# Experimental Report



**Proposal:** **8-02-684** **Council:** 10/2012

**Title:** Role of curvature inducing lipids on the structural changes in model membranes caused by multivalent protein binding and receptor clustering

**This proposal is continuation of: 8-02-626**

**Researh Area:** Biology

**Main proposer:** **WATKINS Erik**

**Experimental Team:** WATKINS Erik

**Local Contact:** WATKINS Erik

**Samples:**
sialyloligosaccharide
lyso-PC (phospholipid)
CTxB (protein binding subunit)
DPPC (phospholipid)

| Instrument | Req. Days | All. Days | From | To |
|---|---|---|---|---|
| FIGARO | 4 | 4 | 16/05/2013 | 20/05/2013 |

**Abstract:**

We propose a series of experiments to investigate the interplay between receptor clustering and the presence of positive spontaneous curvature lipid species on the structure of planar model membranes. These experiments are designed to elucidate the mechanism driving raft associated endocytosis as well as lead to the development of a switchable biomemetic signaling device. Using neutron reflectivity (NR) and in-situ impedance spectroscopy NR, we will investigate structural changes induced by CTxB binding and resulting receptor clustering on lipid membranes containing lyso-PC lipids. Preliminary NR experiments found that CTxB binding increased the bilayer thickness and decreased the bilayer coverage on the substrate (by ~10%) and that these two parameters changed in such a way as to conserve the overall lipid volume of the sample. This preliminary work will be extended to confirm the hypothesized membrane strcutral changes and to measure the associated transmembrane electrical responses which may be exploited for future use in biomemetic devices.

**Role of curvature inducing lipids on the structural changes in model membranes caused by multivalent protein binding and receptor clustering**

Neutron reflectivity (NR) was used to investigate the influence of CTxB binding to membranes containing GM1 glycolipids and how the resulting receptor clustering impacted the selective recruitment of curvature inducing lipid species to the leaflet with bound protein. These experiments were designed to elucidate structural changes in the membrane related to clathrin independent endocytosis mechanisms. Supported membranes were prepared in the liquid-ordered / liquid-disordered co-existence region of the phase diagram and were composed of 38.5 mol% DPPC, 38.5 mol% DOPC and 23 mol% cholesterol. GM1 was included at 10 mol% in the exterior bilayer leaflet to enable CTxB binding. Membranes containing deuterated DPPC were compared to membranes containing hydrogenated DPPC to enable spatial localization of the membrane components. Our hypothesis was that CTxB binding would lead to the specific recruitment of lipids to the bound leaflet thereby altering the phase state of each leaflet to promote curvature.

These measurements showed significant changes to the reflectivity following protein binding. However, the aim of the experiment is to identify and characterize a more subtle effect originating from changes in the membrane structure. To accomplish this, we have written neutron reflectivity software specifically designed to analyze these measurements. This software parameterizes the constituent molecules by their known molecular volumes and scattering lengths and constrains the molar ratios of components before and after protein binding. Four data sets (two contrasts before binding, and two contrasts following binding) are then simultaneously co-refined to obtain fits that conserve the overall number of molecules but allow the lipid species to have differing distributions between the two leaflets. At this stage, the software has been successfully applied to the bilayers containing mixtures of DPPC and lyso-PC lipids. However, cholesterol does not span the entire length of a lipid leaflet so bilayers including cholesterol require further development of the software to be successfully corefined. Preliminary fits also suggest that lateral phase separation of the liquid ordered and liquid disordered regions of the film may necessitate modelling the data as the incoherent sum of two surface structures. Such technical challenges have impeded the analysis of the data for this project. Additional microscopy experiments need to be conducted to determine the length scales associated with the lateral phase separation. Following these experiments, the specific data analysis challenges inherent in these systems can be continued.