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Supplemental Information

Structural Transitions in Ceramide Cubic Phases during Formation of the Human Skin Barrier

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Supporting information

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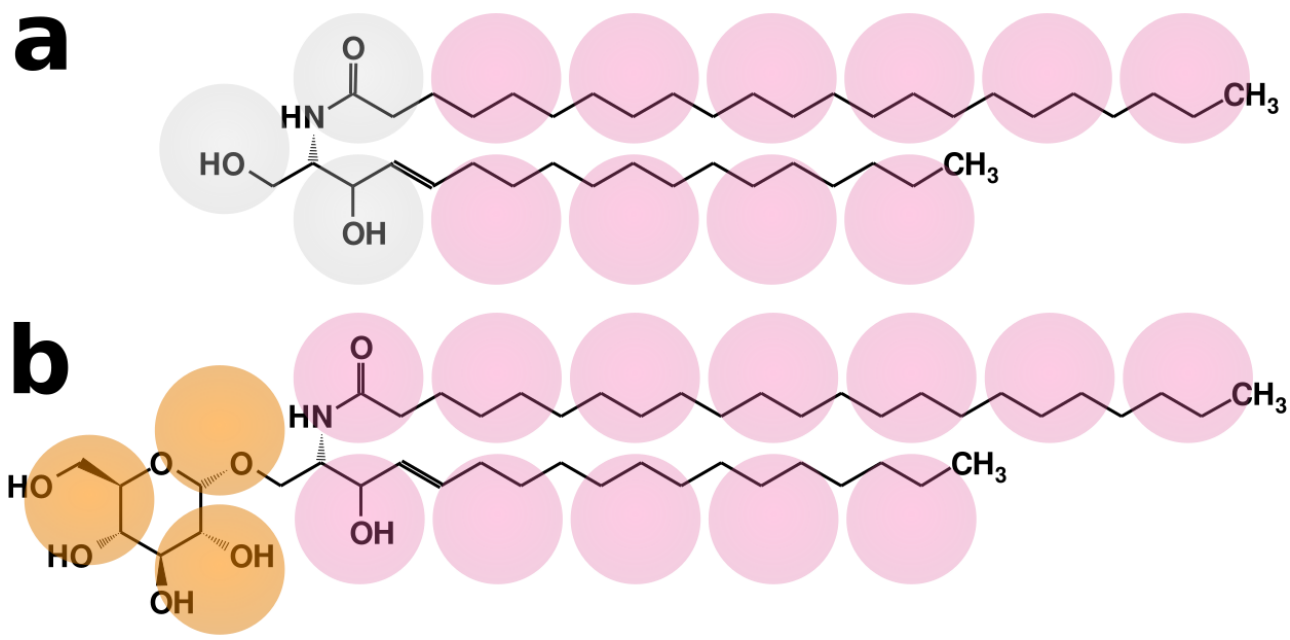

Fig S1. Simulated molecules and coarse-grained representation. The molecules simulated in this work are either ceramide NS (a) or glycosylceramide NS (b). The coarse-grained MARTINI force field utilized for the simulations uses a four-to-one mapping of heavy atoms. Most of the visualizations in this paper only display the headgroup-particles. The colors utilized in all images in the main article are as follows: The water structure is displayed in blue and yellow, the ceramide headgroup region in white, the glycosylceramide headgroup region in orange and the hydrocarbon tail regions in pink. The simulation box is outlined in blue.

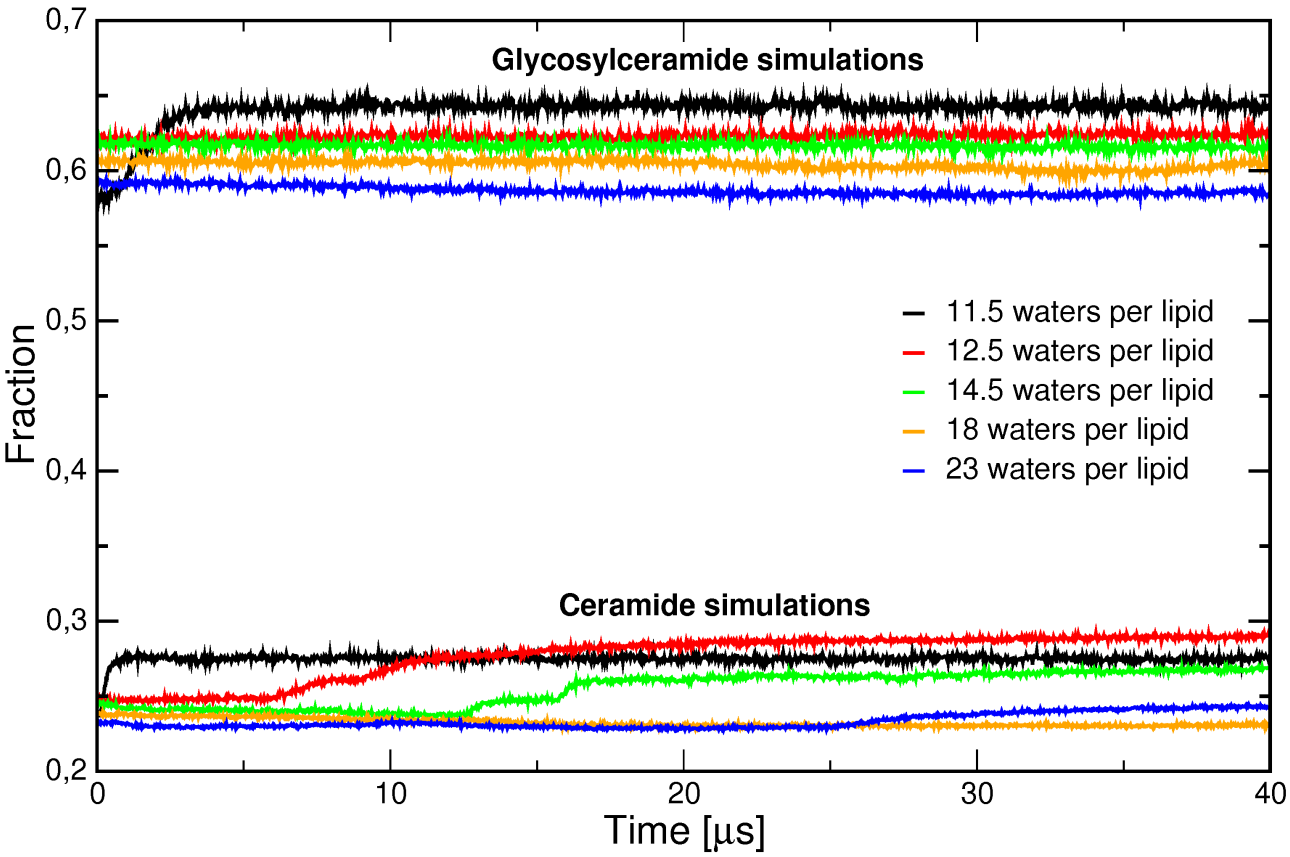

Fig. S2. Inter-headgroup potential energy divided by inter-tail potential energy in the simulated systems, displayed as a per-molecule average over 40 µs of simulation.
The interaction between the hydrophobic tails is relatively similar between the simulated ceramide and glycosylceramide systems. There is a large discrepancy in the interaction strength between the headgroups, which gives a large difference when comparing the potential energy of the inter-headgroup interactions as a fraction of the corresponding potential energy inter-tails interaction.

