EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

# Experiment Report Form

**The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF**.

Once completed, the report should be submitted electronically to the User Office via the User Portal:
*https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do*

## Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

### Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

## Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

### Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

### Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

### Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

| | | |
|---|---|---|
| ESRF | **Experiment title:**<br>Nanostructural characterisation of protein interactions with lipid bilayer membranes: basis for biosensor development | **Experiment number**:<br>LS-3092 |
| **Beamline**:<br>ID10 | **Date of experiment**:<br>from: 22/11/2022 to: 24/11/2022 | **Date of report**:<br>05/01/2023 |
| **Shifts:**<br>9 | **Local contact(s)**:<br>JANKOWSKI Maciej | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):

Mme Beatrice Barletti* (Institut Laue-Langevin)

Dr Marco Maccarini* (CNRS, TIMC)

Dr Giovanna Fragneto (Institut Laue-Langevin)

Mr. Jean-pierre Alcaraz (TIMC)

Professor Donald Martin (TIMC)

| | | |
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| **Beamline**:<br>ID10 | **Date of experiment**:<br>from: 29/11/2022 to: 01/12/2022 | **Date of report**:<br>05/01/2023 |
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**Report:**

The aim of the project is to study at nanostructural level the interaction between the biomarker, soluble Vascular-Endothelial cadherin (sVE), and biologically relevant lipid bilayer membranes using X-ray scattering techniques, and particurlarly to identify how different lipids can affect this interaction to build a potential biosensor, in which this interaction is enhaced, that is aimed for early detection of scarce biomarkers in blood samples. These fundamental results will better elucidate the mechanism of interaction between protein and lipids and will set the basis for the development of biosensors aimed to optimise the sensitivity and reliability of biosensing techniques for biomarkers of tumours.

In this context it is important to understand the effect of glycosylation since the majority of biomarkers of tumours are glycosylated. As reported in a previous related study [1], the glycosylated state of proteins leads to easy formation of protein/lipid complexes with either HDL or LDL particles. To discriminate the effect of glycosylation we tested two different proteins, soluble Vascular-Endothelial cadherin (sVE) and Bovine Serum Albumin (BSA), with supported lipid bilayers (SLBs) of different lipid compositions. sVE is the glycosylated extracellular domain of the Vascular-Endothelial cadherin and is cleaved from endothelial cells following inflammation or infection, leading to loss of the endothelium's barrier function [2,3,4], whereas BSA is a non-glycosylated protein belonging to the serum albumins family.

The experiment at ID10 was successfully performed. To study protein/lipid bilayer interactions, we performed X–ray scattering measurements (XRR and GID) on three different lipid bilayers with and without the presence of proteins to obtain information on structural changes, such as thickness, roughness and hydration as a function of lipid mixtures, and the amount and average localisation of proteins. In particular, results on three different lipid compositions of SLBs (pure POPC, POPC/DOTAP 70:30 and POPC/SM/CHOL 60:30:10) with two different proteins (sVE and BSA) were obtained.

For each system we acquired various scans in different positions of the empty silicon substrate, the bilayer and the bilayer after the injection of the protein. The kinetic of absorption/insertion of proteins was also studied. Examples of reduced and normalised reflectivity curves are reported in Figure 1.

