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Computer Simulation of a Hexagonal Assembly for a Branched Chain Glycolipid

Teoh T. Chong[# 1], **Thorsten Heidelberg**[1*], **Rauzah Hashim**[1] and **Richard A. Bryce**[2]

Present address: Stamford College (PJ), Lot 7A Jalan 223 Section 14, 46100 Petaling Jaya, Selangor Malaysia
[1] Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur Malaysia
* heidelberg@um.edu.my (corresponding author)
[2] School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester M13 9PL, United Kingdom
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ABSTRACT Glycolipids are surfactants that assemble in ordered structures both in the presence and absence of a solvent, e.g. water. Branched chain glycosides with long chain length display stable columnar assemblies, which are commonly hexagonal packed. We here report on a molecular dynamics simulation study of a Guerbet-type maltoside involving a long symmetrically branched chain alcohol. A hexagonal columnar model-assembly involving 224 glycolipid and 560 water molecules with a total of almost 27,000 atoms was constructed and monitored over a period of 5 ns at 300 K using Amber. The simulation indicates stability for the hexagonal structure and classifies the water as bound water.

(Molecular dynamics, lyotropic glycolipid assembly, Guerbet glycoside, hexagonal columnar)

INTRODUCTION

Glycolipids are amphiphilic compounds comprising of a hydrophilic sugar domain and a hydrophobic alky chain region. The incompatibility of the two molecular regions leads to molecular assembly based on a microphase separation [1]. The phenomenon is commonly known for surfactant systems. While industrial surfactants comprise a single alkyl chain only, biological analogues usually involve two alkyl chains. This difference in molecular structure has impacts on the assembly behaviour [2]. Single chain surfactants usually favour lamellar assemblies, while double chain surfactants exhibit more complex phases involving lamellar, columnar and bicontinuous cubic phases depending on temperature and water concentration. Amphiphiles with long double alkyl chains are likely to form columnar phases. For Guerbet glycosides, the tendency to form columnar phases increases with increasing chain length [3].

Molecular dynamics (MD) studies on assemblies can provide insights of molecular interactions within aggregations, thus leading to an enhanced understanding of the driving forces. Besides, a comparative study of structurally related compounds [4] enables structure property correlations, which may in the future be used for material optimization based on a target assembly. Simulation studies on glycolipid assemblies so far are related to micellar [5], [6] and lamellar [4], [7] phases only (Figure 1 left and right). The latter aims for a study of cell membranes and is related to more extended simulation studies for phospholipids [8], [9]. An example for a biologic related study targets on the gating of an ion channel [10]. Columnar assemblies (centre in Figure 1) have been studied less intensely. For glycolipids in a columnar phase no MD has been reported so far. A possible application for a columnar assembly can be a grid supported molecular filtration devices, where filtration is achieved through the columnar channels of the assembly. Such a device would enable not only a separation based on size but on polarity as well (Figure 2).

Branched chain glycolipids can be easily obtained from common Guerbet alcohols, which are technical products obtained by a catalytic conversion of primary alcohols or aldehydes (Figure 3). Due to the two different chain lengths at the branching carbon, the alcohols are chiral.

Commercially available products are racemates, which upon glycosylation lead to diastereomeric glycosides (Figure 4). The diastereomeric products give rise to problems in simulation studies. Due to limited CPU power, infinitely long MD observation windows are impossible. In fact, the size of the assembly already requires the use of a multi-CPU cluster to provide results within a reasonable time. Based on the limitation of CPU time, a self assembly of molecules based on a random arrangement of molecules cannot be achieved. Instead MD studies are based on a prearranged assembly. This input configuration, however, will affect the outcome of the production. The arrangement of different diastereomers in an assembly provides a huge number of combinations, which likely will differ in energy. In order to avoid this obstacle, a symmetric Guerbet alcohol was chosen instead. This selection limits the comparison of simulation results with experimental measurements, since symmetrical Guerbet alcohols are not commercially available and require a multi-step synthesis. With respect to the increasing stability of hexagonal assemblies for long chained Guerbet glycosides, we selected the literature unknown 2-cetyl-stearyl β-maltoside (Figure 5) as our model glycoside.

