DEGENERACY IN MOLECULAR SCALE ORGANIZATION OF BIOLOGICAL MEMBRANE

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1. Principal Component Analysis and spectrum of non-affine modes

Principal component analysis is done to convert the projection of the data points from the current space to subspace where the co-ordinates are eigenvectors along which variance of the data is maximum. We perform a PCA on the χ^2 values for the DPPC/DOPC/Chol, PSM/DOPC/Chol and PSM/POPC/Chol systems to study the nature of spread of χ^2 values for the systems. PCA performed on χ^2 values, P atom positions and distance between the P atom sites is shown in Figure S1 - S4. The difference between the in-vitro like and in-vivo like lipid mixtures is not captured in PCA done on P atom sites and the distance between the P atom sites (Figure S2 – S3). The PCA analysis performed were done using the scipy package in python and MODE-TASK tool in python (after appropriately modifying it for lipid systems: using P atom positions and distances instead of CA in the python code.)for analyzing normal modes and principal components. [1]

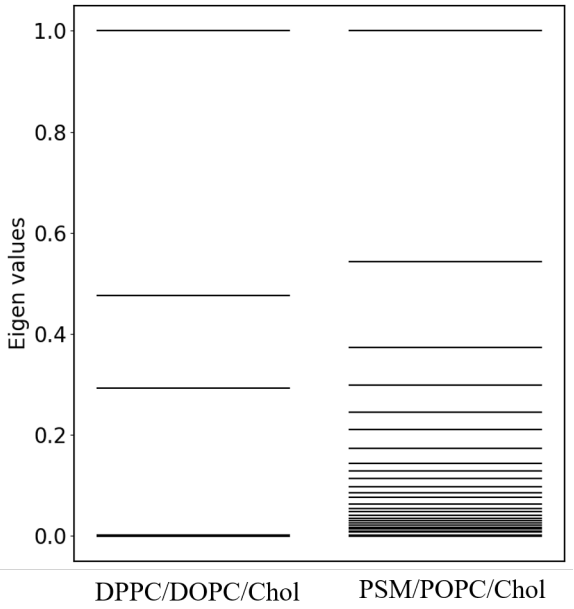

Figure S1. Distribution of eigenvalue values for DPPC/DOPC/Chol and PSM/POPC/Chol systems. Eigen spectrum of non-affine values do indeed carry very unique and interesting information. In fact, clear phase separating system, with no degeneracy, have mode far apart –and clearly only a few modes contribute. While system showing degeneracy in organization have very well calibrated – well spaced levels – suggesting that multiple spectrum contribute towards the patterns seen on the surface of the surface of the bilayer.

