Report

In this set of experiments we studied 2D assemblies of the cell adhesion protein *VE-cadherin.* The reference of the study was a monolayer of a fragment composed of the four extracellular domains 1 to 4 and a histidin-tag on C-terminal, *VE-Cad1-4His,* anchored to nickel-chelating lipids. The aims of the experiments were then

1) to study the location of VE cadherin domains along the protein. This was done by specular Reflectivity using three VE-cadherin shorter fragments (*VE-cad1-3*, *VE-cad2-4* and *VE-cad3-4*).
2) and to visualise the discrete hexameric structures using Grazing Incidence Diffraction on 2D crystallized samples.

The measurements were performed at 8 keV in a home built cell. The cell was filled with helium in order to reduce air scattering. The Teflon® troughs containing the protein samples were placed on a translation stage to keep fresh protein layer under the beam. A Level-O-Matic (NanoFilm Technologie GmbH, Germany) was used to counter evaporation and keep water level centred in the beam within 10 µm. Mixtures of VE-cadherin fragments *VE-Cad1-4His* with *VE-cad1-3*, *VE-cad2-4* or *VE-cad3-4* in 100 excess were made prior to the protein folding to promote heterotypic associations. The *VE-Cad1-4His* concentration was kept constant in all experiments.

The GID measurements did not show particular interests, as we could not reach the conditions for the formation of 2D-crystals.

X-ray reflectivity measurements were more successful. They were performed first on pure lipid monolayers and then on the lipid-cadherin layers. Taking into account our experience on making C-cadherin monolayers and on analysing X-ray reflectivity curves (reports on SC 694 and SC 828[*]), we obtained information on the VE-cadherin monolayer formation and the association of different VE-cadherin fragments.

First, the fragment *VE-Cad1-4His* was bound to *Ni-NTA-DLGE* lipids (see **Figure 1**). Short oscillations in the reflectivity curves #13 and #20 are an evidence for the presence of the protein. The corresponding electron density profile shows that the protein layer is about 16nm thick. Taking into account a length of 18 nm for the *VE-Cad1-4His* [1], this proves that the cadherin is not rigid and normal to the surface but has a mean tilt angle of 30°.

Figure 2 and **Figure 3** show the effect of the various mixtures of cadherin fragments. Only the mixture of *VE-Cad1-4His* with the fragment *VE-cad1-3* anchored to the lipids. The presence of the protein is proved here also by the shorter oscillations in the reflectivity curve (**Figure 2**, #14). Two alternatives arise: 1) either the complexes interact with the lipids or 2) the association between the fragment was weak or did not occur and *VE-Cad1-4His* bound alone to the lipids. The electron density profile need further analyse to distinguish between these possibilities. Regarding the mixture of fragments *VE-cad2-4* and *VE-cad3-4* (see **Figure 3**), the absence of protein on the surface provides evidence that these fragments did associate in the subphase and that the assembly prevented the histidin-tag to be accessible to the lipid.

In conclusion, we observed the formation of monolayers of only two of the available VE-cadherin fragments, *VE-Cad1-4His* and *VE-cad1-3*. We have shown that the association of fragments containing domain 4 prevent the binding of the cadherin to the lipid, suggesting that this domain is located closely to the histidin-tag. The complex could therefore be thought as parallel complexes of VE-cadherin, dimers or hexamers. This results need to be confirmed by further biological study of fragment associations, e.g. chemical reticulation.

Concerning the structural study, the next step will be to look at the calcium dependence of the assemblies in order to confirm that the complexes that were formed are biologically relevant.

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[*] The studies corresponding to the proposal SC-828 result in an article: L. Martel, C. Johnson, S. Boutet, R. Al-Kurdi, O. Konovalov, I. Robinson, D. Leckband, J.-F. Legrand, **X-ray reflectivity investigations of two-dimensional assemblies of C-cadherins: first steps in structural and functional studies**, *J. Phys IV,* in press.

