	Experiment title: Interaction of HIV-1 Viral protein R and p6	**Experiment number**: **SC-5178**
Beamline: ID02	**Date of experiment**: from: 04/02/2022 to: 06/02/2022	**Date of report**:
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Report:

During the HIV-1 replication cycle the Gag pp is known to mediate the assembly and release of progeny virions from an infected cell membrane. The Gag pp C-terminal p6 domain (52 amino acids, 5.8 kDa) facilitates virus release from the plasma membrane and mediates incorporation of the accessory protein Vpr into the virion.
In aqueous solutions p6 presents a weak secondary structure that strengthens itself into two helical domains connected by a flexible hinge region under hydrophobic conditions simulating membranes. The p6 binding site for Vpr has been identified just next the C terminal alpha helix. Then the interaction between p6 and Vpr can be modified by the proximity of cellular membrane.
We first measured the proteins in solution.
While p6 curve shows that p6 has a predominant monomeric shape, more compact in presence of ionic strenght, Vpr shows an aggregated conformation, indicating that the protein has undergone deterioration (Fig.1). Thus the experiment of p6-vpr interaction has been biased by the non-monomeric condition of the protein.

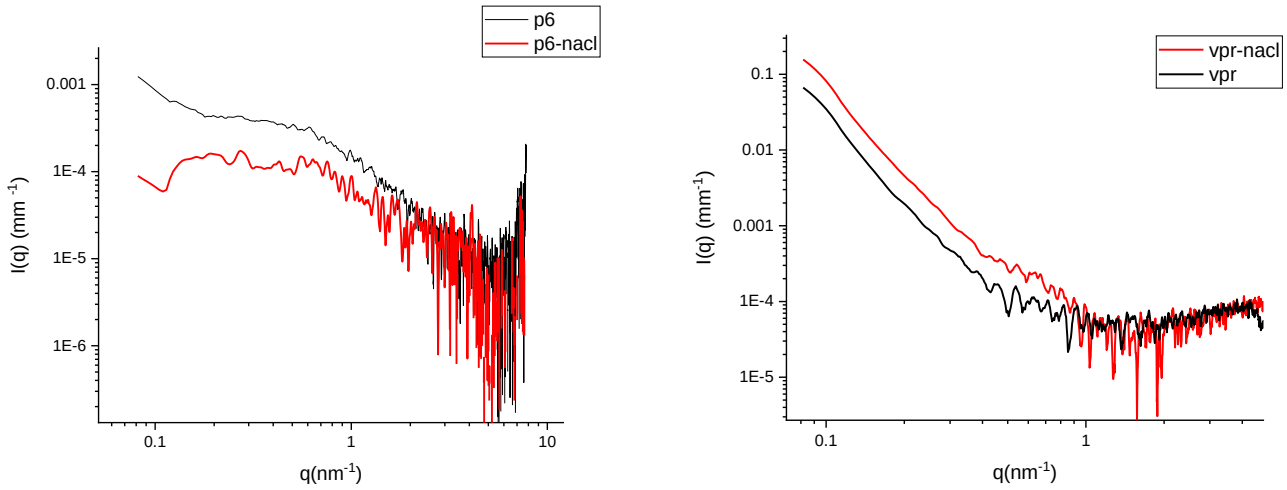

Figure 1: p6 (left) and Vpr (right) in buffer with low (black) and high ionic strenght (red, NaCl 150mM).

Preliminary reflectometry results suggested that the interaction of p6 with supported lipid bilayers is favoured by the presence of small amount of glycolipids, namely GM1 ganglioside, bearing a huge saccharidic headgroup and a sialic acid. We investigated the effect of the ganglioside on the lipid-protein interaction. We observed the interaction of p6 with micelles of dodecylphosphorylcholine (DPC) with GM1 and Gd1a.

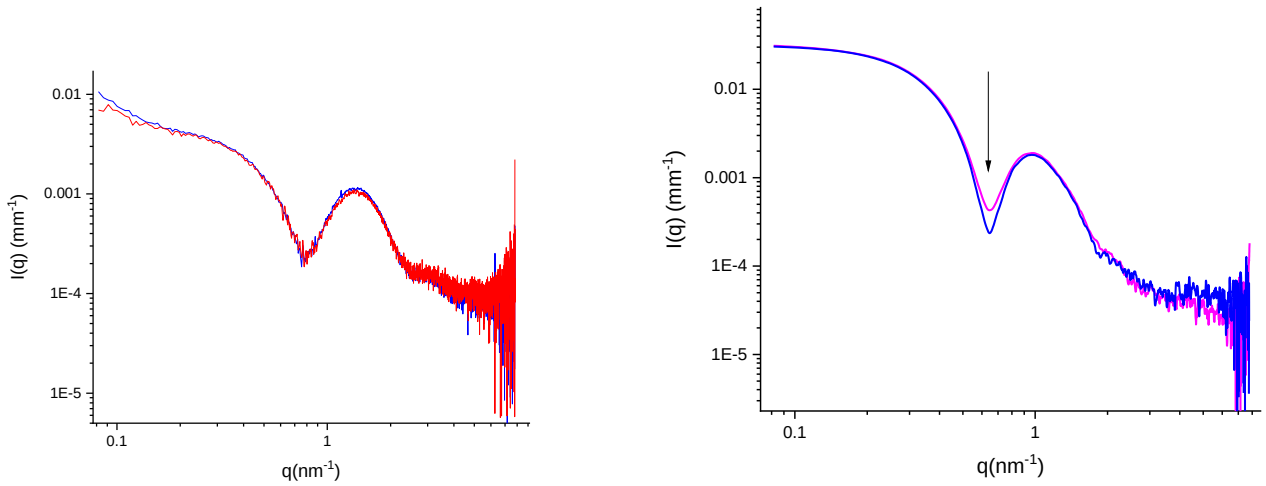

Figure 2: DPC micelles with Gd1a and p6 (blue curve) and the expected non interacting spectrum (red curve).

At this DPC/ganglioside concentration (1:4 DPCmicelles-GM1 molar ratio) there is no observable interaction of p6 with the micelle. The same result has been obtained by adding Gd1a to DPC micelles (Fig. 2, left). However we tested the effect of p6 on a whole GM1 micelle. The GM1 micelle is affected by the presence of p6. As it can be seen in Fig.2, right, the minimum at 0.65 nm^{-1} is modified due to p6 binding. The experimental result behaves differently from the theorical curve, given the low amount and the small dimension of p6 could not affect the GM1 micelle profile.
Further analysis will allow to interprete this results determining the interaction of p6 with GM1 micelles.