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: Mapping the Structure-Function Relationships of the Tear Film Lipid Layer by Grazing Incidence X-ray Diffraction on its central Lipid Species**	**Experiment number**: SC5281
Beamline:	**Date of experiment**: from: 3.5.2022 to: 9.5.2022	**Date of report**: 8.9.2022
Shifts:	**Local contact(s)**: Maciej Jankowski	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report: GIXD and XRR reveal the molecular structures of the tear film lipid layer components and their mixtures

We conducted Grazing Incidence X-ray Diffraction (GIXD) and X-ray Reflectivity (XRR) experiments on synthetic tear film lipid layer (TFLL) components, representing all the main TFLL lipid species and their most relevant mixtures. The experiments were carried out using a sealed Langmuir trough with a Wilhelmy balance and thermostat bath at the beamline ID10-EH1. We used saline subphase in the trough and were able to study the film structures and dynamics of the TFLL lipids close to their physiologically relevant conditions, at two different temperatures (25° C and 30° C) and as a function of the surface pressure (between 4 to 40 mN/m). The lipids studied included the pure samples of two O-acyl-omega-hydroxy fatty acids (OAHFA) representing different chain lengths (with long and very long chains), behenyl oleate (representing long chain wax ester), iso-methyl branched wax ester (iso-WE), iso-methyl branched cholesteryl ester (iso-CE) and long chain diester (DiE). These lipids are considered to represent the lipid types which are found in abundance in intact TFLL. In addition, carefully chosen mixtures of these components were characterized, including different ratios of iso-WE, iso-CE and OAHFAs and one mixture of the primary components mimicking closely the composition of the natural TFLL (OAHFA (5%) Iso-WE (47.5%) Iso-CE (47.5%)).

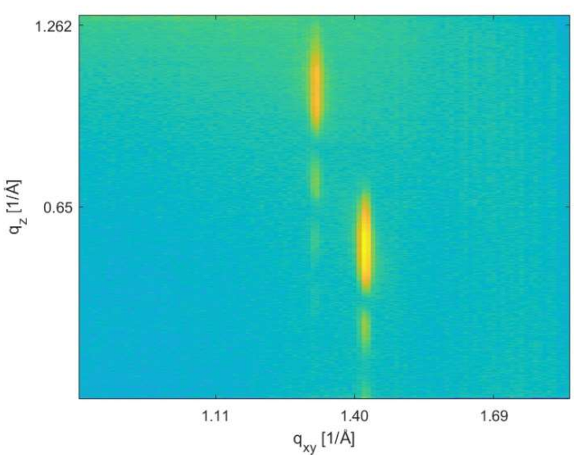

Fig. 1: The GIXD pattern of the sample iso-WE at surface pressure of 10 mN/m and temperature 30º C. Based on the two maxima, the lattice represents rectangular lattice with NNN tilt.

Clear diffraction peaks were observed in the GIXD patterns of all the lipid samples studied when the surface pressure was above 4 mN/m. Thus, it can be concluded that all the primary TFLL lipids characterized in this study formed ordered, crystalline structures. Based on the changes of these peaks, structural differences as a function of the mixture composition, temperature and the surface pressure were determined. Based on the GIXD patterns, most of the TFLL lipids studied presented rectangular lattices with either NN or NNN tilt (Fig. 1), the exact phase depending on the surface pressure and temperature. This corresponds well to previous GIXD results on intact meibum and pure lipid monolayers found in the literature. The various TFLL lipids had various phase behaviors: it was observed that the lattice spacings were decreased as a function of increasing surface pressure in OAHFAs (Fig. 2), whereas there were no changes observed in iso-WE.

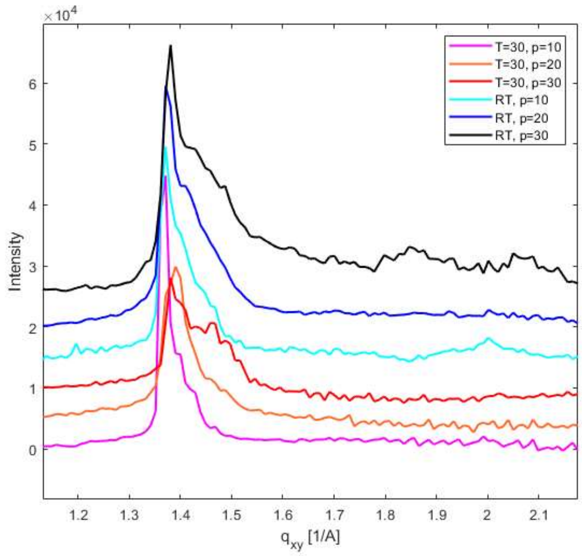

Fig. 2: integrated GIXD intensities of the pure OAHFA at two temperatures (room temperature (RT) and 30º C) and different surface pressures. A shift in the position of the main diffraction peak around 1.38 1/Å can be observed.

These insights gleaned from the GIXD measurements on the lattice structure are combined and complemented with the analysis of the XRR data yielding the electron density profile and film thickness (Fig. 3). Thus, a detailed view on the molecular structure of the films is obtained.

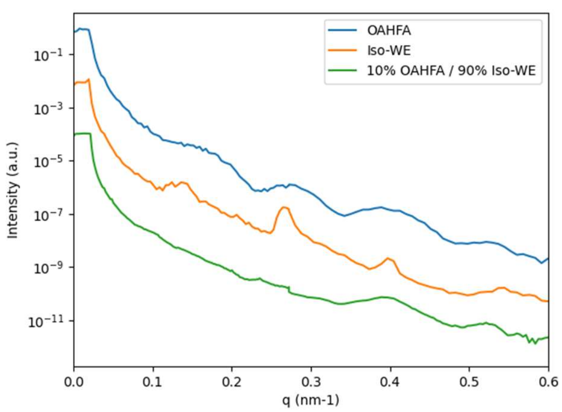

Fig. 3: A comparison of the measured XRR curves for pure OAHFA, pure Iso-WE and a mixture of both. Distinct features are observed in each of the spectra: the pure OAHFA features correspond to a monolayer, whereas the pure iso-WE features correspond to multilayers.

Cholesteryl and wax esters are known to be the key components of the TFLL; as an example of this, lower CE/WE-ratios have been connected to e.g. the dry-eye-disease. Interestingly, a clear structural difference between the molecular organization of the pure iso-WE and iso-CE and any other lipids studied could be observed in our GIXD results. Based on the Debye rings observed in the GIXD patterns of the pure iso-CE, these lipid molecules formed 3D crystallites in the higher temperature studied (at 30°C) and at higher surface pressures (above 10 mN/m). However, in the mixtures, e.g. in the case where 10% OAHFA was mixed with iso-CE, similar kinds of very clear Debye rings were not observed. This might be connected to the TFLL function and the iso-CE's role in it.

Our results indicate that the lipid compositions and surface pressure play important roles in the behavior and function of the TFLL lipids. The structural results by GIXD and XRR will be combined with the surface properties determined by surface pressure, evaporation and Brewster angle microscopy. The full analysis of all the data is still on-going and the results will be published in two separate scientific articles (in preparation). Based on the results of these primary TFLL components, we are now planning further studies, which will be approaching even closer composition of the native TFLL, i.e. the intact meibum composition by adding the phospholipids into the picture.