Experimental Report

Proposal: **TEST-2429** **Council:** 4/2014

Title: Insulin adsorption to hydrophobised surfaces

This proposal is a new proposal

Researh Area:

Main proposer: **CARDENAS Marite**

Experimental Team: CARDENAS Marite
HEDEGAARD Sofie

Local Contact: BARKER Robert

Samples: Silicon
Insulin

Instrument	Req. Days	All. Days	From	To
FIGARO	1	1	03/12/2014	04/12/2014

Abstract:

Experimental report

TEST-2429: Insulin adsorption to hydrophobised surfaces

This project is 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. In bulk samples 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.

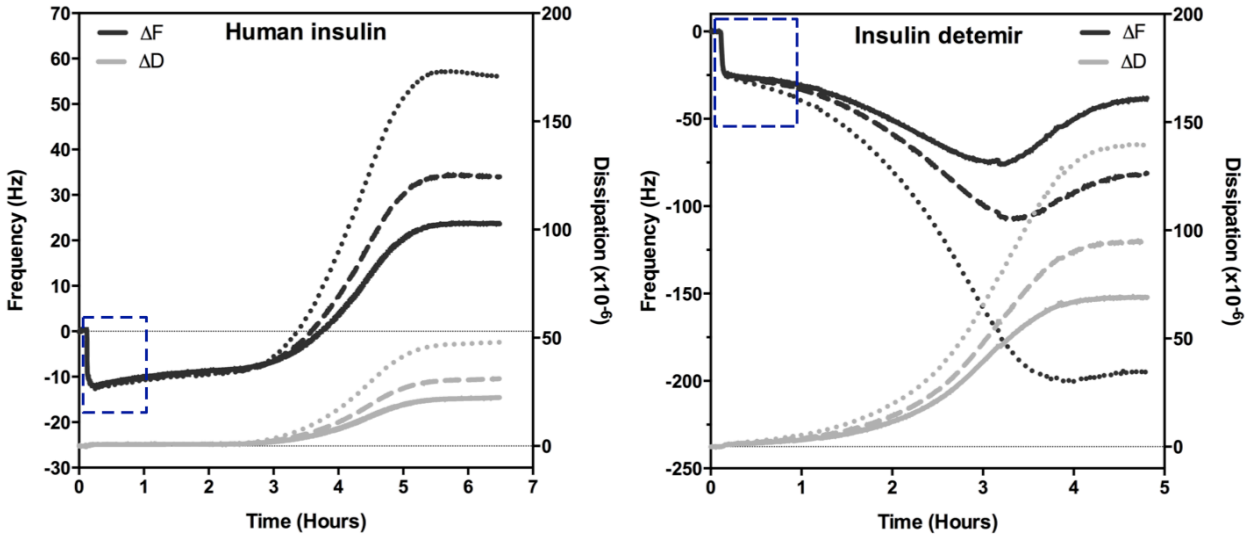

Figure 1: Representative QCM-D traces from a typical experiment of human insulin at 45 °C (left) and insulin detemir at 35 °C (right), respectively. The frequency (ΔF) and dissipation (ΔD) shifts for overtones 5 (dotted line), 7 (dashed line), and 9 (solid line) are displayed as a function of protein 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.

The neutron reflectivity profiles on human insulin suggest minor adsorption of protein, even after 8 hours of incubation at 45 °C (Fig. 2). The measurements were performed in H_2O solvent, thus the protein-solvent contrast is limited under these conditions. A second measurement in D_2O solvent would most likely increase the contrast and the confidence of the fit considerably, however, this change in condition could affect the fibrillation process in an unpredictable manner, and was therefore not performed. It was not possible however with only the H_2O contrast, applying a three-layer model (Si/SiO_2/silane/protein/solvent) to fit both protein layer thickness and coverage at the same time. Therefore, fixing the protein layer coverage to 15 Å based on our QCM-D results, the coverage was fitted to be less than 18% after 8 hours. In this fit the roughness was fixed to 1 Å, as the fit was not sensitive to changes in roughness below 5Å.

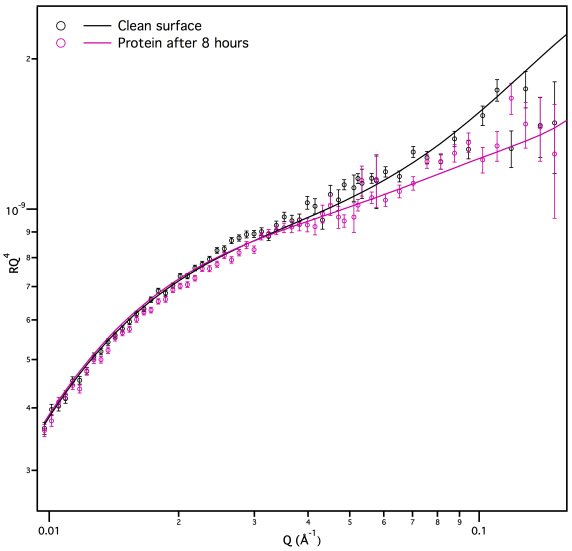

Figure 2: Neutron reflectivity profiles of a silane-modified surface in H_2O contrast, before and after adsorption of 2.5 mg/ml human insulin. The lines are best corresponding fits to the data.