

Simulation Studies of the Structural and Thermotropic Phase Behavior of Pure Ceramide Bilayers



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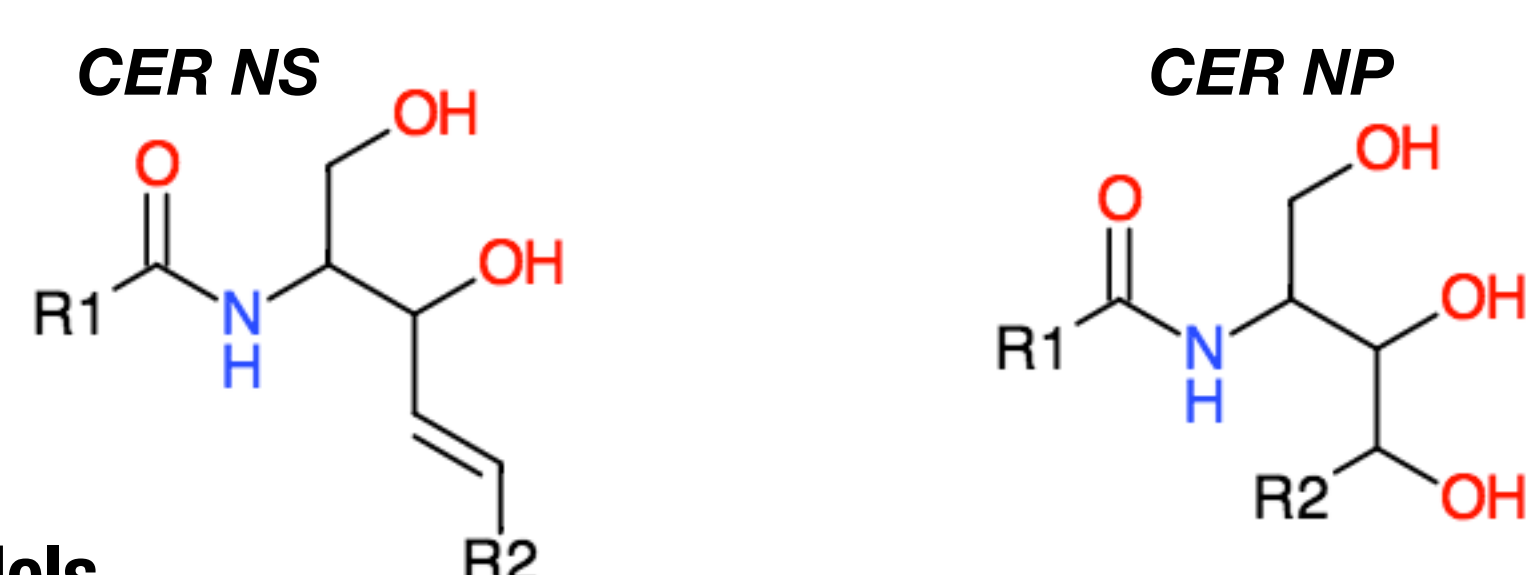
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Overview

Ceramides are a key component of the stratum corneum (SC), the outermost layer of the skin that controls its barrier function. Although the lipid profile of the SC is known, experiments have not yet been able to discern its molecular level structure. In this regard, molecular simulation is an attractive tool to study skin lipid systems, as the position of every atom is known during the course of the simulation. This fact allows us to gain insight into the lipid chemistry and/or composition dependence of lipid organization.

