# CHOLESTROL CONCENTRATION EFFECT ON BILAYER MEMBRANES AND ITS ROLE IN DESIGNING EFFICIENT LIPOSOMLAL DRUG DELIVERY SYSTEMS

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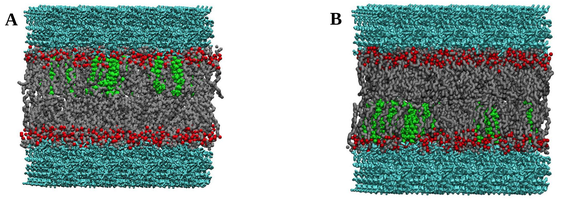
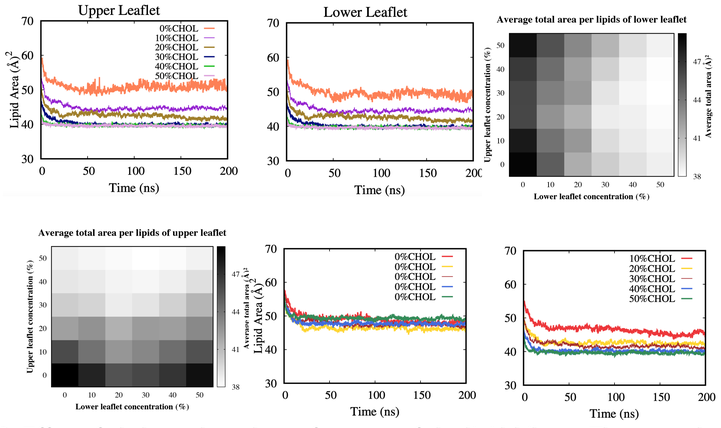
## INTRODUCTION

- Lipid bilayers are fascinating materials because of their unusual physical properties and their role as the foundation of biological membranes. The behavior of pure bilayers and mixed bilayers continues to be an important topic of ongoing research. Cellular membranes can contain dozens of different types of lipids, which give rise to complex spatial organization and lipid-specific effects on membrane proteins. This chemical diversity, however, makes it difficult to systematically investigate the physical effects that emerge when different types of lipids are mixed in a single bilayer. The study of model membrane systems that contain only a small number of lipid types has therefore been a fruitful approach to investigate mixed bilayers.
- Liposomal drug delivery systems provide a versatile therapeutic platform for treating numerous diseases. Cholesterol, as a common component of many liposomal drug delivery systems, plays a crucial role in enhancing mechanical strength and decreasing the permeability of biomembranes. However, the ratio required for a proper formulation is poorly recognized. In this study, all-atom molecular dynamics simulations are performed to characterize the effect of cholesterol concentration in bilayers. Furthermore, it has been quantified how a range of bilayer properties, including area per lipid, leaflet interdigitation, membrane thickness, and lipid Scd order parameters, are altered by variation in concentrations of lipid and cholesterol. Therefore, the most suitable amount of cholesterol and lipid to prepare stable and controlled drug release vehicles is obtained by screening the lipid and cholesterol ratio arrangement. These results provide a better understanding of the fundamental characteristics of the structure and dynamics of cholesterol-containing membranes.
- By revealing molecular details of interactions between cholesterol and phospholipids, these simulations accurately represent atomic-level characteristics of the planar and spherical bilayers. The simulations presented here shed light on the workings and limitations of liposomal drug delivery technology and establish a computational framework for the rational design of liposomes in drug delivery systems. This study contributes to the overall effort to understand the structural and dynamical phase behavior of phospholipid bilayers with cholesterol.

