Experimental report

10/01/2024

Proposal: 9-13-1048 **Council:** 10/2022

Title: Nanostructural characterisation ofsVE interaction with lipid bilayermembranes: basis for biosensor development

Research area: Biology

This proposal is a new proposal

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Samples: POPC solution
AM199 lipid mixture (DPEPC:GDPE 70:30)
POPC/POPS 90:10 solution
POPC/DOTAP 70:30 solution
POPC/SM/CHOL 60:30:10 solution
sVE-cadherin solution
DSPE-PEG-NHS tBLM

Instrument	Requested days	Allocated days	From	To
D17	3	0		
FIGARO	3	3	06/06/2023	09/06/2023

Abstract:

We propose to draw a mechanistic understanding of the molecular mechanisms responsible for protein/lipid interactions by performing nanostructural characterisation with neutron reflectometry. This proposal is an integral part of an INNOVAXN PhD grant, which was awarded in 2021 to Mme Beatrice Barletti for the development of a novel biosensor based on nanostructured biomimetic lipid membranes. The results from this proposal will provide essential structural characterisation for the interpretation of our initial functional investigations that demonstrated the interaction of a blood protein biomarker, soluble VE-Cadherin (sVE), with a biologically relevant lipid bilayer membrane. sVE is the glycosylated extracellular domain of the Vascular-Endothelial cadherin and is cleaved from endothelial cells following inflammation or infection, leading to a breakdown of the endothelial junctions and a loss of the endothelium¿s barrier function. Hence, the presence of sVE in the blood provides a sensitive biomarker of vascular endothelium pathophysiology and can be used for early detection of diseases associated with vascular permeability.

Experimental report for experiment 9-13-1048:

Nanostructural characterisation of sVE interaction with lipid bilayer membranes: basis for biosensor development

The aim of the experiment is to study at nanostructural level protein/lipid bilayer interactions using neutron reflectometry to understand how different lipids and glycosylations affect this interaction. NR provided high-resolution and depth-sensitive information probing structure and composition of complex layered systems.

The experiment was successfully performed on 4 different supported lipid bilayers of pure and mixed lipids (POPC, POPC/POPS 8:2, POPC/DOTAP 7:3, POPC/SM/CHOL 6:3:1) before and after the inclusion of aliquots of **alpha-fetoprotein** and **BSA** in the liquid subphase, using 2 contrasts for the bare silicon blocks, 3 water contrasts for the pure lipid membranes and 3 water contrasts after protein addition (100 % D_2O, 66% D_2O and 100% H_2O). The results in D_2O contrast for alpha-fetoprotein are reported in Figure 2.

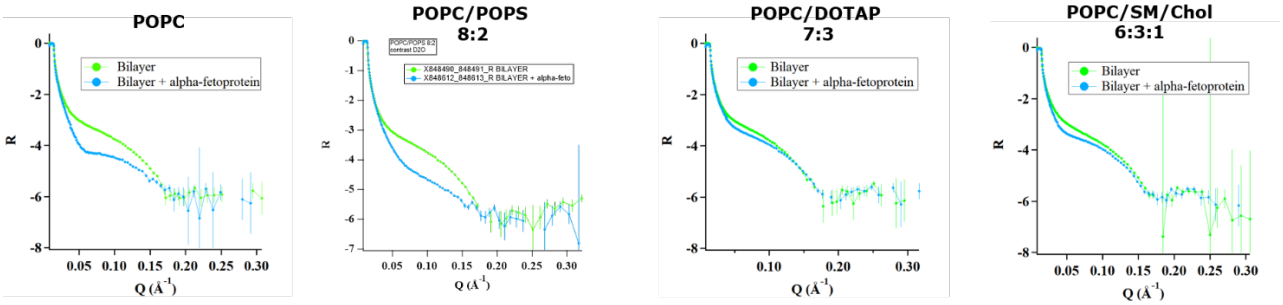


Fig.2 NR results on different SLB compositions in presence of alpha-fetoprotein in D_2O contrast.

The results show significant changes in the lipid bilayer after the injection of alpha-fetoprotein, while very small changes are reported in presence of BSA (Fig.3).

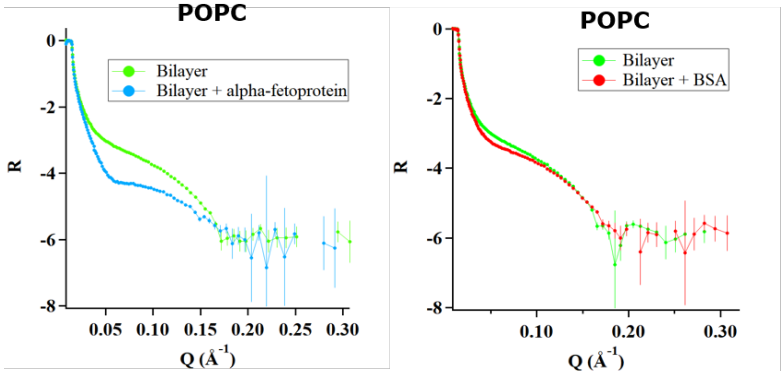


Fig.3 Comparison of the interaction behavior of alpha-fetoprotein and BSA with a POPC bilayer resulted from NR data.