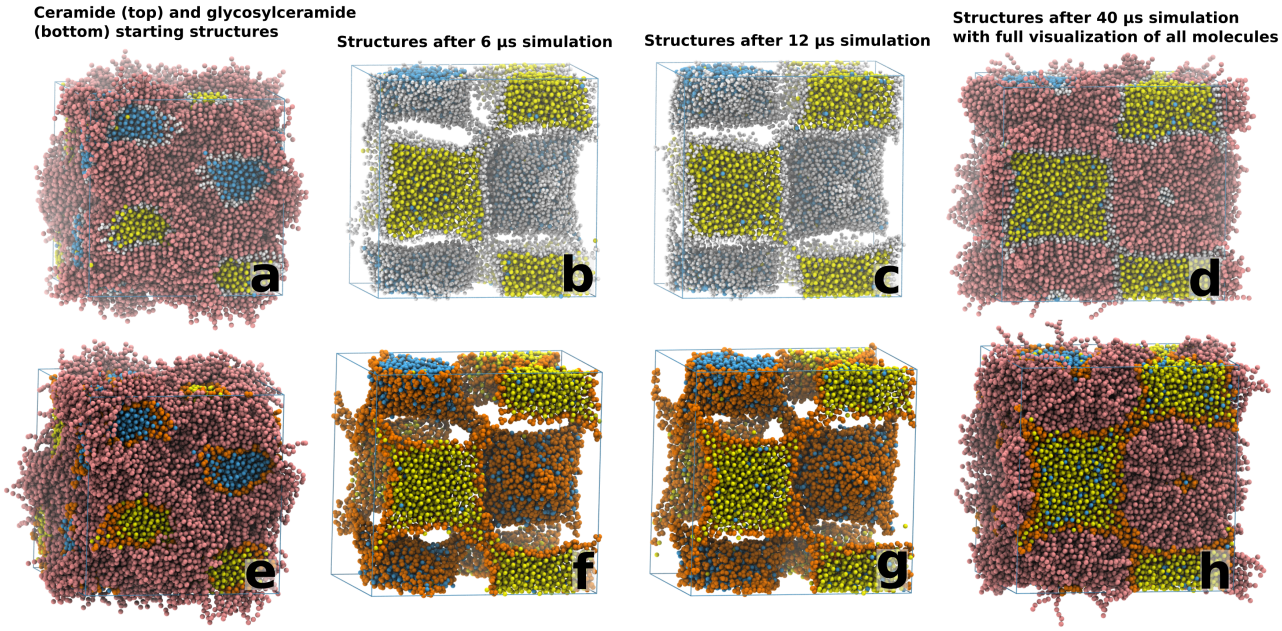

Fig. S3. Snapshots of the cubic structures with a hydration of ~11.5 waters per lipid after 0, 6, 12 and 40 µs of simulation time. In both the ceramide (a-d) and glycosylceramide (e-h) systems there is a structural rearrangement into a structure similar to an inverted micellar phase. The starting (a and e) and final structures (d and h) are displayed using a full representation of all simulation particles. The (originally) bicontinuous water structure is displayed in blue and yellow, the ceramide headgroup region in white, the glycosylceramide headgroup region in orange and the hydrocarbon tail regions in pink. The simulation box is outlined in blue.

