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## Supplemental Information

# Regimes of Complex Lipid Bilayer Phases Induced by Cholesterol Concentration in MD Simulation

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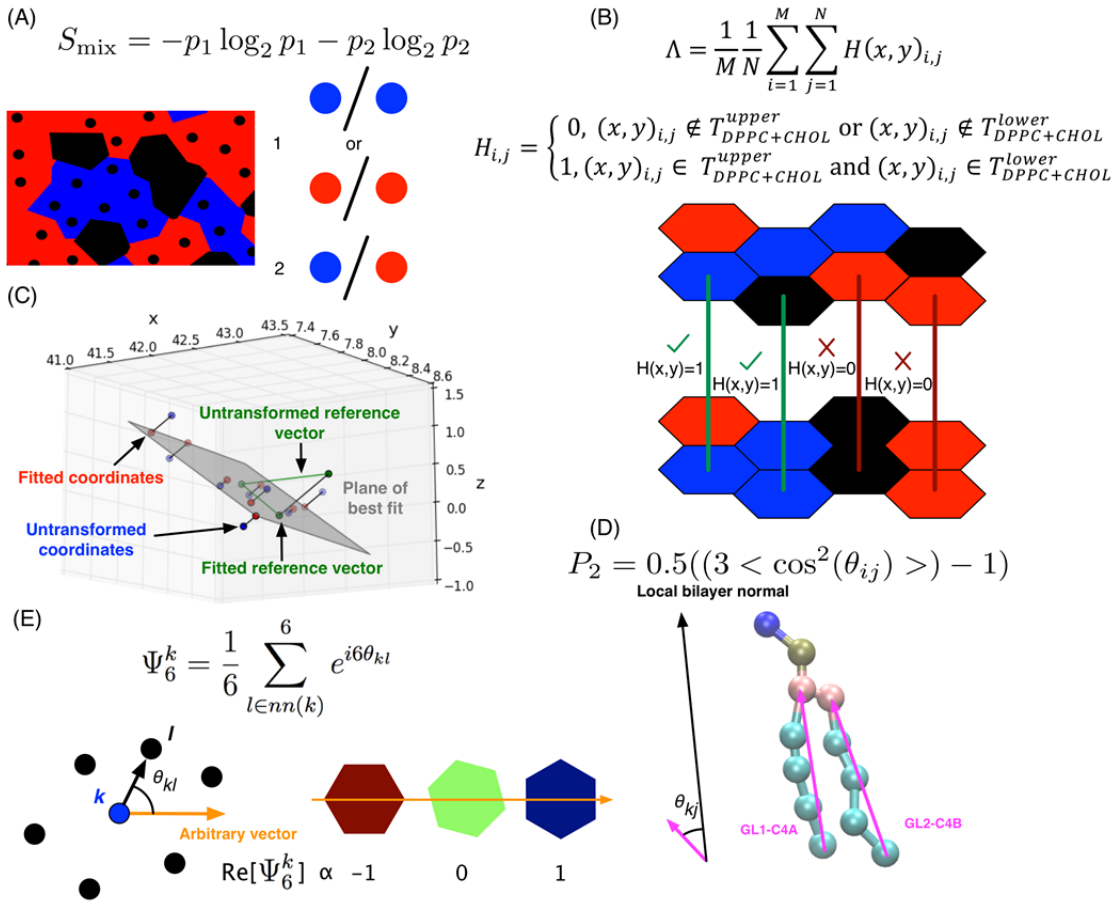


Figure S1. Visual explanation of (A) mixing entropy and (B) domain overlap measurement. Example Voronoi tessellations show DPPC (blue), DIPC (red), and Chol (black). (C) lipid plane-fitting used to determine local director vector used in computing (D) $P_2$ and (E) $\Psi_6^k$ order parameters.

## Relation of Mixing Entropy to 50% Miscibility

Miscibility temperatures and compositions drawn on most ternary lipid phase diagrams (the spinodal) typically represent the point at which 50% of the maximum signal coming from the labelled lipid type is present. We relate this 50% miscibility point to our mixing entropy, which also only considers the two lipid species.

