Mean and Gaussian Curvature of Lipid Mesophases measured using molecular dynamics

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Abstract

The ability to measure the mean and Gaussian curvature in lipid mesophases is important in our understanding of their formation and properties, and can be achieved both experimentally and computationally. Here we show that curvature can be measured using Molecular Dynamics simulations, however the reliability of the results is highly dependent on the choice of fitting algorithm, the atoms within the lipid membrane that are selected for fitting and the number of atoms that are included in the fit. We compare the results of our method to the $\text{L}_\alpha$ mesophase as previously studied, and subsequently extend the method to the $Q_{II}^D$ and $H_{II}$ mesophase, not previously studied in this way computationally, but whose curvatures are known both analytically and experimentally. By systematically comparing our results, we demonstrate a robust method which can be used in general to measure curvature.

Graphical TOC Entry

Keywords

Coarse Grained Molecular Dynamics, Membrane Curvature
Membrane curvature is a fundamental property of membrane systems, and is known to play a role in membrane fusion, membrane protein activity, and lateral membrane organisation. The curvature of membranes arises from the global restraint of molecular composition, and is a key factor when considering the elasticity of membranes. The mean (H) and Gaussian (K) curvatures of a surface are defined by the mean and product of the principal curvatures respectively. The Helfrich Hamiltonian, describing the energy density of membranes, is dependent on both measures, indicating that the dynamic behaviour - of both model and real membrane systems - is significantly influenced by the underlying geometry of the surface around which the lipid bilayer is formed.

Lipid mesophases may be characterised by their mean and Gaussian curvatures, from the flat planar lamellar ($L_\alpha$), to the highly curved inverse hexagonal ($H_{II}$), where cylindrical water channels are arranged in a hexagonal pattern. One of the most studied non-planar mesophases is the Diamond cubic phase ($Q^{D}_{II}$), based on triply periodic minimal surfaces, mathematical surfaces defined by zero mean curvature, and negative Gaussian curvature. $L_\alpha$ mesophases have been extensively studied in silico for their physiological importance.

Non-lamellar mesophases, on the other hand, have been the subject of fewer computational studies, especially in comparison to experimental studies where they have been studied extensively, particularly using small angle X-ray scattering techniques to investigate the nature of mesophase transitions. However, in recent years, there has been increased interest in the simulation of non-lamellar lipid mesophases, driven by the need to understand their role in processes such as drug delivery, templating, and membrane protein crystallisation.

For sufficiently large and simple systems, curvatures can be calculated directly, for example using microscopy or dynamic light scattering. For a spherical liposome, the principal curvatures are identical, and the curvature is simply the reciprocal of the radius experimentally measured. However, for mesophases with more complex topologies, such as the inverse bicontinuous cubic phases, curvatures are dependent on the dimensions of the unit cell.
Figure 1: The coarse-grained Martini lipids used a) Monoolein after Johner et al., b) DOPE, and c) DPPC. d) A 2D sketch of the method used. A search is carried out on a selected green atom (with a red outline) for surrounding atoms of identical type at two different radii, $r_1$ (blue circle) and $r_2$ (orange circle), to create a point cloud. After finding the principal axes of the point clouds, the atoms found are fitted to Equation 1, the results of which are shown as two curves in the corresponding colours. Here, the green atoms could represent terminal molecule atoms, and the purple atoms (not used to form the point clouds), the atoms further towards the headgroups on either side of the bilayer.

Measurements of membrane curvature from simulated lipid systems have been limited, with the most significant method posited by Yesylevskyy and Ramseyer, who fitted the generic quadric surface described by:

