Hexagonal Substructure and Hydrogen Bonding in Liquid Ordered Phases of Palmitoyl Sphingomyelin

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SUPPLEMENTAL METHODS AND RESULTS

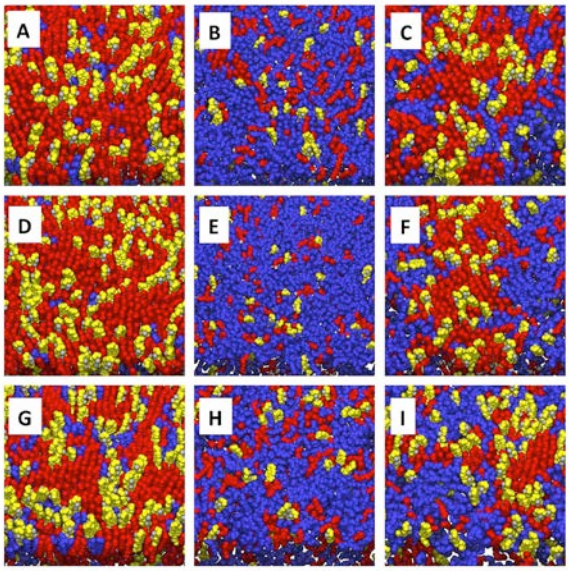

Figure S1. Final configuration of each simulation shown in Fig. 1 of the main text, but in space filling representation. Only one leaflet is shown, and the head groups are removed to reveal the packing in the hydrocarbon chain region. Column order is: L_o phases (left), L_d phases (middle), coexistence systems (right). Row order is: PSM/POPC/Chol (top), PSM/DOPC/Chol (middle); DPPC/DOPC/Chol (bottom). Red indicates PSM or DPPC, blue indicates DOPC or POPC, yellow indicates cholesterol.

Definition of local hexagonal lattices. For each saturated chain lipid, a best-fit local hexagonal lattice was selected. The local lattice was parameterized by its center (in two dimensions) and an angle that determined the orientation of the lattice in the plane. The lattice length constant was fixed at the average chain-chain spacing selected from the histogram in **Fig. S3**.

Forming clusters. A cluster here is a contiguous set of lipids that use one of the cluster members' local lattice as the best-fit lattice for the group. The lattice selected is that one that minimizes the sum of squared distances of each member from its site on the lattice (the lattice chi-squared value). Clusters are formed using a Monte Carlo algorithm to minimize the sum of lattice chi-squared values. In addition to the sum of lattice chi-squared values, a biasing constant is added to the sum for each lipid which is in the same cluster as its neighbor. At zero bias each lipid will belong to its own best-fit local lattice. At large

negative bias a cluster of all lipids will be formed. For this qualitative analysis, an intermediate low, negative bias was selected that overcame noise in the local lattices, but that did not make egregious errors assigning poor matches. Given that the L_o phase contains both order and significant disorder there is no unique definition of the local lattice, and therefore cluster sizes.

