# Experimental report

11/02/2021

**Proposal:** 9-13-898 **Council:** 10/2019

**Title:** Effect of the saponins aescin and glycyrrhizin on hydrogenated and fully deuterated phospholipid membranes extracted from yeast P. pastoris

**Research area:** Soft condensed matter

**This proposal is a new proposal**


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**Samples:** Phospholipid + aescin
Phospholipid + glycyrrhizin

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| D11 | 4 | 3 | 04/09/2020 | 07/09/2020 |
| D22 | 4 | 0 | | |
| D33 | 4 | 0 | | |


## Abstract:

Biological membranes can interact with saponins, a class of biological amphiphiles. The interaction between lipid membranes and saponins has not been fully understood, especially in case of membranes which resemble natural systems. Therefore, we want to examine in this work the effect of the saponins aescin and glycyrrhizin on membranes of small unilamellar vesicles composed of phospholipids extracted from yeast P. pastoris. This phospholipid extract shows a higher similarity to living organisms than the usual model membranes consisting of only one kind of lipid. Furthermore, we want to examine the effect of the two saponins on fully deuterated phospholipids, which were also extracted from yeast. The use of fully deuterated phospholipids leads to a variation of the contrast and therefore allows to examine the distribution of saponin embedded into the membrane. The experiment is proposed for the D11 SANS spectrometer. If this spectrometer is not available, we propose your experiment for the D22 or D33 SANS spectrometer.

## Aim of Experiment

By small angle neutron scattering (SANS) the structural information of a system composed of a phospholipid yeast extract[1] and the saponin aescin can be obtained. Membranes based on this lipid extract were already successfully investigated by neutron diffraction by other groups[2]. A saponin content dependent study is conducted for aescin to investigate the general phase behaviour. Moreover, a contrast variation experiment shows the distribution of the aescin molecules in the lipid membrane. Therefore, a fully deuterated yeast phospholipid extract is used, and the lipid membrane is completely contrast matched with the solvent. The remaining scattering signal arising from the hydrogenated aescin molecules will therefore gives information about possible cluster formation inside the membrane.

## Description of Experiment

Unilamellar yeast extract phospholipid-vesicles (lipid mass concentration: 10 mg/mL) with different amounts of aescin (0-30 mol%) as well as deuterated yeast extract phospholipid-vesicles (lipid mass concentration: 10 mg/mL) with aescin (0-30 mol%) were prepared by extrusion through a membrane with a pore size of 500 Å. At least 15 extrusion steps were performed. Samples with less lipid mass concentration (5 mg/mL) are obtained by dilution with the same buffer.

All measurements were performed at two temperatures, 10 and 25 °C. The scattering intensity was measured using three configurations with different sample-detector-distances (6 Å, 1.4000 m; 6 Å, 7.9980 m; 6 Å, 38.9450 m). The covered $q$-range of $1.56 \cdot 10^{-3}$ – 0.4 $Å^{-1}$ allowed to reach the plateau of the vesicle form factor at low $q$(see fig. 1).

## Results and data evaluation

The measurements show that samples with yeast extract phospholipids create a vesicle-like form factor (fig. 1). Measurements at two different temperatures, 10 & 25 °C, show only small changes or no changes at all in the form factor. Measurements of diluted previously measured samples containing yeast extract phospholipid-vesicles show less intensity, especially at low $q$-values. At hight $q$-values the intensity differences between diluted and non-diluted samples differs so that the intensity of the diluted samples is not necessarily lower. In both cases, diluted and non-diluted samples, the form factor slightly changes with increasing aescin amount, which indicates changes in vesicle and bilayer size with increasing aescin amount.

To study the behaviour of aescin incorporated in the bilayer, samples with aescin and deuterated yeast extract phospholipids were measured. The measured form factors show almost no differences when measured at 10 and 25 °C. Also, in this case, the dilution of the samples leads to a decrease of the measured intensities. In contrast to hydrogenated samples, the form factors of the deuterated samples show concise changes depending on the aescin amount. The curvy form at 0.03 – 0.1 $Å^{-1}$ which is prominent in the form factor for the sample containing 30 mol% aescin, can also be seen in a less pronounced way in the form factor of the sample containing 5 mol% aescin. The form factors for all measurements of the yeast extract deuterated phospholipid samples show a greater deviation in the scattering data than the hydrogenated samples. This is due to the deuteration of the whole lipids, the scattering only takes place at the hydrogen atoms of the aescin. The great deviation could not be eliminated by increasing the measuring time, which was already 85 minutes per sample (153 min for dilutes samples).