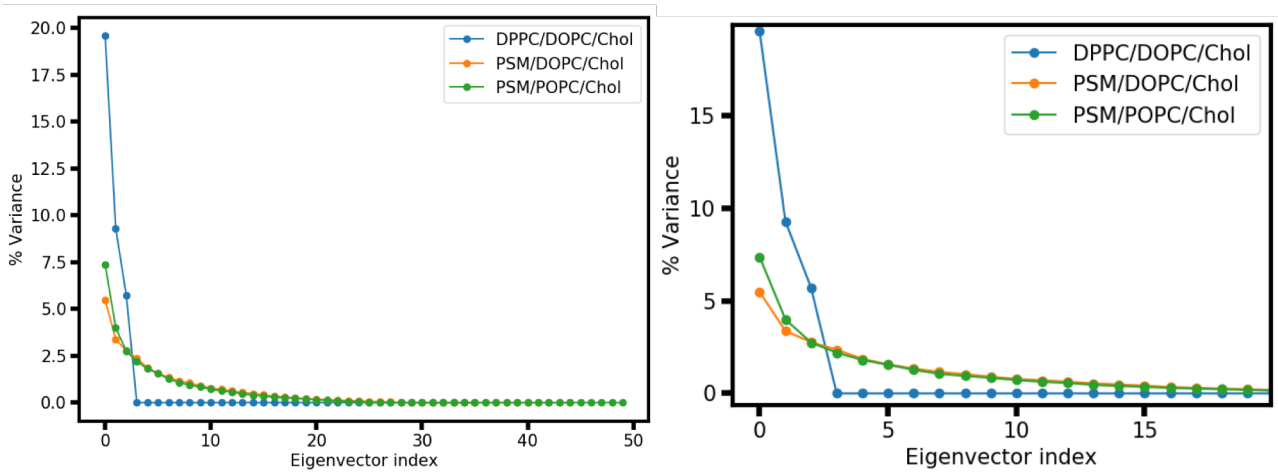

FIGURE S2. shows eigenvalue spectrum plot where the eigenvalues are arranged in decreasing order of their contribution. The in-vitro to in-vivo like nature of the system has the following trend: DPPC/DOPC/Chol > PSM/DOPC/Chol > PSM/POPC/Chol. PCA done with χ^2 shows that for DPPC/DOPC/Chol system the difference between the first two PCs is larger than the PSM/DOPC/Chol and PSM/POPC/Chol systems. This means the χ^2 values for PSM/POPC/Chol system are reasonably dense in comparison to DPPC/DOPC/Chol. In addition, PC1, PC2 and PC3 are sufficient to project the χ^2 values with minimal loss of information for the DPPC/DOPC/Chol, system, while that is not the case for PSM/POPC/Chol system. (left) Full eigen spectrum, (right) zoomed lower eigen modes.

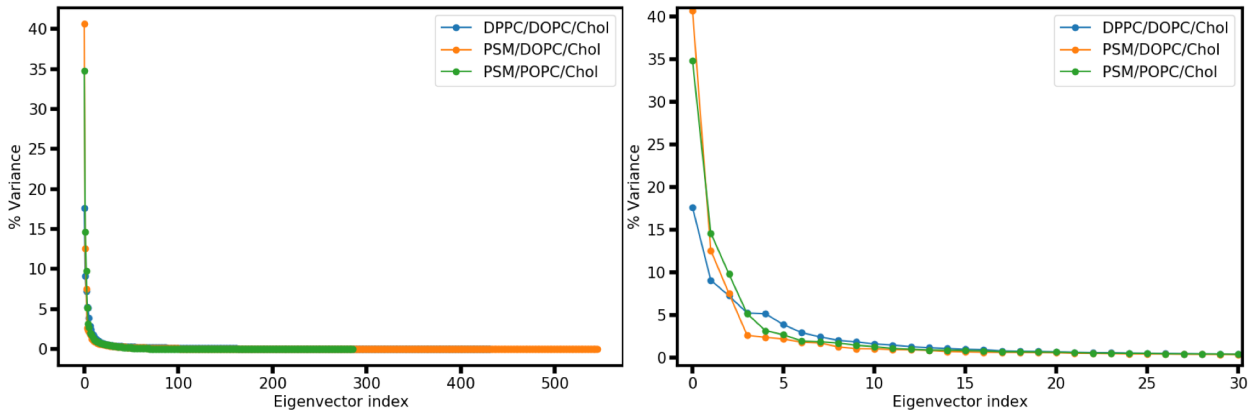

FIGURE S3. shows eigenvalue spectrum plot where the eigenvalues are arranged in decreasing order of their contribution. PCA done with position of the P atom sites. (left) Full eigen spectrum, (right) zoomed lower eigen modes.