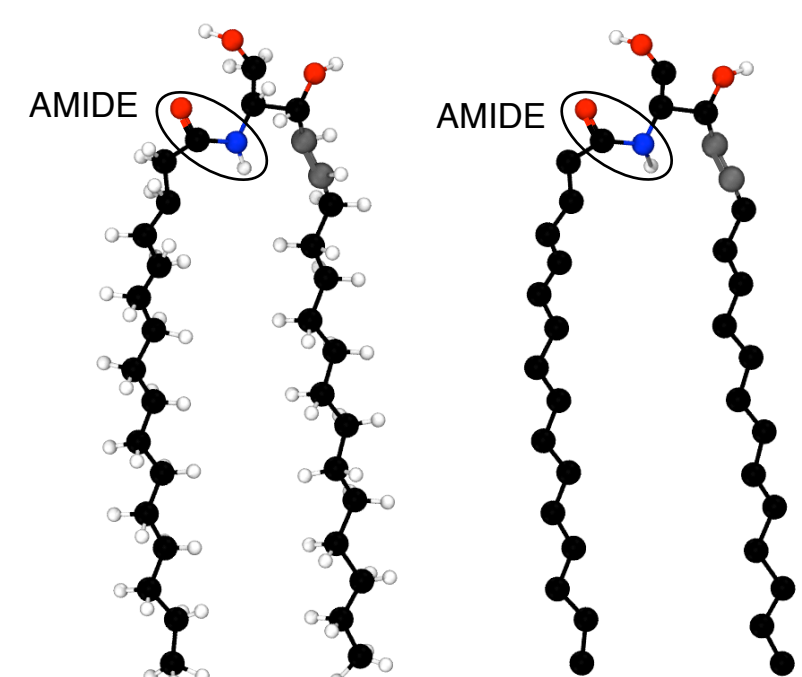
In this work, molecular dynamics (MD) simulations are used to examine the behavior of ceramide bilayers, focusing on non-hydroxy sphingosine (NS) and non-hydroxy phytosphingosine (NP) ceramides. A modified version of the CHARMM force field is proposed for ceramide simulation, which is directly compared to the more commonly used GROMOS-based force field of Berger.¹ While both force fields are shown to closely match experiment from a structural standpoint at the physiological temperature of skin, the modified CHARMM force field is better able to capture the thermotropic phase transitions observed in experiment.

The role of ceramide chemistry and its impact on structural ordering is also examined by comparing ceramide NS to NP, using the validated CHARMM-based force field. These simulations demonstrate that the addition of an extra OH group changes the hydrogen bond structure in the lipid headgroups. The additional OH in the headgroup results in the formation of a distinct hydrogen bonding network, that is ultimately responsible for shifting the gel-to-liquid phase transition to higher temperature, in direct agreement with experiment.^{2,3}



Models

The all-atom CHARMM and the united-atom GROMOS force fields were used. Despite their abundant use in the lipid bilayer literature, neither forcefield has been parameterized for ceramides. We used *ab initio* calculations to calculate the missing amide group parameters for CHARMM. The Berger-modified version of the GROMOS force field was used for the GROMOS ceramides. The water models used were TIP3P for CHARMM and SPC for GROMOS.



CHARMM (left) and GROMOS (right) models of CER NS.

Force Field Validation

Simulation Procedure

CHARMM simulations were run with the LAMMPS simulation package, using a 1 fs timestep using PPPM long-range electrostatics and Nose-Hoover temperature/pressure coupling. The GROMOS simulations were run with the GROMACS package with a 2 fs timestep, cutoff electrostatics and Berendsen temperature/pressure coupling.

