# Experimental Report



**Proposal:** **8-02-672** **Council:** 10/2012

**Title:** Neutron reflectivity studies of the interaction between human antimicrobial peptides and gram negative membrane models

**This proposal is a new proposal**

**Researh Area:** Biology

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BELLO Gianluca
BODIN alice

**Local Contact:** BARKER Robert

**Samples:** Lipopollysaccharide e7
Lipopolysaccharide D21

| Instrument | Req. Days | All. Days | From | To |
|---|---|---|---|---|
| FIGARO Langmuir trough | 5 | 4 | 19/07/2013 | 23/07/2013 |

**Abstract:**

Lipopolysaccharide (LPS) molecules released from the outer membrane (OM) of infecting Gram negative bacteria induce an uncontrolled inflammatory response in humans which can lead to severe septic shock. LPS molecules also act as the bacterium's permeability barrier making it therefore less susceptible towards external agents and drugs. Human antimicrobial peptides (hAMPs) of diverse 3D structure have the capacity to disrupt the bacterial cell membrane. The mechanism of action for this phenomenon is not fully understood yet and might be affected by different chemotypes of LPS; hence further studies need to be performed in order to gain deeper insight into this interaction. Neutron reflectivity studies at the air/water interface will help to clarify these interactions between human antimicrobial peptides and outer membrane models.

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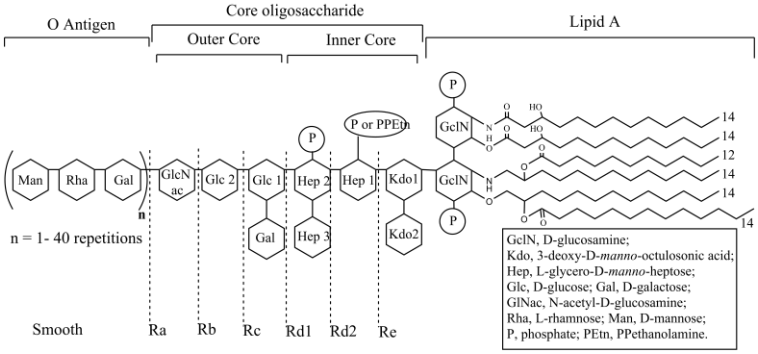


**Figura 1 Chemical structures of different chemotypes of LPS expressed by different strains of *E. coli* Gram negative bacteria.**

**Background:** Endotoxin or Lipopolysaccahride (LPS) is the main constituent of the outer membrane (OM) of Gram negative bacteria (Figure 1) all together with lipids and proteins. It covers up to 75% of the OM surface, acting as permeability barrier [1] towards external agents such as cationic antimicrobial peptides (CAPs), hence interfering with their mechanism of action. In consideration of an increased antimicrobial resistance toward common antibiotics [2,3] we are studying by neutron reflectivity (NR) at the air/liquid interface the interaction of two amphiphatic CAPs of mammalian origin with different 3D structure (human cathelicidine LL37, lactoferricine LFb) with *E. coli* Gram negative bacteria monolayer model membranes containing chemically different LPS chemotypes (Figure 1). The model consists of mixtures of deuterated or hydrogenated DPPC and either Rough Ra EH100 LPS or Rough Rc J5 LPS.