Consider the mixing entropy of a perfectly immiscible system of *N* lipids where the experimental signal would be maximum. Between the two domains on a 2D hexagonal lattice with square periodic boundary conditions, there are two linear interfaces of $\sqrt{N}$ lipids. Each of these interfacial lipids shares 2/3 of its edges with lipids of the same type, at each interface contributing $\frac{2}{3}\sqrt{N}/N$ to $p_1$ and 1/3 of its edges with lipids of the opposite type, contributing $\frac{1}{3}\sqrt{N}/N$ to $p_1$, making the $p_1 = (N - 2\sqrt{N} + 2\frac{2}{3}\sqrt{N})/N$ and the $p_2 = (2\frac{1}{3}\sqrt{N})/N$. This system is

illustrated in Figure S2.

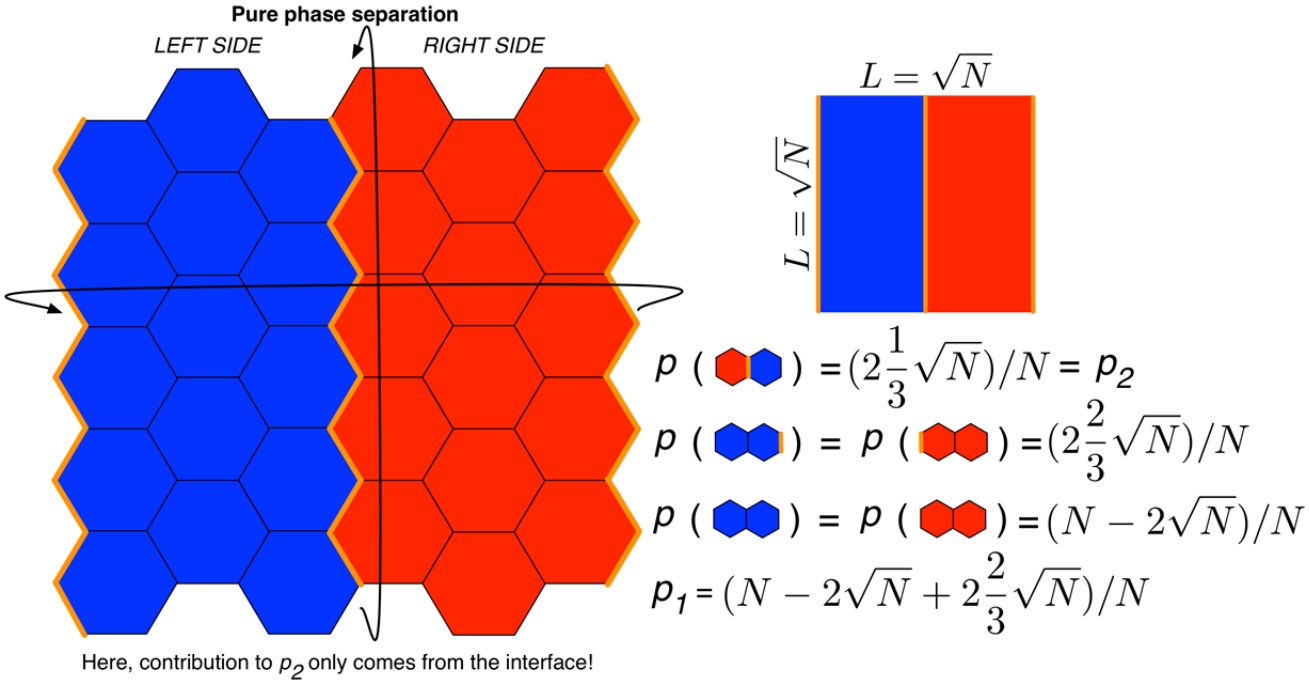


Figure S2. Illustration of a pure binary phase separation on a 2D hexagonal lattice in square periodic boundary conditions. The two types of lipids are represented in red and blue, respectively, and the interfaces between domains are drawn with a bold orange line.

