Experimental Report

Proposal: **9-13-543** **Council:** 4/2014

Title: Cytochrome P450 Reductase in Nanodiscs: determination of parameter determining its change in conformational equilibrium

This proposal is continuation of: 8-05-413

Researh Area: Soft condensed matter

Main proposer: **CARDENAS Marite**

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Samples: nanodiscs
NADPH
DTT

Instrument	Req. Days	All. Days	From	To
D17	1	1	01/10/2014	02/10/2014

Abstract:

The cytochrome P450 Reductase (CPR) is a membrane bound enzyme that acts as an electron donor in a NADPH-dependent manner to all microsomal CYPs. We have shown earlier that we can extract the conformational equilibrium between the open and closed conformations of CPR in nanodiscs films using neutron reflection. Moreover, this conformational equilibrium is dependent on the presence of NADPH. However, it is not known yet whether the change in conformation is due to the actual binding of NADPH to the protein, to the change in oxidation state induced by NADPH or to both oxidation state change and NADPH binding. We have explore these issues and we still require 1 more day of beam time to unravel this question.

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Due to lack of sample availability at the last minute, we were forced to use the beam time allocated to test a new project dealing with insulin adsorption onto hydrophobic surfaces, and the subsequent fibrillation process under accelerated conditions of acidic pH and elevated temperatures. Our aim is to compare the fibrillation kinetics, extent and fibrillar structures as a function of the presence of a hydrophobic lipid tail attached to insulin. Thioflavin T assays shows that the lipid tail accelerate the kinetics of fibrillation, and moreover, QCM-D result suggests that both the extent and kinetics of fibrillation on a hydrophobic modified surface is highly dependent on the presence of the lipid tail. We see major differences in the initial protein monolayer formed at the surface showing a Sauerbrey thickness of 16 Å and 35 Å for insulin without and with lipid tail respectively.