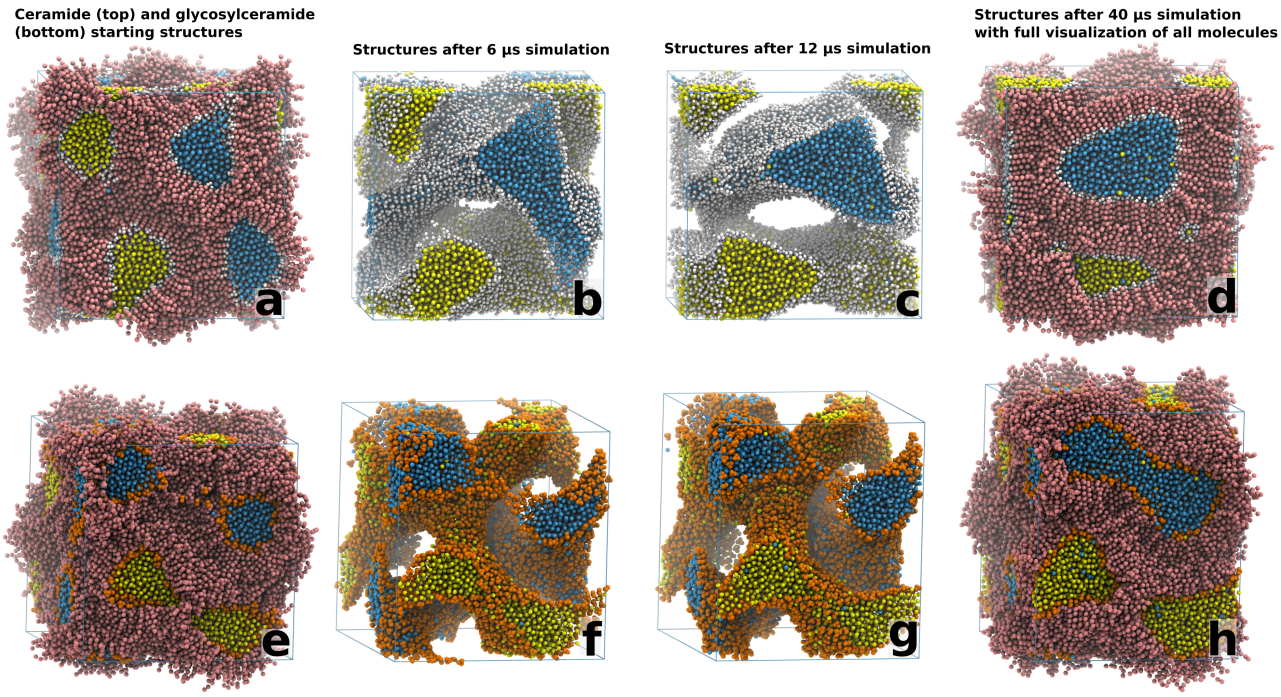

Fig. S4. Snapshots of the cubic structures with a hydration of 12.5 waters per lipid after 0, 6, 12 and 40 µs of simulation time. In the ceramide (a-d) system there is a structural rearrangement towards a lamellar phase. In the glycosylceramide system (e-h) the gyroid geometry is maintained throughout the simulation. The starting (a and e) and final structures (d and h) are displayed using a full representation of all simulation particles. The bicontinuous water structure is displayed in blue and yellow, the ceramide headgroup region in white, the glycosylceramide headgroup region in orange and the hydrocarbon tail regions in pink. The simulation box is outlined in blue.

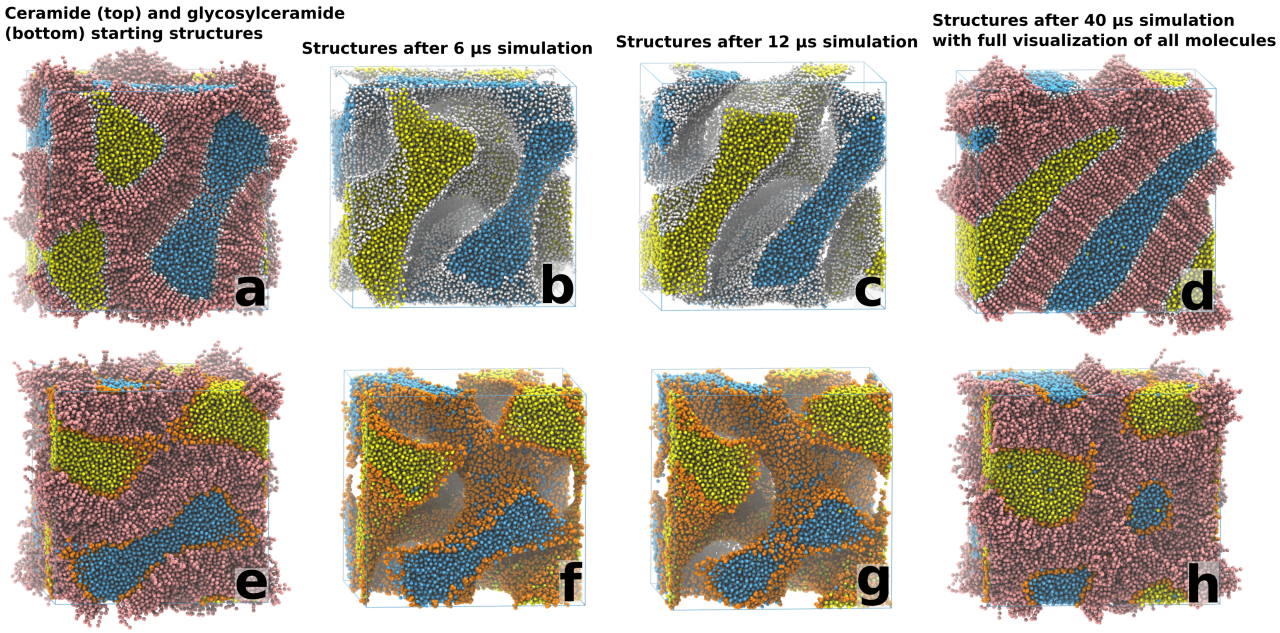

Fig. S5. Snapshots of the cubic structures with a hydration of ~18 waters per lipid after 0, 6, 12 and 40 µs of simulation time. In the ceramide (a-d) system there is a structural rearrangement towards a lamellar phase. In the glycosylceramide system (e-h) the gyroid geometry is maintained throughout the simulation. The starting (a and e) and final structures (d and h) are displayed using a full representation of all simulation particles. The bicontinuous water structure is displayed in blue and yellow, the ceramide headgroup region in white, the glycosylceramide headgroup region in orange and the hydrocarbon tail regions in pink. The simulation box is outlined in blue.

