Experimental report

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Proposal: 8-02-711 **Council:** 10/2014

Title: Effect of protein lipidation on adsorption and surface-associated protein fibrillation

Research area: Other...

This proposal is a new proposal

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Samples: proteins
Acetic acid
buffers

Instrument	Requested days	Allocated days	From	To
FIGARO	2	2	24/05/2016	26/05/2016
D17	2	0		

Abstract:

Lipidation of proteins is used in the pharmaceutical industry to improve the therapeutic efficacy of proteins. However, there is a limited knowledge on the physicochemical properties of these lipidated proteins. They may form irreversible multilayers upon adsorption onto solid surfaces, which in turn may catalyze the formation of surface-associated protein fibrillation. Fibrillation of proteins is considered to be a cause of multiple serious diseases. Insulin detemir, a human insulin analogue, with a fatty acid chain attached to the protein, was used as model compound, and compared to human insulin. Initial AFM and QCM-D results have shown that the lipid chain significant increases the initial adsorption and subsequent formation of fibrils onto hydrophobic surfaces. NR data can give a unique structural insight into the initial adsorbed (multi)-layers, density and thickness of these layers as well as for surface-associated fibrils. This structural insight will be of great significance in exploiting lipidation of proteins as a safe method to improve the therapeutic activity of proteins and understand the impact of the lipid chain in the adsorption process and subsequent fibrillation.

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This beam time was postponed from a previous beam time cycle, and due to this delay, we had to chance plans, and allocate this beam time to a new project dealing with the interaction of fluorophore labeled cell-penetrating peptides (CPPs) with supported lipid bilayers (SLB). Detailed investigations of the effect of the fluorophore moiety on the membrane binding properties of short hydrophilic CPPs is crucial in order to fully understand the consequences of using fluorophores in drug delivery efficacy assessment by CPPs; an extremely interesting point given the wide use of fluorescence-based techniques applied for biomolecular interaction and cellular uptake and trafficking studies.

The aim of the present work is to elucidate the impact that the fluorescent moietiy RhB have on lipid membrane interaction, when N-terminally conjugated to the CPP penetratin (PEN). The effect of RhB is compared with the un-labeled penetratin.

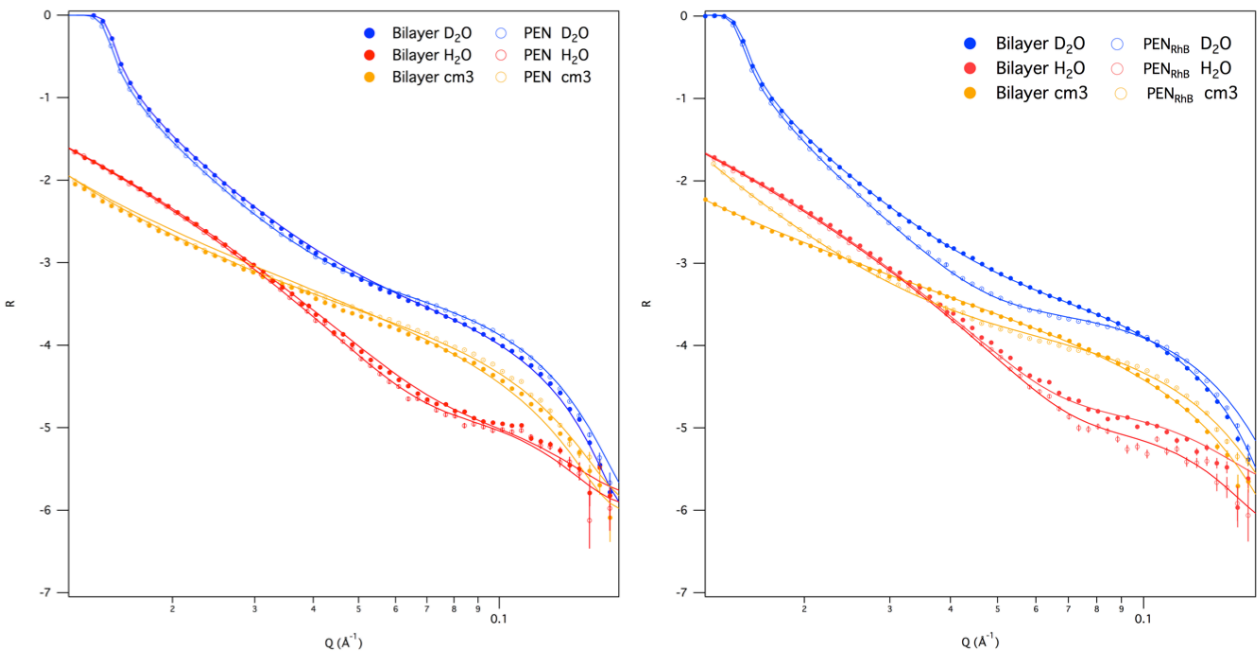

Figure 1: The neutron reflectivity profiles of a POPC:POPG (80:20 mol%) bilayer incubated with 5 μM PEN (left) and 5 μM PEN-RhB (right) in three isotropic contrasts. The solid lines represent the best fit of the data.

For construction of the SLB, a lipid vesicle solution of POPC:POPG (80:20 mol%) was allowed to incubate on the silicon crystal for minimum 15 min. before rinsing with H_2O contrast 10 mM HEPES buffer pH 7.4 to remove excess lipids. The SLBs were characterized using three buffer contrasts (H_2O, cm3 and D_2O). Subsequently, using a syringe pump, 30 ml of 5 μM peptide in H_2O buffer were applied with a flow rate of 3 ml/min to the SLB for 10 min. Kinetic measurements were performed during the first hour after peptide injection, where after a full characterization was carried out. The sample cell was rinsed with HEPES buffer of three contrasts (H_2O, cm3 and D_2O) prior to full characterizations. The clean crystal was fitted to a two-layer model (Si-SiO_2), whereas the best fit for the bilayer was obtained with a three-layer model consisting of head groups-lipid tails-head group.

The un-labeled penetratin was observed to locate as a fourth layer between the silicon surface and the SLB fitted to a thickness of 5.5Å with 66 % coverage. No penetration into the lipid core of the bilayer was observed and only a minor penetration degree of 3 % peptide was located in the head group area of the lipids. No effect of bilayer thickness was detected upon interaction with penetratin. In contrast, fitting the data from the RhB labeled penetratin, a clear effect on the bilayer penetratin degree and bilayer thickness was observed. A fourth layer of 5.4Å peptide was again observed between the silicon layer and the SLB, but with a low coverage of 5 %. In the head group area of the bilayer a penetration degrees of 25-30 % peptide could be fitted. Even minor penetration of peptide into the lipid core could be observed in the fit. The thickness of the bilayer decreased from 45Å to 38Å upon interaction with PEN-RhB, and also lipid removal could be observed.

Thus, from these results it is evident that the labeling of penetratin with the fluorophore moiety RhB significantly alters the bilayer interaction by integrating deeper into the lipid bilayer and affecting the thickness of the bilayer.