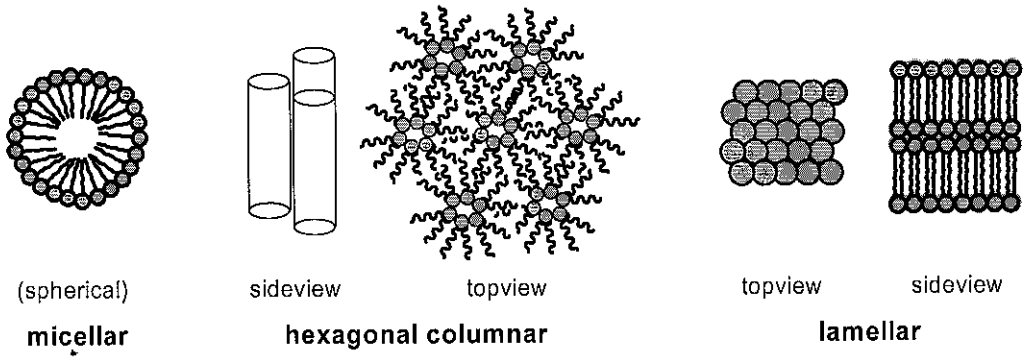

Figure 1. Surfactant assembly types

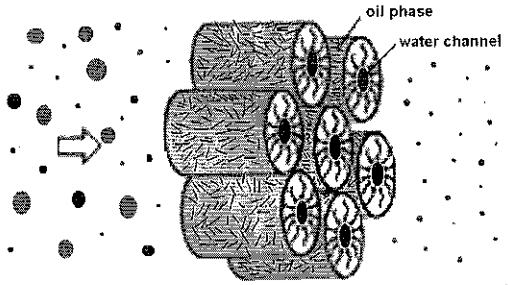

Figure 2. Hypothetical filtration device based on a columnar phase

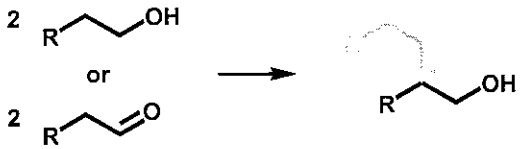

Figure 3. Guerbet reaction – a technical route to racemic branched chain alcohols

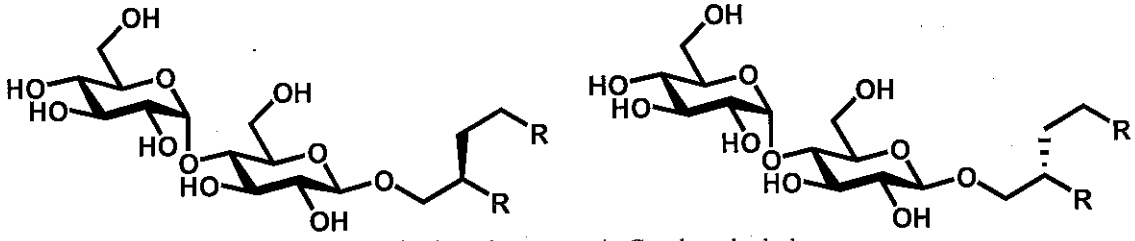

Figure 4. Diastereomeric maltosides based on racemic Guerbet alcohols

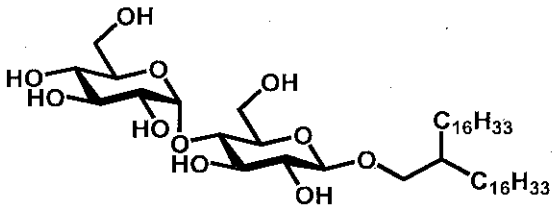

Figure 5. Model compound – 2-cetyl-steryl β-maltoside

METHODS AND MODELING

The starting conformation of the glycolipid was obtained by energy minimization using Hyperchem [11]. The molecule was transferred into an Amber readable format and assigned with all-atom Glycam 2000 force field [12]. Eight glycolipid molecules were arranged to form a channel structure based on an interdigitated two layer head to head arrangement of four molecules, as displayed in Figure 6a. Seven of these assemblies were arranged in a hexagonal lattice (Figure 6b) and the whole structure was multiplied four times in channel direction. A total of 560 molecules of water with TIP3P potential [13] were added to the carbohydrates head groups to form the starting configuration as displayed in Figure 6c. This reflects a water concentration of 5 wt%. The assembly was minimized until the energy gradient convergence criterion of 1×10^{-4} kcal/(mol Å) was met. The temperature was set to 300 K and the simulation was carried out under constant pressure (NpT) using Amber 7. First, an equilibration of 1200 ps with gradual decreasing group harmonic constraint from 500 to 0 kcal/(mol Å) was applied, followed by a 5 ns production time in 1 fs time steps. Simulation results were recorded every 1 ps, thus 1 ns trajectory contains 1000 frames. The computer facilities were provided by UM CAD-CAM Geranium CRAY cluster, comprising 16 Intel Xeon processors, and MIMOS BIOGRID cluster, comprising 16 AMD Opteron processors. A simulation of 1 ns required about 48 h to complete.

