Experimental report

14/09/2016

Proposal: 8-04-772 **Council:** 4/2016

Title: Influence of Cholera Toxin SubunitB binding on lipid membrane fluctuations

Research area: Biology

This proposal is a new proposal

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Samples: cholera toxin subunit B
POPC:GM1 vesicles
DSPC:GM1 vesicles
Live sperm

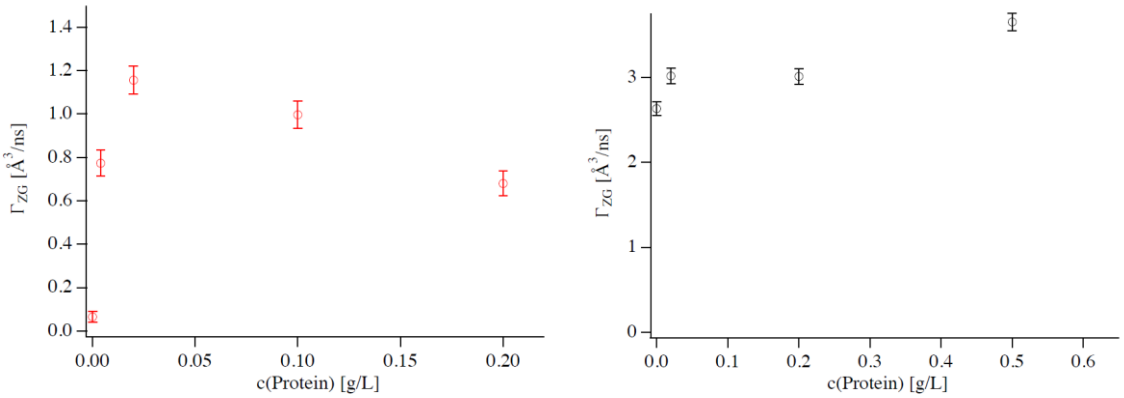
Instrument	Requested days	Allocated days	From	To
D33	1	1	13/06/2016	14/06/2016
D11	1	0		
IN15 Standard	4	4	17/06/2016	21/06/2016
D22	1	0		

Abstract:

The membrane binding subunit of cholera toxin CTxB has previously been shown to strongly affect lipid membrane ordering in gel phase membranes upon binding to GM1 inducing lipid texture. We expect the structural changes observed to be accompanied by large changes in membrane dynamics upon binding as regions of the membrane become pinned by the defined geometry of the binding sites in GM1. We propose to measure using neutron spin-echo the changes in membrane fluctuation upon binding. We plan on using samples in the previously investigated (using grazing incidence X-ray) diffraction gel phase as well as in the fluid phase where investigation using GIXD is not feasible.

Preliminary Experimental Report

CTB interaction with lipid vesicles was studied by SANS and NSE spectroscopy. These experiments have been by and large successful with results that can be concisely described as being that CTB interaction with fluid phase vesicles leads to membrane stiffening and with the gel phase vesicles a large decrease in membrane stiffness is observed. SANS shows only small changes upon CTB binding unless membrane coverages comparable to our previous neutron reflectivity measurements are used where large changes occur which are accompanied by very large changes in turbidity. NSE spectroscopy seems really incredibly sensitive to the changes in this system with large changes observed for the addition of even miniscule amounts of protein.



Left: Effect of CTB binding on DPPC:GM1 gel phase vesicles.
Right: CTB binding on POPC:GM1 fluid phase vesicles.

In terms of the completeness of our result and the current state of the work, all NSE data has been successfully fit using the Zilman-Granek model (results displayed above) with additional constraint by ex-situ DLS as the in-situ system on IN15 unfortunately blew its power supply during these measurements. Fitting of the SANS data is ongoing as this is rather complex and is being informed by additional USAXS-SAXS measurements. However in the originally proposed role as a pre-characterisation method for the NSE part of the measurement this worked as planned.

We are proposing to continue this experiment with further NSE and SANS measurements. This is for a number of reasons:
1) To perform repeat measurements of a limited number of data points in order to confirm reproducibility. The now repaired in-situ DLS would further assist with this.
2) The effect on membrane rigidity with the gel phase is large and the amount of protein required to induce this change is rather small we believe this could be an excellent measurement to highlight the extreme sensitivity that may be achieved by NSE in a contrast optimised system.
3) The charges in this vesicle system also act as the receptor molecules for the protein. In the higher protein concentration samples significant changes occur in the turbidity and the solution stability is affected. We wish to measure a range of samples with further charges added (these vesicles have already been characterised by SAXS) where vesicles formed by the same method of those used in this experiment are stable on the order of months. Another reason for desiring to do this is due to the effect of charge neutralisation by binding of the oppositely charged protein.

A more complete experimental report is due to be submitted as the analysis progresses.