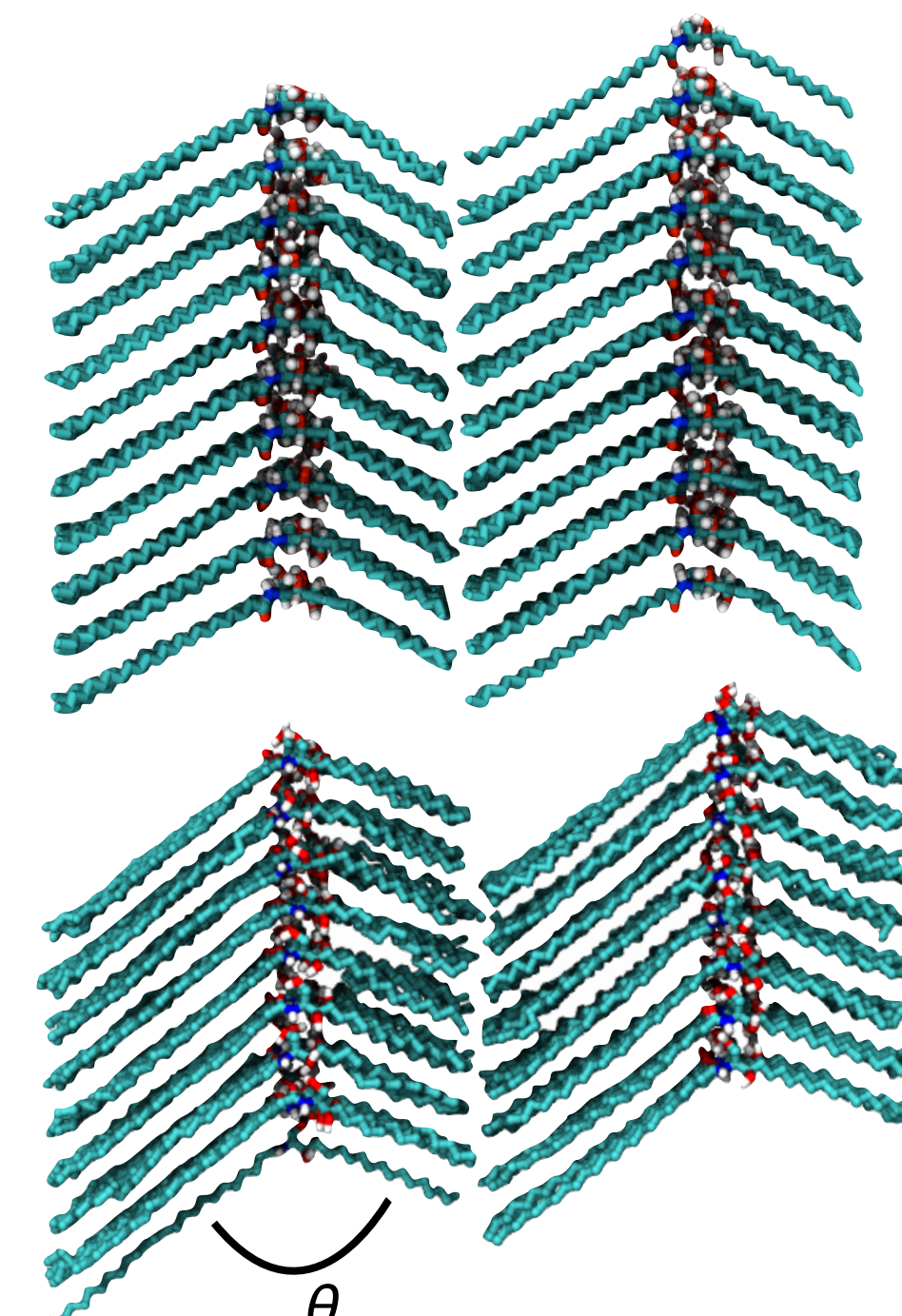
Crystal simulations: Starting from the known crystal structure, simulations were run in the constant stress ensemble at 298 K and 1 atm, allowing the simulation cell to change size and shape.

Bilayer Simulations: preassembled bilayers consisting of 100 lipids per leaflet and 10,000 water molecules were constructed and used as the initial configurations. The constant temperature simulations were run for 100 ns at 305 K, and data collected over the last 30 ns. The temperature scans were done at a rate of 5 K/ns from 305 K until bilayer rupture, and then cooled from just before rupture back to 305 K.

Results: Crystal Structure

The crystal structure of CER NP C24:0 was simulated using each force field and compared to known values. We found that the CHARMM parameters predict a structure that better matches the known experimental value. The GROMOS model favors a more well-ordered crystal structure with a larger splay angle.