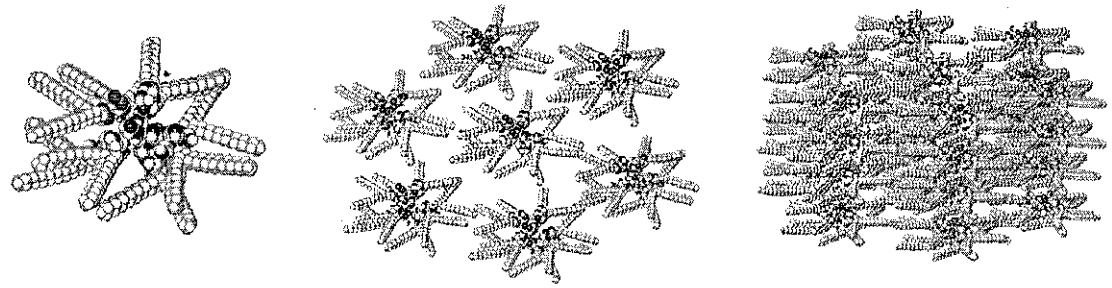

Figure 6. Model building of the assembly, (a) eight glycolipids form the channel core, (b) basis of the hexagonal lattice, (c) full glycolipid assembly containing (8×7×4)=224 glycolipids and 560 molecules of water after minimization

RESULTS AND DISCUSSION

Figure 7 displays the modelling system during the equilibration with decreasing group harmonic constraints. Within the first 800 ps the constraint was reduced from 500 to 0.01 kcal/(mol Å) (Figures 7a - d). The structure shows gradual relaxation but only minor contraction, indicating significant remaining vacuum gaps. Upon reduction of the group harmonic constraint from 10^{-2} to 10^{-3} kcal/(mol Å) (Figures 7d and e) the assembly significantly shrinks to fill remaining vacuum gaps. The fully equilibrated structure is displayed in Figure 7f, when the group harmonic constraint finally reached 0 kcal/(mol Å). During the remaining 200 ps there is no significant change to be seen.

The assembly remains intact during the whole production time. No significant changes were noticed between the input structure and the final frame. This supports the expectation of a stable hexagonal assembly for the studied formulation. During the simulation periodic near-escape events of surfactant molecules were observed, as described by Israelachvilli [14]. This results in a rough surface, with protrusion of glycolipids. Typically the glycolipid molecule adopts a surface orientation which enables movements of both, sugar domain as well as alkyl chain region.

Subsequent interaction with other molecules avoids a full escape of the molecule. While experimental results indicate escapes of surfactants from assemblies in μs scale [15], no such event was detected over the production time.

