Experimental report

14/02/2018

Proposal: 8-02-704 **Council:** 4/2014

Title: Magnetic contrast reflectometry toresolve transmembrane potential effects on the binding of MinD-MTS toa floating bilayer

Research area: Physics

This proposal is a new proposal

Main proposer: Simon TITMUSS

Experimental team: Simon TITMUSS
Laura MCKINLEY

Local contacts: Robert BARKER

Samples: d62 DPPG
gold/permalloy/silicon
d62DPPC
MinD-MTS

Instrument	Requested days	Allocated days	From	To
D17	4	4	25/09/2014	26/09/2014
			31/07/2015	04/08/2015

Abstract:

Magnetic contrast neutron reflectometry will be used to resolve the effect of an applied transmembrane potential on a floating bilayer and its interaction with the MinD-MTS peptide which is involved in the control of bacterial cell division. The floating bilayer will be deposited on a gold layer capping a permalloy under layer on a silicon block. The gold layer will serve as the working electrode in an electrochemical cell, permitting application of transmembrane potentials in the range -100mv to -1V, which span the range normally found across the cytoplasmic membrane of bacterial cells. We aim to demonstrate that such an applied potential leads to a change in the lipid tilt and area per molecule in the bilayer, that effectively increases the fluidity (lowers the lateral pressure), providing a structural mechanism to bridge between the observations that the binding of fluorescent MinD in cells is affected by transmembrane potential and MD simulations of changes in bilayer fluidity with applied potential.

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Experimental details

In this experiment we used the newly developed ILL solid/liquid electrochemsitry cell to apply a membrane potential in the range ± 500 mV across a floating bilayer of composition 3:1 DPPC/DPPG deposited onto a thiol-lipid functionalized gold surface on a permalloy coated silicon block. The presence of the permalloy film acts as a magnetic reference layer that can be exploited to simultaneously measure two magnetic contrasts, without changing the deuteration level of the sub-phase. We challenged this bilayer with the membrane targetting sequence of the MinD, bacterial cell division protein at different membrane potentials, and observed small differences in the specular reflectivity (only one spin state is shown in the figure). The presence of a second reflectivity curve measured under the same solution conditions from the other spin state has greatly helped to constrain the fitting procedure, giving us greater confidence in the small changes in bilayer structure that we observe with applied membrane potential.

Preliminary data analysis

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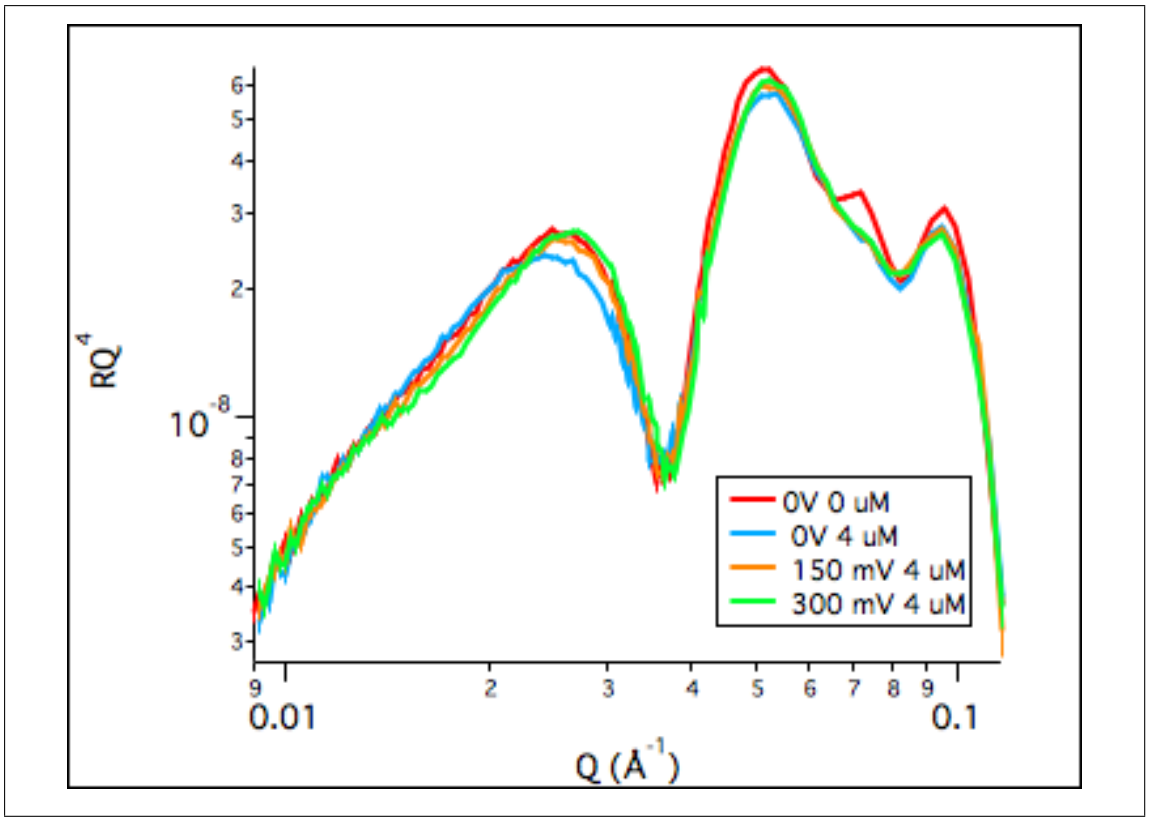

Figure 1: Neutron Reflectivty from 3:1 DPPC/DPPG floating bilayers deposited on thiol-lipid functionalized gold/permalloy/silicon with 0 and 4 μM MinD (bacterial membrane peptide) at different applied transmembrane potentials in the range 0 to 300 mV.

During the experiment we received a lot of help from Thomas Saerback, who produced a first version of the data reduction software.

Outcomes

This contributed to the PhD work of Laura McKinley (awarded November 2017) and the results are currently being written up as a manuscript for submission to Physical Review Letters.