Experimental report

23/10/2019

Proposal: 8-02-849 **Council:** 10/2018

Title: Interaction of HIV-1 p6 protein with raft mime membranes by neutron reflectometry

Research area: Biology

This proposal is a new proposal

Main proposer: Elena DEL FAVERO

Experimental team: Giovanna FRAGNETO
Miriam GRAVA
Laura CANTU
Elena DEL FAVERO

Local contacts: Giovanna FRAGNETO

Samples: LIPIDS
p6 small protein

Instrument	Requested days	Allocated days	From	To
D17	3	3	04/07/2019	07/07/2019

Abstract:

The 52 amino acid HIV-1 p6 protein is synthesized as the C-terminal part of the group specific antigen Gag polyprotein Pr55. In this context p6 is known to promote virus release, but during HIV-1 life cycle it is cleaved from Gag polyprotein and its function in this mature form has not been investigated yet. Recently HIV-1 p6 has been found to interact with plasma membrane and eventually to form pores. Aim of this proposal is to study the interaction of the small p6 protein with model membranes to investigate the structural changes induced by the interaction of this small soluble protein on model membranes as a function of membrane composition, from phospholipid bilayers to more complex biomimetic membranes (raft-mime).

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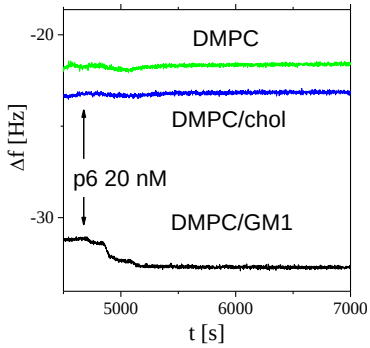
We performed test measurements with a QCM-D apparatus (PSCM laboratories) to verify the propensity of p6 protein to interact with three different supported model membranes: phospholipid DMPC, DMPC:CHOL and DMPC:ganglioside GM1. Protein solution (20 nM) was injected in the solution contacting the deposited membranes, to a final protein/ lipid ratio 1/1000, in order to be consistent with biological results. As the total mass of the small p6 protein was very low, we performed parallel experiments (contemporary in membrane deposition, protein injection and signal detection) on the three membranes for better comparison. Although very small, a difference is visible in the frequency shift Df of the three model membranes. The membrane containing GM1 seems to be more sensitive to the presence of p6.



QCM results on model membranes after addition of p6 protein

We investigated the same systems by NR, namely DPPC, DPPC:CHOL bilayer 10:1.25 molar ratio,DPPC:GM1 bilayer (10:0.5). After characterization of the host membrane we added the p6 solution to the membranes directly in the measuring cell (1:100 protein:lipid mole ratio). After incubation (1 h at 47°C, above the chain melting transition of lipids) we characterized the mixed systems obtained.
Preliminary results indicate that both propensity and extent of interaction of p6 with model membranes depend on membrane lipid composition. The presence of cholesterol hinders the penetration of p6, while the presence of ganglioside GM1 favours p6-membrane interaction.