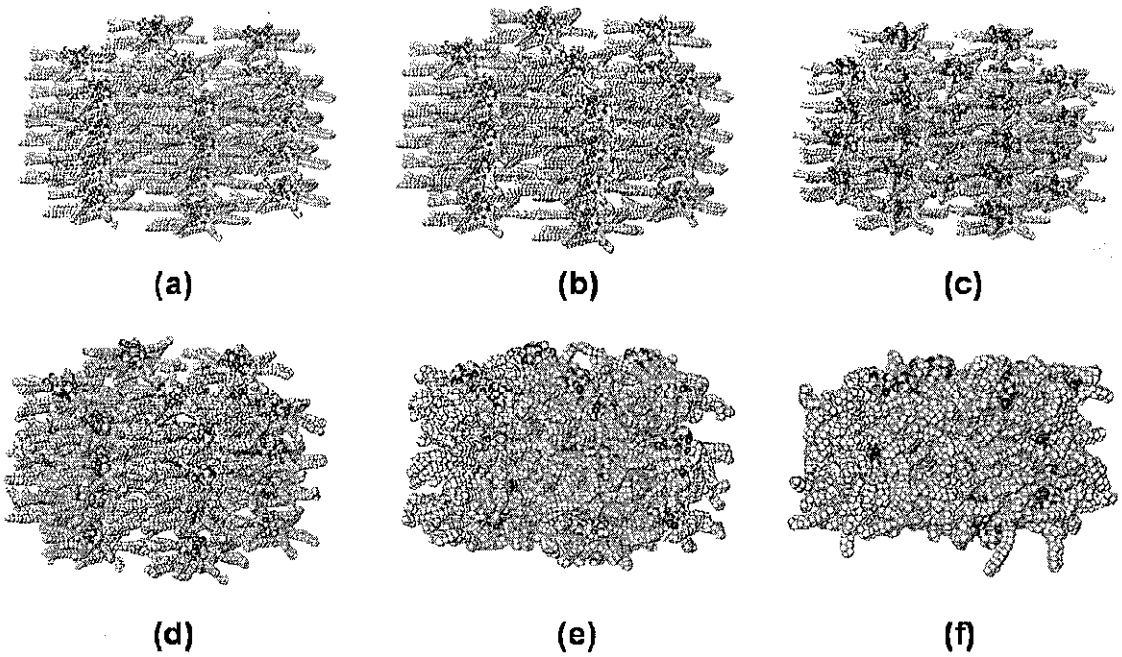

Figure 7. Snapshots of the modelling system during the equilibration; (a) 100 ps, (b) 200 ps, (c) 700 ps, (d) 800 ps, (e) 900 ps, (f) 1000 ps (black = oxygen, grey = carbon, white = hydrogen)

The local density profile (LDP) for the surfactant regions and the water is displayed in Figure 8. With respect to the geometry of the assembly, the LDP was determined in a 2D radial mode from the centre of the hexagonal assembly. The remaining third dimension, i.e. the direction of the channels, is insignificant for the LDP and was ignored. While the shape of the LDP matches the expectations, i.e. alternating maxima and minima for the polar and non-polar molecular regions, the densities are significantly lower than expected. The polar region, comprising of sugar domain and water is expected to display a density around 1.5 g/mL, while the density of alkyl chain region should be around 0.8 g/mL. The determined values of ~ 0.95 g/mL for the polar domain and ~ 0.55 g/mL for the paraffin domain are about 35% lower. This discrepancy may partially be due to the rough surface of the assembly, which leads to an overestimation of assembly volume. However, the deviation seems too high to be completely explained this way. Analyses on lamellar assembly simulations [4], which also led to an underestimation of densities, suggest a possible systematic error.

Due to the radial overlay of polar and non-polar regions for the non-central channels, the LDP cannot be applied to prove the complete separation of the domains. Figure 9, which only displays the alkyl chains of the assembly, provides a good visualization of the separation. Correspondingly Figures 10a and b show the polar region only. The polar region comprises of sugar domain and water. In order to analyze the distribution of both, Figure 10c only shows the water molecules. From the images in Figure 10 it can clearly be deduced, that water is attached to the sugar domains rather than forming a water channel in between them. However, the attachment of the water to the sugar does not provide any information regarding the possibility of water molecules to migrate within the sugar-water channels. In order to determine, whether the water is sugar bound or freely moving inside the polar domains, we monitored the movement of water molecules inside the assembly over the production time. The results are summarized in Table 1.