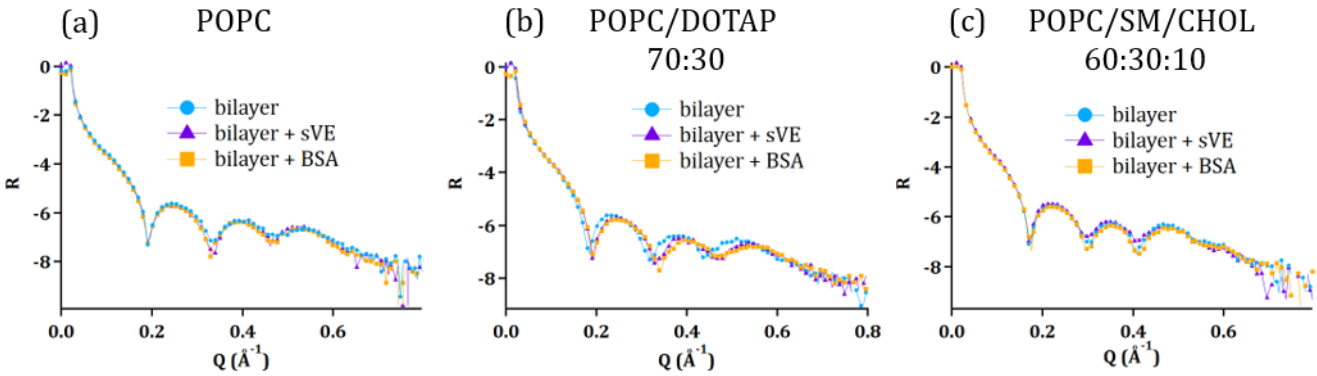


**Figure 1.** (a) Reflectivity curve for the pure POPC bilayer (light blue line) and with the presence of sVE (purple line) or BSA (yellow line) in PBS. (b) Reflectivity curve for the POPC/DOTAP 70:30 bilayer (light blue line) and with the presence of sVE (purple line) or BSA (yellow line) in PBS. (c) Reflectivity curve for the POPC/SM/CHOL 60:30:10 bilayer (light blue line) and with the presence of sVE (purple line) or BSA (yellow line) in PBS.

The results show different effects on protein interaction with different lipids in the membrane model. A similar interaction behaviour is observed for sVE and BSA with the pure POPC bilayer. A greater interaction for both proteins is highlighted with the SLB of composition POPC/DOTAP 70:30. The presence of the cationic lipid in the mixture favoures the interaction with proteins which are negatively charged at physiological pH of the working buffer. In addition, a bilayer with composition POPC/SM/CHOL 50:25:25 was tested in the presence of BSA. GID scans of this last cited system show interesting results demostrating the presence of different lateral distribution depending on the position.

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, for the fit of the bilayer a 4 slabs model have been used, whereas 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.

So far, an in-depth analysis of the data has been performed for POPC and POPC/DOTAP SLBs in presence of BSA. The model that seems to describe this 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 for which we report the example below (Figure 2). For this model we have tested two different variants, one considering symmetric tails (model 1) and one considering asymmetric tails (model 2)