CHARMM CER NP
C24:0 crystal structure.
Hydrogens not shown for clarity



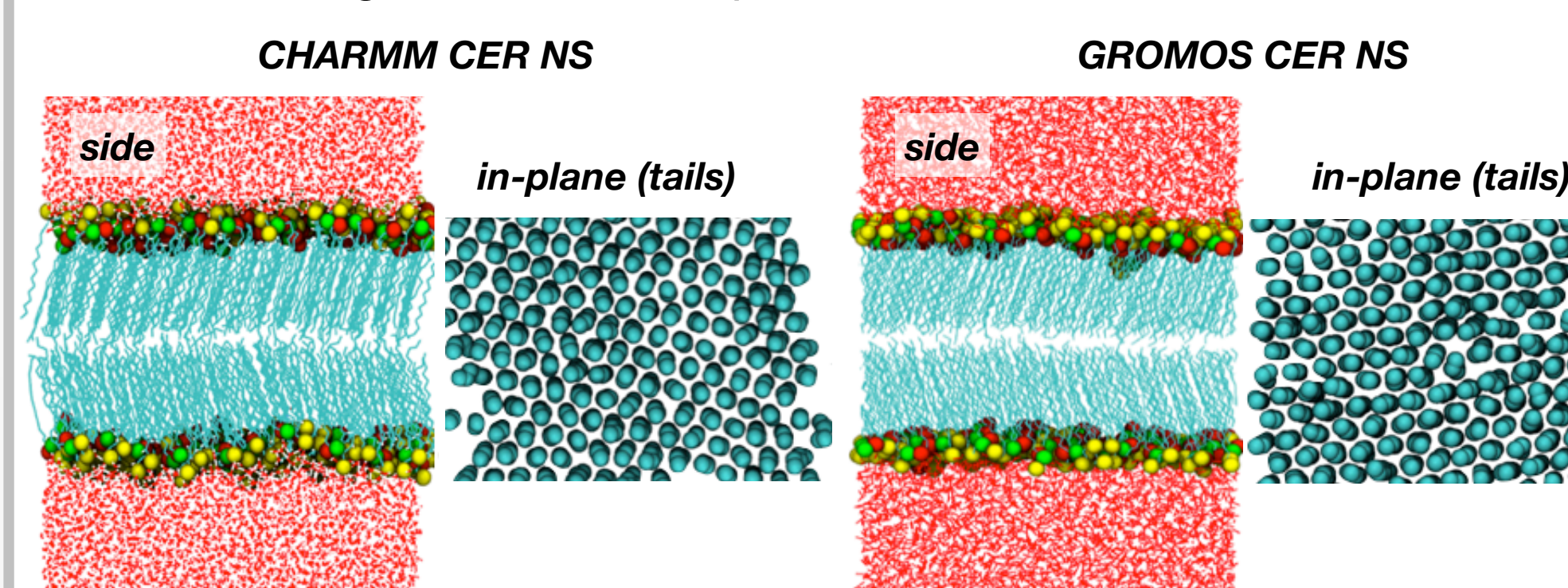
GROMOS CER NP
C24:0 crystal structure.
Hydrogens not shown for clarity

	Experiment ⁴	CHARMM	GROMOS
a (Å)	6.181	6.2	5.2
b (Å)	4.929	5.0	4.7
c (Å)	37.278	36.7	44.3
α (°)	90.96	88.6	81.7
β (°)	91.3	91.4	77.9
γ (°)	105.51	105.9	111.0
θ (°)	101	102.2	110.4

Unit cell parameters for CER NP C24:0 from experiment, CHARMM, and GROMOS

Results

Pure CER NS bilayers were simulated at skin temperature using the CHARMM and GROMOS models. We found that both models predict stable, well-ordered bilayers, with area per lipid (APL) values that agree well with experiment.

