ESRF	Experiment title: **Interactions between cadherins ectodomains investigated by grazing incidence X-rays at the air/water interface.**
SC-694 **Beamline**: ID 10 B	**Date of experiment**: from: June 21, 2000 to: June 27, 2000
Shifts: 18	**Local contact**: **Oleg Konovalov**

Names and affiliations of applicants (* indicates experimentalists):

Jean-François LEGRAND* and Laurence MARTEL*,
UMR SPrAM (CEA-CNRS-Université J. Fourier), DRFMC/SI3M, CEA Grenoble

Stéphanie BIBERT*, Danielle GULINO*, Pierre LEGRAND* and Thierry VERNET
Laboratoire d'Ingénierie des Macromolécules, IBS, Grenoble

Deborah LECKBAND* and Sanjeevi SIVASANKAR*
Dept. of Chemical Engineering, Univ. of Illinois at Urbana Champaign, USA

Report:

After setting the undulator gap and the two diamond monochromator angles to a wavelength of 1.385 Å, the deflection Ge <111> crystal was aligned for reflectivity studies on liquid surfaces. The incident beam size was set to 100 μm (vertical) x 1 mm (horizontal) and the intensity of the reflected beam was analysed using a 1D Position Sensitive Detector, which permits a clear separation between the signal and the background.

We used a specially constructed sample cell filled with He gas and containing two Teflon troughs (80 mm x 60 mm) on a horizontal translation stage. One trough is needed for incubating a monolayer sample at the air-water interface, while a reflectivity curve is being mesured on the other one. The trough are moving step by step through the beam to avoid radiation damage. A water level controlling system (Nanofilm Technologie) was installed on top of the working trough to compensate for evaporation. The whole cell was mounted on antivibration active pads.

Monolayers of polyhistidin tagged C-cadherin ectodomains were prepared using the following procedure : a solution of nickel chelating lipids was spread onto the surface of the water buffer and after evaporation of the solvent, the protein was injected via a peristaltic pump to a final concentration of 5μM. As shown by ellipsometry measurements, it takes a few hours to obtain a dense monolayer of C-cadherins. A similar procedure was used with VE-cadherin ectodomains constructed with a cystein at the C terminus and with ligand-lipids functionalised by a maleimid group able to form covalent bonding with a cystein.

Preliminary analyses of the results are displayed in two representative figures:

Figure 1 shows the reflectivity data of the monolayer consisting of 100% of nickel chelating lipid, adjusted by a model whose density profile is displayed in the inset : it essentially consists of two layers :

- one of thickness L_1= 14.5Å and of electron density ρ_1= 0.42 e/Å^3 for the C_{12} aliphatic tails

- one of thickness L_2= 16.6Å and of electron density ρ_1= 0.49 e/Å^{3}for the head group (Ni NTA + ethylene oxide spacer)

This is only a model which is used in the next steps to analyse the lipid + protein layers.

Figure 2 shows the reflectivity data obtained after incubating for 5 hours the Ni-chelating lipid monolayer on a solution of His_6-C-cadherin. The multiboxes model used to fit the data shows a first peak for the lipid heads and a layer of about 170 Å of thickness for the proteins. The protein layer exhibits several bumps and an average electron density ρ_4= 0.39 e/Å^3 This last value corresponds to a layer of density 1.19 g/cm^3, a relatively high value indicating a dense packing of the cadherins. Taking into account the total length of the 5 domain cadherin of roughly 225 Å, these results suggest that the protein is not perfectly aligned perpendicular to the surface but, instead slightly tilted and/or bent.

A more detailed analysis of the electron density profiles taking into account the strucural data of the protein is necessary before being able to interprete the subtile changes observed after exchanging the subphase by a solution of C-cadherin fragments containing only the domains 1-2-3-4 and no His tag, with the aim of localizing the domains that are involved in the adhesive interactions between the cadherins.

With the VE cadherins the reflectivity results where even more complicated to interpret as it appears that unspecific adsorption also occurred during the incubation. Thus the binding tests with the different fragments could not be performed. A new ligand-lipid construction has therefore to be worked out.

Figure 1
Ni-NTA-DLGA Lipids

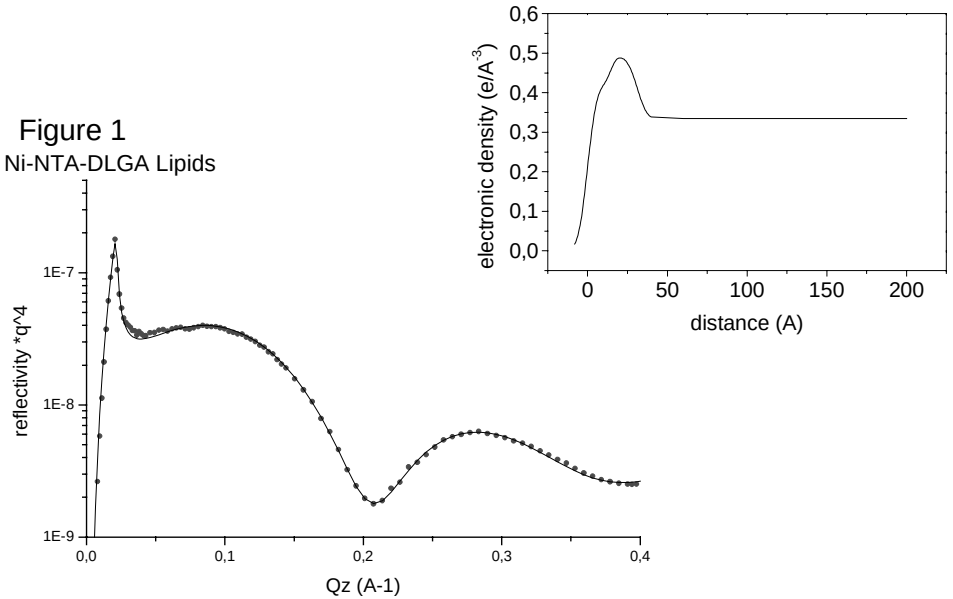

Figure 2
C-Cadherin layer + Ni-NTA-Trig-DLGE lipids