Experimental Report

Proposal: **9-13-490** **Council:** 10/2012

Title: Specificity of Beta Arrestin - Lipid Membrane Interactions

This proposal is a new proposal

Researh Area: Biology

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Samples: Beta-Arrestin
Lipids

Instrument	Req. Days	All. Days	From	To
FIGARO User-supplied	4	4	29/04/2013	03/05/2013

Abstract:

Beta-arrestins are proteins thought to turn off GPCR receptor signaling by assisting the recruitment of clathrin leading to endocytosis of GPCRs, where the receptor is removed from the plasma membrane and the activating extracellular ligands. Our recent fluorescence microscopy and QCM-D studies suggest beta-arrestins are recruited specifically by phosphoinositide lipids, but that adsorption also is affected by strong non-specific binding. In order to determine how specific and non-specific binding contributes to interaction between beta-arrestins and membranes, we need to use neutron reflection to monitor and characterize protein binding to supported lipid bilayers. This will be achieved by studying a set of membranes composed to isolate different types of interaction, titrating them with a relevant range of protein concentration, and using appropriate contrast matching. The results here obtained will permit us to identify the mechanism of interaction of beta-arrestins with lipid bilayers and thus identify the critical steps in the biological function of this protein.

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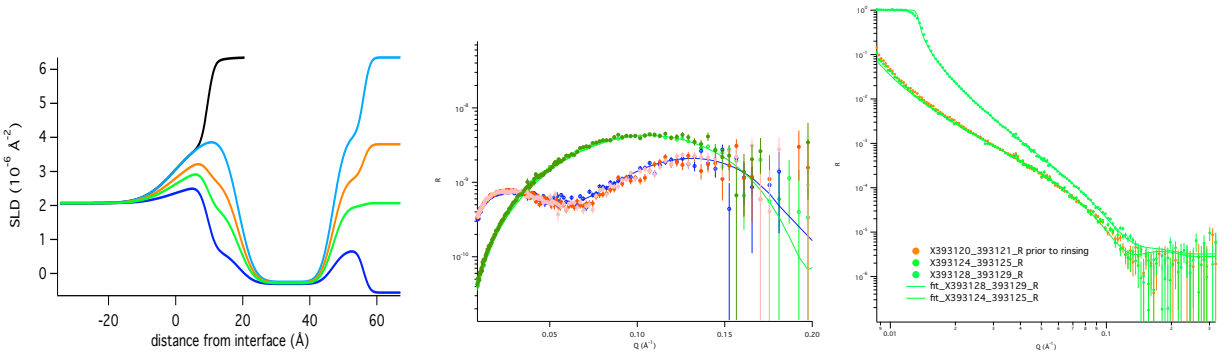
Experimental team: Achebe Nzulumike, Marité Cárdenas, Tommy Nylander and Robert Barker

From our first NR experiment at D17 in November 2012, we learnt that hArrestin did bind to hPIP3 bilayers but we observed mainly a change in the hydration in the lipid headgroups due to interaction with the protein. We could model a protein layer on top with a very low coverage 2% and thus there is a high uncertainty in the structural parameters of this layer. However, we had problems with incomplete bilayer formation and one of our PIP3 containing bilayers had ~20% defects while the hPOPC had a rather poor coverage of ~50%. We observed major change in the hPOPC bilayer, which could correlate with protein adsorption on the empty spots of the surface but had no time to run a protein adsorption on silica.

The first thing we did in the Figaro April 2013 experiment was to repeat the formation of the POPC membrane and expose it to 0.5 uM dArrestin (at D17 we added 1 uM hArrestin but QCM-D show similar adsorption extent above 0.5 uM which we decided to use to save on protein sample). On cell 1 we loaded hPOPC and formed a SLB with perfect coverage with perfect structural parameters (see figure below to the left). Here we used 10 mL of 0.025 mg/mLextruded 10x in HEPES buffer (20 mM) pH 7.5, enriched with 200 mM NaCl and 1 mM EDTA.