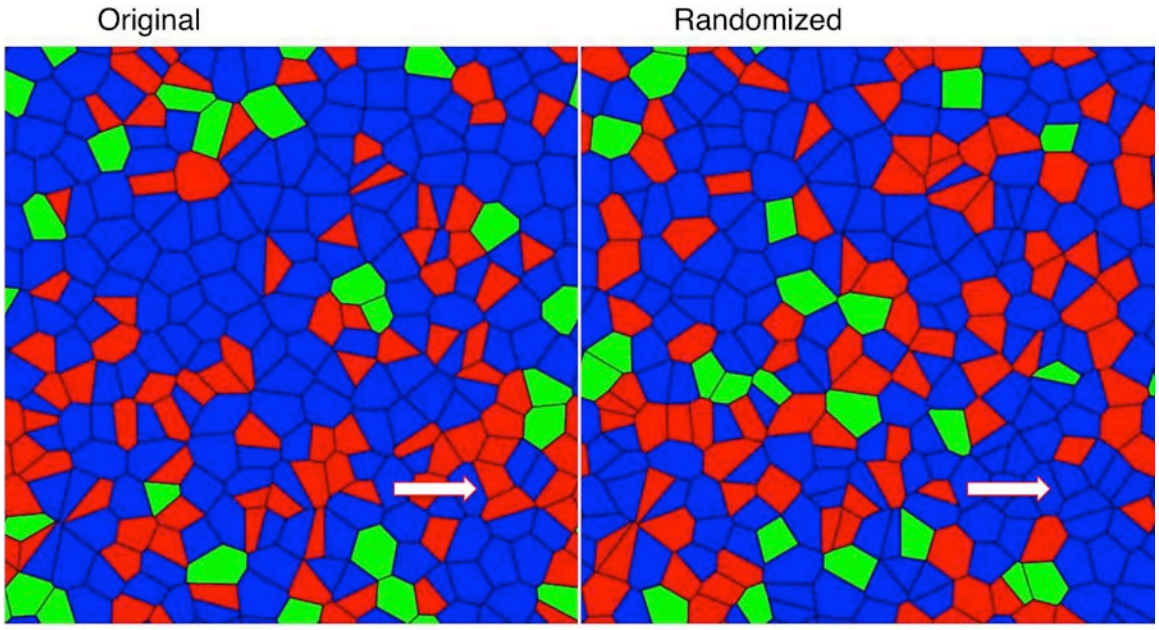

Figure S2. On the left is a Voronoi tessellation of a DPPC (blue), DOPC (green), and Chol (red) configuration. On the right is the same configuration, but with the labels randomly swapped. The randomization will, in general, change the total length of the borders between each pair of colors/lipids. For example, the cholesterol polygons indicated by the arrow are changed to DPPC on the right. This change decreases the red/blue length boundary, giving a negative contribution to the change in boundary length between cholesterol and DPPC. In this example, randomization therefore indicates that there are more DPPC/Chol neighbors than would be expected for an ideal mixture. In Tables 2-4, the sign of these contributions are flipped, so that positive contributions indicate favorable interactions. Lipid borders are scaled by factors (near one) computed to keep the average border length of, for example, cholesterol the same even after randomization.

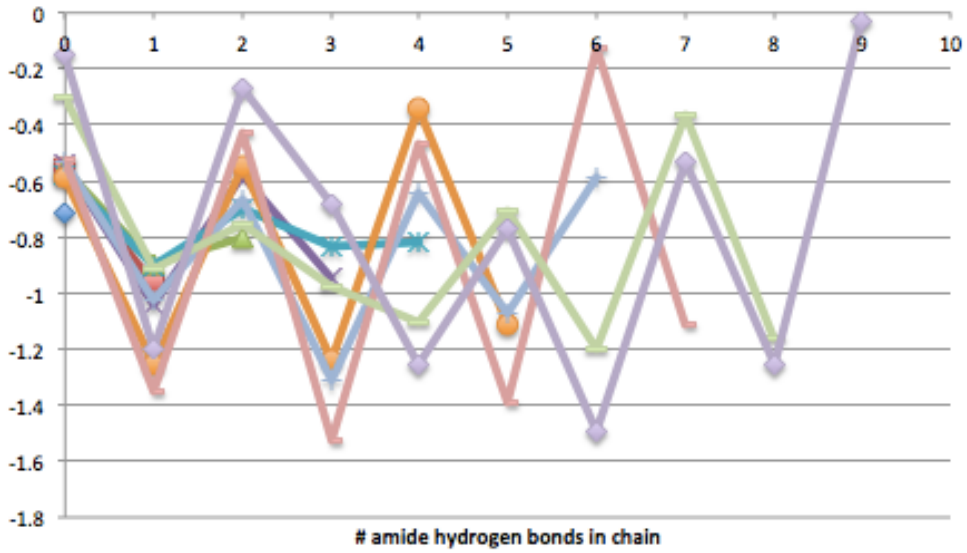

Figure S3. Orientation of the amide plane along a chain of hydrogen bonded sphingomyelin. Each curve is the average of a set of chains with the same number of PSMs linked by hydrogen bonds between the amide planes. Each point is the average angle (in radians) that the amide plane makes relative to the bilayer normal, with one point per PSM amide plane. The longest chain is 9 PSM in length.