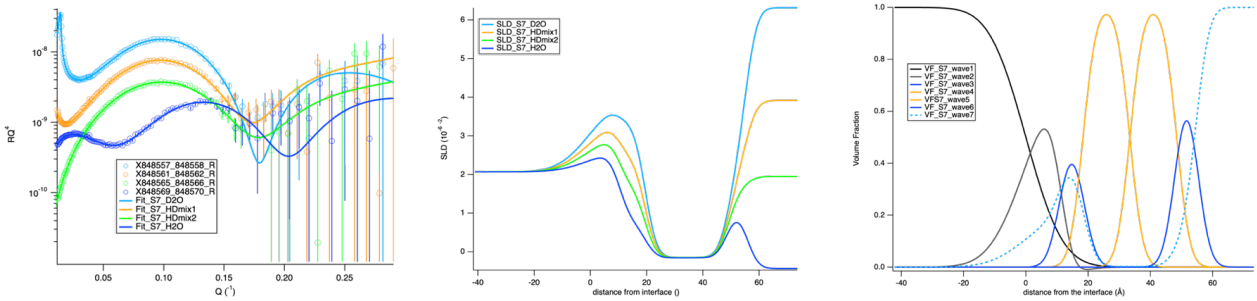
We discovered that the kinetic has an important role for the interaction. The interaction behavior of alpha-fetoprotein is influenced by the lipid composition of the system and the protein interacts faster and stronger in presence of POPS.

Data analysis is in progress and is performed using both the Motofit plug-in for Igor Pro and the software Refnx to check the consistency of results between programs. The sample systems are being modelled as a series of homogeneous slabs, each slab will be described by the four parameters thickness, SLD, roughness and solvent percentage or hydration in the layer. In particular, various slabs models for the bilayers in presence of proteins have been tested. The different models tested for protein/lipid bilayer interaction take into account the possibility of a protein adsorbed layer onto the SLB, a partial penetration of proteins into the outer headgroup layer and also a full penetration of

proteins in the SLB with also an additional layer of proteins on top. The data analysis is performed by simultaneous co-refinement of data at different water contrast.

First results of fit on SLB

POPC/DOTAP 7:3



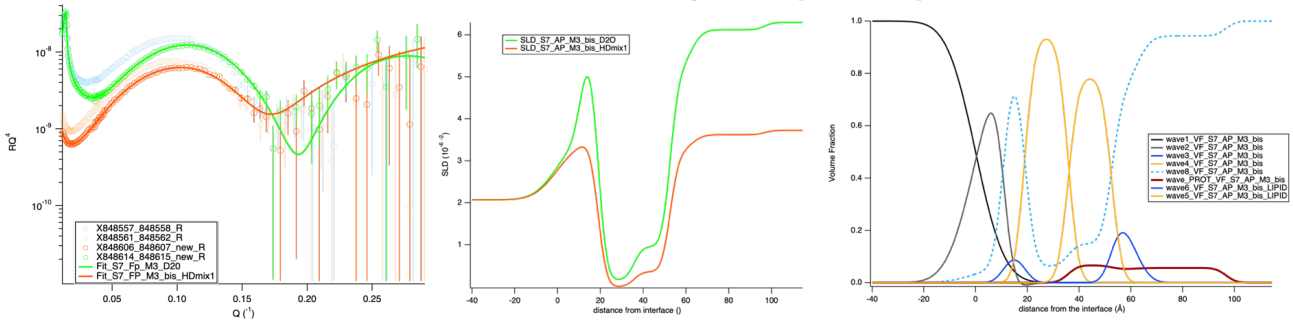
Layer	Thickness[Å]	SLD[×10⁻⁶Å⁻²]	Solv [%]	Rough[Å]
Si	-	2.07	-	-
SiO2	11.1±0.4	3.47	0.215±0.009	9.2±0.6
Head inner	7.2±1.3	1.5± 0.1	0.5±0.4	3.6±0.2
Tail inner	15.1±0.5	-0.13±0.03	0.02±0.002	3.6±0.2
Tail outer	15.1±0.5	-0.13±0.03	0.02±0.002	3.6±0.2
Head outer	6.3±0.5	1.5±0.1	0.14±0.04	3.6±0.2

The model that seems to describe the data most appropriately is the one considering not only the adsorption of the protein on the bilayer, but a partial incorporation of the protein in the outer head region of the bilayer.

First results of fit on SLB + proteins

Model 3: Proteins adsorbed and incorporated in the outer leaflet (outer head and tail)

POPC/DOTAP 7:3 + 0.1 mg/mL alpha-fetoprotein



Layer	Thickness[Å]	SLD[×10⁻⁶Å⁻²]	Solv [%]	Rough[Å]
Si	-	2.07	-	-
SiO2	11.1	3.47	0.215	9.2
Head inner	8.0±0.5	1.67	0.89±0.08	3.19737±0.3
Tail inner	16.8±0.3	-0.243569	0.06±0.03	3.19737±0.3
Tail outer	16.8±0.3	-0.243569	0.06±0.03	3.19737±0.3
Head outer	9.05226±0.03	1.67479	0.7±0.1	3.19737±0.3
Vol Fr Protein	0.056436			