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NhaA protein incorporation in tethered lipid bilayer membranes studied by neutron reflectometry and impedance spectroscopy

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The study of active membrane proteins requires an environment which is as close as possible to their natural environment to retain protein function, while at the same time keeping the system as simple as possible to allow for an experimental characterization and to be able to identify factors which influence the system. Tethered lipid bilayers (tBLMs) represent an experimentally accessible and stable model for biological membranes that offers a high level of control over the structure and can form a more natural environment for membrane protein incorporation than the widely used solid supported bilayers [1]. We report the use of a tBLM system to investigate how the structural factors of the surrounding membrane influence the incorporation and subsequently the activity of the NhaA protein, which is the main sodium proton antiporter of *Escherichia coli*. NhaA serves as the means for *E. coli* to maintain sodium homeostasis and for pH control [2]. Here we present a combined neutron reflectometry (NR) and electrochemical impedance spectroscopy (EIS) study on the incorporation of NhaA into PEG-tBLMs on gold surfaces.

We describe how lipid composition and incorporation methods affect the resulting tBLM-protein system. NR allowed us to determine the nanostructure of the membrane-protein system to monitor bilayer dimensions, completeness and to precisely determine the amount of incorporated NhaA protein. EIS provided functional characteristics like electrophysiological properties related mainly to ion permeability and indicated defects induced by protein incorporation.

The combination of these two methods enables us to correlate structural and functional information of the NhaA-membrane system in order to understand the mechanisms behind these dependencies.

[1] M. Maccarini et al., *Langmuir*, 2017, 33, 9988-9996

[2] Etana Padan, *BBA-Bioenergetics*, 2014, 1837. Jg., Nr. 7, S. 1047-1062

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