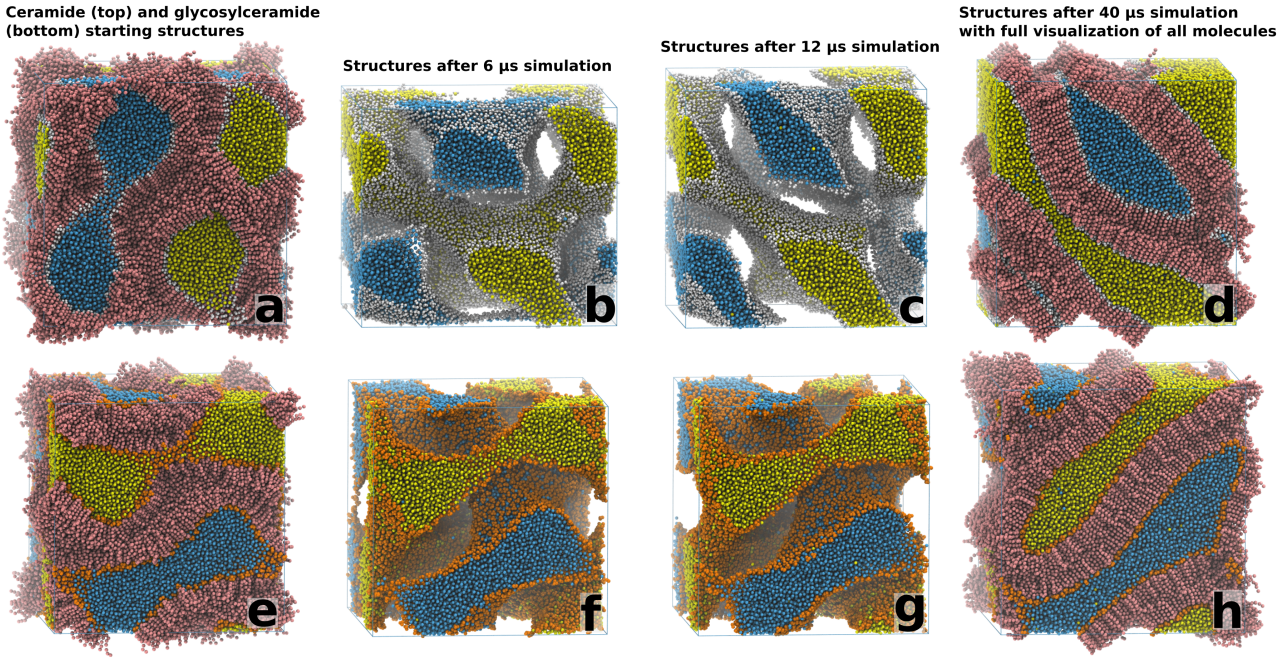

Fig. S6. Snapshots of the cubic structures with a hydration of ~23 waters per lipid after 0, 6, 12 and 40 µs of simulation time. In both the ceramide (a-d) and glycosylceramide (e-h) system there is a structural rearrangement towards a lamellar phase. The starting (a and e) and final structures (d and h) are displayed using a full representation of all simulation particles. The bicontinuous water structure is displayed in blue and yellow, the ceramide headgroup region in white, the glycosylceramide headgroup region in orange and the hydrocarbon tail regions in pink. The simulation box is outlined in blue.

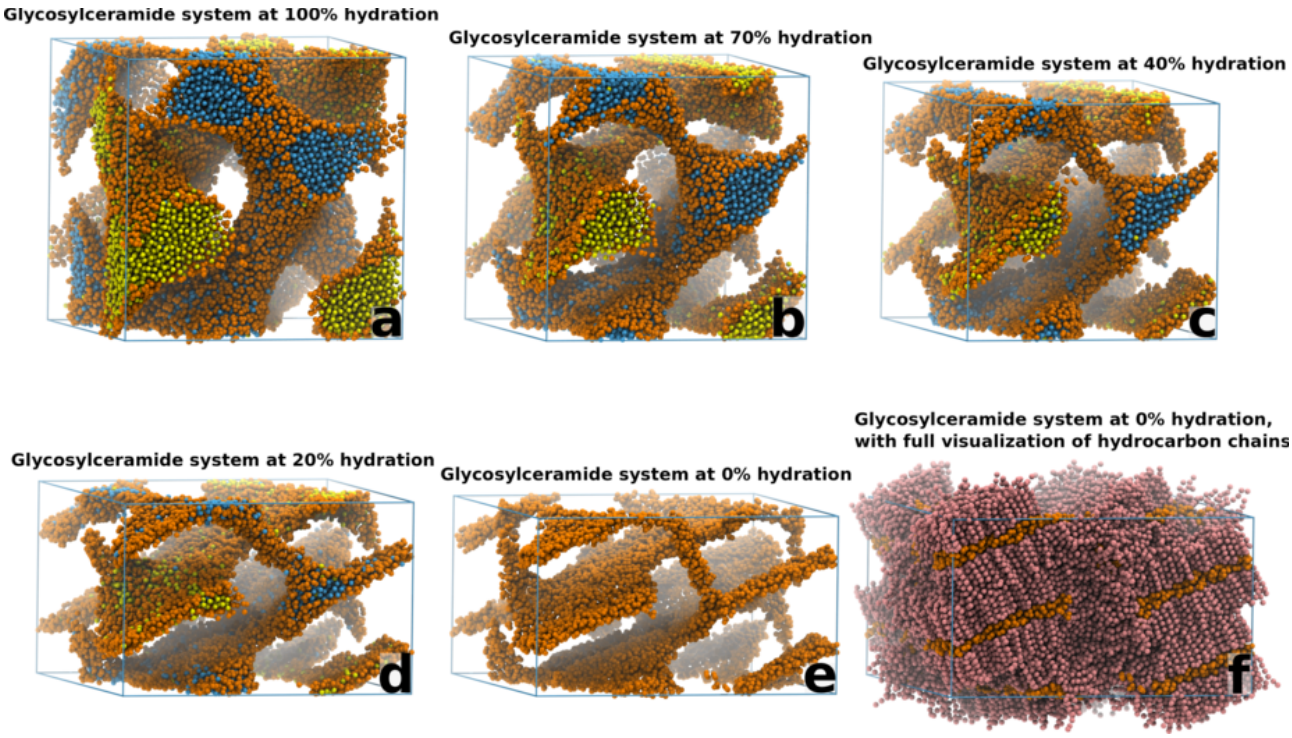

Fig. S7. The final structures of the glycosylceramide system in Fig. 2 at a water content (compared to the original system) of 100, 70, 40, 20 and 0 % (a-f). The gyroid organisation of the glycosylceramide structure is maintained as water is removed from the system. Although the final system contain areas with lamellar structures (as seen in e-f), the overall structure still resembles the bi-continuous gyroid geometry of the original system (a). Water is shown in blue and yellow, glycosylceramide headgroups region in orange and glycosylceramide hydrocarbon tails in pink. The simulation box is outlined in blue.

