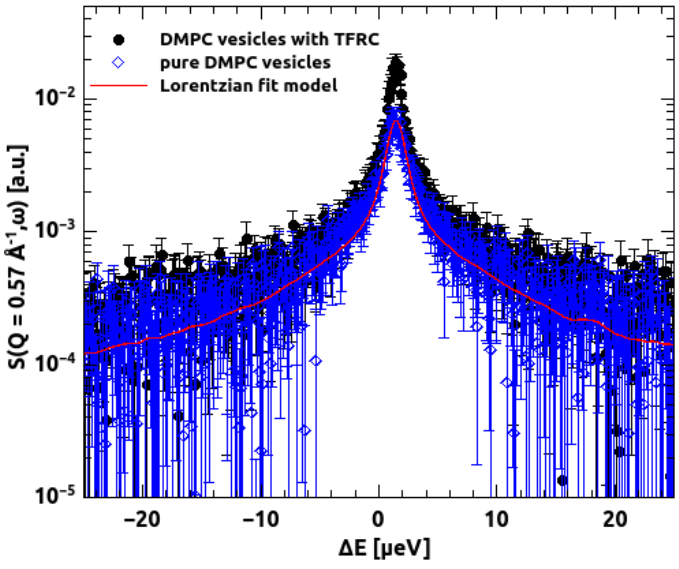

Figure 1: QENS data of a pure DMPC vesicle (blue diamonds) and the lipid vesicles with TFRC protein (black circles) at 310 K, respectively. The red curve represents the best Lorentzian fit.

Using the backscattering spectrometer IN16B (ILL, Grenoble, France) quasielastic neutron scattering (QENS) experiments were performed on phospholipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) vesicles at 310 K with and without the transmembrane sequence of the TFRC protein to study his influence on the lipid dynamics.

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In this experiment, special emphasis were also laid on the dynamics of the protein TFRC itself. Therefore, chain-deuterated DMPC-d54 vesicle with and without TFRC were measured as well to distinguish between the lipid and the protein dynamics.

The first step was to analyse the lipid motions in the pure lipid vesicles. In order to describe the lipid dynamics, two fit models consisting of a convolution of a function describing the long range motion (Lorentzian for a diffusion and Gaussian for a flow-like motion) with two broad Lorentzian functions modelling the fast internal molecular motions and with the resolution function of the spectrometer determined by a Vanadium measurement were tested (for detailed description of the fit models see [4]). Both fits can describe the data almost equally well. Figure 1 displays the pure DMPC lipid vesicles with the Lorentzian fit model and also the DMPC vesicles with the TFRC protein for the same *Q*-value.

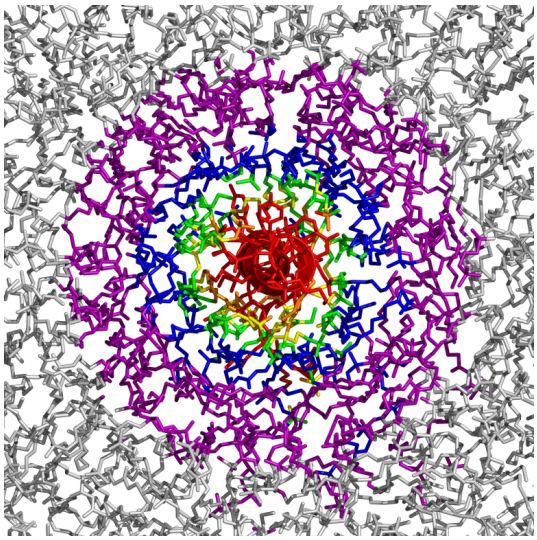

Figure 2: Top-view of a MD simulation of the transferrin receptor protein (red) in a phoshpolipid bilayer. The different colors represent the lipids at certain distances to the protein for which the diffusion constants were calculated. It was found that the diffusion constant increases with increasing distance to the protein. At a distance of about 2 nm to the protein, the values of the calculated lipid molecule diffusion were comparable with the values of lipids in a pure bilayer without a protein.

First results indicate that the slower proteins mainly contribute to the elastic line and seem to restrict the lipid mobility compared to the 'free' lipids in the pure DMPC vesicles. The measurements of the deuterated DMPC-d54 lipid vesicles will now be used to best possible distinguish between the contributions of the proteins and that of the lipids to the scattering signal. Out of these results it should be possible to estimate how far the influence of the proteins reaches on the surrounding lipid molecules.

Finally, the results of the QENS experiment will be compared to MD simulations which also displayed a restricted motion of the nearest lipids which surround the proteins. Figure 2 displays a top-view on a simulated lipid bilayer with embedded TFRC protein (red) and the coloured lipids from which the diffusion coefficients were calculated (further description see figure 2).

[1] T. Moos *et al*., *Cell. Mol. Neurobiol.*, 2000, **20**, 77-95
[2] M. Srivastava *et al.*, *Biophysical Chemistry*, 1998, **75**, 201-211
[3] S.J. Singer *et al.*, *Science*, 1972, **175**, 721
[4] S. Busch *et al.*, *J. Am. Chem. Soc.*, 2010, **132**, 3232-3233