To define the 50% miscibility point, consider a case where pure domains coexist with an ideally mixed domain that composes 50% of the system (see Figure S2). Each interface between pure domains and mixed domains will contribute $\frac{7}{10}\sqrt{N}/N$ to $p_1$ and $\frac{3}{10}\sqrt{N}/N$ to $p_2$. The ideally mixed domain contributes $(\frac{1}{4}N_D/N)$ to $p_1$ and $p_2$, where $N_D = N - 3\sqrt{N}$, the number of lipids not located at any of the interfaces (one of the pure-pure domain interfaces disappears when introducing the mixed domain). Combining these contributions together leads to equations 2 and 3 (see Methods in the main text) used to map binary mixing entropy to a definition of miscibility similar to the practical definition commonly employed in experimental work.

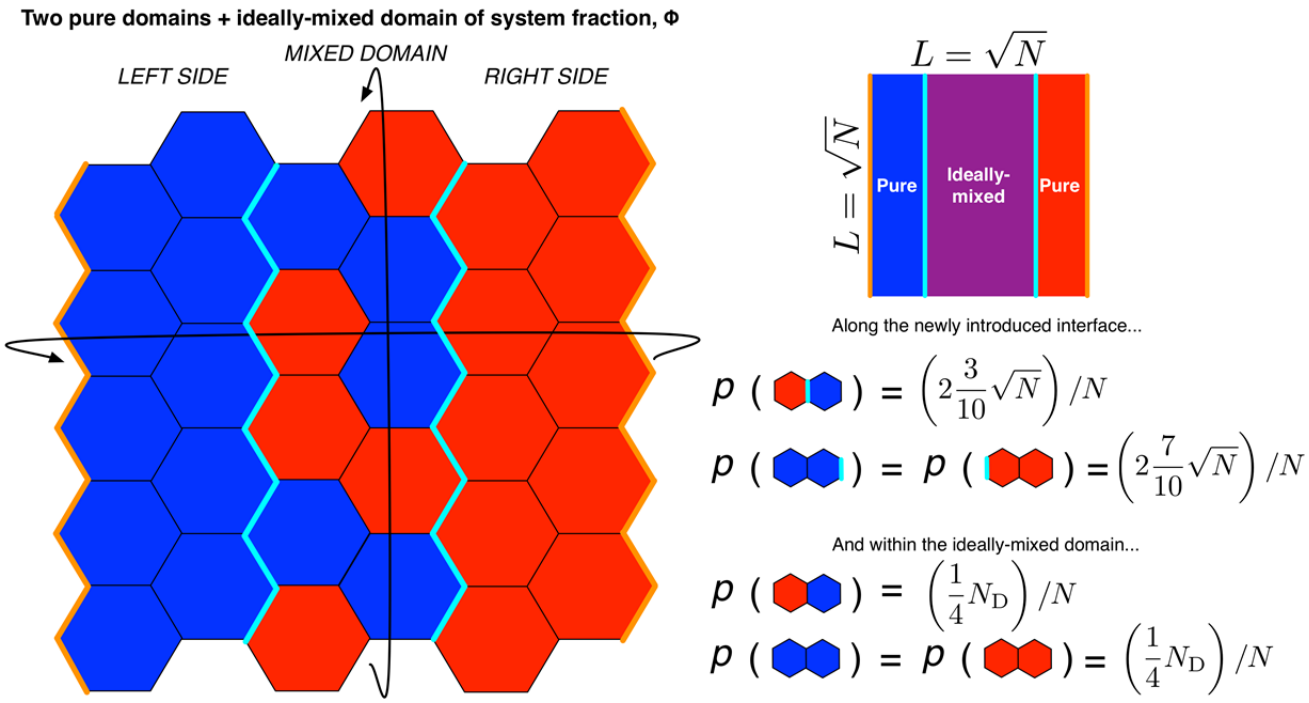


Figure S3. Illustration of pure domains coexisting with an ideally mixed domain composing 50% of the system. The two types of lipids considered are represented in red and blue, the interfaces between pure domains are drawn with an orange line, and the interfaces between ideally mixed and pure domains are drawn with a cyan line.

