Supporting Information

Arrangement of Ceramides in the Skin: Sphingosine Chains Localize at a Single Position in Stratum Corneum Lipid Matrix Models

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Tables

Table S1:

Neutron sample list including composition and lipid ratio. Deuterated components and molar ratios are highlighted in bold.

Model	Composition	Ratio
Simple	CER EOS/CER NS/ CHOL/FFA5	0.4/0.6/1/1
CER NS-d7 Simple	CER EOS/**CER NS-d7**/ CHOL/FFA5	0.4/**0.6**/1/1
CER NS-d47 Simple	CER EOS/**CER NS-d47**/ CHOL/FFA5	0.4/**0.6**/1/1
Porcine	CER EOS/CER NS/CER NP/CER AS/CER NP(C16)/CER AP/ CHOL/FFA7	0.4/0.36/0.11/0.03/0.06/0.04/1/1
CER NS-d7 Porcine	CER EOS/**CER NS-d7**/CER NP/CER AS/CER NP(C16)/CER AP/ CHOL/FFA7	0.4/**0.36**/0.11/0.03/0.06/0.04/1/1
CER NS-d47 Porcine	CER EOS/**CER NS-d47**/CER NP/CER AS/CER NP(C16)/CER AP/ CHOL/FFA7	0.4/**0.36**/0.11/0.03/0.06/0.04/1/1

Methods

Method S1:

Using the formula for each of the models, F_0 was calculated using the NIST neutron activation and scattering calculator[1]. Calculated values of F_0 in 100% D_2O were as follows;

Model	F_0 x10^{-6} (A^{-2})
Simple	0.39
CER NS-d7 Simple	0.506
CER NS-d47 Simple	1.26
Porcine	0.39
CER NS-d7 Porcine	0.476
CER NS-d47 Porcine	0.93

The relative absolute scaling values were determined as previously described by Wiener et al.[2] and Mojumdar et al [3]. Initially the SLD peak height (SLD_{inital}) and area (SLD_{area}), with a known number of deuterium atoms per lipid is fitted (in the case of CER NS-d7 samples each deuterated peak represents 7 deuterium per lipid). The difference in the SLD_{area} between the deuterated and protiated model was determined. This area was attributed to the number of deuterium atoms present in the model. Calculating the difference in the SLD value

due to this isotope exchange (SLD_{dif}), the relative absolute SLD value ($SLD_{corrected}$) for that peak can be determined;

$$SLD_{corrected} = \frac{SLD_{inital} * SLD_{dif}}{SLD_{area}}$$

And from the ratio between SLD_{inital} and $SLD_{corrected}$ the scaling factor between the deuterated and protiated samples can be determined and applied to the F_n values.

1. NIST Center of Neutron Research, https://www.ncnr.nist.gov/resources/activation/ (Accessed:May 2020)
2. Wiener, M. C., et al. (1991). "Structure of a fluid dioleoylphosphatidylcholine bilayer determined by joint refinement of x-ray and neutron diffraction data. I. Scaling of neutron data and the distributions of double bonds and water." Biophysical Journal **60**(3): 568-576.
3. Mojumdar, E. H., et al. (2013). "Localization of Cholesterol and Fatty Acid in a Model Lipid Membrane: A Neutron Diffraction Approach." Biophysical Journal **105**(4): 911-918.

Figures

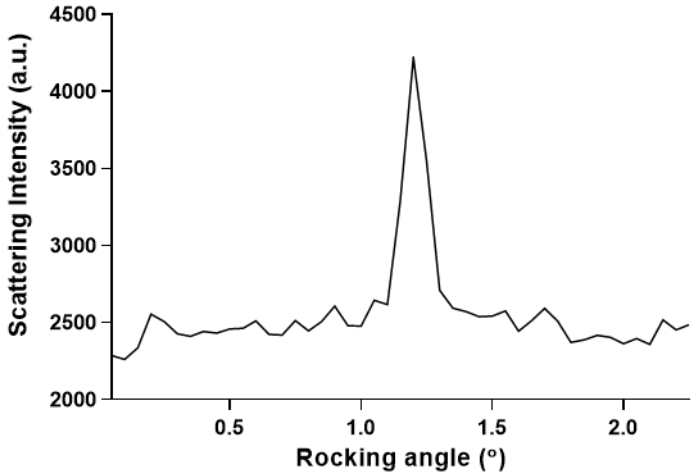

Figure S1:

Example of the sample rocking plot intensity over a specified at a limited 2θ range. In this plot the 2θ range was set between 1.8 -2.0 to determine the specular scan number (±0.1°) of the 1st order (q=0.044 nm^{-1}) of the porcine model hydrated in 100% D_2O.

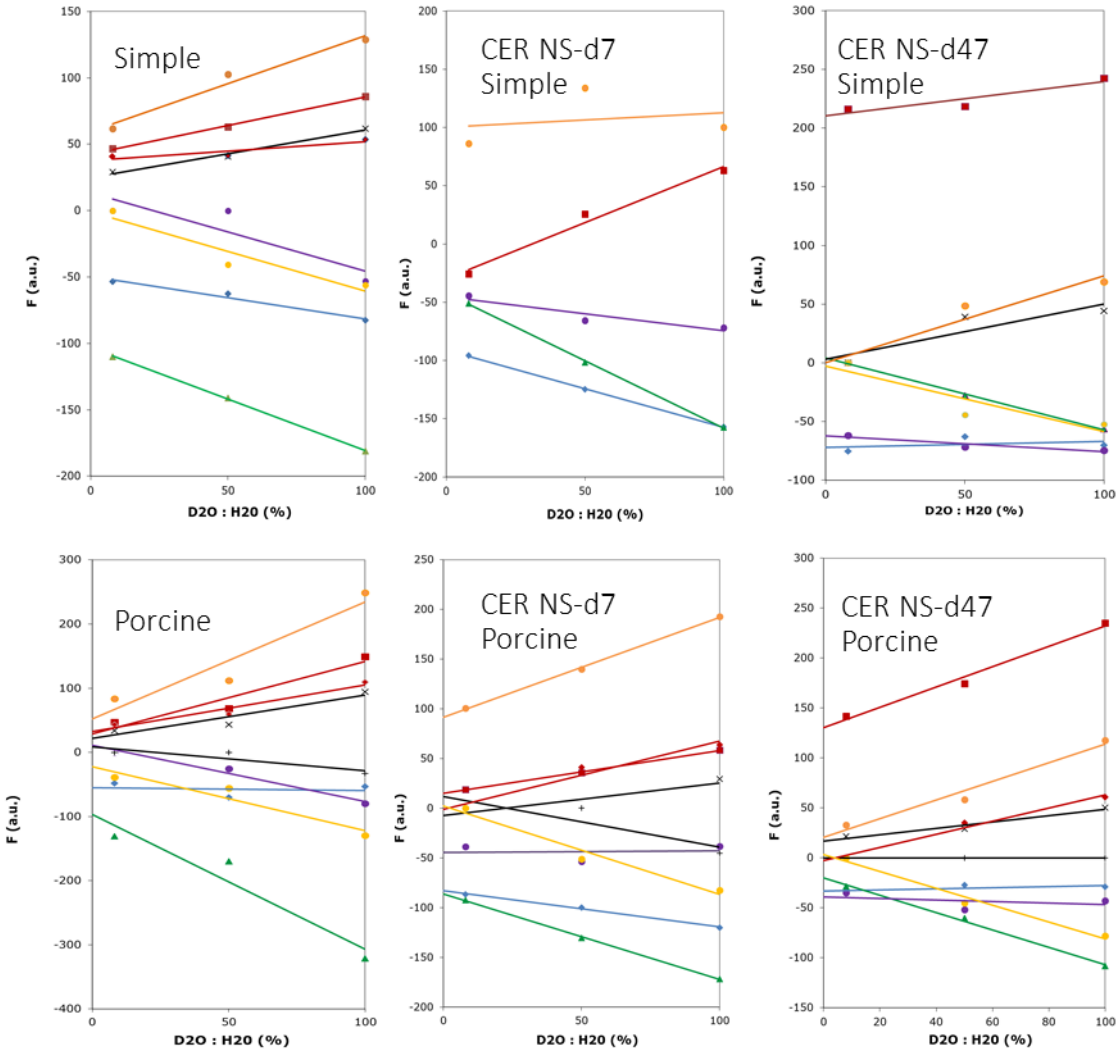

Figure S2:

Relative structure factor of the various diffraction orders, as a function of D_2O/H_2O volume ratio. The Bragg orders for each sample are represented at 1st (blue, ♦), 2nd (red, ■), 3rd (green, ▲), 4th (black, X), 5th (purple, •), 6th (Orange, •), 7th (black, +), 8th (red, ♦), and 9th (yellow, •). The linear difference in the structure factor against the solvent ratios indicates that the samples were sufficiently hydrated for contrast variation measurements.

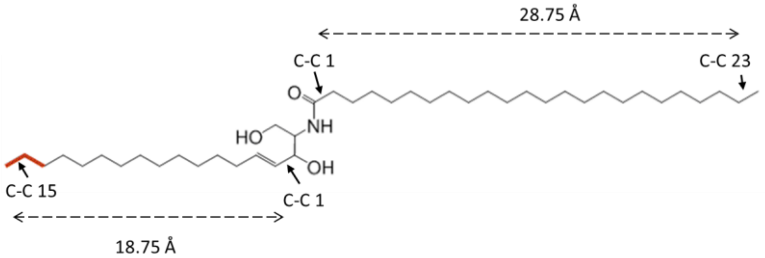

Figure S3:

Molecular arrangement of CER NS when in a linear extended conformation. In this arrangement a C18 sphingosine chain extends along 15 C-C bonds, equating to a length of 1.875 nm. The acyl chain has a length of 23 C-C bonds, at a length of 2.875 nm. The deuterated component of the sphingosine chain is highlighted in red, lengths were calculated assuming a C-C bond length of 1.25Å.