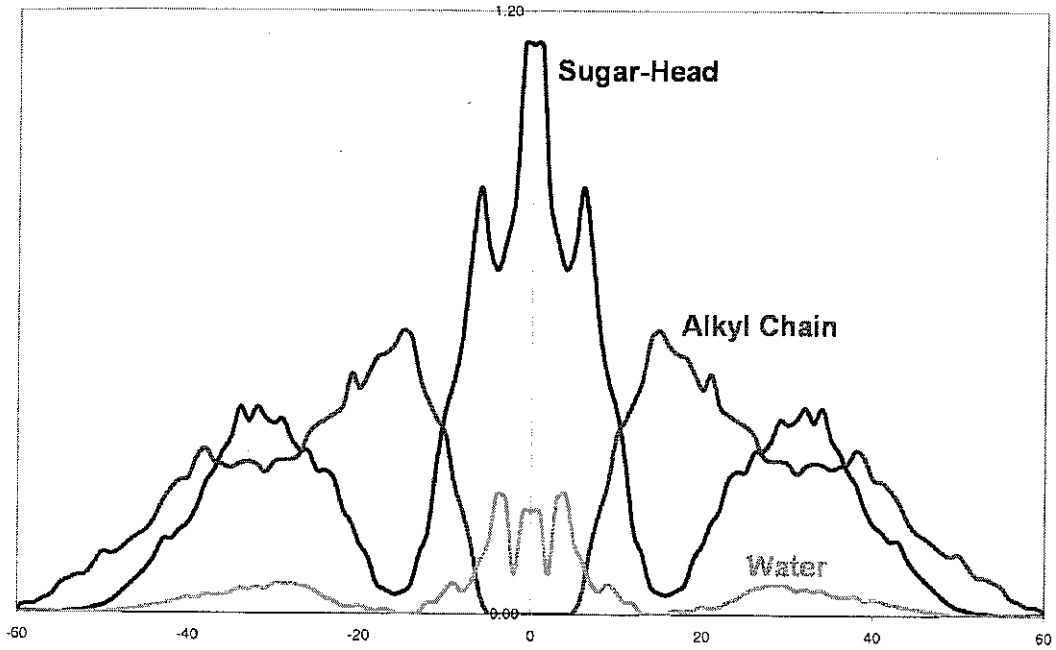

Figure 8. Local density profile for final frame

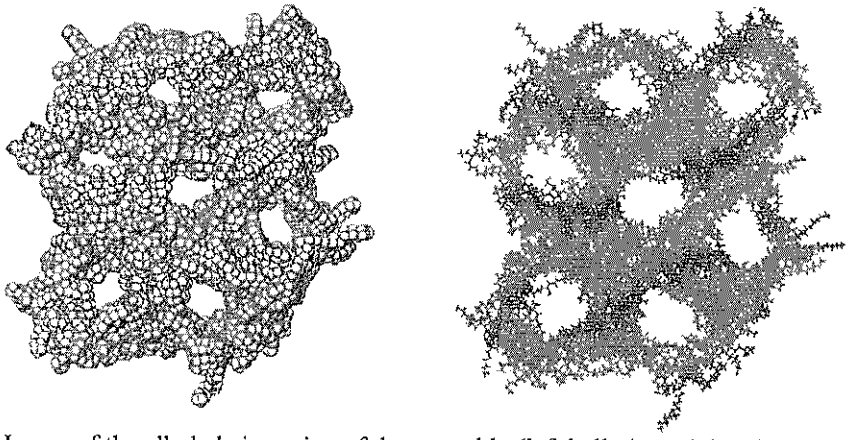

Figure 9. Image of the alkyl chain region of the assembly (left ball view, right wire view)

Figure 10. Image of the polar region of the assembly, (a) sugar-domain and water (top ball view), (b) sugar-domain and water (side wire view), (c) water only

Table 1. Water mobility analysis

Δt [ns]	Δz [Å] CHANNEL DIRECTION		Δ(x,y) [Å] RADIAL TO CHANNEL	
	AVERAGE	MAX	AVERAGE	MAX
1	1.4 ± 1.6	10.7	2.2 ± 1.8	9.9
2	1.9 ± 1.9	12.9	2.8 ± 2.2	15.4
3	2.2 ± 2.2	12.5	3.2 ± 2.3	12.9
4	2.4 ± 2.4	14.7	3.6 ± 2.6	14.5

Table 1 clearly indicates the water as bound. The mobility of water is negligible and, further more, does not show any preferred direction, as would be expected due to the radial movement restriction set by the diameter of the polar region channel. Conclusively a columnar phase comprising the glycolipid under investigation containing a water content of up to 5% cannot be considered for filtration purposes as displayed in Figure 2.

The shape of the polar channels, as displayed in Figure 9, is not circular but slightly ellipsoidal. Geometric analysis of the two ellipse axes leads to a ratio of about 5:6. This coincides with a distortion of the cylinder assembly from a regular hexagonal shape, with a channel distance ratio of about 4:5 in different directions. The deviations from circular and hexagonal geometry may be taken as an indication of a non-optimized assembly. Due to the time restriction for the molecular dynamics, the global minimum of the assembly may not be reached, if the input configuration deviates too much. A non regular hexagonal cylinder assembly results in more complex scattering spectra due to different repeating distances. However, experimental SAXS measurements of shorter branched chained maltosides [3] only show regular hexagonal patterns. The axial ratio of the ellipsoid suggests, that either the number of glycolipids in the basis unit of the channel should be reduced, or the water content be (significantly) increased in order to match the energy minimum. The simulated structure is, therefore, expected to deviate from experimental results.

