Experimental Report

Proposal: **9-13-450** **Council:** 4/2012

Title: Effect of dendrimer chemistry on the interaction mechanisms with fluid supported lipid membranes

This proposal is a new proposal

Researh Area: Soft condensed matter

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Samples: Dendrimers
Single crystal silicon
Lipids (dPOPC) purchased from Avanti Lipids Inc.

Instrument	Req. Days	All. Days	From	To
FIGARO User-supplied	3	3	27/11/2012	30/11/2012

Abstract:

Novel anti-microbial peptide dendrimers (AMPDs) are believed to act on membranes in a non-specific manner by electrostatic and hydrophobic interactions, which decreases the risk of resistance inducement via mutations and transfer of resistance genes between bacteria. In this study, we will investigate the interaction mechanism of four chemically different dendrimers on fluid phase SLBs. The chosen dendrimers differ in level of branching by changes in stereochemistry and by a chlorine unit. They thus have specific properties in terms of shape, functional group accessibility and polarity - properties that may have an impact on the activity towards SLBs. NR is the only technique that provides detailed structural information in the direction perpendicular to the interface, thus enabling us to unambiguously conclude where the dendrimers are located in the lipid bilayer, if they translocate through it, and whether or not the bilayer is solubilized via a detergent-like mechanism. These studies will allow for a systematic investigation of AMPD dendrimers and identification of the dendrimer properties that affect activity and selectivity towards bacteria and the toxicity towards host cells.

Effect of dendrimer chemistry on the interaction mechanisms with fluid supported lipid membranes

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Naturally occurring antimicrobial peptides (AMPs), which are part of the innate immune response in many organisms, are likely to become one of the answers in the fight against antimicrobial resistance. They act on membranes in a non-specific manner via electrostatic and hydrophobic interactions and thus decrease the risk of antibiotic resistance.[1, 2] The prospect of exploiting the dendritic structures as scaffolds for AMP-based antibiotic agents with increased activity have led to the synthesis of novel antimicrobial peptide dendrimers.[3] In this study we have investigated the interaction between an amphiphilic, branched analogue of lysine (BALY[4]) dendrimer and lipid bilayers composed of DPPC lipids at temperatures above and below the lipid melting temperature (42 °C).

The neutron reflection data resolved the mode of BALY interaction with membranes at the molecular level and showed that the mechanism depends on the fluidity of the membrane. Partial penetration of the dendrimer is observed in homogeneous fluid or gel phase membranes, with fluidity determining the extent of structural rearrangement in the lipid bilayer. These data sets were needed to complement earlier results based on POPC and phase separated membranes obtained at ILL and ISIS and have recently been published in BBA Biomembranes.[5]

Deuterated and hydrogenated DPPC lipids were purchased from Avanti Lipids Inc. Under a stream of flowing nitrogen lipids were dried onto the walls of a glass vial. The lipid films were resuspended in PBS (10 mM PBS, 100 mM NaCl, pH 7.4) to a concentration of 500 µg/ml. Small unilamellar vesicles (SUVs) were prepared by sonication of the suspension at 50 °C until clarity. The vesicles were diluted to 200µg/ml in PBS.

After characterization of the clean silica surface in D_2O and H_2O, supported lipid bilayers were deposited *in situ* by vesicle fusion at the FIGARO beamline. After rinsing the membranes with excess PBS, they were characterized in H_2O, D_2O and a 1:1 mixture of these. After successful bilayer formation was confirmed, BALY dendrimers in a concentration of 6 µM were introduced to the membranes in order to follow the interaction behaviour. The dendrimer interaction was characterized in the same three contrast environments as the bilayers. Several experiments were carried out in order to probe the effect of fluidity of the membranes for dendrimer interaction as clarified in Table 1 below.

Table 1: experimental conditions for the model system. At 25 °C the DPPC membrane is in the gel phase and at 50 °C it is in the fluid phase.