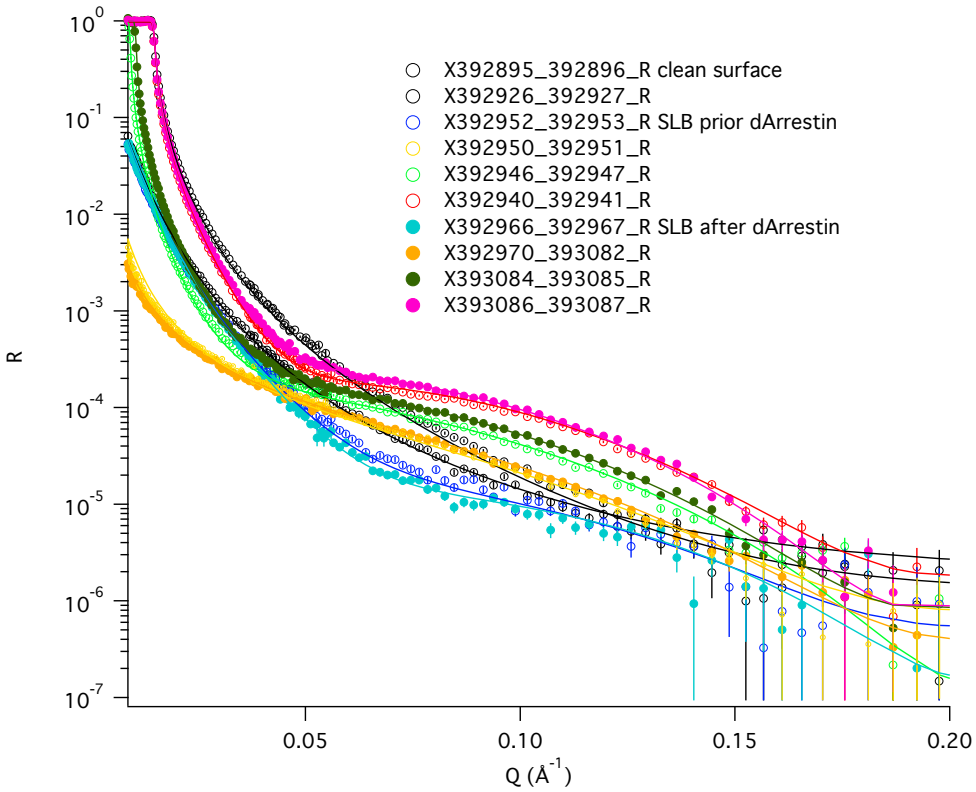


Addition of 0.5uM dArrestin showed no binding at all (see figure above middle). Thus, we can be sure that the changes observed for the PIP3 membrane with high coverage are indeed due to specific protein binding to the lipid membrane.

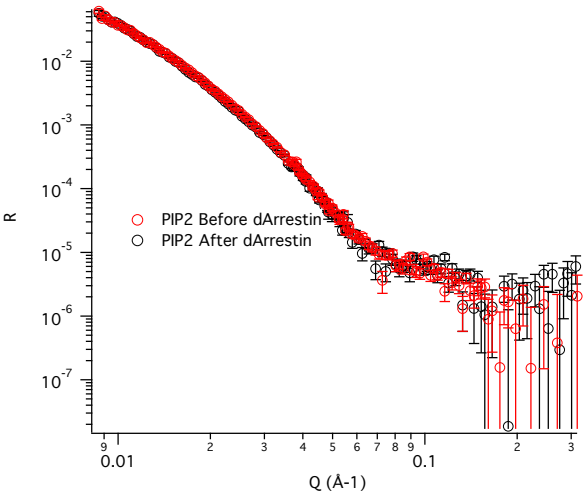
On cell 5 we added 0.5 uM dArrestin on a pure substrate and we indeed saw some irreversible adsorption (see figure above right). Here, the d-Arrestin SLD values were calculated to 6.5, 6.9, 7.15 and 7.5 in H2O, cm2, cm4 and D2O. We observed protein adsorption forming a monolayer of thickness 44 +/- 4 Å and surface coverage of 30 +/- 3 %, which is typical for soluble proteins on this type of substrate. This explains why there was so much changes in our D17 experiment for dPOPC bilayers were we had ~80% coverage only.

A POPC-DP-PIP3 SLB was then formed using vesicle extrusion (x10) on cell 3 and added in the same buffer as for the POPC experiment, but the coverage was only down to 60%. The lipid solution was re-extruded (x10) and added once more under slow flow. The coverage now increased slightly to 81% (see figure below). Note that in this case the lipid concentration was only 0.01 mg/mL instead of 0.025 mg/mL as for POPC SLB. The structural parameters are similar to those obtained in our last D17 experiment although the interfacial roughness for each

sub-layer increased considerably from 2-3 Å to 5-7 Å in these fits. Addition of 0.5 uM d-Arrestin led to significant changes in the water contrast as it should be due to the presence of deuterated protein in the layer (see figure below). Indeed this could again be fitted to ~3% protein adsorption on the defects of the SLB.



Finally, a POPC-DO-PIP2 membrane was also formed in which we expect no specific lipid-protein interactions. The membrane was formed using vesicles that were tip sonicated instead of extruded and pre-mixing with 2 mM CaCl2 to attempt to improve the coverage of the SLB. We obtained about 98% coverage in this case and the structural parameters were similar as for the SLB formed by PIP3. Addition of 0.5 uM d-Arrestin lead to ~3% protein desorption but no significant change in the H2O contrast was observed showing lack of protein adsorption (figure to the right). High interfacial roughness is also found in this case.



In summary, better SLB were formed in the presence of Calcium and d-arrestin binding to SLB can be observed only in the presence of PIP3. However, it mainly occurs due to the presence of defects on the SLB. Thus, we cannot tell so far any concrete about the specificity of dArrestin binding to the membrane.