Experimental Report

Proposal: **9-13-453** **Council:** 4/2012

Title: What are the implications of changes in lipid membrane curvature induced by beta Arrestins?

This proposal is resubmission of: 9-13-406

Researh Area: Biology

Main proposer: **NYLANDER Tommy**

Experimental Team: CARDENAS Marite
LIND Tania
NZULUMIKE Achebe
NYLANDER Tommy

Local Contact: BARKER Robert

Samples: lipids
protein
salts and buffers

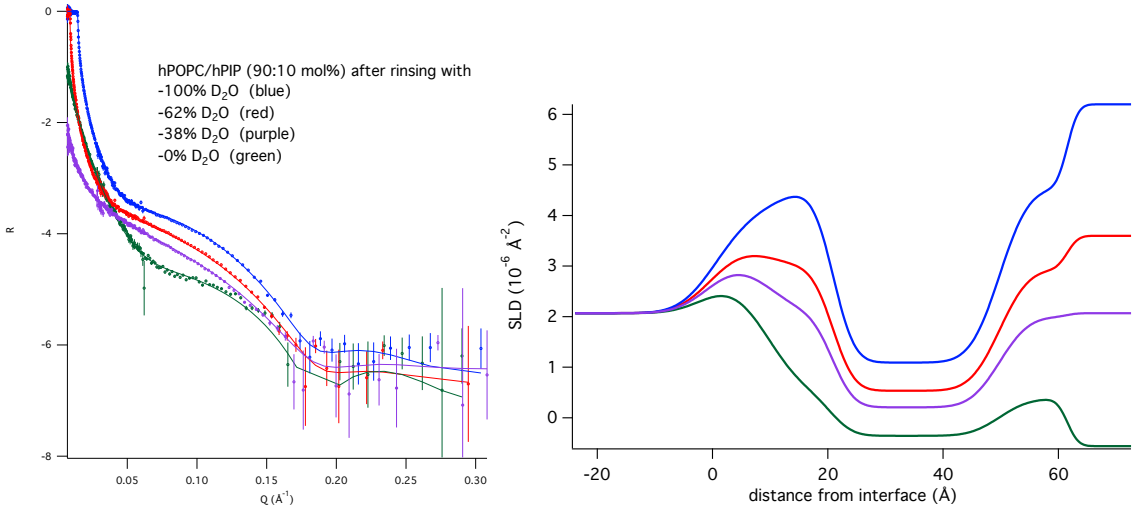
Instrument	Req. Days	All. Days	From	To
D17	4	3	26/11/2012	29/11/2012

Abstract:

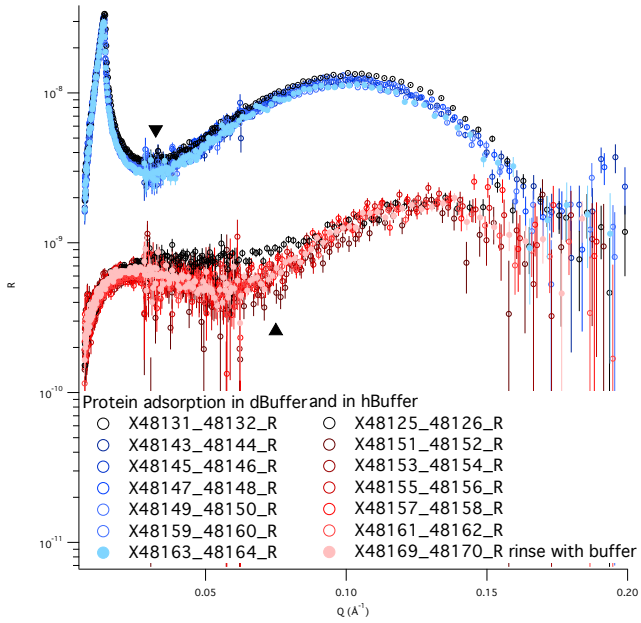
beta-Arrestins are proteins thought to turn off GPCR receptor signaling by assisting the recruitment of Clathrin leading to endocytosis of GPCRs, where the receptor is removed from the plasma membrane and the activating extracellular ligands. Our recent fluorescence microscopy studies suggest that beta-Arrestins may have a so far unknown function in nature: to induce changes in the bilayer membrane that would aid the endocytosis of the GPCR receptors. In order to determine how beta-Arrestin induces such distortion of the lipid bilayer we need to use Neutron Reflection to monitor the binding of beta-Arrestins to supported lipid bilayers and thus determine not only the positioning of beta-Arrestin within the bilayer but also the level of perturbation it induces to the lipid bilayer structure. This will be achieved by a careful study in which the protein concentration and the supported bilayer composition will be varied, and proper contrast matching. The results here obtained will permit us to identify the mechanism of interaction of beta-Arrestins with lipid bilayers and thus confirm a new biological function of this protein.

What are the implications of changes in lipid membrane curvature induced by beta Arrestins? Proposal Number: 9-13-453. Experimental team: Achebe Nzulumike, Marité Cárdenas, Tommy Nylander and Robert Barker.

In this experiment we attempted for the first time to produce supported lipid bilayers of POPC and DOPIP3 via vesicle fusion. Two attempts for formation for samples containing 10 mol% PIP were made using hydrogenated lipids and two attempts were made using a combination of one chain deuterated POPC and DOPIP3. The lipid vesicles were prepared by tip sonication in 20 mM HEPES, pH 7.5 enriched with 200 mM NaCl and 1 mM EDTA (in water) at a total concentration of 10 mg/L and the cells were flushed with 20 mL of such solution. We used motofit to treat the NR data and the results from fits to a three-layer model with symmetric head group structure are given in the graph below for the fully hydrogenated membrane. The headgroup layer is 12 Å as compared to typically 7 Å for pure POPC bilayers, and the coverage of the bilayer varied between 80-99% or ~3.5-4.0 mg/m^2. The tail region was 29-31 Å, very much in agreement with a pure POPC bilayer.