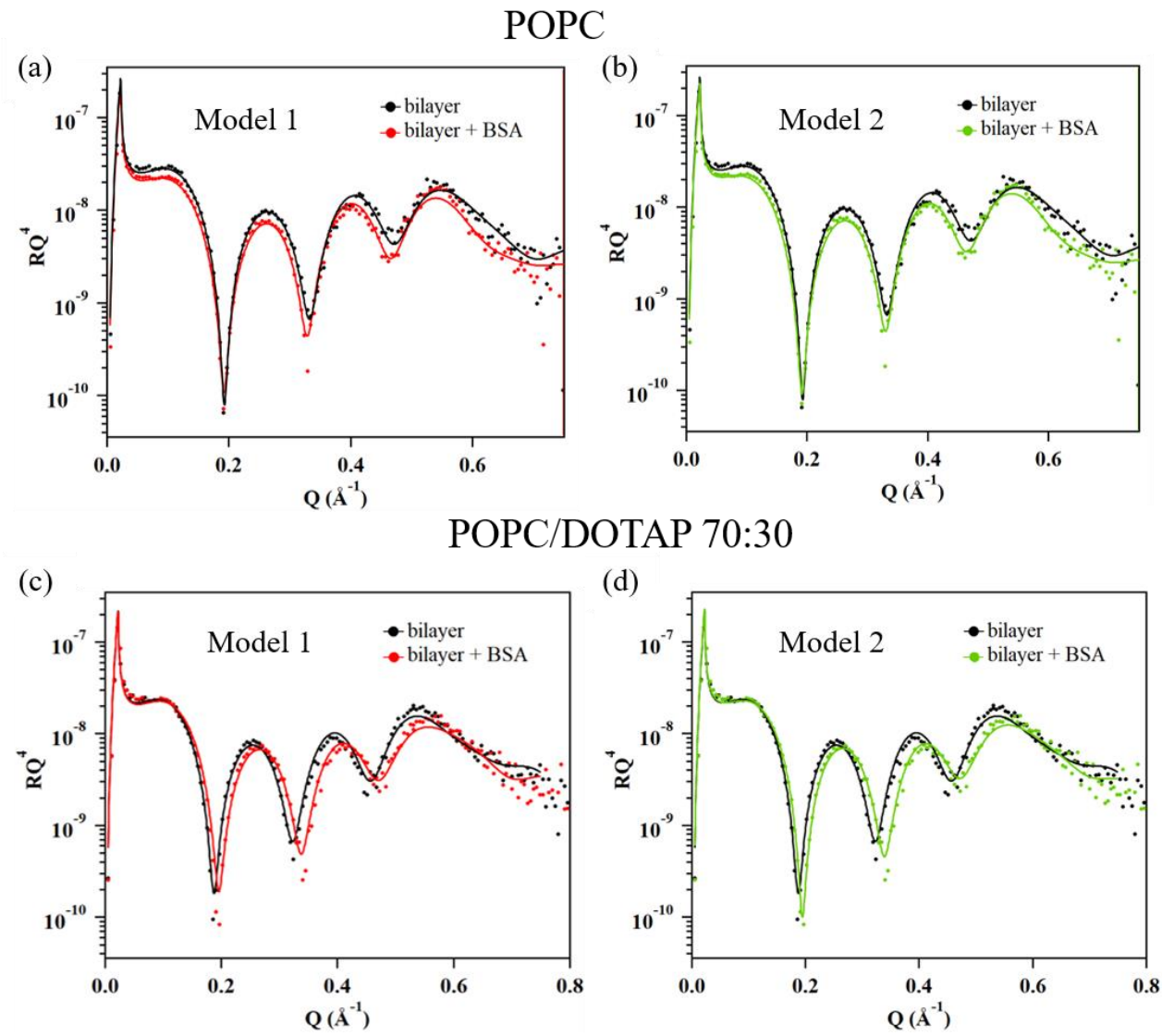


**Figure 2.** Examples of fitting results for different models.

**Complementary measurements**

Complementary measurements with sVE on the last lipid composition tested (POPC/SM/CHOL 50:25:25) are still necessary.

To further discriminate the effect of glycosylation on the interaction, we are willing to perform new synchrotron XRR experiments using different glycosylated proteins, such as alpha-fetoprotein (biomarker for liver cancer) and glycosylated BSA. The interaction with SLBs will be characterised using the same parameters and lipid compositions tested for the previous proteins. The results with the four different proteins will be compared and elucidation on the effect of glycosylation and impact of different lipids will be highlighted.

## References

[1] A. Khalil-Mgharbel, H. Polena, P.K. Dembélé, Md. M. Hasan Sohag, J-P Alcaraz, D.K. Martin, and I. Vilgrain, A *Biomimetic Lipid Membrane Device Reveals the Interaction of Cancer Biomarkers with Human Serum Lipidic Moieties*, Biotechnology Journal (2018), 13:1800463.

[2] Blaise, S., Polena, H. & Vilgrain, I. Soluble vascular endothelial-cadherin and auto-antibodies to human vascular endothelial-cadherin in human diseases: Two new biomarkers of endothelial dysfunction. *Vasc. Med.* 20, 557–65 (2015), DOI: 10.1177/1358863X15591201

[3] Flemming, S. *et al.* Soluble VE-cadherin is involved in endothelial barrier breakdown in systemic inflammation and sepsis. *Cardiovasc. Res.* **107**, 32–44 (2015), DOI: 10.1093/cvr/cvv144

[4] Vilgrain, I. *et al.* Evidence for post-translational processing of vascular endothelial (VE)-cadherin in brain tumors: Towards a candidate biomarker. *PLoS One* **8**, (2013), DOI:10.1371/journal.pone008005