[1] P. Legrand, S. Bibert, M. Jaquinod, C. Ebel, E. Hewat, F. Vincent, C. Vanbelle, E.Concord, T. Vernet et D. Gulino, *J. Biol. Chem.*, 276, 3581-3588, (2001)

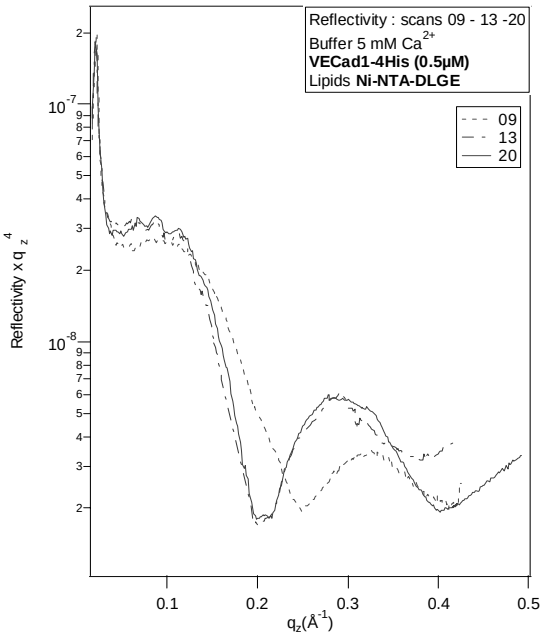

Figure 1: Reflectivity curves showing the binding of *VE-cad1-4His* to nickel-chelating lipids (#13 and #20) compared to the bare lipid monolayer (#09).

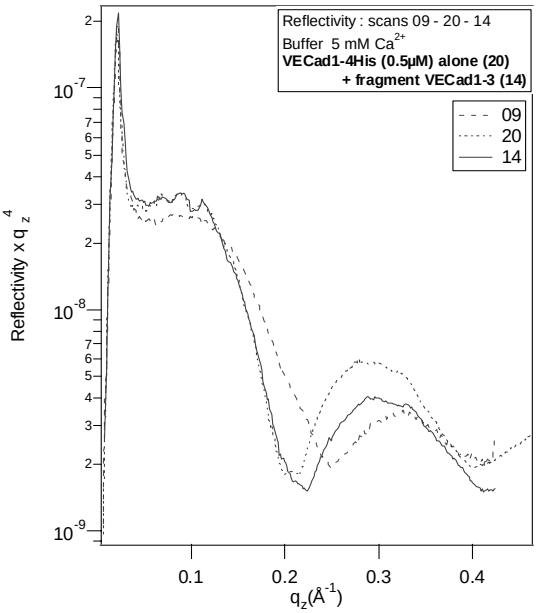

Figure 2: Reflectivity curves of the mixture *VE-cad1-4His* with the shorter fragment *VE-cad1-3 (*#20*)* binding to nickel-chelating lipids. The curves of lipids alone (#09) and with *VE-cad1-4His* alone (#20) are shown for comparison.

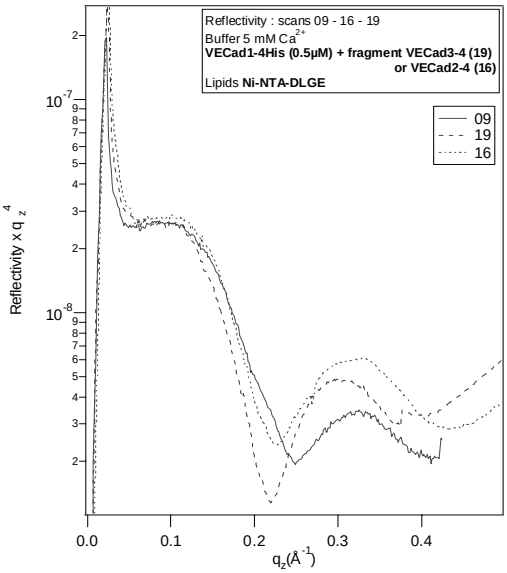

Figure 3: Reflectivity curves of the mixture *VE-cad1-4His* plus short fragments under nickel-chelating lipids: curve #16 for *VE-cad2-4* and curve #19 for *VE-cad3-4*. Curve of lipids alone (#09) is shown for comparison.