Experiment	Lipid deposition temperature/°C	Measuring temperature/°C	Dendrimer addition temperature/°C	Measuring temperature/°C
1	50	50	50	50
2	50	25	25	25
3	50	25	25	50

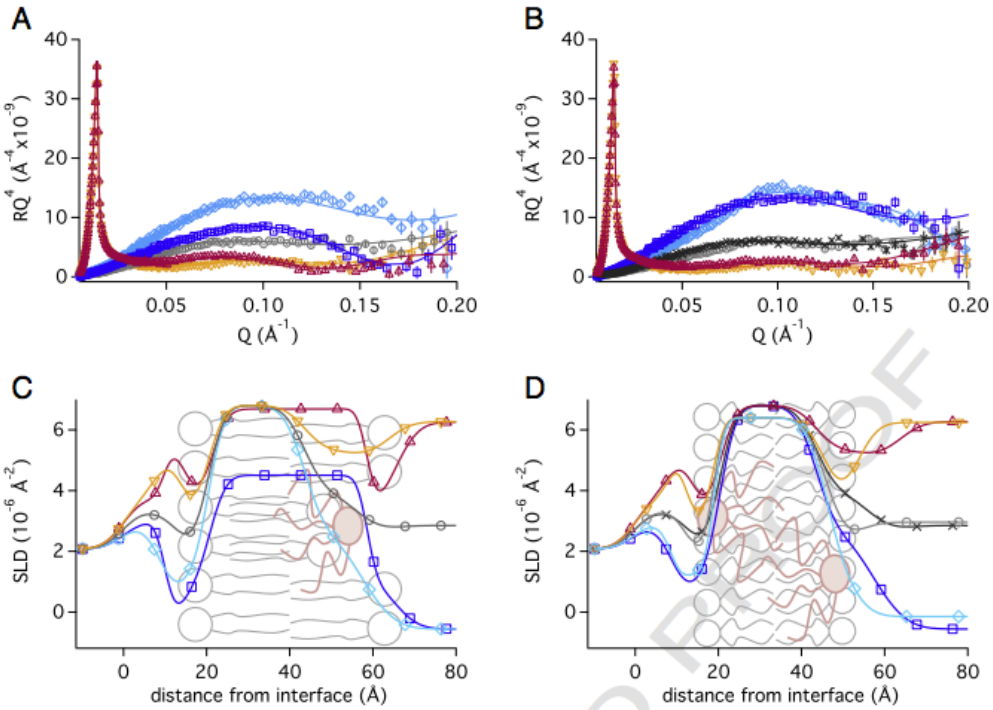

Figure 1: Neutron reflection and SLD profiles of deuterated DPPC before/after interaction with BALY at 25 °C in the gel phase (A, C) and at 50 °C after heating above the main phase transition temperature (B, D). The contrasts were (i) D2O: red triangle up (before interaction) and orange triangle down (after interaction), (ii) H2O: dark blue square (before interaction) and light blue diamond (after interaction), and (iii) 1:1: dark gray cross (before) and light gray circle (after).

BALY inserted into the upper leaflet of fluid DPPC bilayers (experiment 1, data not shown) and did not translocate through the membrane. In gel phase membranes, membrane thinning was observed after interaction with BALY (experiment 2 and figure 1 A+C). The decrease in thickness could arise from induced structural changes such as local melting or interdigitation of the lipid leaflets, both of which have been observed for small surface active molecules such as alcohols and anesthetics.[6] Once the BALY molecules were inserted in the gel phase membrane, subsequent heating of the membrane to above the lipid transisition temperature (experiment 3 and figure 1 B+D) led to further thinning of the membrane, and the dendrimer penetrated evenly throughout the membrane reaching to the proximal leaflet. The results show clear differences in the interaction mechanism of the dendrimer depending on the lipid membrane fluidity, and suggest a role for lipid phase separation in promoting its antimicrobial activity

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