Title

Phase behavior of skin lipid mixtures: effect of cholesterol on the lipid organization

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Figure S1: The thermotropic CH_2 scissoring vibration frequency curves as a function of temperature for the various sCER containing lipid mixtures. The spectra are shown from 20 to 60 °C temperature region. The spectra of the lipid mixtures in the absence of CHOL exhibits scissoring vibration with a peak position of 1467 cm⁻¹ indicating a high level of hexagonal packing compare to the CHOL containing lipid mixtures. At low CHOL levels, the gradual increment of CHOL enhances the level of orthorhombic packing in the lipid mixtures. Overall a reduced level of orthorhombic packing is observed in the SPP compared to the LPP as described in the results section and also evident from the OR/HEX peak ratio presented in table 2 & 3.



Figure S2: The FTIR CH₂ scissoring vibration spectra at 25 °C for the sCER containing lipid mixtures and their fitted Lorentzian spectra. Original spectra – solid lines, Lorentzian fitted spectra – dashed lines, individual components contributing to the fitted Lorentzian spectra are denoted as 1, 2 and 3. The figure title describes the composition of the lipid mixtures. The contribution of the peak area of component 1 and 3 are considered to be the orthorhombic contribution to the lateral packing, while the peak area of component 2 is a measure for the level of hexagonal packing present in the spectra of the lipid mixtures. From the areas under the peak values, the OR/HEX peak ratio was calculated. The level of orthorhombic packing is more pronounced when CHOL is present at higher levels in the lipid mixtures compare to the absence of CHOL. The OR/HEX peak ratio values are provided in table 3.



Figure S3: SAXD diffraction profiles of the various sCER containing lipid mixtures (prepared in the absence of CER EOS). The figure title describes the composition of the different lipid mixtures. In the SAXD profiles, the roman numbers (I, II...) indicate the diffraction orders attributed to the SPP. The peak originating from the crystalline CHOL domain is indicated by the asterisk (*). Peaks not attributed to the SPP are denoted by hash sign (#). The lipid membranes in the absence of CHOL show several other phases that cannot be attributed to the SPP. The gradual increase in CHOL level diminishes the presence of these phases and at a level of 0.2 molar ratio CHOL in the sCER/CHOL/FFA (1:0.2:1) mixture, only the SPP is observed. At the diffraction curve of sCER/CHOL/FFA (1:1:1), a diffraction peak of phase separated CHOL is detected.