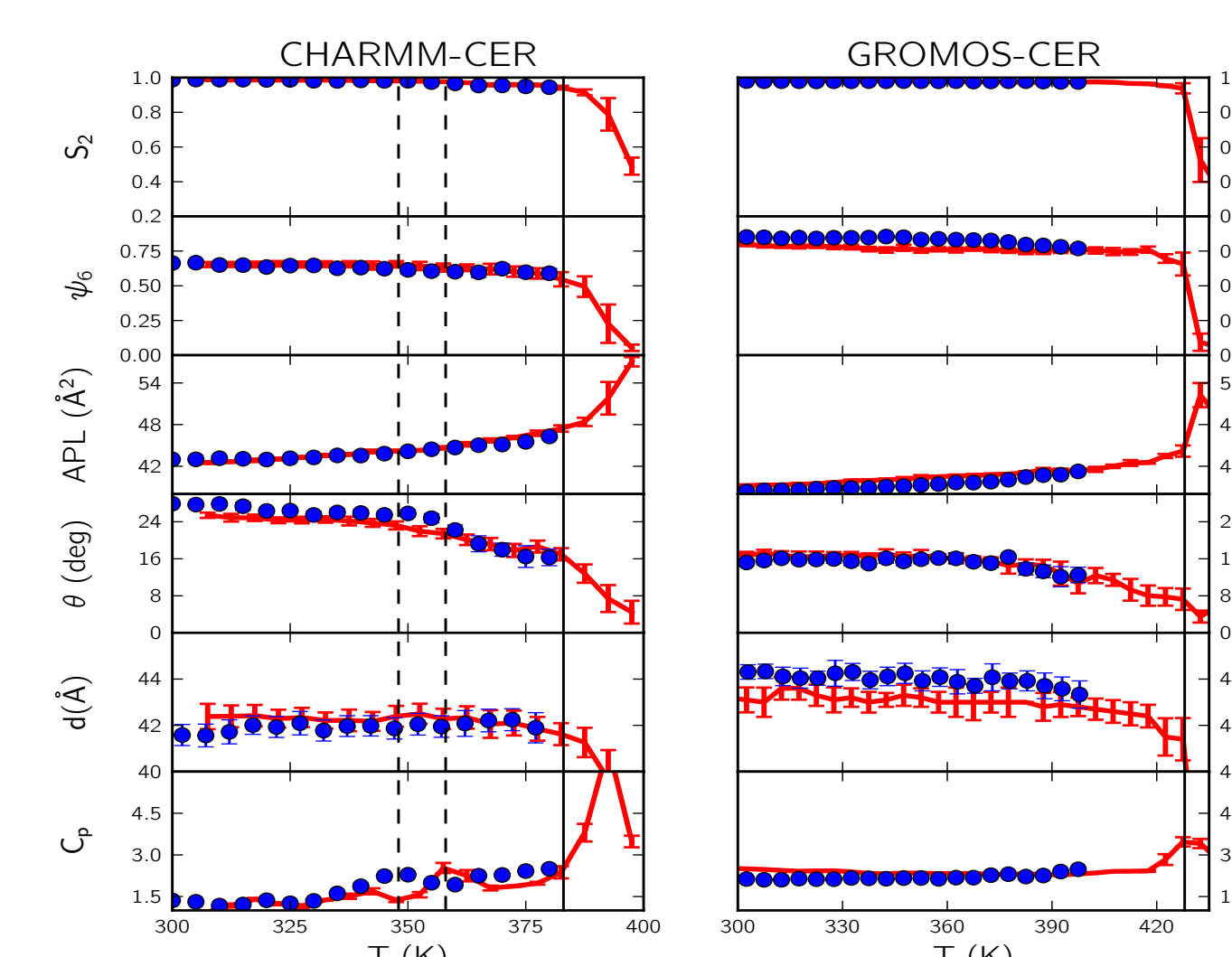


	Experiment	CHARMM	GROMOS
APL (Å ²)	37.8 - 42	42.4 ± 0.2	39.8 ± 0.2
Thickness (Å)	46.9	42.5 ± 0.5	43.7 ± 0.4
Tilt angle (°)	-	24.3 ± 0.3	17.0 ± 1.0

Bilayer properties at 305 K

Results: CER NS Phase Behavior

The phase behavior was explored by heating the bilayer and calculating several structural properties as a function of temperature. The CHARMM model predicts a main order-disorder transition (ODT) around 390 K, while the GROMOS model predicts an ODT around 430 K; Chen *et al.*⁵ find a main ODT of 366.1 K experimentally. The change in APL, chain tilt angle and the peak in Cp suggest a secondary transition near 358 K for the CHARMM bilayer, consistent with the work of Shah and Atienza⁶ and Chen *et al.*⁵ This secondary transition is not seen in the GROMOS system. As such, the CHARMM force field predicts the phase behavior of ceramide bilayer systems in better agreement with experiment than GROMOS.