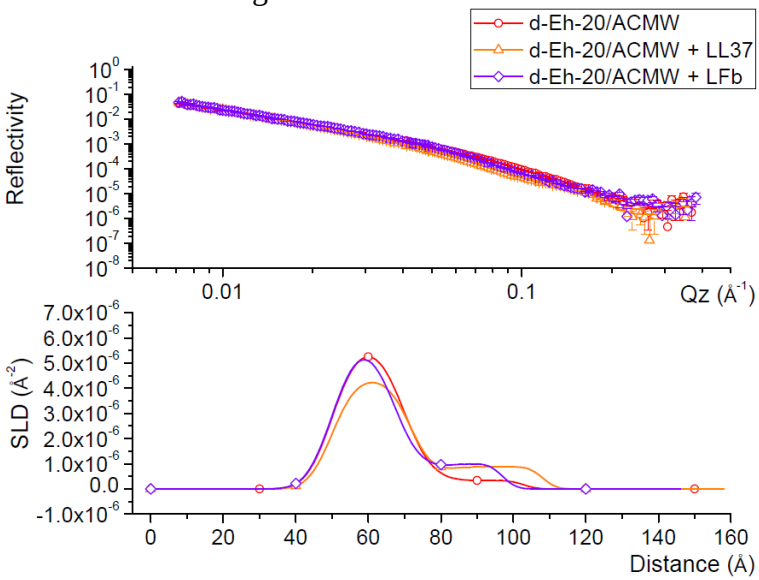


**Figura 2 NR fitted data of deuterated Eh-20 monolayer on ACMW subphase at 22C before and after the subphase injection of either LL37 or LFb peptides. Upper plot: reflectivity profile. Lower plot: SLD profile.**

**Results:** The mixed monolayers formed by 80% deuterated or hydrogenated DPPC and 20% LPS Eh100 (Eh-20) or J5 (J5-20) are stable at 22C on ACMW, $D_2O$ or $H_2O$ subphases; this allowed the subphase injection of the peptides, either LL37 or LFb, and the acquisition of the scattering and reflectivity profiles (Figures 2 and 3). Despite its bulkier headgroup, the

monolayers containin LPS EH100 seem to be more susceptible to the penetration of both the amphiphatic CAPs tested compared to monolayers containing LPS J5.

LL37 induces a greater stretching of the hydrophobic chains of the Eh-20 monolayers (Figure 2, orange line), whereas its effect on the J5-20 monolayers (Figure 3, orange line) is limited to the interfacial region between acyl chains and hydrophilic headgroups. The peptide LFb has limited effect on the headgroup region of the monolayers containing LPS J5 whereas it presents a greater penetration activity on the monolayers containing LPS EH100, with a shortening effect of the hydrophobic acyl chains.

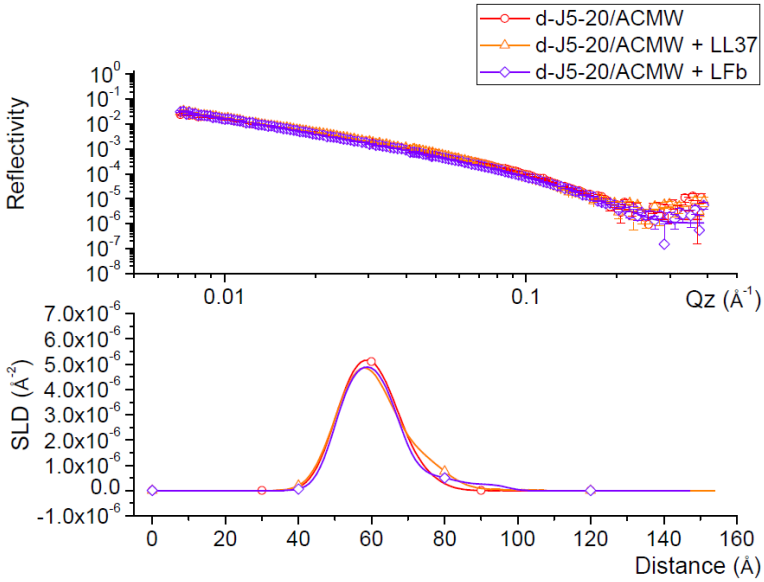


**Figura 3 NR fitted data of deuterated J5-20 monolayer on ACMW subphase at 22C before and after the subphase injection of either LL37 or LFb peptides. Upper plot: reflectivity profile. Lower plot: SLD profile**

In general the α-helical LL37 peptide is interacting more than the β-sheet LFb peptide with LPS-containing monolayers. The Eh-20 monolayers are more susceptible to the penetration of CAPs probably due to a higher disordering effect on the monolayer due to its extended core region (Figure 1) that more likely expose the negative charges of the interface. The LPS J5 forms otherwise a tighter interface resulting in an ordered monolayer, which impedes the penetration of CAPs.
These findings suggest that the outer membrane of Gram negative bacteria is susceptible to the membrane-perturbing activity of CAPs and such susceptibility is dependent on the 3D structure of the peptides and the chemical structure of the LPS as well.

[1] Vaara M, Antimicrobial agents and chemotherapy, Vol 37, 1993
[2] European Center for Disease Prevention and Control (ECDC). Annual epidemiological report 2011.
[3] Karami N, Journal of antimicrobial and chemotherapy, Vol 60, 2007