Studying the lateral chain packing in a ceramide bilayer with molecular dynamics simulations

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Abstract. In this work, we present a novel technique, based on molecular dynamics simulations, that allows the study of the lateral chain packing in a lipid bilayer. It utilizes the radial distribution function of the alkyl chains to determine the arrangement of the chains along the bilayer plane. The positions of the mass centres of the chains are projected onto the bilayer plane and a 2D radial distribution function is calculated for these projections. The proposed technique can be particularly useful for lipid bilayers in the gel (solid) phase where the chains present a limited degree of mobility. As a case study, we have examined a bilayer that consists of ceramide NS 24:0. Ceramide bilayers can be found in the lipid domain of the skin where they have a significant role in its barrier function. The specific bilayer was found (at 300 K) to adopt a strictly hexagonal chain packing with a separation distance between the chains of 0.466 nm, in good agreement with the available experimental data.

1. Introduction

Stratum corneum is the outermost layer of the skin and it is responsible for the mechanical properties of the skin as well as for its barrier function (i.e. control of the flow of substances into or out of the body). It mainly consists of dead cells (corneocytes) that have an elongated shape and are filled with keratin. Corneocytes are surrounded by a lipid domain forming a “brick-and-mortar” structure. This lipid domain comprises of lipid bilayers consecutively placed the one next to the other. In this way, the lipid phase is contiguous and offers the most probable route for a substance to penetrate the stratum corneum.

Concerning its composition, the skin lipid domain is an approximately equimolar mixture of ceramides, cholesterol and free fatty acids. Ceramides are a special class of lipids that contain sphingosine, an unsaturated aminoalcohol. In ceramides, sphingosine is bonded to a fatty acid via a peptide bond. As a result, each ceramide molecule contains a polar head with a protein-like chemical structure and two alkyl chains with unequal lengths (Figure 1). Various ceramide types exist depending on the exact geometry of the polar head, the length of the alkyl chains and the presence of polar groups or unsaturated bonds in them.

At room temperature, ceramide bilayers form a gel phase where each chain may oscillate around a fixed position but without diffusing among the other chains. This is the main difference between ceramide bilayers and phospholipid bilayers that are found in the cell membranes. Since the alkyl

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chains are in a solid phase, their positions follow a specific crystal lattice (usually hexagonal or orthorhombic). Temperature changes or other reasons may induce phase transitions from one crystal structure to the other. Such phase transitions dramatically affect the transport properties and the overall functionality of the skin.

Figure 1. Chemical structure of ceramide NS 24:0.

2. Methodology
For the study of ceramide bilayers we have performed a series of molecular dynamics simulations. The examined system is a lipid bilayer that contains 128 ceramide molecules. Only one ceramide type is used, namely NS 24:0 (Figure 1). The bilayer is confined between two water slabs each containing 2493 water molecules. The initial structure is geometrically optimized and then it is allowed to equilibrate for 140 ns. In this equilibration stage an annealing process is followed: the system is heated from 300 to 360 and then cooled back to 300 K in steps of 20 K with a relaxation time of 20 ns per step. The production run lasts for 20 ns. Atomistic interactions are described with the approach proposed by Berger et al. [1]. More detailed description of the simulations can be found in Ref. [2].

Among several properties examined in the aforementioned system, the lateral packing of the alkyl chains is one of the most interesting and main the subject of this work. For the rigorous study of this property, we have developed a novel technique that uses the radial distribution functions (RDFs) of the chains to quantitatively describe their relative positioning. The RDF shows the probability of finding two objects at a specific distance. In our case, these objects are the projections of the mass centers of the alkyl chains on the bilayer plane. The overall shape of the RDF reveals the type of arrangement (e.g. liquid, hexagonal, or orthorhombic) while the positions of the peaks can be used for the estimation of the characteristic distances of the crystal lattice.

For the RDF calculation, first the position of the mass center of each chain is calculated. Since lipids have limited mobility in the lamellar direction (z-axis) and we are interested in the lateral structure, the positions of the chains are calculated on a 2D basis by considering only the x and y coordinates. Formally, this RDF is calculated as:

$$g(r) = \frac{1}{2\pi rN} \left\langle S(t) \cdot \frac{\delta N(r,t)}{\delta r} \right\rangle$$

where $N$ is the total number of chains, $S(t)$ the xy cross-section area of the simulation box as a function of time $t$, and $\delta N(r,t)$ is the number of chains located in a circular shell of radius from $r$ to $r + \delta r$. The angle brackets $\langle \rangle$ denote averaging over the total number of chains and total simulation time.