The simulation indicates a diameter of 20 ± 2 Å for the polar domain cylinders and an average d-spacing of 36 ± 1 Å between neighboured columns. The values are stable throughout the whole simulation. Since the model compound has not been investigated experimentally so far, comparisons with experimental data are restricted to similar homologues, in this case a Guerbet β-maltoside with a C_{24} carbon chain ($d_{160°C}$ = 34.2 Å [3]). With respect to differences in alkyl chain length and temperature some extrapolations are required. The experimental d-spacing is converted into the cylinder distance according to:

$$x = \frac{2}{\sqrt{3}} \times d = 41.1\,\text{Å} \qquad (1)$$

The polar column diameter is determined based on the volume fraction of the polar domain and a regular hexagon unit cell, which surface area can be calculated according to:

$$A = \frac{3}{2} \times \sqrt{3} \times \tan^2(30°) \times x^2$$
$$= 2 \times \sqrt{3} \times \tan^2(30°) \times d^2$$
$$= 1463\ \text{Å}^2 \qquad (2)$$

The calculation assumes independent densities for the polar and non-polar region. Reference densities are based on maltose (1.5 g/mL) and Guerbet alcohols (0.8 g/mL). Thermal expansion coefficients are based on literature value for aqueous sucrose solutions [16] and paraffins [17]. Calculations are summarized in Table 2.

Table 2. Determination of volume fractions for a C_{24} maltoside

DOMAIN	FORMULA	MASS [AU]	DENSITY [g/mL]		V = M/D [mL/mol]		VOLUME FRACTION	
			20 °C	160 °C	20 °C	160 °C	20 °C	160 °C
Sugar	$C_{12}H_{21}O_{11}$	341	1.5	1.42	227	240	35%	32%
Parafin	$C_{24}H_{49}$	337	0.8	0.62	421	544	65%	68%
	$C_{34}H_{69}$	477	0.8	-	596	-	-	-

With the volume fraction and the unit cell area at hand, the area of the polar domain can be calculated as:

$$A_{polar} = V\%_{\mathbf{polar}} \times A \quad = 468\ Å^2 \qquad (3)$$

leading to a column diameter according to:

$$2r = \sqrt{\frac{A_{polar}}{\pi}} = 24.4\ Å \qquad (4)$$

For extrapolation of the experimental data on a C_{34} chain at room temperature, a volume correction of the non-polar region is required. The volumes for a C_{34} chain at 20°C and a C_{24} chain at 160°C in Table 2 lead to a correction factor of 1.16. This correction factor applies for a 3D-analysis. With respect to our 2-dimensional analysis approach the value has to be adjusted according to:

$$(1.16)^{2/3} = 1.08. \qquad (5)$$

The column distance for a C_{34} maltoside based on extrapolation of experimental data is hence 44 Å, or 43 Å, if the contraction of the sugar domain due to the lower temperature is considered as well. Both values clearly exceed the simulation based result of 36 ± 1 Å. The simulation based value of the diameter of the polar domain, i.e. 20 ± 2 Å, however, matches reasonably good with the estimation of 24 Å, especially, if a temperature contraction of ~1 Å is considered. Based on these results, we conclude that the simulated structure requires corrections, but provides a reasonable first impression on the assembly of a C_{34} Guerbet maltoside.

CONCLUSION AND OUTLOOK

The simulation suggests a stable hexagonal-columnar assembly for a 5% water formulation of 2-cetyl-stearyl β-maltoside and determines the water as bound, i.e. stationary. The geometric specifications of the modelled assembly most likely require adjustments in order to match experimental results. This conclusion is based both on the non-circular but ellipsoidal shape of the hydrophilic channel cross, which indicates a non-optimal packing, as well as on comparison with extrapolated experimental results. More extensive molecular dynamics should be preformed under periodic boundary conditions to enable a proper correlation of refraction patterns with experimental small angle X-ray scattering (SAXS) results, which also requires prior synthesis of the symmetric Guerbet glycoside.

ACKNOWLEDGEMENTS

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