Supplementary Material for

Lipid Sorting by Ceramide and the Consequences for Membrane Proteins

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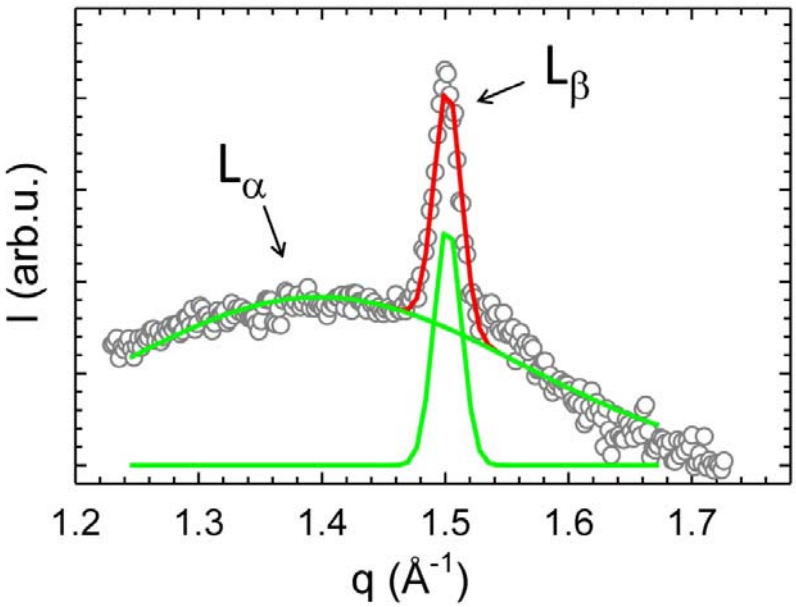
Analysis of coexisting L_α/L_β domains in the WAXD regime



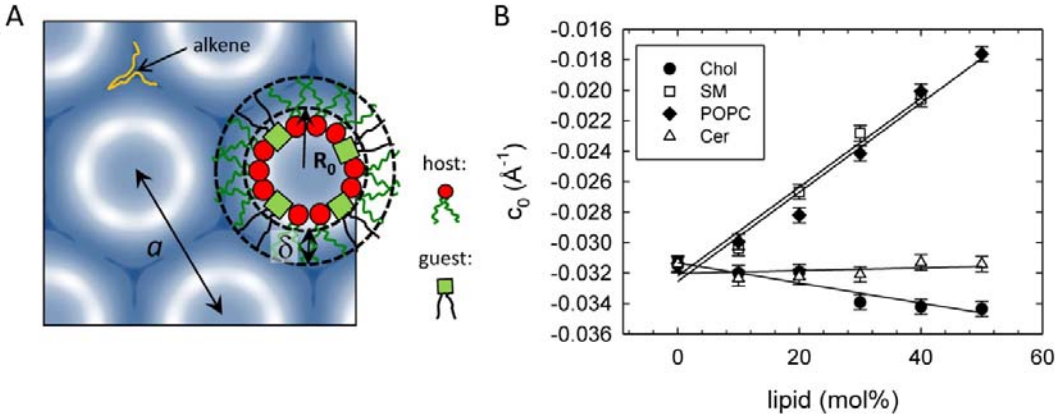
Supplemental Figure S1: Analysis of WAXD patterns exhibiting L_α / L_β phase coexistence. The broad peak is due to short-range order of hydrocarbons in the L_α domains and the sharp peak signifies the presence of L_β domains. Peaks were fitted using Lorentzian functions.

Determination of Spontaneous Curvatures

Spontaneous curvatures of diverse lipids were estimated according to (1) in the inverted hexagonal phase H_{II} of a host lipid system using SAXD (for review, see (2)). It is convenient to choose dioleoyl-phosphatidylethanolamine (DOPE) as the host lipid system, as it exhibits the H_{II} phase at room temperature. We further added 12.5 wt% of 9-*cis*-tricosene to the lipid systems, which inserts in the interstitial regions of the hexagonal cylinders (Fig. S1 *A*) and thereby reduces packing frustration energy of acyl chains to an almost stress-free state (3). DOPE was purchased from Avanti Polar Lipids (Birmingham, AL) and 9-*cis*-tricosene was from Sigma-Aldrich, AUT.

The spontaneous curvature, c_0 needs to be determined in the pivotal plane, where bending and stretching modes are decoupled (4). Here, we assume that the pivotal plane lies at a constant of δ = 9 Å (Fig. S2 *A*) from the terminus of the acyl chains, as justified previously for DOPE (1). Then, $c_0 = -R_0^{-1} = -2(a - 2\delta)^{-1}$, where a is the lattice constant of the H_{II} phase (Fig. S2 *A*), determined by SAXD experiments (5). All experiments were performed at 25°C at the Austrian SAXS-beamine (Elettra, Trieste, Italy) with the same set-up described in the manuscript, but using a Pilatus 100K detector (Dectris, Baden, Switzerland).