$$z = Px^2 + Qy^2 + Rxy + Sx + Ty + C$$  \hspace{1cm} (1)$$

where $\{x, y, z\}$ are an orthogonal coordinate system and $\{P, Q, R, S, T, C\}$ are parameters to be optimised, to point clouds of coarse-grained lipid atoms at positions in a simple system. This is illustrated in 2d in Figure 1d), where point clouds are generated by a particular cutoff radius, transformed according to their principal components, and fitted. Considering the generality of Equation 1 this method should be able to measure the curvatures of any lipid mesophase, and measure even local variations within them. Here, we further explore the method by evaluating its performance on two lipid mesophases with significant non-zero
curvatures, the $H_{II}$ and the $Q^{D}_{II}$. As detailed in the methods and SI, the $Q^{D}_{II}$ and the $H_{II}$ are formed spontaneously in water by monoolein (MO) and dioleoylphosphoethanolamine (DOPE) respectively. Coarse grained Martini models of these are shown in Figure 1a) and b). We also studied the $L_\alpha$ mesophase, with both mean and Gaussian curvatures equal to zero, formed by dipalmitoylphosphatidylcholine (DPPC) (Figure 1c)). Additionally, we have explored how the measurements of curvatures using Equation 1 vary between fitting methods as implemented in the Python library SciPy. SciPy is a widely used scientific computing library in Python, which has an array of function optimisation routines. These range from the local Least Squares optimisation, to global optimisation algorithms such as Differential Evolution.

In the work where Yesylevskyy and Ramseyer first fitted Equation 1 to point clouds of lipid atoms, the Least Squares method was used. Here, we chose to investigate the effect that the choice of fitting algorithm has by comparing the results of 5 optimisation functions. The functions were Least Squares, Minimize, Differential Evolution, Simplicial Homology Global Optimization (SHGO), and Dual Annealing. As the $L_\alpha$ mesophase is based on a flat plane, the results should show a distribution around 0 for both the mean and Gaussian curvature measures, by any measure of the curvature. Ideally, the surface fitted by each method would be identical, and the curvatures calculated would match exactly in every case.

An example of the outcome of comparing these results in an $L_\alpha$ mesophase is seen in Figure 2 where we show the results for Gaussian and mean curvatures compared for the Differential Evolution and SHGO close to 0. For a full comparison of all the fitting methods examined here, we have plotted every pair plot comparing methods in Figure S1 of the SI. In this region, there is a very high agreement between the results, as shown by the high $R^2$ values in Figure 2c) and d). Additionally, for the measures by SHGO there is a sharp peak centered close to 0 for both curvatures. In comparison, the distributions of Gaussian curvature measured by Differential Evolution is much broader, and for the mean curvatures,
Figure 2: How the measures of Gaussian (a, c) and mean (b, d) curvature varies between two optimisation methods (Differential Evolution, and Simplicial Homology Global Optimization) on an $L_\alpha$ mesophase at a cutoff radius of 10 Å. a) the distribution of Gaussian curvature between the two measures. The median values of the distributions are $1.07 \times 10^{-2}$ and $9.85 \times 10^{-3}$ for Differential Evolution and SHGO respectively. b) the distributions of mean curvature according to the different methods. The medians for Differential Evolution and SHGO are $2.02 \times 10^{-2}$ and $1.81 \times 10^{-2}$ respectively. c) and d) show the correlation between the measurements of Gaussian and mean curvature respectively. The distributions have $R^2$ values of 0.66 and 0.92 respectively.
there is not a clear peak. As Figures S1 and S2 of the SI further demonstrate, the agreement between these two global optimisation methods is the highest among any of the possible comparison. In contrast, Least Squares fitting has a very poor level of agreement with any other method.

In Figure 3, we show that the choice of fitting method further effects the length of time of the calculation, the value of the objective function, and the termination tolerance status of the routine. Figure 3 used 1253 fits to point clouds in a single frame from a simulation of 4 Lα unit cells. Considering these factors, SHGO was determined to be the best method for the fit. Compared to the Least Squares method, it always terminated fits within its tolerance, with a smaller valued objective function, and in far faster timescales. Furthermore, as we show in both Figure 2 and S1 & S2 of the SI, the median value of the mean and Gaussian curvatures in the Lα mesophase using SHGO is the closest to zero.

