# Experimental report

04/05/2022

**Proposal:** 9-13-939 **Council:** 4/2020

**Title:** Investigating the molecular basis of inflammatory skin conditions

**Research area:** Soft condensed matter

**This proposal is a new proposal**


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**Samples:** Ceramide mixture/fatty acid mixture/cholesterol

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| D16 | 6 | 6 | 07/10/2021 | 11/10/2021 |


## Abstract:

The tri-layered structure of the long periodicity phase (LPP) is unique to the stratum corneum (SC) and is critical to the skin's barrier function. Small changes in the LPP composition can greatly reduce its barrier properties and can lead to inflammatory skin conditions such as atopic dermatitis and psoriasis. Some of the major pathologically relevant changes involve a decreased concentration of the ceramide nonhydroxy(N)-(tetracosanoyl)-phytosphingosine (CER NP) and an increased concentration of the ceramide N-(tetracosanoyl)-sphingosine(CER NS). Using judiciously formulated / LPP-mimicking mixtures of ceramides, free fatty acids, and cholesterol, we aim here to determine (a) the extent and nature of any differences in structure between simple and more complex LPP model systems, and (b) the effect on the structure of our more complex LPP model caused specifically by changes in the relative proportions of CER NP and CER NS. By extrapolation from these latter observations we aim to shed light on the effect of such changes in the SC of patients suffering from inflammatory skin conditions.

# Effect of ceramide loss on the lipid ordering within the long periodicity phase of the stratum corneum

Proposal: 9-13-939
Beamline: D16
Local Contact: Bruno Deme
Published manuscript: C. Beddoes et al., Biochimica et Biophysica Acta (BBA) - Biomembranes, Volume 1864, Issue 6, 1 June 2022, 183886

## Background

The stratum corneum (SC) lipid matrix is critical for the skin's barrier function (1). Composed of primarily ceramides (CERs), cholesterol (CHOL) and free fatty acids (FFAs), these lipids form a long periodicity phase (LPP), which consists of a unique tri-layer unit cell structure, primarily in an orthorhombic phase (2-4).

For the synthesis of these lipids, an enzyme driven pathway is implemented (5). If these enzymes are down- or upregulated as found in inflammatory diseases, the final lipid composition is affected, often altering the barrier function.

## Aim of this experiment

In this study, we mimicked the effects of the down regulation of enzymes involved in the synthesis of the sphingosine and measured its effects on the LPP structure. In a simple four lipid LPP model, we substituted CER N-(tetracosanoyl)-sphingosine (CER NS) with FFA C24 and C18 to simulate the loss of the sphingosine headgroup.

These models were then investigated on D16 with complementary studies with FTIR and SAXS to identify if alterations in the LPP had occurred.

## Method

The lipid composition used in our model comprised CER EOS, CER NS, CHOL and FFA C24 in a 0.4:0.6:1:1 molar ratio and mimics important aspects of the lipid organization in the SC, including the formation of the LPP and a primarily orthorhombic lateral packing. The CER EOS concentration was increased from native concentrations (around 12%) to 40 mol% of the CER content, to ensure the LPP would form exclusively (6).

Table 1 shows the models measured where the lipid composition was changed by substituting a single CER NS with a FFA C24 and FFA C18 (25% FFA sub). In all models, the carbon chain number and length remained consistent. For the small angle neutron scattering (SANS)

measurements, CER NS-d7, CER NS-d47 and FFA C18-d35 were substituted into the model, replacing their protiated counterparts.

**Table 1**: LPP models used in this study.

| Model | Composition | Ratio |
|---|---|---|
| 0% Sub | CER EOS:CER NS:CHOL:FFA C24 | 0.4:0.6:1:1 |
| 25% FFA-sub | CER EOS:CER NS:FFA C18:CHOL:FFA C24 | 0.4:0.45:0.15:1:1.15 |

Samples were measured at 25°C in the humidity chamber available at D16, with measurement times of 5-6h depending on the signal to noise ratio. Further details on the sample preparation can be found in the published article.

## Results

To probe if there was any change in the LPP structure in the 25% FFA-sub models, the lipid arrangement in the unit cell of the LPP was investigated with SANS. The intensity vs q curves for all models demonstrated that the only lamellar structure present was the LPP, with the only additional structure being phase-separated crystalline CHOL. The repeat distances were determined by least square fitting of all peak positions. The mean repeat distance determined for the 25% FFA-sub model was 12.6 ± 0.1 nm.

The location of the deuterated chains was determined by the subtraction of the scattering length density (SLD) profile for the non-deuterated sample from that of the profile for the sample involving deuterated lipid, both hydrated in 8% $D_2O$ solvent. Fig 1 shows the SLD profile for the 25% FFA-sub model. The blue curve shows the location of water at the unit cell boundary at 2.1 nm from the cell centre and at the cell boundaries. The position of the terminal sphingosine chain of CER NS-d7 (green curve) is located 4.2 nm from the centre of the LPP unit cell, while the acyl chain of CER NS-d47 (red curve) is distributed between the central and outer lipid layers of the unit cell. These positions imply that the CER NS in the centre remains in an extended conformation while a proportion of the CER NS is also present in the outer regions. The SLD profile of the free FFA C18 (black curve) shows no regions of higher SLD intensity throughout the unit cell implying the FFA C18 is distributed evenly throughout.

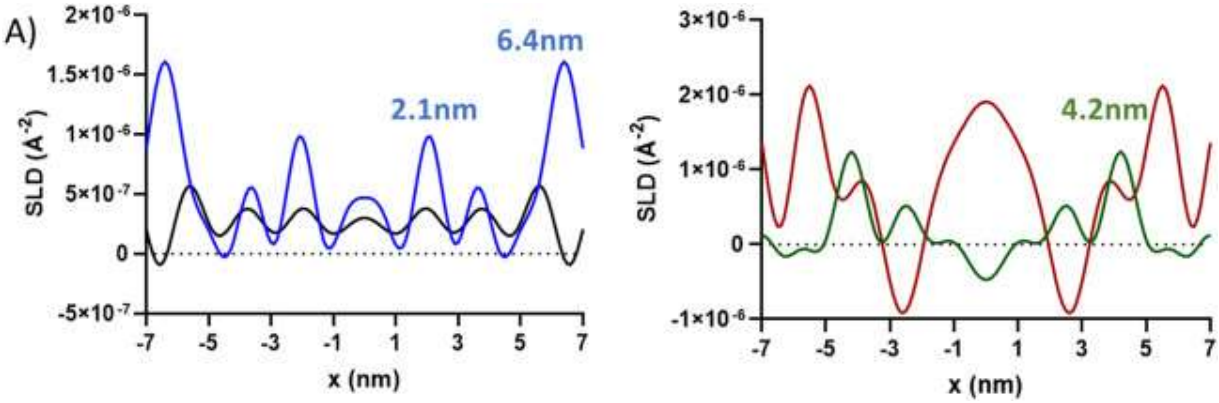


**Figure 1**: SLD profile of 25% FFA-sub, where 25% of CER NS is substituted with a FFA C24 and FFA C18. A) The water profile (blue) and the position of the entire carbon chain of the FFA C18 (black). B) The terminal position of the CER NS's sphingosine chain (d7, green), and the length of the acyl chain (d47, red).

**References**

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