ESRF	**Experiment title:** **Influence of perfluorocarbons on protein adsorption and adsorbates**	**Experiment number**: SC-5423
Beamline: ID10	**Date of experiment**: from: 03.05.2023 to: 09.05.2023	**Date of report**:
Shifts: 11	**Local contact(s)**: Maciej Jankowski	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Susanne Dogan-Surmeier[1], Jaqueline Savelkouls[1], Michael Paulus[1], Christian Albers[1], Eric Schneider[1], Gordon Scholz[1]

[1]Fakultät Physik/DELTA, Technische Universität Dortmund, Maria-Goeppert-Mayer-Str. 2, D-44227 Dortmund, Germany

Report:

Experiment SC-5423 was a combined grazing incidence X-ray diffraction (GID) and X-ray reflectivity (XRR) study of the effect of aerolised and gaseous perfluorocarbons on protein adsorption at Langmuir films with different surface-active proteins and surface pressures. The lateral and vertical structure of model membranes made of lung surfactant lipids such as anionic 1,2-dipalmitoyl-*sn*-glycero-3-phosphate (DPPA) and zwitterionic 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) before and after protein adsorption and the influence of an overlying perfluorocarbon atmosphere should be examined.

The experiment was performed at beamline ID10 with a photon energy of 22 keV. In this experiment, we investigated the influence of the aerolised perfluorocarbon perfluorodecaline (F-Decalin) on the structural changes of the DPPC and DPPA monolayers at different initial surface pressures in a custom-build gas pressure sample cell with a circular sample plate and Wilhelmy balance for surface pressure measurements. Due to time constraints, the influence of other perfluorocarbons could not be studied. The influence of F-Decalin on the adsorption of the surface-active proteins human serum albumin (HSA) and lysozyme to the Langmuir films was considered. All samples were measured *in situ* at ambient temperature and pressure.

Preliminary results are presented below. As an example, the DPPA-Langmuir film is shown at a surface pressure of 20 mN/m on an aqueous buffer solution with a pH value of 3. At this surface pressure, the lipid film is in the tilted liquid-condensed phase. Besides the influence of F-Decalin on structural changes of the monolayer, the influence on the adsorption of human serum albumin is also analysed. Figure 1 shows the corresponding results. Figure 1(a) represents the fresnel-normalised reflectivities and figure 1(b) the resulting electron density profiles. Figure 2(a) illustrates the Bragg reflections from the GID measurements and figure 2(b), the resulting lattice spacing a and crystalline domain sizes L.

From the electron densities in Figure 1(b), it can be seen that the protein HSA forms a protein layer under the lipid head groups after approx. 45 min due to electrostatic attraction. Furthermore, it can be concluded that HSA also accumulates between the head groups. As a result of flushing with F-Decalin, the electron densities of the individual layers increase. These observations indicate an adsorption of F-Decalin in the hydrophobic alkyl chains of the lipid as well as on the free water surface. In addition, it can be assumed that F-Decalin adsorbs into the hydrophobic bags of the protein. Furthermore, a faster adsorption of the protein at the interface could be expected as a result of the more hydrophobic environment. Moreover, the surface tension decreases, which is shown by a quick increase of the surface pressure by 3 mN/m. Besides the increasing surface pressure, the growth of the chain length by $\Delta d \approx 3$ Å indicates a slightly different configuration of the lipids with an even more vertical arrangement. After opening the sample cell, it can be seen that the electron densities in the

adsorption regions as well as the length of the alkyl chains decrease again, which indicates a desorption of the gas molecules from the interface.