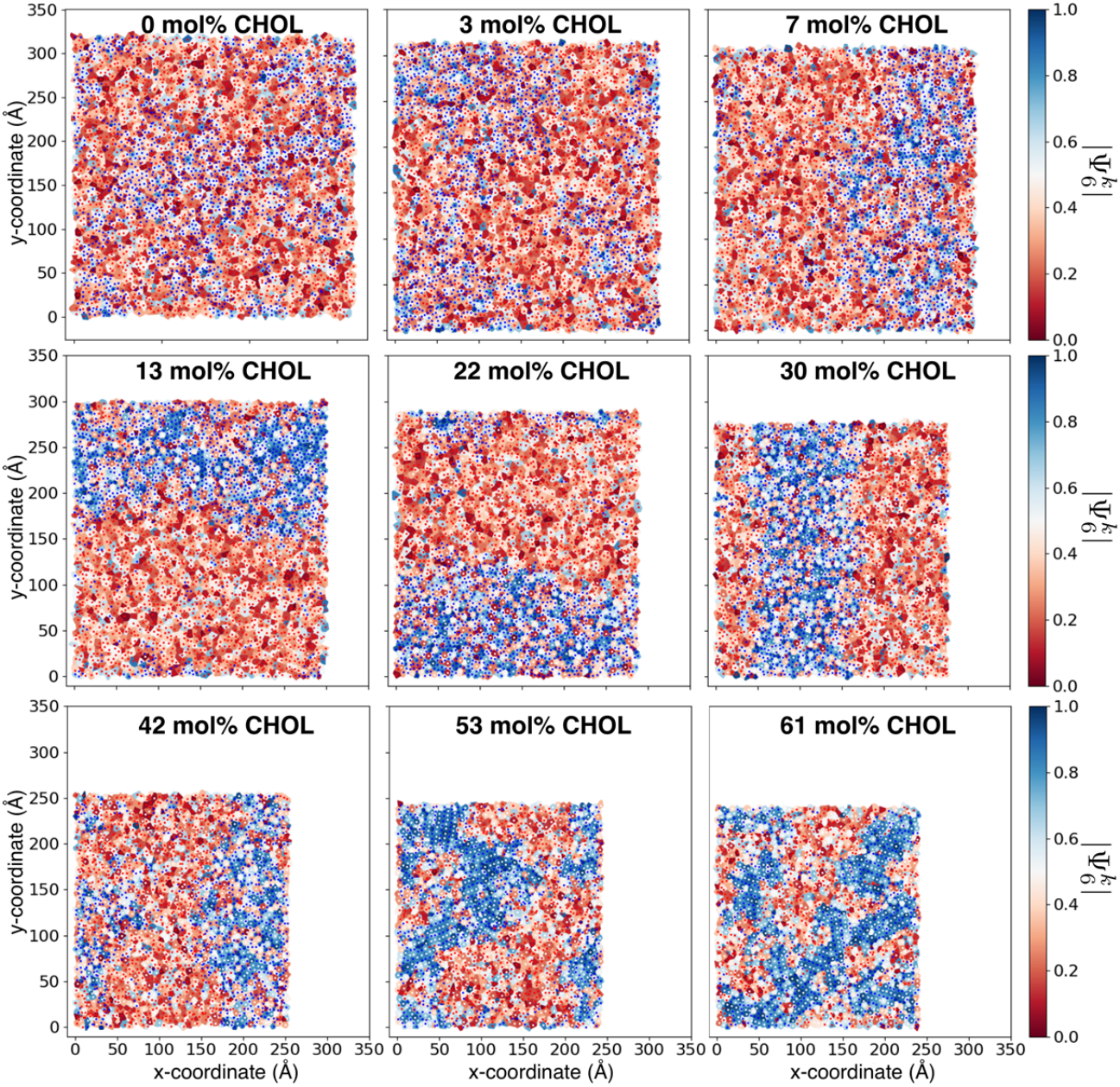


Figure S4. Voronoi tessellations of lipid and Chol tails in upper leaflets of simulated membranes at the last frame of each trajectory. DPPC (blue), DIPC (red), and Chol (white) dots represent tails. Voronoi cells are colored according to the absolute value of lipid tail bond-orientational order parameters. Phase regimes II and III are characterized by the order observed in 13-61 mol% Chol.