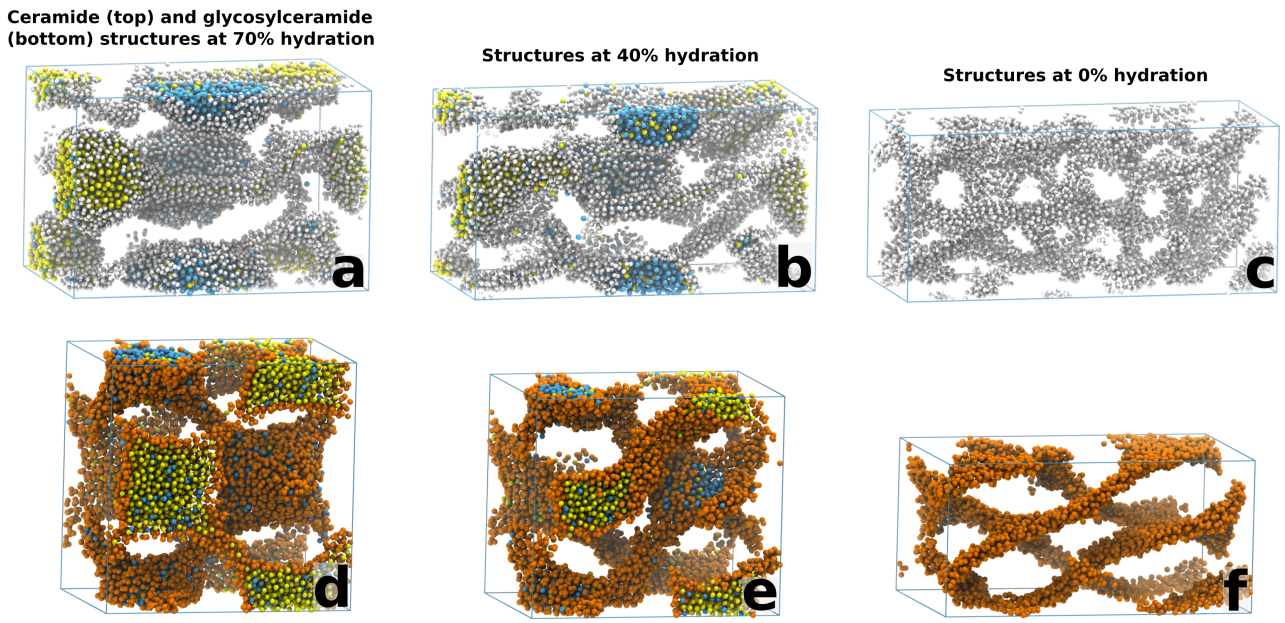

Fig. S8. The final structures of the systems with a hydration of ~11.5 waters per lipid at a water content (compared to the original system) of 70, 40 and 0 %. The ceramide system (a-c) undergoes a transition to a structure resembling a disorganized lipid mixture as water is removed. Comparatively the glycosylceramide system (d-f) maintains the structure present from the original simulation, and at the end the dehydrated structure is similar to those observed at other hydration levels. Water is shown in blue and yellow, the ceramide headgroup region in white, the glycosylceramide headgroup region in orange and the hydrocarbon tail regions in pink. The simulation box is outlined in blue.

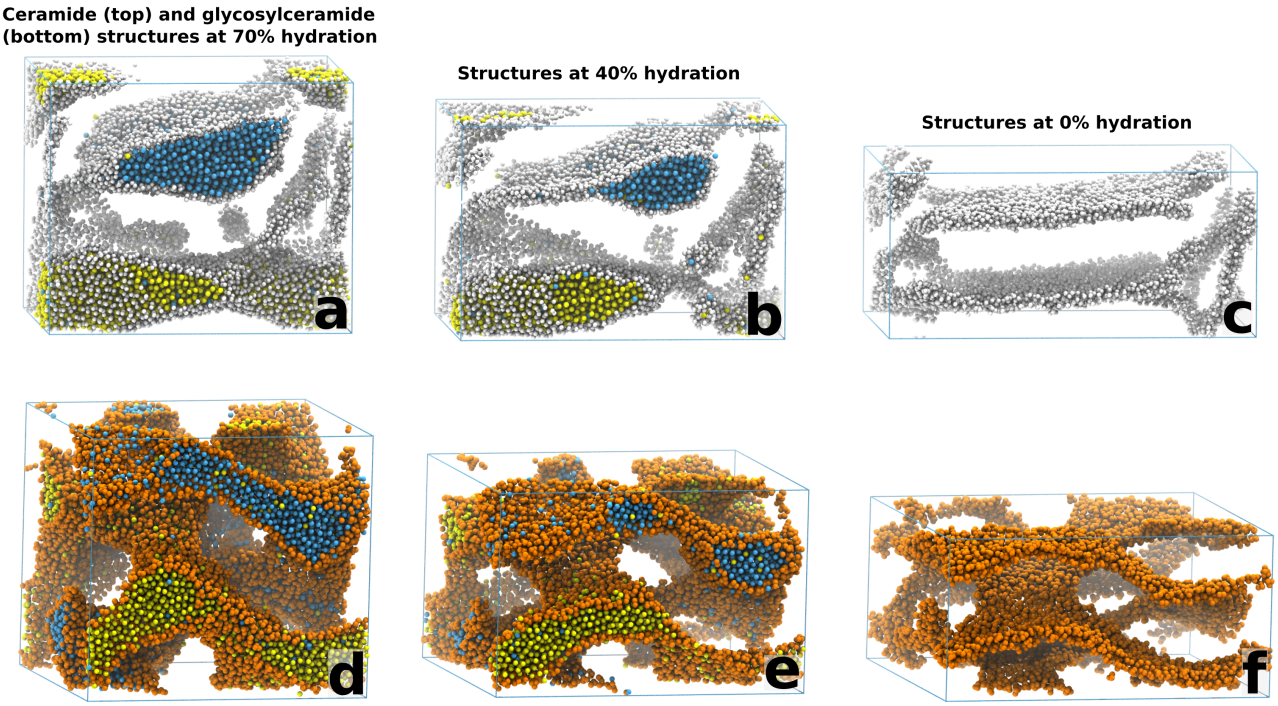

Fig. S9. The final structures of the systems with a hydration of 12.5 waters per lipid at a water content (compared to the original system) of 70, 40 and 0 %. The ceramide system (a-c) transforms into a stacked lamellar system as water is reduced, although some irregularities still exist at the end of the last dehydration step. The gyroid organisation of the glycosylceramide structure (d-f) is maintained as water is removed from the system. Water is shown in blue and yellow, the ceramide headgroup region in white, the glycosylceramide headgroup region in orange and the hydrocarbon tail regions in pink. The simulation box is outlined in blue.

