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Giulio Preta, Marija Jankunec, Frank Heinrich, Sholeem Griffin, Iain Martin Sheldon, Gintaras Valincius

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Tethered bilayer membranes as a complementary tool for functional and structural studies: the pyolysin case

Giulio Preta[1], Marija Jankunec[1], Frank Heinrich[2], Sholeem Griffin[3], Iain Martin Sheldon[3] and Gintaras Valincius[1].

[1] Department of Bioelectrochemistry and Biospectroscopy, Institute of Biochemistry, Vilnius University, Vilnius, Lithuania.

[2] NIST Center for Neutron Research, Gaithersburg, MD, 20899, USA

[3] Institute of Life Science, Swansea University Medical School, Swansea, SA2 8PP, United Kingdom.

ABSTRACT

We demonstrate the use of tethered bilayers (tBLMs) as an experimental platform for functional and structural studies of membrane associated proteins by electrochemical techniques. The reconstitution of the cholesterol-dependent cytolysin (CDC) pyolysin (PLO) from *Trueperella pyogenes* into tBLMs was followed in real-time by electrochemical impedance spectroscopy (EIS). Changes of the EIS parameters of the tBLMs upon exposure to PLO solutions were consistent with the dielectric barrier damage occurring through the formation of water-filled pores in membranes. Parallel experiments involving a mutant version of PLO, which is able to bind to the membranes but does not form oligomer pores, strengthens the reliability of this methodology, since no change in the electrochemical impedance was observed. Complementary atomic force microscopy (AFM) and neutron reflectometry (NR) measurements revealed structural details of the membrane bound PLO, consistent with the structural transformations of the membrane bound toxins found for other cholesterol dependent cytolysins. In this work, using the tBLMs platform we also observed a protective effect of the dynamin inhibitor Dynasore against pyolysin as well as pneumolysin. An effect of Dynasore in tBLMs, which was earlier