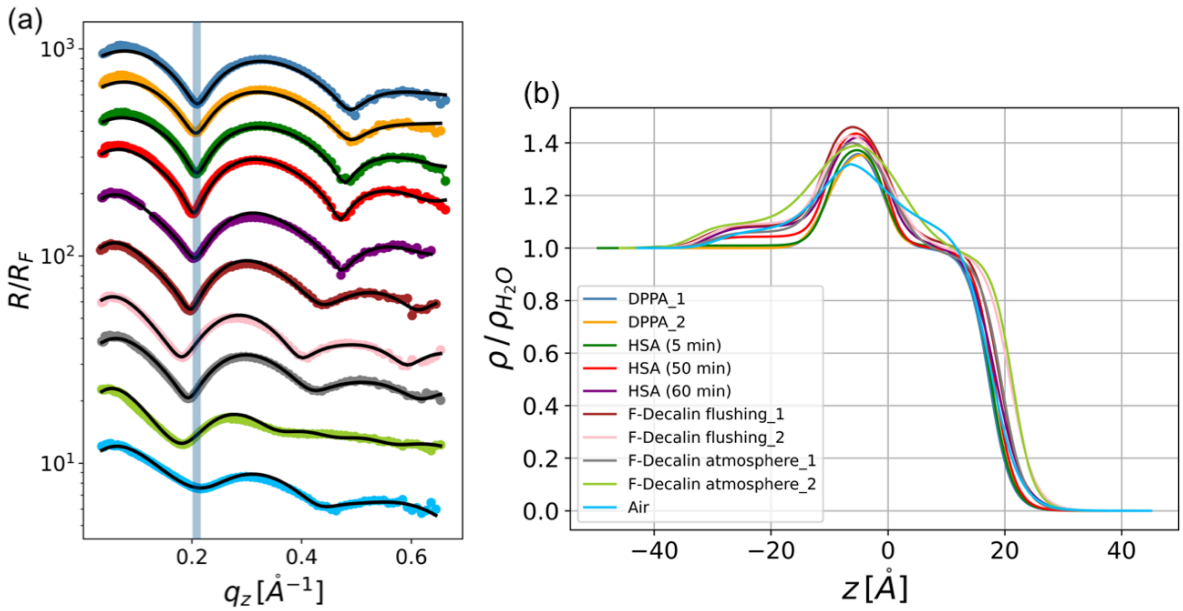

Fig. 1: Data of DPPA films in contact with F-Decalin and human serum albumin at initial surface pressure of 21 mN/m. (a) Fresnel normalized reflectivity data. Fits to the data are shown as black solid lines. (b) Electron density profiles.

The corresponding GID data in figure 2(a) and 2(b) show that the lattice spacing a changes not significantly from the initial value $a = 4.786$ Å to the final value $a = 4.783$ Å during the measurement. The small change suggests a stable DPPA monolayer with high lateral order. On the other hand, the size of the crystalline domain L decreases by almost $\Delta L \approx 40$ Å (see figure 2(c)). Consequently, the crystalline domains are compressed by islanding of the gas molecules, which leads to fluidisation of the membrane.

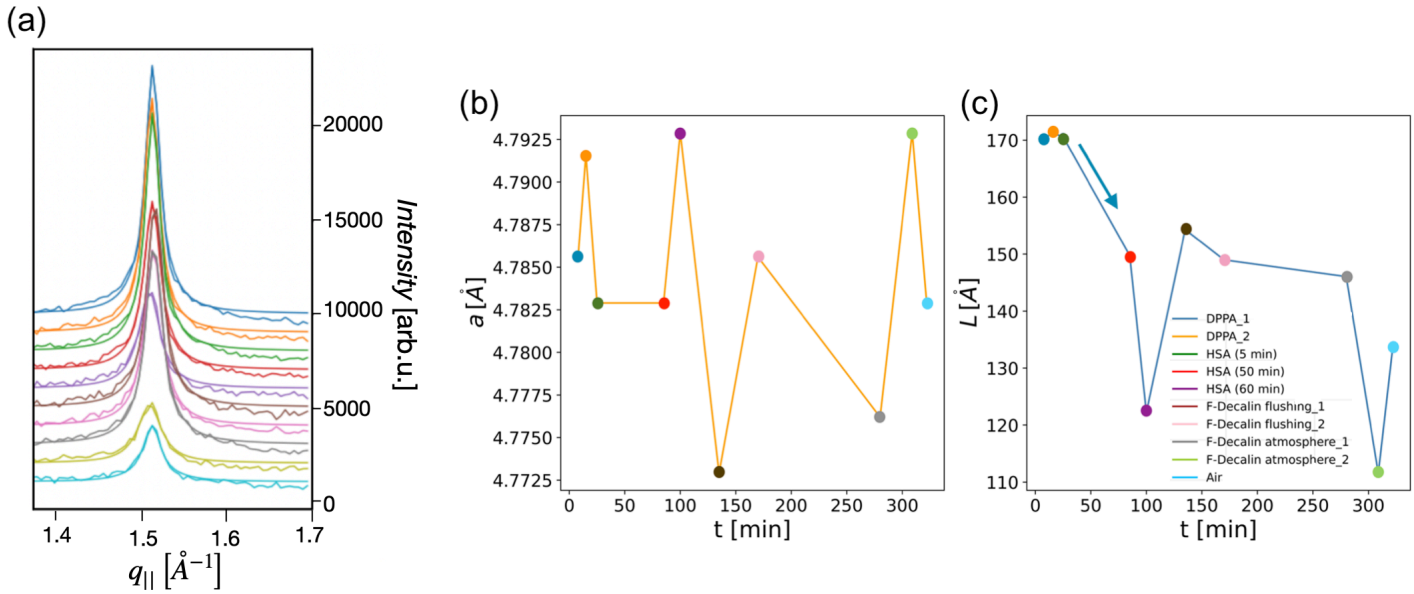

Fig. 2: Data of DPPA films in contact with F-Decalin and human serum albumin at initial surface pressure of 21 mN/m. (a) Underground adjusted GID data. (b) Extracted lattice constant a. (c) Extracted crystalline domain size L.

In summary, surface-active proteins adsorb to the lipid membrane either with and without a perfluorocarbon atmosphere. F-Decalin itself adsorbs to the interface between the head and tail groups of the lipid monolayer as well as to the hydrophobic regions of the lipid and protein. This leads to a compression of the lipid and protein layer. Perfluorodecalin reduces the size of the crystalline domains, the surface tension of the Langmuir films and induces a fluidisation of the lipid monolayer. This effect is seen for DPPA as well as DPPC monolayers with initially high surface tensions. For monolayers with lower surface pressure, this behaviour is only observed for DPPC films and not for DPPA films.