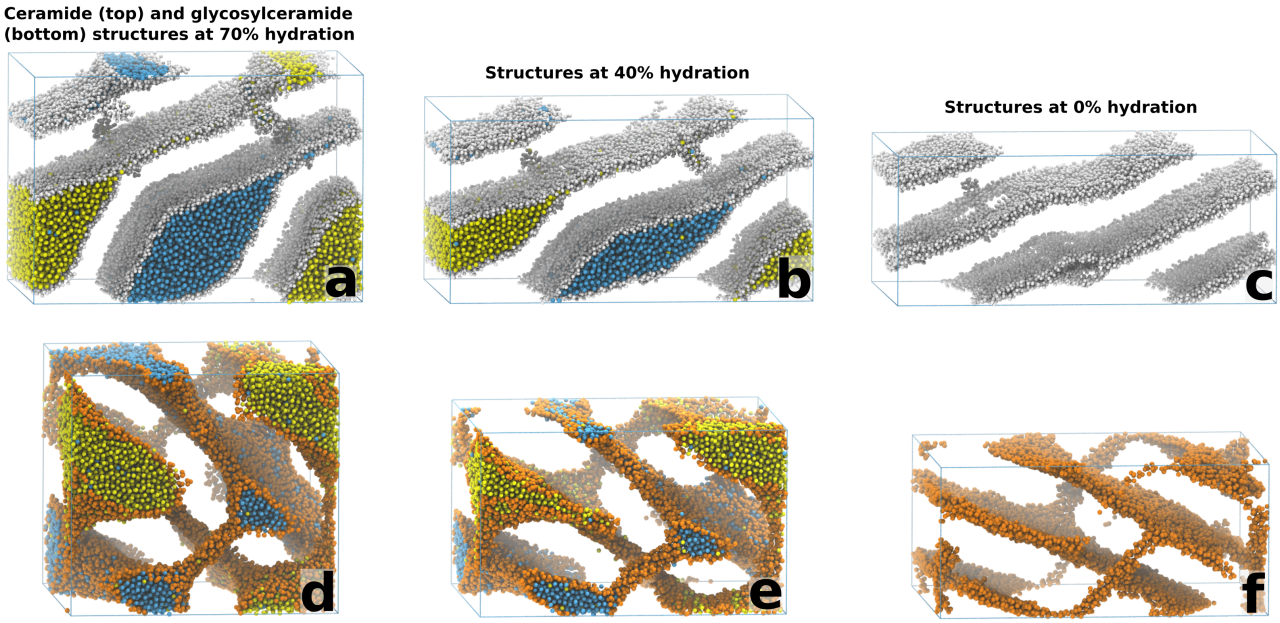

Fig. S10. The final structures of the systems with a hydration of ~18 waters per lipid at a water content (compared to the original system) of 70, 40 and 0 %. The ceramide system (a-c) transforms into a stacked lamellar system as water is reduced. The gyroid organisation of the glycosylceramide structure (d-f) is maintained as water is removed from the system. Although the final system contains areas with lamellar structures (similar to Fig. S7), the overall structure still resembles the bi-continuous gyroid geometry of the original system. Water is shown in blue and yellow, the ceramide headgroup region in white, the glycosylceramide headgroup region in orange and the hydrocarbon tail regions in pink. The simulation box is outlined in blue.

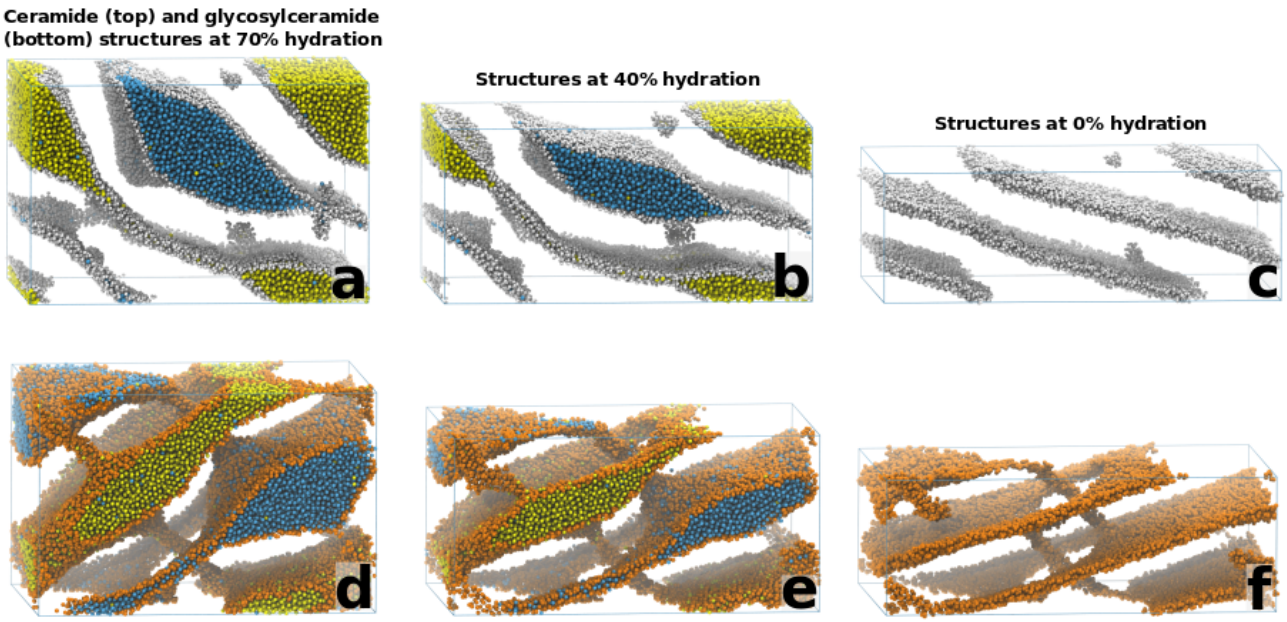

Fig. S11. The final structures of the systems with a hydration of ~23 waters per lipid at a water content (compared to the original system) of 70, 40 and 0 %. Both the ceramide system (a-c) and glycosylceramide system (d-f) transforms into a stacked lamellar system as water is reduced. Although, the glycosylceramide structure still maintains remnants of the gyroid structure present in the original system at the end, similar to Fig. S10f. Water is shown in blue and yellow, the ceramide headgroup region in white, the glycosylceramide headgroup region in orange and the hydrocarbon tail regions in pink. The simulation box is outlined in blue.