Having established that SHGO was the optimal method for fitting Equation 1 we investigated the effect of varying the location in the membrane to measure curvature at an
Figure 4: The distributions of (a-c) Gaussian and (d-f) mean curvatures in the $L_\alpha$ (a, d), $Q_{II}^D$ (b, e), and $H_{II}$ (c, f) mesophases, with the surface described in Equation 1 fitted at different locations in the membrane. For every fitting routine, the cutoff radius for point cloud formation was 40 Å. Dotted lines in f) indicate the position of the expected peak in mean curvature based on measurements of the radius of the C1A cylinder in the simulation frames. Mean curvature is distributed about 0 as a feature of sign convention; negative mean curvature has no particular physical significance. The labels C1AB and C4AB indicate fits to point clouds formed of both the C1A and C1B atoms indicated in the molecule models in Figure 1.
arbitrary cutoff radius of 40 Å, the results of which are seen in Figure 4. For the Lα and HII trajectories, curvatures were measured at the initial and terminal carbons. For measurements of curvature in the QDII simulation, we measured curvature through independent point clouds of all 5 carbon atoms. Additionally, we trialled the method of Yesylevskyy and Ramseyer in taking the positions of both the terminal carbons and headgroups, to fit a surface inbetween them.

In Figure 4, we firstly note the proportion of measured Gaussian curvature data which has been fitted in the range seen within the figure. This necessarily arises from comparison with the calibration for the Gaussian curvature in the QDII mesophase, where the distribution is known from the size of the simulated cell. While the proportion included in the measurements for the Lα and HII mesophases is close to 100%, there is significant variation in the QDII measurements. The proportion fitted within the expected range is far greater when point clouds of atoms closer towards the centre of the bilayer are used. This data is itself plotted for two cutoff radii in Figures S4 & S5 of the SI, in which we also show that at shorter cutoff lengths, the proportion included in the expected range for the QDII mesophase measurements is significantly reduced.

In the Lα and HII mesophases, the Gaussian curvature is expected to be 0. The mean curvature of the Lα mesophase is also 0, but in the HII phase it is non-zero, as it is measured at the (cylindrical) pivotal plane. For an HII mesophase, the pivotal plane is located around the C1A/C1B atom location in the system. The results presented in Figure 4 show that while Gaussian curvature is distributed around 0 as expected regardless of the location in the membrane it is measured, there is a more significant variation in the measurements of mean curvature. This is particularly evident in the case of the Lα mesophase, where taking the C1A/C1B beads of the system has resulted in a skewed distribution. However, for the HII mesophase, the measurement of mean curvature shows a peak close to the expected magnitude.

For measurements of the Lα system, the discrepancy in mean curvature measurements
is likely due to the chosen cutoff of 40 Å in Figure 4. This cutoff radius is larger than the bilayer thickness, which results in both sides of the bilayer being captured to fit, and so a non-planar fit is usually found, for example in Figure S6 of the SI. Indeed, as seen in Figure S7 of the SI, at smaller cutoff radii, the measurement of mean curvature in the Lα is averaged around 0, albeit with a comparatively wide distribution of values.

In comparison, the measurements of mean curvature in the QDII mesophase are distributed around 0 regardless of the location within the membrane to fit. The QDII surface is defined by the property of having zero mean curvature, so in this regard, the measurement is as expected. The measurement of Gaussian curvature in the QDII mesophase varies far more significantly. The location of the neutral plane in cubic phases is expected to be close to be close to the polar/apolar interface of the bilayer, and so one might reasonably expect the measurements closest to there to most closely resemble the curvature of the QDII minimal surface. However, our measurements in Figure 4 b) indicate that the opposite is true, and, while no measurement conditions perfectly resemble the theoretical distribution of Gaussian curvature, taking point clouds of terminal carbon atoms closely resembles it, with the vast majority of the data within the expected range for the unit cell size.

Following the technique of Yesylevskyy and Ramseyer, we additionally measured Gaussian curvature in the QDII phase using point clouds formed of both the terminal carbon C5A atom and the GL1 headgroup atom together. As Figure 4 b) shows, this is perhaps the poorest measure of any. In this case, the first stage of the fitting routine often does not identify the principal axes of the point cloud correctly, and so Equation 4 cannot be fitted correctly to it (for example, see Figure S8 of the SI).