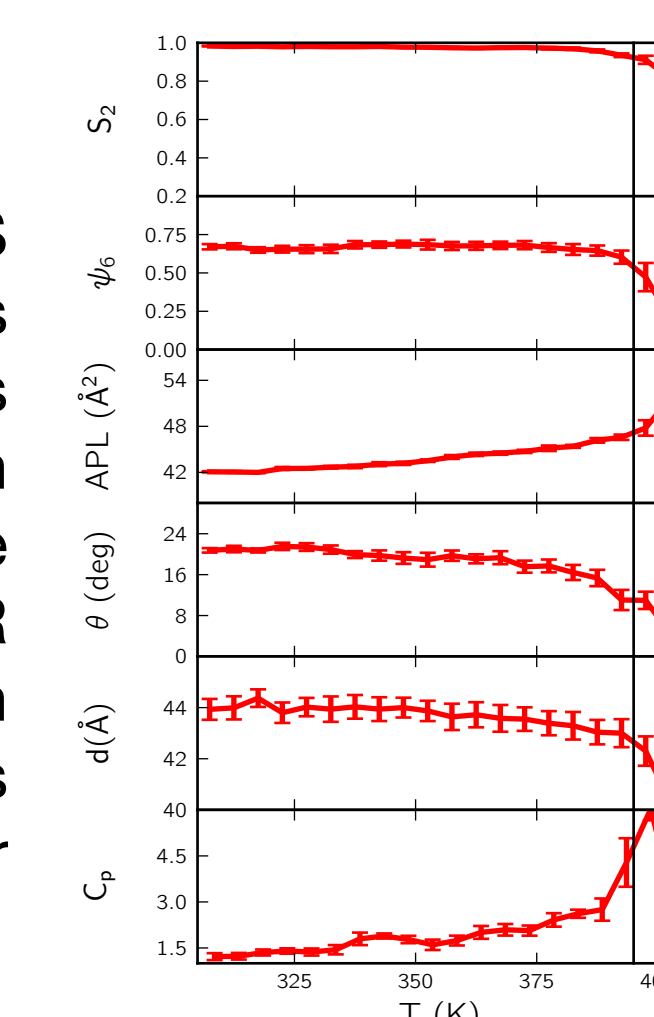
Nematic order parameter (S_2), global hexagonal order parameter (ψ_6) of alkyl tails, area per lipid, chain tilt angle w.r.t. bilayer normal, bilayer thickness (d) and dH/dT as a function of temperature for heating (red) and cooling (blue) scans.

Headgroup Comparison

To examine the structural effect of the extra OH group in CER NS vs CER NP, the phase behavior of bilayers composed of CER NP were compared to those composed of CER NS using the CHARMM force field.