3. Results and discussion

3.1. Lateral chain packing from the RDF
The calculated RDF for the examined system is shown in Figure 2. The first observation on this RDF is that the first peak is sharp and symmetric without any indication of overlapping peaks (e.g. shoulder). This indicates that the chains are placed on a hexagonal lattice. In an ideal hexagonal lattice, each chain is surrounded by 6 other chains (first neighbor shell of chains) and all of them are
located at the same distance $d$. This arrangement gives rise to a unique peak in the RDF. In any other lattice type, some of the surrounding chains would be at different distance from the central chain than some others. In this case, we would expect more than one peaks in the first neighbor shell which is clearly contradicted by the calculated RDF. Moreover, the rest of the peaks that correspond to more distant neighbor shells also follow the same hexagonal pattern.

In the next step, the exact position of each peak is calculated with fitting of a Gaussian function. For peaks that are closely placed, a sum of two Gaussian functions is used so as to more accurately determine the positions of the overlapping peaks. The calculated positions for the 5 first peaks (3 first neighbor shells of chain) are shown in Table 1. With simple geometry calculations (and assuming ideal hexagonal geometry), we can calculate the separation distance $d$ between two chains that correspond to each peak. These results are also shown in Table 1. It must be noted that all of the peaks give essentially the same distance with a maximum deviation less than 0.5%. This result not only confirms that lateral chain packing is strictly hexagonal but also allows the separation distance between the chains to be calculated with high accuracy. For the specific system, this value is found to be 0.466 nm.

![RDF of alkyl chains in a ceramide bilayer. Each peak is associated with the corresponding distance in an ideally hexagonal lattice.](image)

**Table 1.** Positions of the peaks in the RDF and calculated distances between two chains.

| Peak No. | Shell | Peak position (nm) | Distance (nm) |
|----------|-------|--------------------|---------------|
| 1        | 1     | 0.465              | 0.4650        |
| 2        | 2     | 0.808              | 0.4665        |
| 3        | 2     | 0.931              | 0.4656        |
| 4        | 3     | 1.236              | 0.4672        |
| 5        | 3     | 1.399              | 0.4662        |

### 3.2. Comparison with experiments

The RDF function of the alkyl chains provides structural information similar to the X-ray or electron diffraction measurements. In these experimental techniques the distance $d_p$ between the parallel planes of chains is used instead of the distance $d$ between the chains. In the examined system, this distance is

$$d_p = \sqrt{3} \cdot \frac{d}{2} = \sqrt{3} \cdot 0.4661\text{ nm} = 0.404\text{ nm}.$$  

Moreover, the experimentally determined distance is the perpendicular distance $d_t$ between the planes while the RDF refers to the projection of this distance on the bilayer plane. These two distances are equal only in the case when the chains are perpendicular.
to the bilayer plane. However, in ceramide bilayers the angle between the chains and the bilayer normal (tilt angle) is usually not zero. In this case, the two distances are related by the Equation:

\[ d_t = d_p \cos(\theta_i) \]  

(2)

where \( \theta_i \) is the tilt angle. For the calculation of the tilt angle, orthogonal regression is used to find the best-fit line of every chain and then these direction vectors are averaged over the total number of chains and the entire simulation time. In this way, the tilt angle for the examined lipid system is found 9.8° and the corrected distance \( d_t \) is 0.398 nm.

Several experimental studies on ceramide bilayers have been reported that examine the chain arrangement but there is no full consensus about the type of lateral packing. This seems to depend on the sample preparation, the exact lipid composition (real specimens usually contain a mixture of ceramides) and the source of the lipids (e.g. synthetic, isolated from human or bovine skin etc.). However, most of the results from different experimental works based on various techniques [3-6] converge to the conclusion that the hexagonal arrangement is the most stable phase of a bilayer consisting of pure CERs. In the work of Pilgram et al [5], the characteristic distance was accurately measured with electron diffraction and it was found 0.399 ± 0.007 nm. This result is in very good agreement with the value (0.398 nm) calculated from our calculations. The quantitative (in terms of the geometry of lateral chain packing) and the qualitative (in terms of distance between the planes of chains) agreement between shows that the method presented above for the study of the lateral structure of a lipid bilayer is accurate and it can be reliably used for the study of similar systems.

4. Conclusions

We have proposed a computational technique that uses the results of molecular dynamics simulations to provide important information about the lateral chain arrangement in lipid bilayers that are in the gel phase. It is based on the RDF function of the alkyl chains. The calculated RDF allows the determination of the lattice type of the lateral chain packing as well as the calculation of the characteristic distance between the parallel planes of chains. The technique was applied on a lipid bilayer comprised of ceramide NS 24:0. The specific system was found to adopt a strictly hexagonal geometry with a characteristic distance of 0.398 nm. The good agreement between the calculated results and the experimental data verifies the reliability of the technique that can be used for other similar systems with limited lipid mobility.

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