Experimental report

12/09/2019

Proposal: 9-13-851 **Council:** 4/2019

Title: Cushioned lipid bilayers: spontaneous formation of intrinsically disordered protein cushions

Research area: Chemistry

This proposal is a new proposal

Main proposer: Marie SKEPO

Experimental team: Marie SKEPO
Amanda ERIKSSON SKOG
Yuri GERELLI

Local contacts: Yuri GERELLI

Samples: Histatin 5
synthetic lipids

Instrument	Requested days	Allocated days	From	To
D17	3	0		
FIGARO	3	3	19/07/2019	22/07/2019

Abstract:

Solid-supported lipid bilayers are widely used for biological and fundamental studies. Since many years, researchers have been working on strategies to decouple the bilayer from the substrate in order to i) reduce the substrate effect on lipid mobility and ii) promote the formation of a hydrated region between the substrate and the bilayer. In particular, the presence of a water gap will allow permeability studies and insertion of transmembrane proteins (that would otherwise denaturate in contact with the solid). We recently observed the spontaneous formation of a protein cushion upon incubation of partially charged lipid bilayers with an intrinsically disordered protein, Histatin 5. Bilayers composed by POPC and POPS resulted to be lifted from the substrate without an loss of structural integrity. We propose now to elucidate the structural properties of this cushion by using fully deuterated phospholipids. This will allow to quantify the amount of protein present in the cushion and to estimate if any protein remains trapped within the bilayer region.

Experimental report for 9-13-851 on FIGARO

Users: Marie Skepö (PI), Amanda Eriksson Skog, Yuri Gerelli
Local contact: Yuri Gerelli
19-07-2019 to 22-07-2019

During the experiment 9-13-851 performed on FIGARO we measured the interaction of a salivary protein, Histadin 5 (Hst5), with model lipid membranes. In previous experiments (confirmed by data collected during some tests on the SuperAdam instrument in September 2019), we have observed that Hst5 penetrates the bilayer and cumulates in the proximity of the solid substrate (silica in this case) (see report 9-13-656). The aim of the current experiment was to investigate the location of the peptide within the bilayer structure and the influence of ionic strength of the peptide-lipid interaction. The first objective was achieved by using tail deuterated lipids instead of partially deuterated ones used previously. In particular, we used d_{54}DMPC and d_{54}DMPS in a 91:9 ratio. In order to keep the bilayer in the fluid bio-relevant phase, the temperature was increased to 37 °C as compared to 20 °C used in the previous experiments (in which fluid POPS and POPS lipids were used). As expected, structural changes resulting from Hst5-lipids interactions, were observed for samples measured at low (10 mM NaCl) and medium (150 mM NaCl) ionic strength while no changes were observed at high ionic strength (500 mM) (see Figure 1).

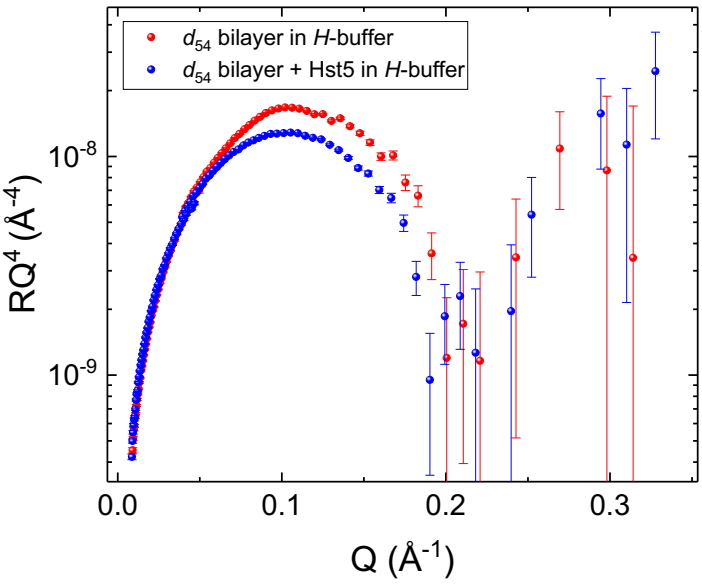

Figure 1. Reflectivity curves for a silicon-supported d_{54}DMPC:d_{54}DMPS bilayer in H-buffer (red symbols) and for the same bilayer after interaction with Hst5 (in 10 mM NaCl). The analysis of the data is still in progress but the differences in the experimental data indicate a slight increase in the bilayer thickness as well as a decrease in overall h-d contrast.

In this last condition, the charges of Hst5 were fully screened also on lengths comparable with histidine-histidine distance within a single protein, thus confirming the electrostatic origin of the interaction.

However, the changes observed resulted to be smaller if compared to what was found for the d_{31}POPC:d_{31}POPS lipid bilayers measured at 20 °C. We estimate that the change in temperature, necessary to keep the bilayer in the fluid phase, affected the Hst5 activity. On the other hand, also the lipid composition (unsaturated vs. saturated) could have an impact on the overall interaction. Anyhow, with the data collected so far it is not possible for us to discriminate between the two situations.