Experimental Report

Proposal: **8-04-696** **Council:** 10/2012

Title: The effect of lipid environment indynamics of peripheral myelin protein P2 and effect of P2 to lipid membrane dynamics.

This proposal is continuation of: 8-04-678

Researh Area: Biology

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Samples: Peripheral myelin proten P2, wild type
Peripheral myelin protein P2, P38G mutated

Instrument	Req. Days	All. Days	From	To
IN6	5	3	04/06/2013	07/06/2013
D16	3	3	04/06/2013	07/06/2013

Abstract:

The myelin sheath is a multilayered membrane wrapped around axons in the vertebrate nervous system; it enables the rapid transmission of nerve impulses. It contains a set of specific proteins, which are involved in myelin formation and linked to neurological diseases. Here we aim to continue studying the dynamics of myelin peripheral protein P2, its orientation in lipid membrane and effects on membrane dynamics. The proposed experiments are part of a PhD project funded by European Spallation Source (ESS). Short- and long-term ILL visits of the PhD student are planned in the framework of the ongoing collaboration between scientists at ILL (IN13) and the University of Oulu in Finland.

Myelin sheath is lipid-rich tightly packed multilayered membrane enriched with a set of myelin-specific proteins. The main goal of this project is to investigate the structure and function of proteins and their affect on the model myelin membranes. The myelin protein P2 is a major protein of peripheral nerve myelin, and a potential antigen in Guillain-Barré syndrome (Ruskamo et al 2014). Human P2 shows significant differences in its CD spectrum in oriented and isotropic samples in the presence of synthetic lipid vesicles. It indicates that the protein adopts a non-random orientation when it binds to a membrane surface.

In this study, the effect of myelin protein P2 into properties of myelin modeling DOPC-DOPS-membrane was examined on D16, IN6 and IN13. Hydrated (D_2O) membranes containing 5% P2 wild type or hyperactive P38G-mutated P2 (Lehtimäki et al. 2012) proteins were prepared on polished silica plates.

D16

Neutron diffraction at λ = 4.4.767 Å was measured in function of temperature in 1 K steps counting 5 x 1 min per image. The empty cryostat was used for collecting background data.

The diffraction from DOPC-DOPS membranes was measured in function of temperature first cooling from 310 K to 200 K and then heating back to 310 K (Figure 1). During cooling, a typical transition temperatures around 260 K were seen whereas during heating there are two transitions at 260 and 275 K. The addition of P2 wild type protein decreases the transition temperatures; P38G-mutated protein has an enhanced effect.

The spacing between membranes above transition is slightly reduced for P2 wild type samples compared to pure lipid sample, but below transition spacing is increased compared to wild type. Again the P38G-mutation in P2 enhances the effect of the protein on membrane properties.

IN6

At IN6, wavelength was set to 5.12 Å. QENS data were collected from the samples in two different orientations, 45° and 135 ° angles towards incoming beam, to get information about molecule fluctuations in membrane plane and out of plane directions at 230 and 302 K. For elastic scattering in function of temperature, scans from 230 K to 320 K were measured using heating rate of 0.5 K / min. Empty cell and 1 mm vanadium samples were used for data correction.

In 135° orientation where in-plane dynamics was observed, addition of P2 protein reduces the quasielastic signal (Figure 2) at high-Q spectra. The effect of hyperactive P38G mutant in membrane stabilization is enhanced.

Interpretation of out-of-plane dynamics (measured in 45° orientation) will be done after further data analysis because of the bragg peaks coming from the periodicity of multilayered sample.