## RESULTS

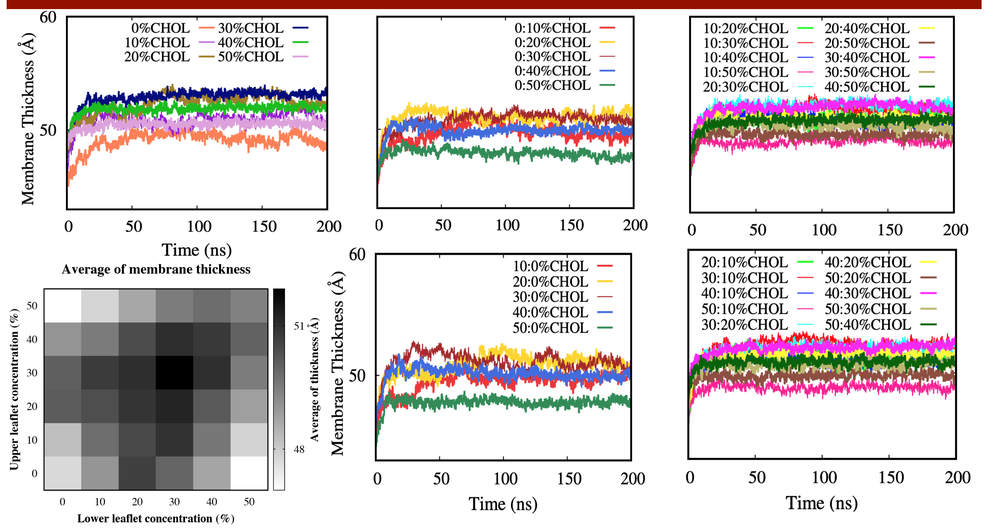



**1. Snapshots of the DSPC bilayers at 310 K with 20% cholesterol at the upper (A) and lower (B) leaflets.**

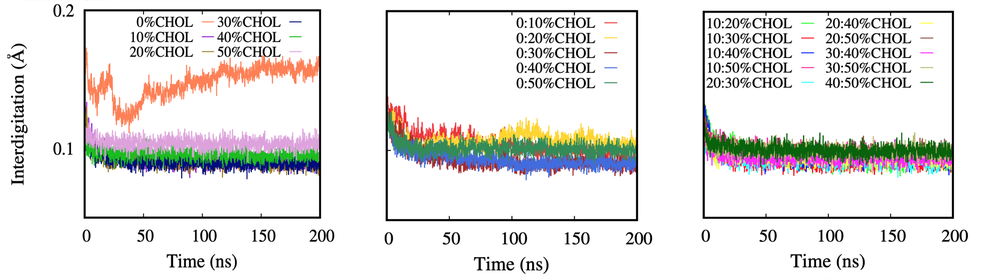



**2. Effect of cholesterol on the surface area of the lipid bilayer. The exposed surface area of DSPC phospholipids is reduced from 0 to 50 mol % cholesterol, corresponding to condensation of the phospholipids and tighter lipid packing. We find a reversal of these effects, observing large decreases in phospholipid surface area for DOPC phospholipids at 20 mol % cholesterol in heterogeneous bilayers. Thus, the large increases in surface area observed at high cholesterol concentrations could be due to a reduction in membrane condensation, or to an increase in exposed headgroup conformations, or a combination of both.**