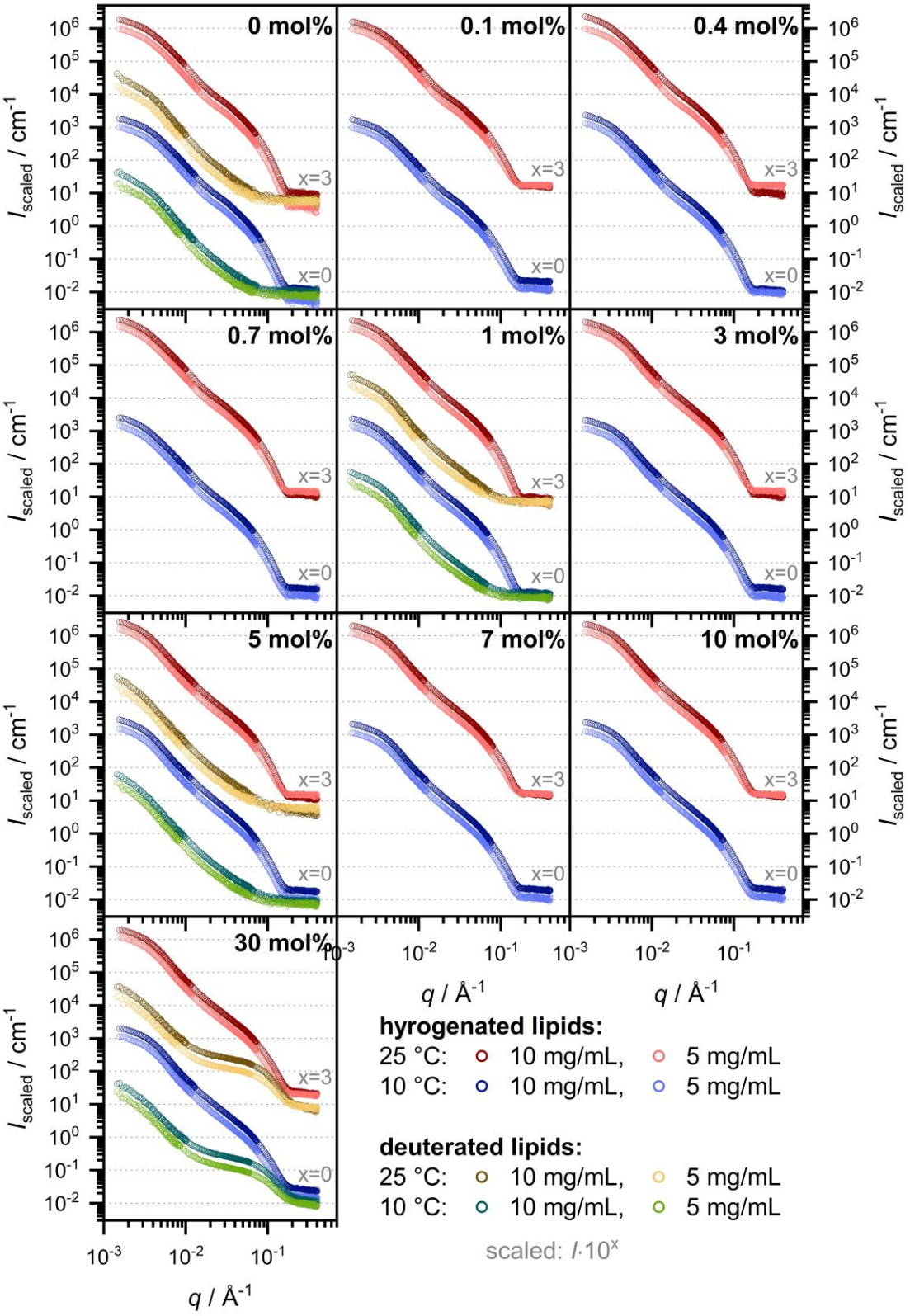


Fig. 1: Form factors of yeast extract phospholipid-vesicles (red and blue) and yeast extract deuterated phospholipid-vesicles (yellow and green) with amounts of aescin from 0 to 30 mol% at two temperatures and two lipid mass concentrations.

## Experimental procedure

To prepare the samples a lab was necessary. We used the lab near the D11 hutch, which was clean and well organized. We had enough space to work properly. For the measurements we were able to use the D11 own round cuvettes to provide the best sample to beam surface. We used a sample holder for 15 samples which was cooled or heated by two baths. The flows of the bath could be switched to change the temperature faster. The temperature was measured with an additional cuvette in the sample holder.

The measurement itself worked properly whereby outstanding support of the local contact Ralf Schweins had a great share. He varied the instrument settings carefully to improve the data and looked carefully at the gained form factor.

## Literature


[1] A. de Ghellinck, H. Schaller, V. Laux, M. Haertlein, M. Sferrazza, E. Maréchal, H. Wacklin, J. Jouhet, G. Fragneto, Production and analysis of perdeuterated lipids from Pichia pastoris cells, *PloS one 9* (**2014**) e92999.

[2] A. Luchini, R. Delhom, B. Demé, V. Laux, M. Moulin, M. Haertlein, H. Pichler, G.A. Strohmeier, H. Wacklin, G. Fragneto, The impact of deuteration on natural and synthetic lipids: A neutron diffraction study, *Colloids and surfaces*. B, Biointerfaces 168 (**2018**) 126–133.