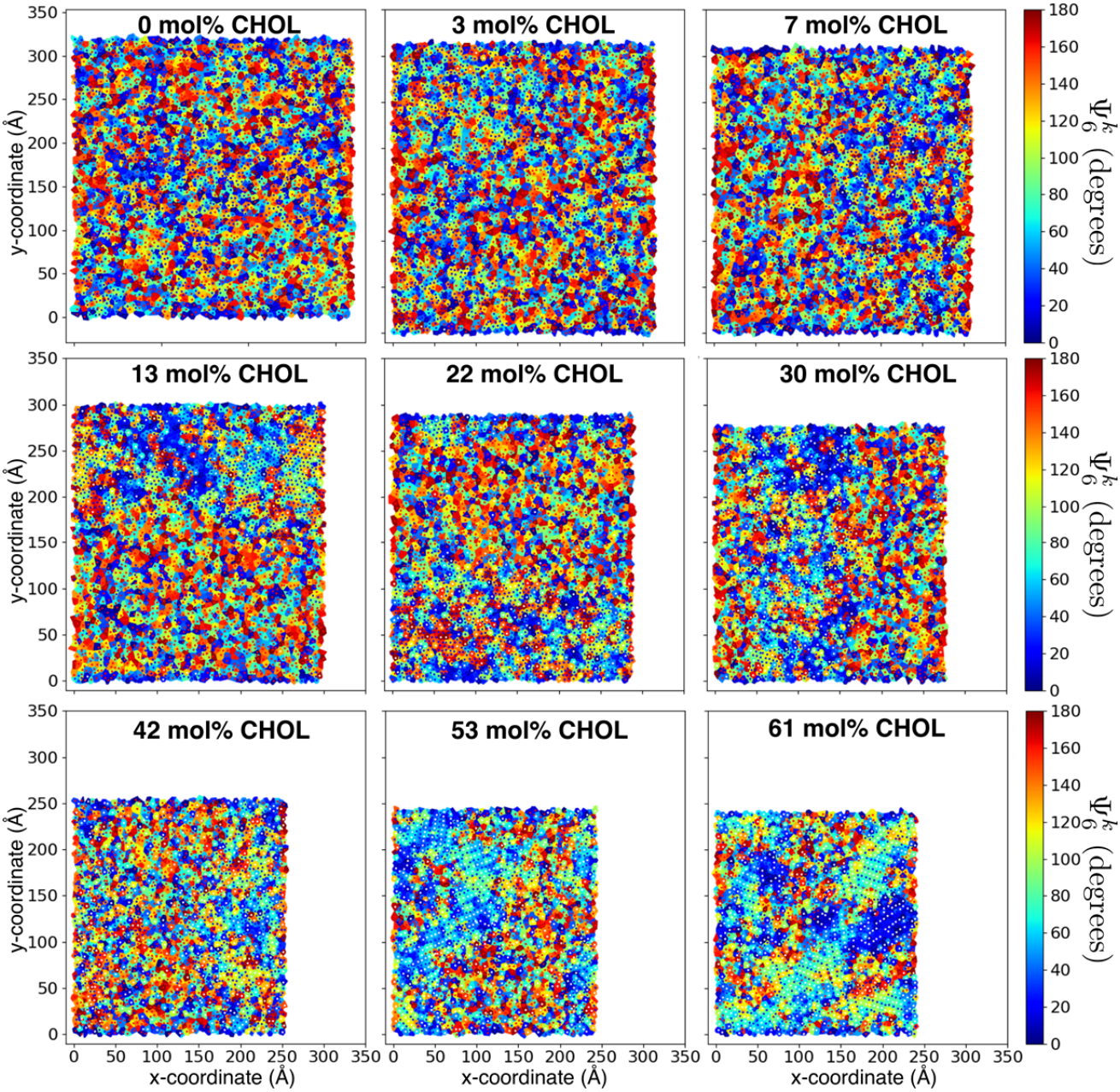


Figure S5. Voronoi tessellations of lipid and Chol tails in upper leaflets of simulated membranes at the last frame of each trajectory. DPPC (blue), DIPC (red), and Chol (white) dots represent tails. Voronoi cells are colored according to the orientation of lipid tail bond-orientational order parameters. Phase regime III is characterized by the order observed in 53-61 mol% Chol.