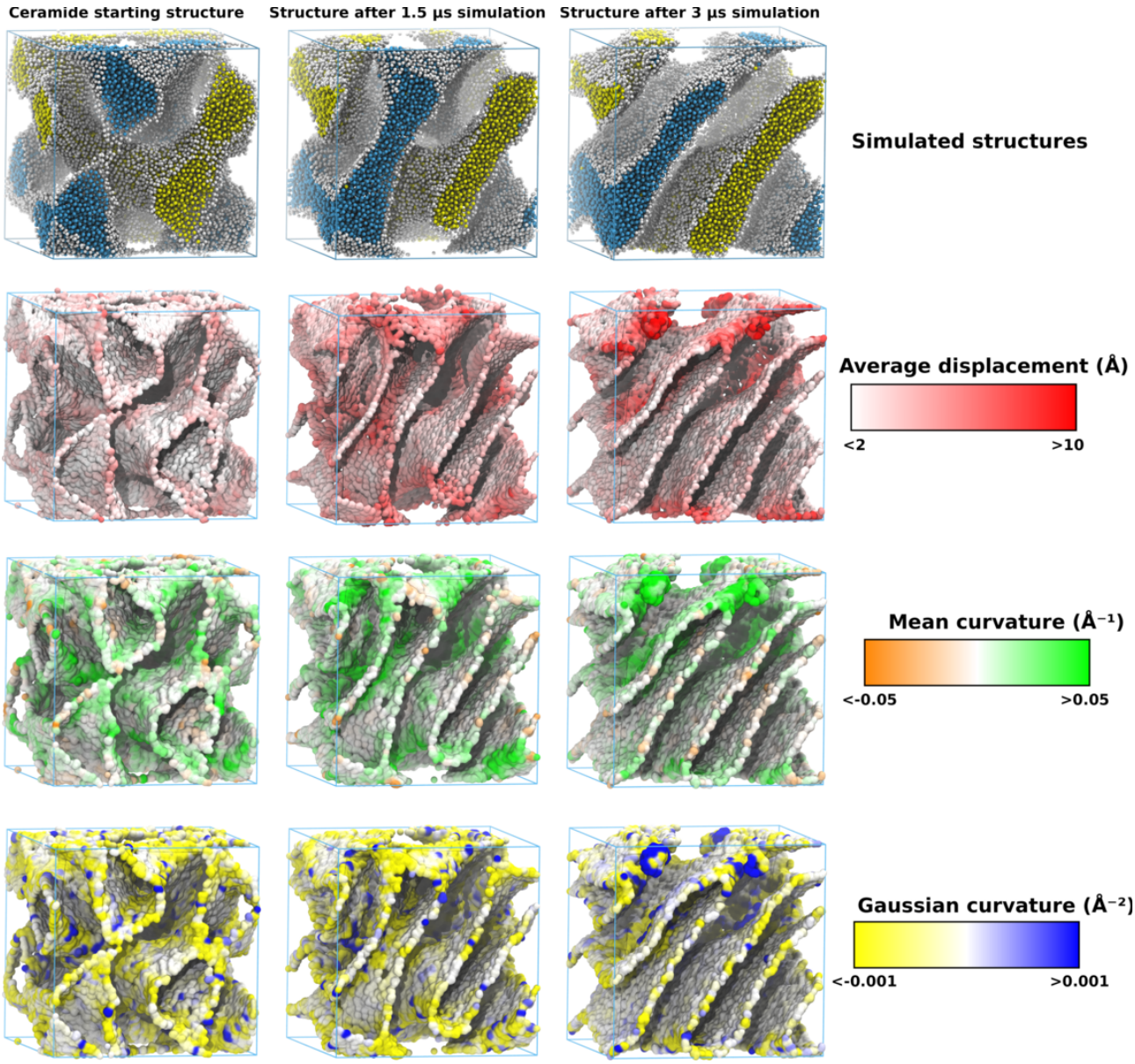

Fig S12. Curvature and average displacement of the ceramide system with a hydration of ~14.5 waters per lipid after 0, 6 and 12 µs of simulation time. The average displacement of each ceramide was calculated as a 3-frame (72 ns) moving average of the average displacement of all simulation particles in each ceramide. The mean and Gaussian curvatures were calculated by first interpolating a surface over the headgroup particles of the ceramides, and then assigning the value of the local curvature (mean or Gaussian) to each triangle in the surface mesh. Each ceramide was then assigned a curvature-value equal to the average of the 5 closest mesh-points in 3D-space.

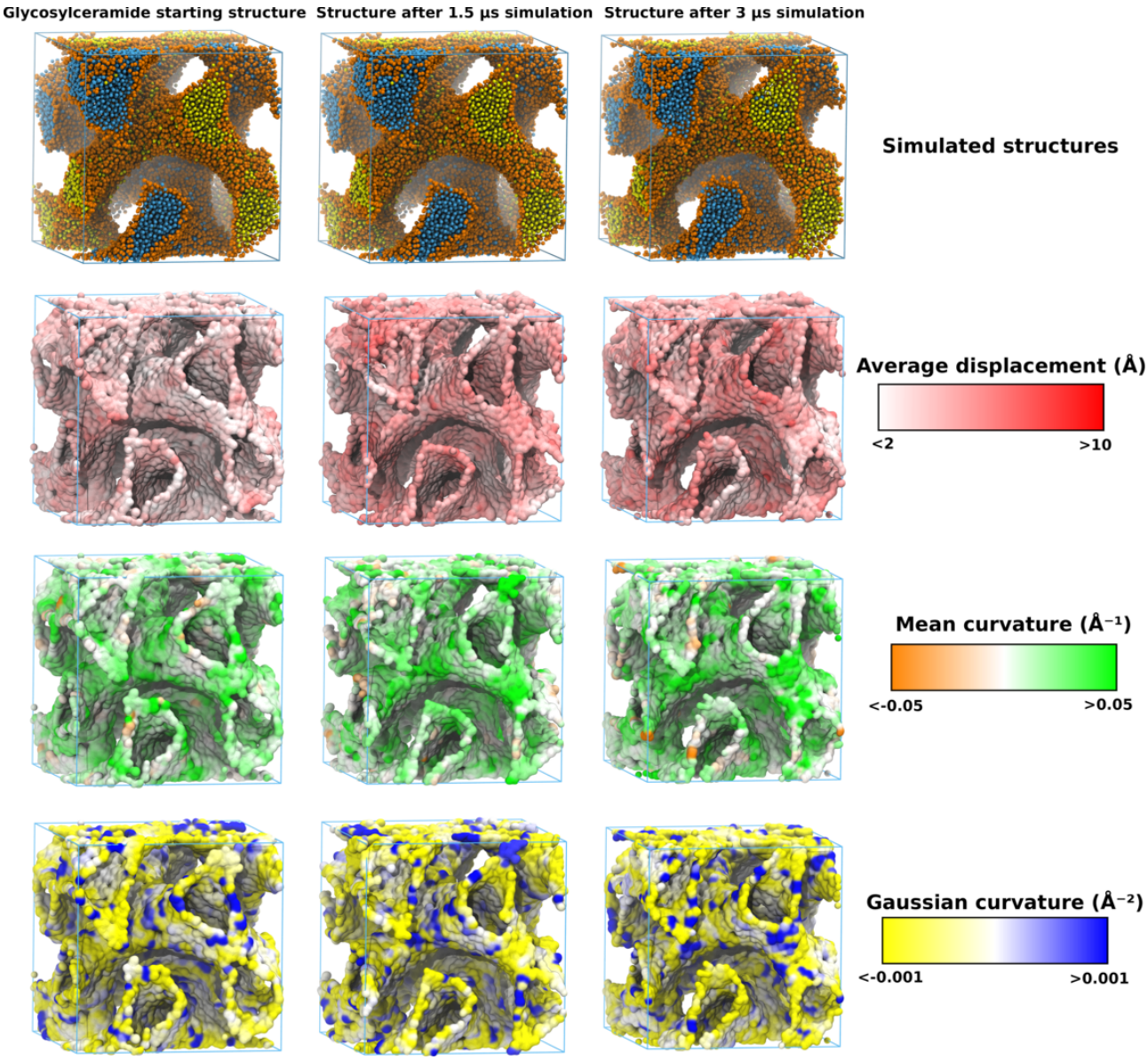

Fig S13. Curvature and mobility of the glycosylceramide system with a hydration of ~14.5 waters per lipid after 0, 6 and 12 µs of simulation time. The average displacement of each glycosylceramide was calculated as a 3-frame (72 ns) moving average of the average displacement of all simulation particles in each ceramide. The mean and Gaussian curvatures were calculated by first interpolating a surface over the headgroup particles of the glycosylceramides, and then assigning the value of the local curvature (mean or Gaussian) to each triangle in the surface mesh. Each glycosylceramide was then assigned a curvature-value equal to the average of the 5 closest mesh-points in 3D-space.