Given that curvatures vary widely with the location in the membrane where they are measured, we further systematically examined the effect of how the results vary with the cutoff radius used, which we show in Figure 5. As might reasonably be expected, the results for the Lα mesophase show that at the cutoff radius increases, and the number of terminal carbons fitted to increases, both the mean and Gaussian curvatures become increasingly
Figure 5: The (a-c) Gaussian and (d-f) mean curvature distributions at varying point cloud cutoff radii in the $L_\alpha$ (a,d), $H_{II}$ (b,e), and $Q_{II}^D$ mesophases. In each case, Equation 1 has fitted to point clouds of the optimum fitting atoms as determined by the analysis in Figure 4. These were the C4A & C4B, C5A, and C1A & C1B atoms for the $L_\alpha$, $H_{II}$, and $Q_{II}^D$ mesophases respectively. Black dotted lines in f) indicate the fitted radius of the cylinder formed by the C1A and C1B atoms.
tightly distributed around the expected mean of 0. Although the same expected pattern is observed for the mean curvatures in the \(H_{II}\) mesophase, the variation in the Gaussian curvature is far more significant. At both small and large cutoff radii, the measurements are distributed around 0, without the peaks at the appropriate values for the radius of the C1A, C1B cylinder indicated by the dashed lines. The fact that these peaks only emerge at intermediate cutoff radii indicates that at smaller and larger values, the surface being fitted becomes planar. At a cutoff radius of 40 Å, the peaks emerge almost exactly at the expected value of H.

In comparison, the distribution of mean curvature in the \(Q_{II}^D\) mesophase is very consistently a sharp peak around 0 irrespective of the cutoff radius. However more than any other, Figure 5 b) demonstrates that careful consideration needs to be given to the cutoff radius used to measure curvatures. While a cutoff of 40 Å has a peak at more negative K as expected, at more extreme cutoffs, the peak completely disappears. As when varying the location in the membrane for the measurements, we also note that the changing the cutoff radius has a significant effect in how much data fall within the expected range not just in the \(Q_{II}^D\) mesophase, but also in the \(L_\alpha\) and \(H_{II}\) in Figure S9 of the SI.

Overall, the results presented here show that measuring curvature in simulated lipid mesophases is far from a trivial task. We have demonstrated here that the optimisation method, membrane location, and fitting cutoff radius all have significant effects on the resultant measurement of mean and Gaussian curvature in simulated lipid mesophases. In particular, for non-planar bilayer mesophases, there is significant variation in resultant curvature distributions when the parameters are chosen incorrectly. While we have found that the optimal measurement of the curvature is not necessarily at the neutral surface of the membrane, the data acquired through the measurement process is sufficient to find the surface in corroboration with experimental data to further molecular dynamic calculations for the physical properties of lipid mesophases. Accurate measures of curvature distributions using the method outlined in this work will enable measures of curvatures across whole sim-
ulations, and across different mesophases. This will enable further physical characteristics - such as the response of different lipids to curvature - to be probed in a way not yet studied.

Methods

Three mesophases were simulated using the coarse-grained Martini force field version 2.1, using the standard simulation parameters for version 2, in Gromacs 2018. The simulations were carried out at a pressure of 1 bar and temperature of 300 K, for more details about the water ratios used, see Table 1 in the SI. For the Q\textsubscript{I\textsubscript{II}} mesophase, an initial equilibration simulation to ensure a well-mixed initial state before self-assembly, after the established method.\textsuperscript{20,39} The final frame of a short initial (150ns) self-assembly simulation were periodically replicated using Ovito, and simulated for a further 1 µs (L\textsubscript{\alpha}, H\textsubscript{II}) or 1 ns (Q\textsubscript{I\textsubscript{II}}).\textsuperscript{20} To compare the effectiveness and agreement between fitting methods, the final frame of the initial (single unit cell) simulation was used. Subsequently, curvatures were measured and averaged from frames of the expanded cell simulation, systematically varying the cutoff radius and measurement location. Details of the lipid/water ratios are in S1 of the SI. The reference Gaussian curvature of the cubic phase was calculated from the fitting of the implicit equation for the nodal approximation of the Diamond minimal surface, and using the parameters of the fitted surface after the method of Goldman.\textsuperscript{11,20,41}

The fitting code is available for open source use at https://github.com/csbrasnett/lipid-md

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Data used available on request.

Supporting Information Available

Simulation details, full method comparison pair-plots, 10 Å cutoff location variation, and data included plots, along with examples of common fitting errors, are shown in the SI.

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