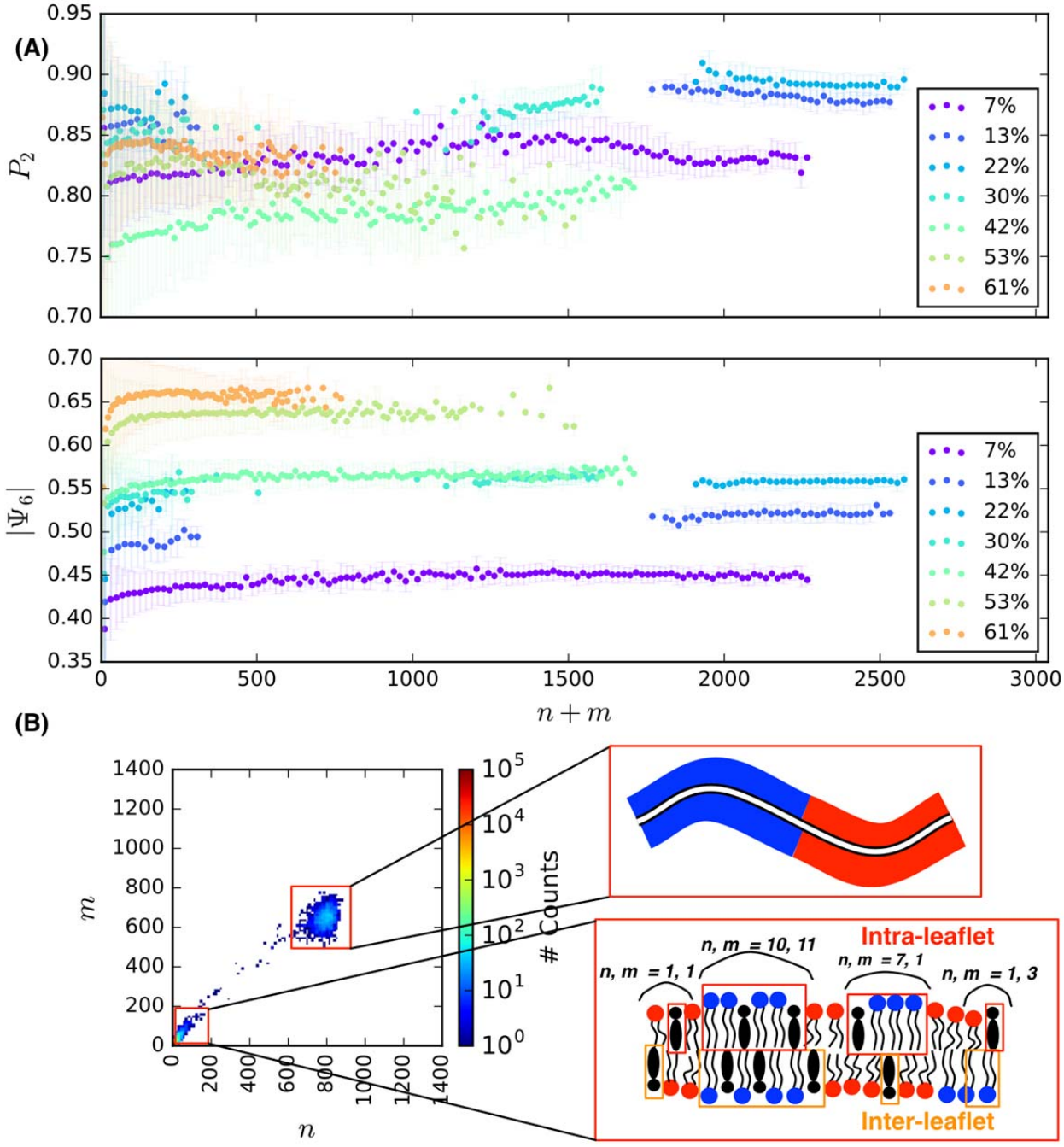


Figure S6. (A) Equilibrium ensemble averaged order parameters of DPPC measured for clusters of *n* intra- and *m* interleaflet DPPC and Chol lipid tails including errorbars . (B) Illustration of how intra- and interleaflet clusters are determined using 30 mol% Chol as an example system condition.