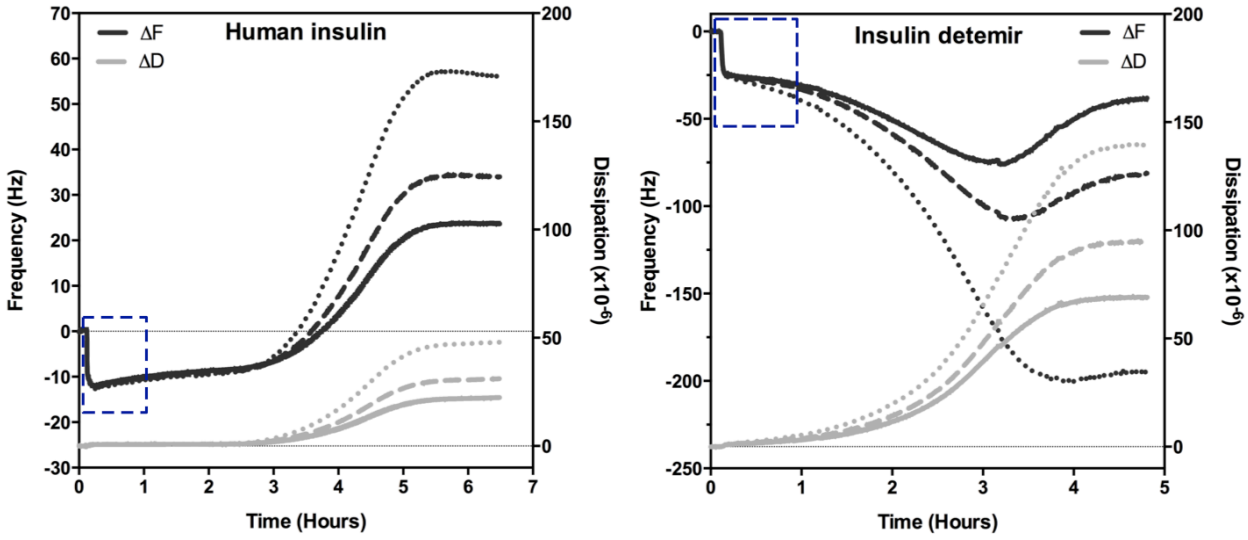

Figure 1: Representative QCM-D traces from a typical experiment of human insulin at 45 °C (left) and insulin detemir at 35 °C (right), respectively. The frequency (ΔF) and dissipation (ΔD) shifts for overtones 5 (dotted line), 7 (dashed line), and 9 (solid line) are displayed as a function of protein exposure time. The protein solutions are continuously flowed through the cells at 100 μl/min for 15 min and then the system is left to equilibrate under no flow conditions. The dashed boxes indicate the level of initial adsorption.

An initial NR experiment was performed in which the lipidated insulin (insulin detemir) was incubated for 1 hour, after which rinsing with solvent was performed. The adsorbed layer is 53 Å thick and thus it suggests a dimeric state of the protein (or a double layer). The data shows that the protein layer can easily be removed from the surface under these conditions. A parallel NR experiment was performed with measurements after the initial adsorption, after fibril development at 6 hours, and finally after a rinsing step with solvent (Fig. 2). Upon initial adsorption of insulin detemir to the surface, the reflectivity profile changes, reflecting the adsorption event. Applying a three-layer model (Si/SiO_2/silane/protein/solvent), the initially formed protein layer thickness of insulin_lip increased from 53 Å to 69 Å upon 6h incubation. The increase in thickness over time of the inner protein layer was not due to a change in surface coverage. It was not possible to obtain a good fit when varying the coverage and fixing the thickness to the initial 53 Å.

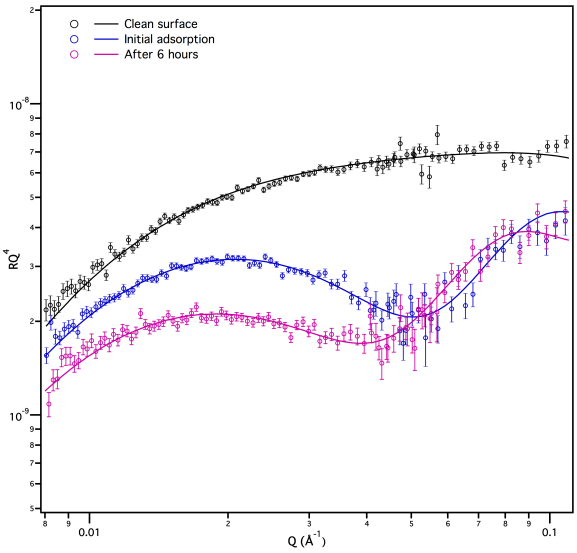

Figure 2: Neutron reflectivity profiles of a silane-modified surface in H_2O contrast, before and after adsorption of 2.5 mg/ml insulin_lip. The lines are best corresponding fits to the data.

The data show only one minimum and thus no obvious structural sign of the presence of a second layer of fibrillar structures after incubation. However by accessing the scattering length density (SLD) changes of the bulk solvent upon incubation, it is possible indirectly to confirm the existence of fibrils in the system. The diffuse fibril layer is likely to be too thick to be within the limit of detection of the neutrons, and thus may be interpreted as a bulk phenomenon by the neutrons, instead of a defined protein layer. From the fit it was found that the fibrils constituted 20 % of the bulk solvent after 6 hours. Upon rinsing with solvent after incubation, the fibrillar content was only decreased by 50 % to a final fibrillar content of 10 % while the inner protein layer is significantly removed. This suggests that the two organizations of protein are probably only weakly connected, and that the fibrils are grown mainly from proteins in solution. Moreover, the fibrils seem not to have any alignment since we did not observe significant off specular scattering, but rather a higher level of noise in the detector image. In summary, two levels of protein organization have been confirmed; an inner protein layer initially formed and indirectly, via the SLD values the existence of fibrils was found as a thick second layer. The aim is to compare these results with NR measurements of the non-lipidated insulin.