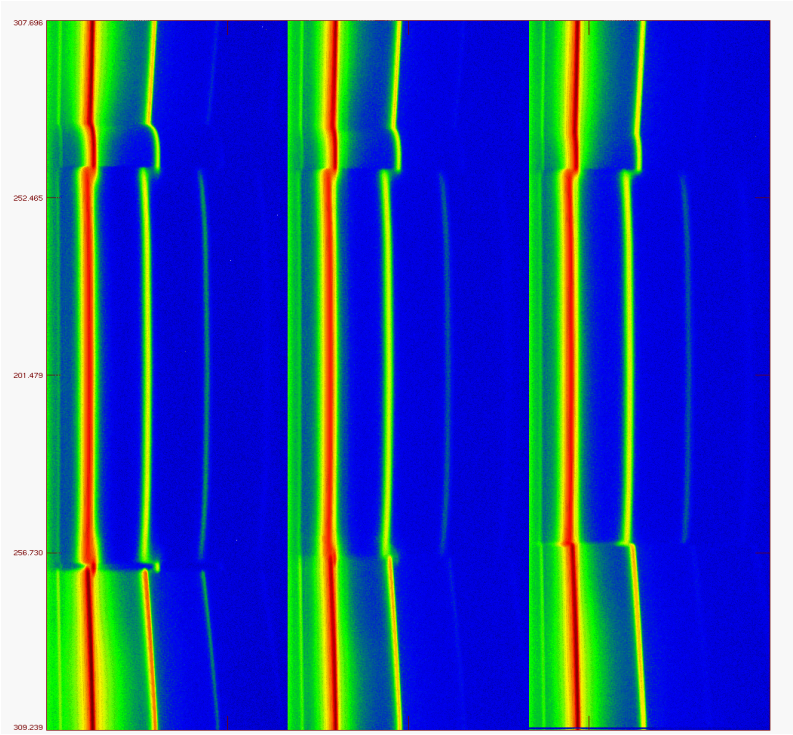

Figure 1. Diffraction of DOPC-DOPS membranes alone (left) and enriched with wild type P2 (middle) and with P38G mutated P2 protein (right). X-axis = 2θ = 0 - 4.7. Y-axis = Temperature from bottom to top cooling from 310-200K and heating 200-310K.

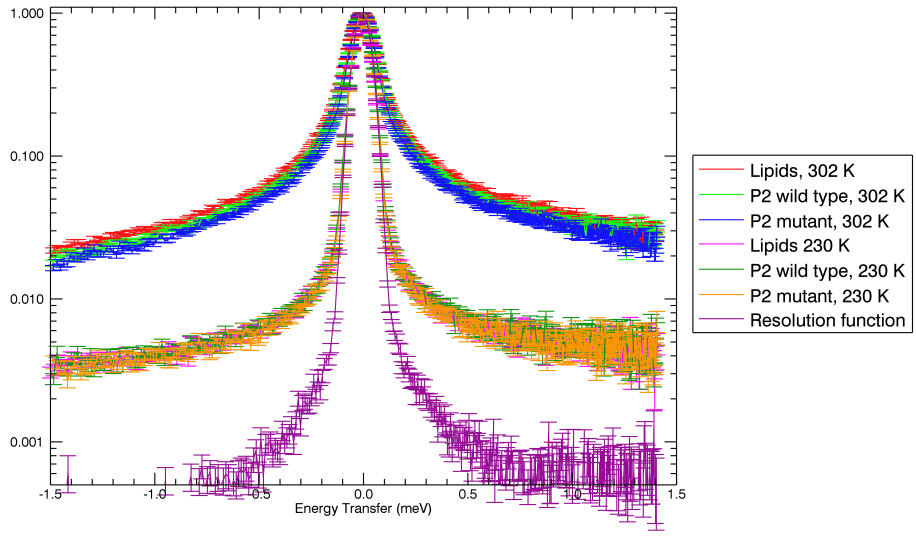

Figure 2. QENS at IN6, in-plane dynamics of P2-enriched DOPC-DOPS membranes, Q = 1.8 Å. Below the lipid transition temperature at 230 K P2 protein addition does not change the high-Q QENS signal but at T = 302 K, the P2 wild type reduces the membrane dynamics, and the P38G-mutated P2 stabilizes the membrane even more.

Conclusions

The used myelin modeling DOPC-DOPS membranes showed predicted diffraction patterns (Figure 1) which verifies from its part that sample preparation was successful. The P2 protein reduces the repeat distance of membrane multilayer at physiological temperatures and reduces the differences between liquid and gel phase membranes. From QENS spectra can be seen that P2 protein stabilizes the membrane dynamics. The P38G-mutation in P2 protein enhances the effect of P2 protein both in structure and dynamics of DOPC-DOPS membranes.

References

1. Ruskamo S., Yadav R.P., Sharma S., Lehtimäki M., Laulumaa S., Aggarwal S., Simons M., Bürck J., Ulrich A.S., Juffer A.H., Kursula I. & Kursula P. (2014) Atomic-resolution view into structure-function relationships of the human myelin peripheral membrane protein P2. Acta Cryst. D 70: 165-176.
2. Lehtimäki M., Laulumaa S., Ruskamo S. & Kursula P. (2012) Production and crystallization of a panel of structure-based mutants of the human myelin peripheral membrane protein P2. Acta Cryst. F 68: 1359-1362.