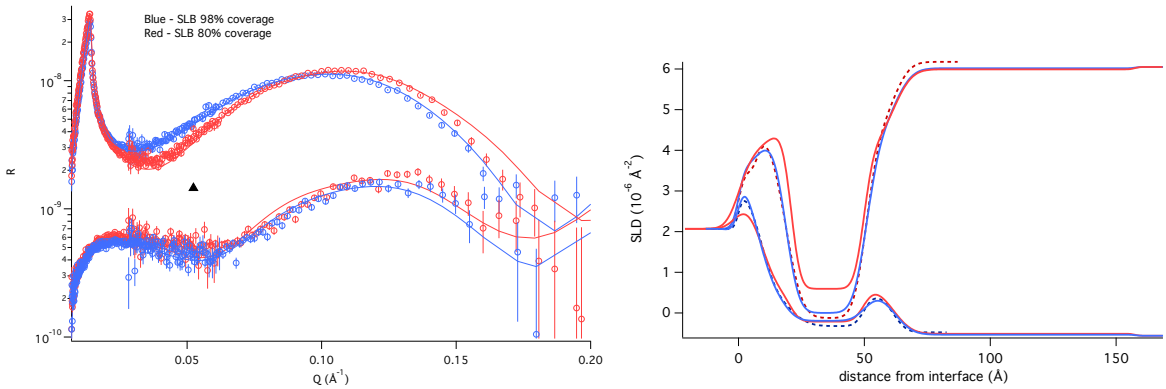


We then proceeded to adsorb beta-arrestin (1uM) on dBuffer in once cell and in dBuffer in a separate cell. We followed the kinetics of interaction over time and observed a slight change in reflectivities in both cases as shown in the figure to the right. Moreover, rinsing with excessive buffer at the end of the experiment did not induce any major changes in the reflectivity, which allowed for complete characterization in both hBuffer and dBuffer on each cell.

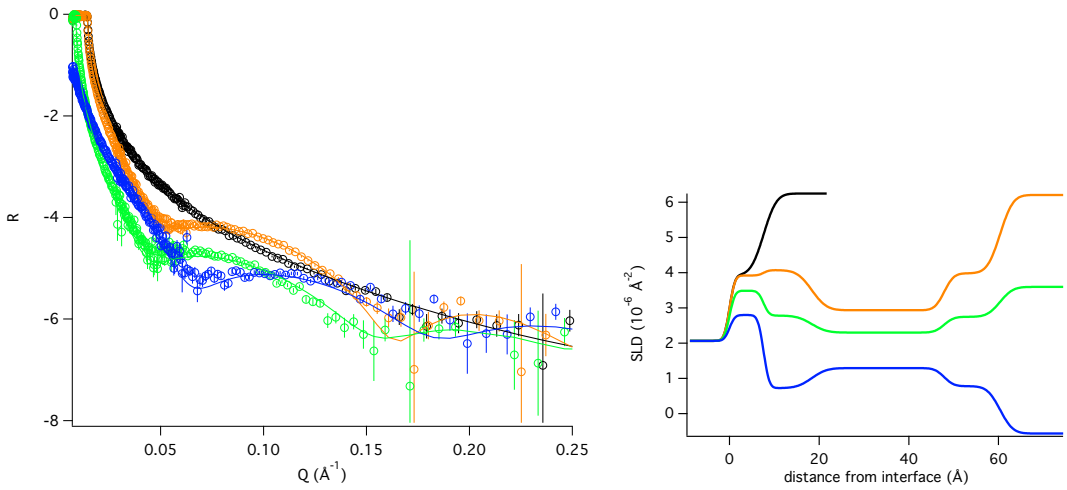


We attempted to fit the protein – SLB data by adding one more layer on top of the SLB. Assuming that Arrestin does not integrate in the lipid bilayer did not allow obtaining any proper fit on the data. In order to obtain a simultaneous fit of both contrasts and constant

protein layer thickness on both sets of data was by allowing an increase in the surface coverage on the bilayer with poor coverage by an additional 10% while the almost perfect bilayer coverage remained intact. However, the coverage of the outer head group region of the lipids increased in both cases by 6-10% depending on the initial coverage of the SLB. The fit improved slightly (chi2 went from 5.04 to 4.84) if we allowed a small change in the SLD of the lipid tail (from -0.3 to -0.15*10-6 Å-2) that corresponds to an incorporation of ~6% protein in the lipid bilayer. Thus, the protein could have been incorporated in the defects of the SLB with poor coverage. Finally the protein protruded by 96+/-12 Å but the coverage was very poor (~2% only) and thus the accuracy in the thickness value is quite poor.



We performed two depositions for the dPOPC-hDOPIP3 SLB formation and we obtained SLB with about 75-80% coverage. Surprisingly, the SLB contained higher content of PIP3 than the nominal composition of the vesicles. We observed 39% PIP3 content in the vesicles for both depositions and the distribution was fully symmetric in the leaflets of the bilayers. The structural parameters were otherwise similar to those obtained for fully hydrogenated membranes. We had however an accident with air bubbles and we partially lost the membrane. We added arrestin and we observed further lipid desorption with what could be a very tiny layer of protein on top of the SLB in agreement with the first series of experiments.



At this point and based on the most successful set up for Arrestin interaction (hPOPC-hDOPIP3) we believe that arrestin interacts with the SLB integrating into the headgroup area. We cannot, however, exclude ~6% penetration inside the SLB due to the presence of defects in the membrane.