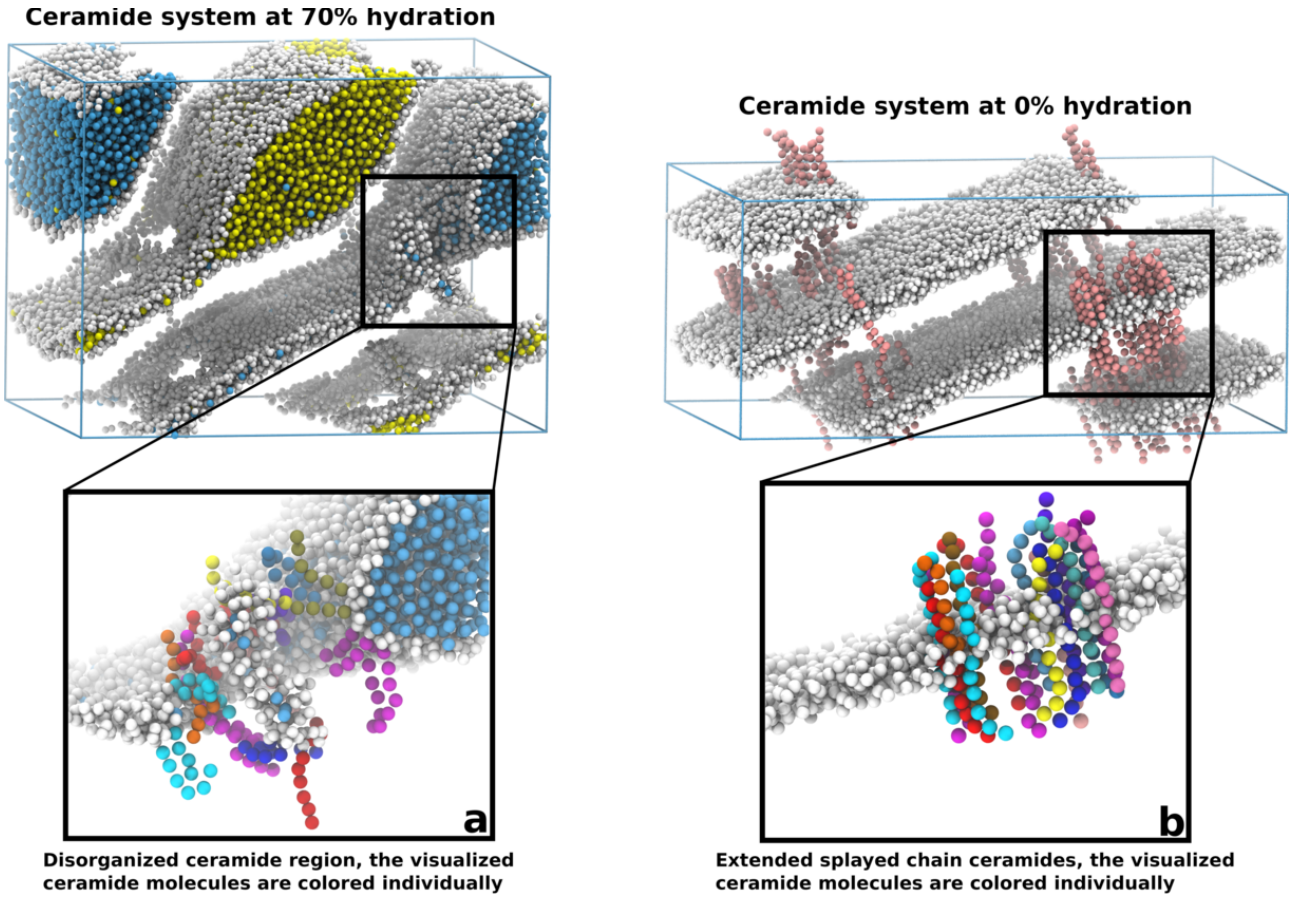

Fig S14. Disorganized regions in the ceramide structure with an original hydration of ~14.5 waters per lipid result in extended splayed chain ceramides. During the cubic- to lamellar transition in the ceramide system shown in Fig. 4f-4h, ceramide-molecules present in the disordered regions (a) eventually relaxed into an extended splayed chain conformation in the final lamellar bilayer structure (b). Water is shown in blue and yellow, ceramide headgroups region in white and ceramide hydrocarbon tails in pink. Ceramides in the insets a and b are colored individually in order to display the transition from an disorganized hairpin conformation (a) into the organized extended splayed chain conformation (b). The simulation box is outlined in blue.

Table S1. Water diffusion coefficients calculated during the first or last 4 μs of simulation in all simulated systems.

System (Molecule, box side length)	Diffusion first 4 μs (cm^2/s)	Diffusion last 4 μs (cm^2/s)
Ceramide, 15 nm	0.0158 ± 0.0082	0.0117 ± 0.0012
Ceramide, 17.5 nm	0.2586 ± 0.0027	0.2365 ± 0.0070
Ceramide, 20 nm	0.3193 ± 0.0043	0.2424 ± 0.0002
Ceramide 22.5 nm	0.3850 ± 0.0030	0.3563 ± 0.0082
Ceramide 25 nm	0.4393 ± 0.0058	0.2973 ± 0.0082
Glycosylceramide, 15 nm	0.0318 ± 0.0136	0.0041 ± 0.0003
Glycosylceramide, 17.5 nm	0.1919 ± 0.0009	0.1756 ± 0.0005
Glycosylceramide, 20 nm	0.2486 ± 0.0005	0.2342 ± 0.0013
Glycosylceramide, 22.5 nm	0.2920 ± 0.0020	0.1776 ± 0.0062
Glycosylceramide, 25 nm	0.3903 ± 0.0013	0.3497 ± 0.0025