Results: CER NP Phase Behavior

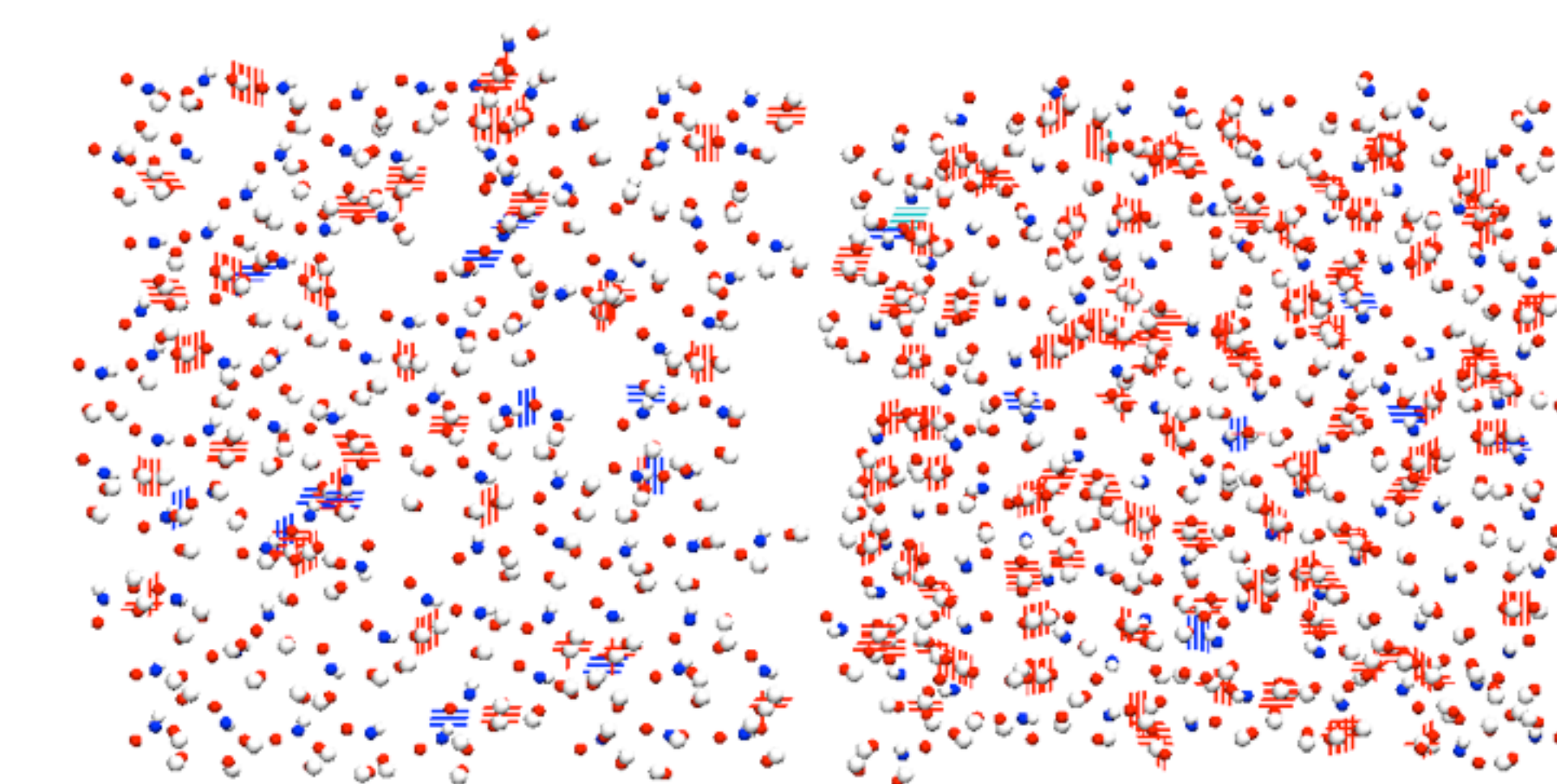
In the same manner as the CER NS systems, a bilayer of pure CER NP was heated, and structural properties measured as a function of temperature. An ODT is seen at a temperature approximately 12 K higher than the CER NS ODT temperature, in agreement with experiment.^{2,3} However, no obvious secondary phase transition is observed for this system.



Results: CER NP Structural Properties

The structural properties of the pure CER NP bilayer and pure CER NS properties were compared at skin temperature (305K). It was found that, like the CER NS system, the tails are very ordered, reflected in the nematic order parameter value close to 1. Despite the extra OH group in CER NP, the bilayer has an APL very close to that of the CER NS bilayer. When considering that the two systems have similar APLs but CER NS system has a larger bilayer height, this suggests that the CER NP system has a larger volume per lipid, consistent with experimental results that suggest phytosphingosines pack less tightly than sphingosines.

The CER NP system has more hydrogen bonds than CER NS, as expected due to the additional OH group. However, the nature of the hydrogen bonding is different for each system. In CER NS, 80% of the hydrogen bonds involve water, whereas this number is only 40% for CER NP. This result shows that CER NP forms a tighter lipid-lipid hydrogen bond network, which appears to manifest itself in a higher ODT temperature, suggesting increased stability of the pure CER NP bilayer as compared to the CER NS bilayer.



Lipid-lipid hydrogen bonds in CER NS (left) and CER NP (right). Only oxygen hydrogen (white), nitrogen (blue) and oxygen (red) shown for clarity.

Acknowledgements

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Conclusions

In this work, we compare the structure and phase behavior of bilayers modeled using two commonly-used force fields, CHARMM and GROMOS. We find that they both do an adequate job describing pure CER NS bilayers at skin temperature. However, CHARMM does a better job predicting the crystal structure and phase behavior, making it better suited to study systems where structural heterogeneity may be present. We then use CHARMM to study CER NP bilayers, and find the extra OH group in CER NP promotes a tight lipid-lipid hydrogen bonding network, resulting in a higher ODT temperature compared to CER NS.

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