Experimental Report

Proposal: **8-02-682** **Council:** 10/2012

Title: Decoupling electrostatic and hydrophobic contributions to the interactions of anti-microbial peptides and model bacterial membranes

This proposal is a new proposal

Researh Area: Biology

Main proposer: **BARKER Robert**

Experimental Team: TITMUSS Simon

Local Contact: BARKER Robert

Samples: Pexiganan
DPPC/DPPG floating bilayers
AMP2
al-PC coated block

Instrument	Req. Days	All. Days	From	To
D17	4	4	25/07/2013	29/07/2013

Abstract:

We propose a further investigation of the interaction of the antimicrobial peptides AMP2 (designed by NPL to prevent oligomerization) and pexiganan (forms dimers via leucine zipper) with floating DPPC/DPPG bilayers as models for bacterial membranes. Our previous investigation, identified different patterns of adsorption behavior depending on the bilayer fluidity and the availability of hydrophobic residues. From this we were able to make inferences regarding the likely dominant contribution (hydrophobically or electrostatically mediated) to the interaction of the peptide with the membrane. Here we propose to selectively deuterate the charged and uncharged lipid components, and make use of the expected differences in the interference effects that would result from the different structures that might be expected for different binding modes to unequivocally identify these modes. This will be of great benefit to the design of future antimicrobials targetted at attacking the societal challenge of acquired antibiotic resistance in bacteria.

Background to project

The aim of this experiment was to decoupling electrostatic and hydrophobic contributions to the interactions of anti-microbial peptides and model bacterial membranes. We focussed on the anti-microbial peptide pexiganan (PXG), which dimerizes via a hydrophobic zipper region and which are previous experiments had suggested interacted primarily with the head group region of the 3:1 DPPC/DPPG floating bilayer. As it is not possible to use salt to screen electrostatic interactions without substantially altering the structure of the floating bilayer, we used an alternative strategy of varying the charge density in the bilayer by constructing both 3:1 and 1:1 DPPC/DPPG floating bilayers. Furthermore, by changing the deuteration level of the two components (d62DPPC/d62DPPG→d75DPPC/hDPPG) it was possible to discern quite subtle changes in the relative scattering length density of the bilayer, which we attribute to a change in the distribution of the two lipid components between the two leaflets of the bilayer. This has necessitated the development of quite a complex fitting model and so data fitting is currently in a preliminary stage, but good progress has been made (the report will be updated once this has been completed in the New Year).

Results

In this experiment we measured at 0.6, 1.2, 2.4 and 4.8 μM PXG to allow an adsorption isotherm to be determined. We observe a Langmuir-like behaviour, whereby the adsorption of PXG to the bilayer increases from 0.6 to 1.2 μM, but then plateaus for the two highest concentrations. A striking feature in the reflectivity profiles measured for the 3:1 d62DPPC/d62DPPG bilayer against an H_2O subphase is the distinct shoulder at 0.1 $Å^{-1}$. This feature is less distinctive in the 1:1 bilayer in which the DPPG is not deuterated. The 1:1 bilayer also shows difference at 0.02 $Å^{-1}$ after the addition of PXG, which increase with increasing PXG concentration that were not visible in the uniformly deuterated bilayer. An explanation consistent with preliminary fitting is that the PXG drives an asymmetry to develop in the bilayer, whereby the outer leaflet is slightly richer in the negatively charged DPPG.

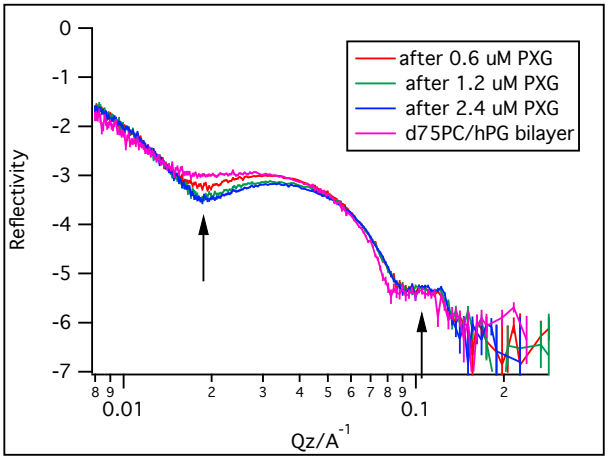

Figure 1: Reflectivity from d75DPPC/hDPPG (1:1) floating bilayer/H_2O sub-phase before/after adsorption of PXG