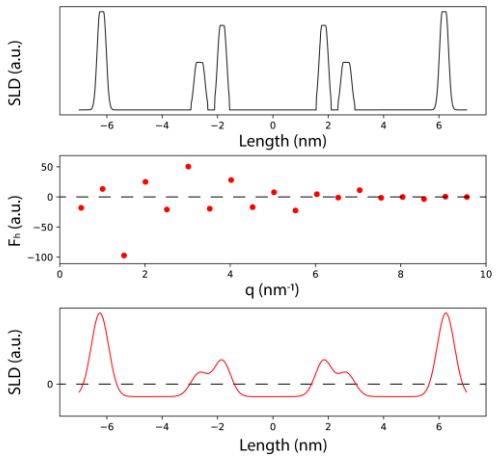

Figure S4:

The baseline of the SLD profiles in Figure 4 contains some small unexpected peaks. In the case of the 8:92 D_2O:H_2O profile, three extra peaks located at ~-4.5, 0, 4.5 nm are observed in both the experimentally derived, and theoretically calculated SLD profiles, which were not accounted for in the unit cell model. These extra peaks are due to the limited number of F_n values used in order to mimic the conditions from the experimental measurements. Figure SI5 shows the same SLD profile as presented in Figure 4, but theoretically calculated with F_n =20, thus significantly reducing the data truncation error. The top plot illustrates the same LPP model used in Figure 4, the central figure shows the first 20 F_n values, of the 20 values calculated. As the F_n number increases, its values, and thus its overall contribution decreases. However by utilizing the values for the first 20 F_n values, the base line of the SLD profile (bottom plot) becomes much clearer, and the extra peaks are no longer present, thus implying that these are not true peaks but rather artifacts from the limited structure factor values.