Experimental report

23/08/2017

Proposal: 8-02-776 **Council:** 4/2016

Title: Using the nano discs approach at surfaces to determine the relation between substrate specificity and the conformation of cytochromes P450 m

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Marite CARDENAS

Experimental team: Tania LIND
Sofie HEDEGAARD
Vivien JAGALSKI

Local contacts: Richard CAMPBELL

Samples: buffer
nanodiscs

Instrument	Requested days	Allocated days	From	To
FIGARO	2	2	01/09/2016	03/09/2016

Abstract:

Nanodiscs films are useful to study the conformation of membrane bound proteins as a function of lipid membrane charge. We have studied systematically the best protocol to form dense nanodiscs films on silica by changing parameters such as the protein belt type, the lipid type, the incubation time and the temperature of incubation. We have now finally obtained a protocol that ensure more than 80% nanodiscs coverage for samples containing membrane bound proteins. This protocol will now allow us to study a series of plant membrane bound enzymes (CYPs) that are involved in several reactions in the production of important metabolites. The protocol already established will now allow to systematically study the overall conformation of the protein in the lipid bilayer (how much it is buried in the bilayer) as a function of bilayer charge, and correlate with the activity of these proteins.

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Due to improper sample environment that did not allow cooling down the sample to 5 celsius, we decided to run a different experiment instead of the originally planned. The results for such experiment are currently being prepared in a manuscript, which deal with the effect of fluorophore modification on penetratin peptide interaction with supported lipid bilayer. Labelling short, flexible, and hydrophilic CPPs with bulky and hydrophobic fluorophore moieties unavoidably alters the physicochemical properties of the CPPs, and can have an influence on the interaction with the lipid membrane. Therefore, it is of high importance to recognise the potential influence of the labelling moieties used in for example mechanistic studies, as these could determine the mode of membrane interaction of the short peptide, leading to erroneous conclusions. We formed supported lipid bilayers (SLB) composed of POPC and POPG. We exposed them to CPPs with different fluorophore modification and neutron reflectivity profiles were recorded in three different isotropic buffer contrasts as presented in 1. In particular, reflectivity profiles from three differently labelled penetratins were measured, as we have already collected data for pure PEN and 2 other labels.

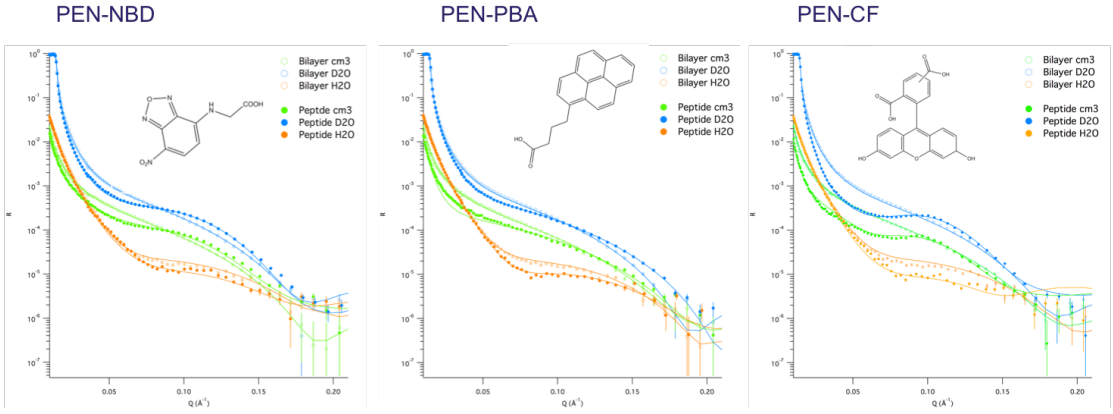

Figure 1: The neutron reflectivity profiles of a POPC:POPG (80:20 mol%) bilayer with A: PEN_{NBD}, B: PEN_{PBA} and C: PEN_{CF} added in a concentration of 5 μM in HEPES buffer, pH 7.4. The SLB's were measured in three isotropic contrasts; blue: D_2O, pink: cm3, and green: H_2O. The solid lines represent the best fit of the data. The molecular structures are the free fluorophores conjugated to penetratin.

Previous results have shown that in the absence of an attached fluorescent label, penetratin predominantly associated to the head groups of the lipid bilayer, and the thickness of the lipid core is only affected slightly by the peptide. For all the fluorophore labeled peptides, one or more layers had to be introduced in the fitting procedure to be able to properly model the data. For PEN_{RhB} an extra peptide layer of ~4 Å was introduced between the underlying surface and the inner head group. Moreover, the peptide also co-localised within the lipid tails, and taken into account that the surface was almost fully covered by the SLB there, some peptide should be incorporated in the hydrophobic core. Peptide incorporation rather than filling defects

is supported by the fact that no change in the SLD due to H/D exchange in the peptide was necessary to fit the data. Similar models and effects were found for $PEN_{PBA.}$ A more complex model with several distinct layers had to be applied in order to fit the SLB affected by the interaction with PEN_{NBD}. The best fit obtained to the data suggests that the peptide did not incorporate into the lipid core of the membrane and furthermore did not associate to the head groups either. Instead PEN_{NBD} loosely attached to the SLB at the same time that lipids were removed from the bilayer. Thus, we assume that mixed lipid/peptide structures formed that attached to the remaining SLB.

In comparison to the described reflectivity profiles, the profiles generated from SLB interaction with PEN_{CF} and PEN_{TAMRA}, show a more pronounced shoulder in the D_2O and cm3 contrasts at a q-value of approximately 0.1 $Å^{-1}$. Similarly to PEN_{NBD}, this suggests significant effects of the peptides on the structural properties of the lipid bilayers. Specific models were, however, not obtained for penetratin labeled with the fluorophores CF due to the complexity of the interfacial structure.

From these results it is evident that the labeling of penetratin with the tested fluorophore moieties significantly alters the bilayer interaction by integrating deeper into the lipid bilayer, affecting the thickness of the bilayer and in some cases also removing lipids from the SLB. Fluorophores for detection of short peptides in various bioassays, should therefore be used with great care, and the possible effect of the hydrophobic label should be taken into account in the data analysis in order to ensure accurate data interpretation.