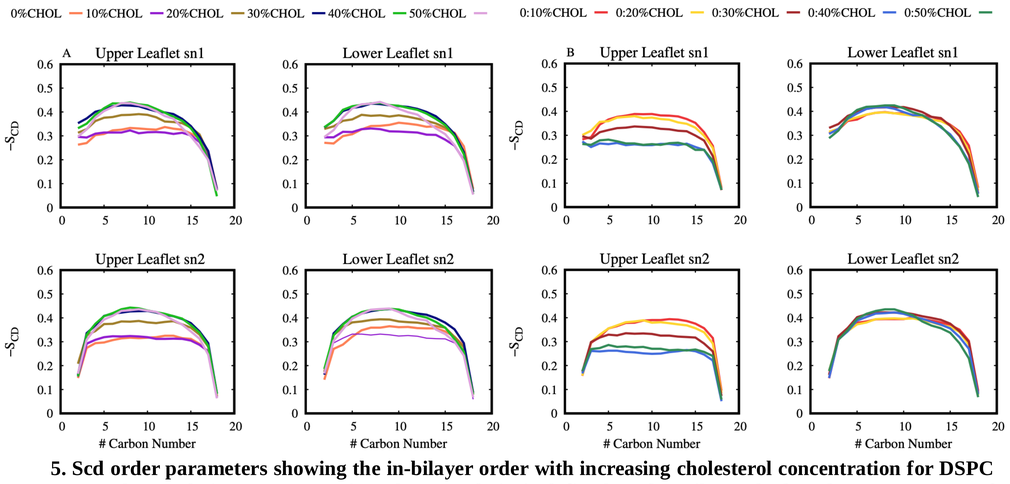
## RESULTS




**3.Membrane thickness of lipid bilayers. This increase corresponds to tighter lateral packing, which is indicative of membrane condensation. Above 30 mol % cholesterol, membrane thickness plateaus and then decreases, indicating looser lateral packing of lipids and a partial decondensation or relaxation of the membrane structure**




**4. Interdigitation of membrane bilayers. As shown ,we find that interdigitation initially decreases with increasing cholesterol content but stays essentially flat for $\geq 10\%$. This suggests that increased interdigitation is not the reason for the slight decrease in bilayer thickness at high cholesterol concentrations.**




**5. Scd order parameters showing the in-bilayer order with increasing cholesterol concentration for DSPC sn-1 and sn-2 chains. By convention, the sn-2 chain is defined as the tail attached to the oxygen atom of the second carbon of the glycerol group; while the other tail is denoted as the sn-1 chain. Compared to membranes without cholesterol, cholesterol in the model bilayers increases chain order in bilayers with the highest order.**

## METHODS

Twenty-one membrane models were developed to study the impact of interactions between cholesterol on bilayer structure. To prepare liposomes, we simulate DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine) phospholipid combined with different molar ratios of cholesterol (100, 80–20, 70–30, 60–40, and 50–50%) in planar bilayer. Each simulated model was built using CHARMM- GUI Membrane Builder, contained 100 lipids evenly distributed in two leaflets with neutralizing ions, and was fully hydrated using the TIP3P model for water. All models were equilibrated on CHARMM using the standard six-step CHARMM-GUI protocol for 225 ps and periodic boundary conditions. The first two steps were run using NVT (constant particle number, volume, and temperature) dynamics, and the remaining four using the NPT (constant particle number, pressure, and temperature) ensemble to slowly reduce various restraints on the system. NAMD software package was used to run 200-ns trajectories of each model to ensure proper equilibration. We ran three replicates of each model with the CHARMM36 lipid force field, which includes the most updated and accurate parameters for PI lipids and sterols needed for this study. Two sets of independent simulations were performed for the DSPC/Cholesterol system..

## CONCLUSION

Cholesterol molecules have a critical role in biological membrane properties and functionality, resulting from their specific structural features. The main functional elements of this molecule are the rigid and planar ring system, the small hydrophilic hydroxyl group, the short flexible tail, and the asymmetric nature due to the presence of the smooth side and the rough side, which includes the methyl groups, as well as the respective existence and the position of double bonds in the ring system and the tail.

- Our study of intermediate cholesterol concentrations in bilayers highlights the importance of interactions between cholesterol and acyl chains in a heterogeneous bilayer.
- Cholesterol could alter the structure and dynamics in phospholipid bilayers.
- Cholesterol concentration has the same effect as increasing chain order and membrane thickness. These results serve both to improve our knowledge base of membrane structure and dynamics and as a further addition to the data set necessary for continuing to advance the accuracy and utility of MD simulation.

Future work will continue to build upon this basis by adding more components to the bilayer system and curving lipid membranes and their interplay with membrane-associated proteins, with the eventual goal of being able to simulate bilayers closer to physiological composition than those of the models presented here for the most in-depth look at membranes possible.

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