Experimental report

13/09/2024

Proposal: 8-02-993 **Council:** 10/2022

Title: NR determination of structural changes of myelin membranes by application of electrical potential gradients

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: Phospholipids
D2O-Buffer (MOPS+NaCl)
Myelin Basic Protein

Instrument	Requested days	Allocated days	From	To
FIGARO	3	3	01/06/2023	04/06/2023

Abstract:

Myelin is an asymmetric multilamellar membrane wrapped around the axons and consists of alternating extracellular and cytoplasmic leaflets. Any structural changes of the myelin sheath, mainly demyelination, are signatures of several inflammatory neurological disorders as Multiple Sclerosis (MS). We suggest generating flat asymmetric myelin membranes with intercalated MBP, which are suitable for Neutron Reflectometry (NR) analysis. We aim to obtain a precise measurement of the vertical membrane layer structure for native myelin. We also plan to analyze the structural changes of myelin membranes by application of electrical potential gradients which emulate neuronal action potentials.

Experimental Report

Experiment N°: 8-02-993
Instrument: FIGARO
Dates of experiment: 01/06/2023 to 04/06/2023
Title: NR determination of structural changes of myelin membranes by application of electrical potential gradients
Experimental team: Julio Martín Pusterla, Alexandros Koutsioumpas and Andreas Stadler
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Myelin constitutes the membrane material surrounding axons [1] and consists of alternating extracellular and cytoplasmic leaflets whose lipidic composition was previously reported [2]. The Myelin Basic Protein (MBP) is one of the main proteins in myelin and plays an essential role in stabilizing and maintaining the myelin structure [3]. It is located only between the 2 cytoplasmic faces, where it acts as an intermembrane adhesion protein and forms the so-called major-density-line.

The bilayers in myelin are in close contact (~3 nm separation between lipid headgroup–water interfaces), creating a low dielectric constant of the myelin sheath that is crucial for rapid saltatory nerve impulse propagation. Deficiencies in myelin assembly and structure can lead to neurological disorders, the most notable being Multiple Sclerosis (MS), a chronic inflammatory disease of the central nervous system characterized by myelin sheath destruction, membrane de-adhesion, swelling, and vesiculation, resulting in nerve conduction failure and neurodegeneration [4]. In MS, both MBP and the lipid composition of myelin membranes exhibit altered physicochemical properties, underscoring the need to better understand their molecular effects and interdependencies. Experimental autoimmune encephalomyelitis (EAE) is a commonly used animal model for MS, characterized by changes in overall myelin lipid composition [5].

In this study, we investigated molecular aspects of the myelin formation process by using biomimetic membrane systems. We first produced supported asymmetric bilayers mimicking native or diseased-like myelin on a silicon substrate and coated it with an MBP layer on top. Each individual measurement was performed at four different contrasts (D2O, H2O, 4MW and silicon matched D2O/H2O) to reduce ambiguity.

The initial results demonstrated that membrane asymmetry cannot be accurately detected when non-deuterated lipids are employed. This is because the small compositional difference between the cytoplasmic and extracellular leaflets effectively results in a very slight difference in SLD (scattering length density) values. Nevertheless, asymmetry can be clearly detected at low temperature (10-15

°C) when one of the leaflets is deuterated with d45-cholesterol (Figure 1, left). The solvent fractions obtained from our fittings demonstrate a fairly acceptable percentage of bilayer coverage (around 75-80%) on the silicon (Si) wafers. The SLD values from the fittings for each leaflet are consistent with those obtained from theoretical calculations and the thicknesses are also reasonable.

The affinity of MBP for the cytoplasmic leaflet is higher when the membrane is asymmetric, compared to a symmetric one (Figure 1, right). This result is an important proof that membrane asymmetry is essential for the adhesion of the MBP protein.

When comparing specifically native and EAE-modified myelin, MBP shows lower affinity for the pathological membrane than for the native one (Figure 1, right). The modified membrane also appears to increase its thickness when binding to MBP, which could indicate destabilization by MBP due to swelling.

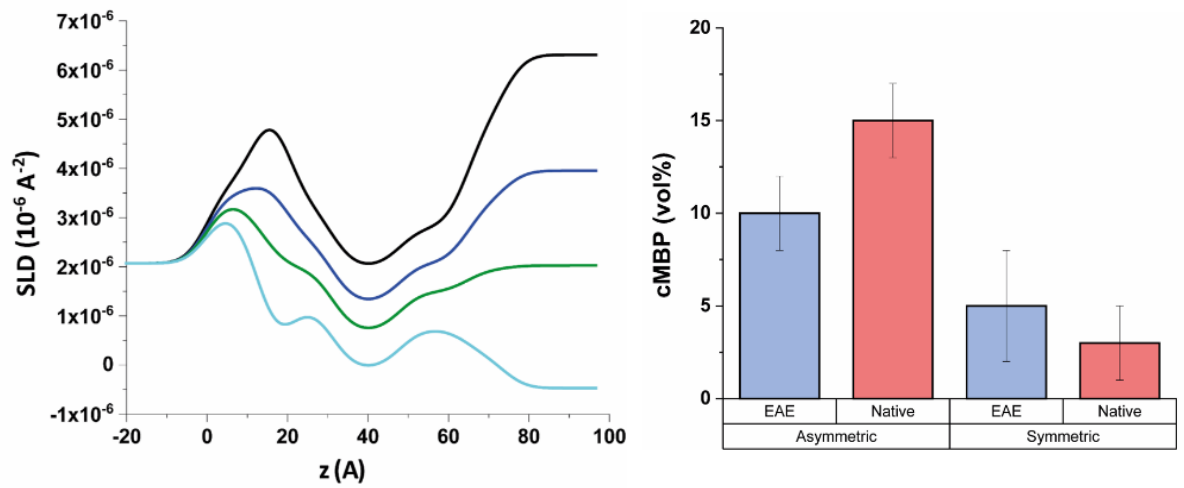

Figure 1: (left) Scattering Length Density (SLD) profiles from NR curves ~~at FIGARO~~ for an asymmetric myelin bilayer with d45-Cho in one leaflet, measured at 10 °C under four contrasts: D_2O buffer (black), 4MW (blue), SiMW (green), and H_2O (light blue). (right) Concentration of MBP adsorbed on the cytoplasmic leaflet of asymmetric and symmetric myelin bilayers with native and EAE-modified composition.