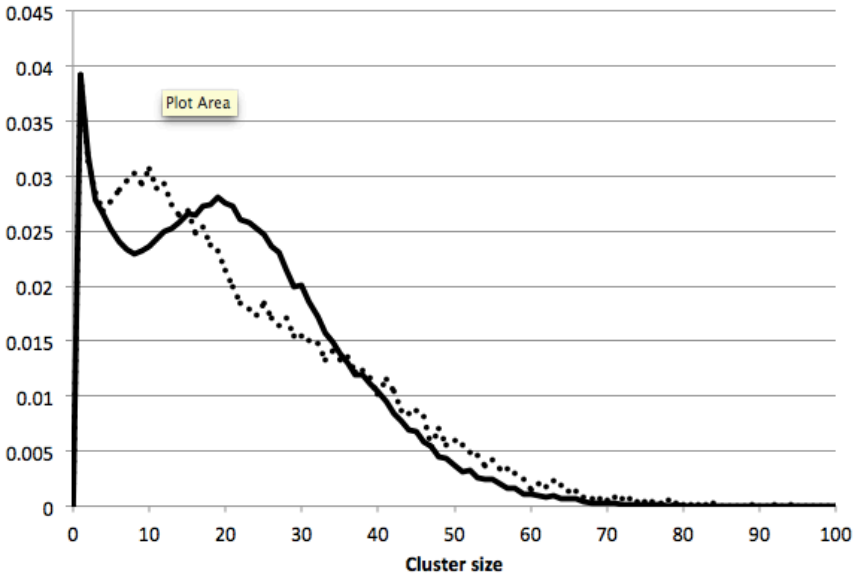

Figure S4. Hexagonal cluster size distributions for the DPPC/DOPC/Chol (solid black line) and PSM/POPC/Chol (dashed line) L_o phases. The peak in the PSM distribution is shifted to a smaller number of chains, relative to the DPPC case, indicating that PSM forms more small clusters. There is a slight enrichment of large clusters in PSM as well. Cluster sizes are based on counting hydrocarbon chains (not lipids); the first sharp peak is comprised of single chains not part of any cluster.

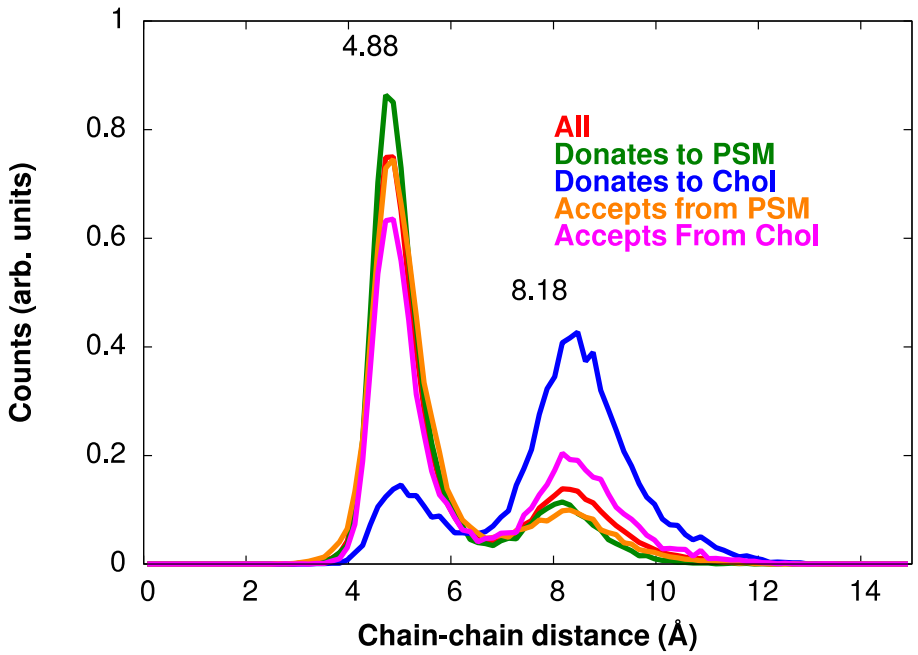

Figure S5. Histograms of chain-chain center of mass distances for PSM in a L_o mixture of PSM/POPC/Chol. Each set is normalized individually. The data are parsed according to hydrogen bonding interactions of the amide plane of PSM . The numerical values are the location of the peak.