The spontaneous curvatures of a given lipid were estimated by preparing several mixtures of DOPE with the lipid of interest over a concentration range from 0 to 50 mol%. Changes of c_0 were then plotted as a function of guest lipid concentration (Fig. S2 *B*) and linearly extrapolated to 100 mol%. This method assumes random mixing of lipids and a linear addition of the c_0's of the host and the guest lipid system (6). The observed linear changes of c_0 with lipid concentration (Fig. S2 *B*) justify this assumption. The planar bilayer forming lipids POPC and SM, exhibited similar swelling of the H_{II} phase, signifying a decrease of the spontaneous curvature. Cer instead exhibited a spontaneous curvature close to that of DOPE and showed no distinct changes in c_0. Finally, the addition of cholesterol lead to a negative slope. All resulting spontaneous curvatures are compiled in Tab. S1. Results for DOPE and Chol are in good agreement with previously reported vales (for review, see, (2)).



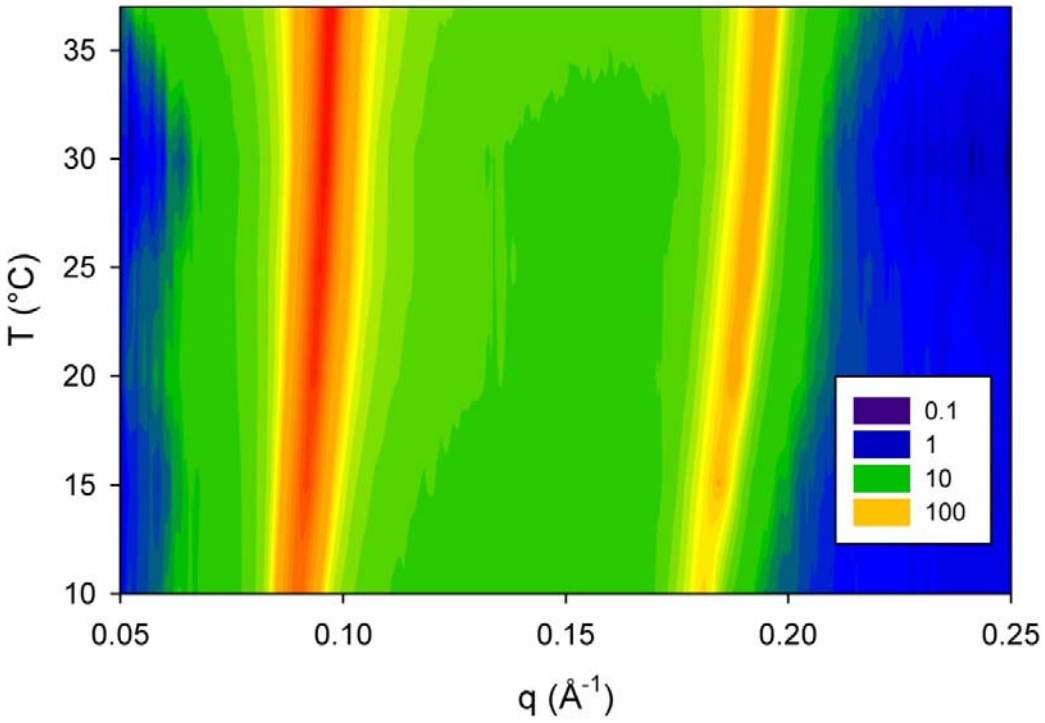
Supplemental Figure S2: Estimation of spontaneous curvatures from H_{II} phase measurements using DOPE as template phase. Panel A shows a cross section of the H_{II} phase determined from an electron density map, including a scheme that explains the structural parameters and the location of 9-*cis*-tricosene. Panel B shows the changes of c_0 with concentration of the guest lipids (Chol, SM, POPC and Cer) in DOPE.

Supplemental Table S1: Spontaneous curvatures of diverse lipids at 25°C.

Lipid	c_0 (Å^{-1})
DOPE	-0.032 ± 0.001
Chol	-0.038 ± 0.002
SM	-0.003 ± 0.003
Cer	-0.031 ± 0.002
POPC	-0.003 ± 0.003

Phase Separation in POPC/SM/Chol

It is well known that phase coexistence in raft-like ternary lipid mixtures depends on temperature (7, 8). Thus, at high enough temperatures, the systems mix homogenously, while lowering temperature induces phase separation. Depending on lipid composition this transition may be of second order (9). Since we did not observe fluid-fluid phase coexistence in fully hydrated MLVs composed of POPC/SM/Chol (45/45/10) at 37°C by SAXD, it is therefore possible that lowering temperature reveals phase coexistence. Hence, we decreased temperature from 37°C to 10°C. Because osmotic pressure helps to bring coexisting domains into registry, this experiment was performed at Π = 1.31 atm. Diffraction patterns shown in Fig. S3 exhibit typical changes in *d*-value and peak width due to lattice expansion and thermal disorder. However, no splitting of the peaks occurred. We, therefore, exclude phase separation in the presently studied system, as supported by fluorescence microscopy (10).



Supplemental Figure S3: SAXD patterns of POPC/SM/Chol (45/45/10) **at Π = 1.31 atm** as a function of temperature. The contour plot shows the first and second lamellar diffraction order on a logarithmic intensity scale.

Supporting References

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