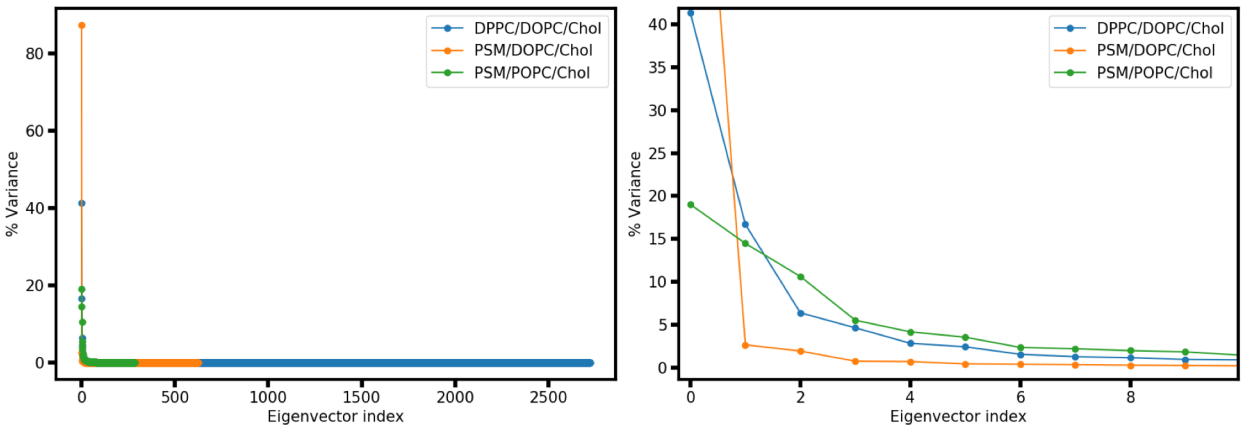

FIGURE S4. shows eigenvalue spectrum plot where the eigenvalues are arranged in decreasing order of their contribution. PCA done with distance between the P atom sites. (left) Full eigen spectrum, (right) zoomed lower eigen modes.

Additionally, we calculate correlation between soft normal modes and non-affine displacement values to characterize the soft modes that gives rise to χ^2. The soft modes of the P atom configurations by calculations of the Debye Waller Factors (DWF). DWF is defined as the variance in particle position during time interval t_{DW}. t_{DW} is taken to be 4.8 ns (20 frames = 20 * 240 ps) for these systems. The structurally soft modes are generally identified by the DWF calculations and play an important role in the dynamics of the system. We observe that DWF in PSM/DOPC/Chol system carries information about the specific lateral rearrangements giving rise to χ^2 in PSM/DOPC/Chol system. This is seen by Figure S5 - S7. In PSM/POPC/Chol system where there are the large energy barriers between the metabasins we find least correlation between the non-affine displacements and DWF (Figure S6). In other words, in PSM/DOPC/Chol system, the modes captured by the DWF play an important role in capturing the rearrangements leading to non-affine displacements.

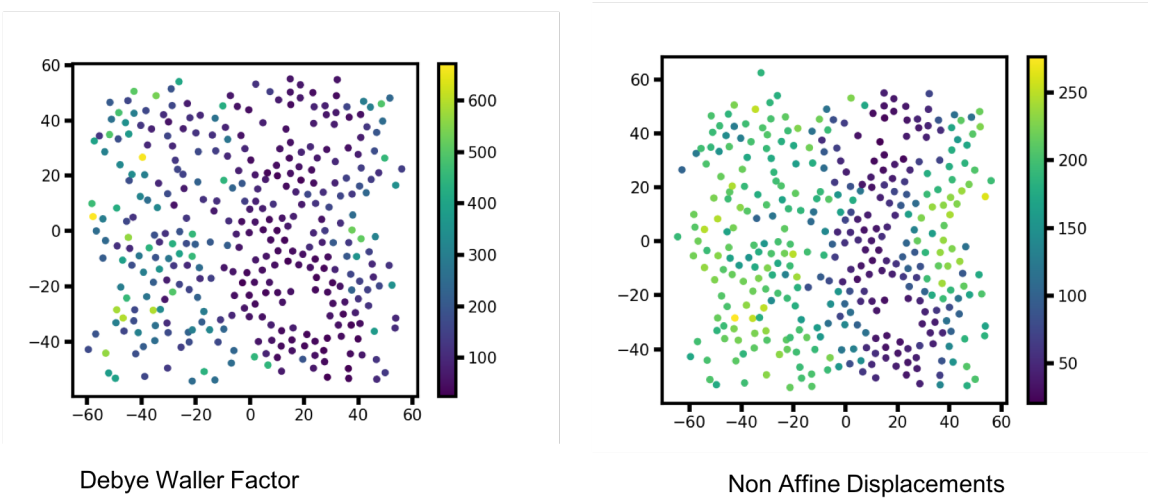

FIGURE S5. Scatter plot showing the P atom sites of PSM/DOPC/Chol system colored according to (left) Scaled DWF values and (right) χ^2 values.

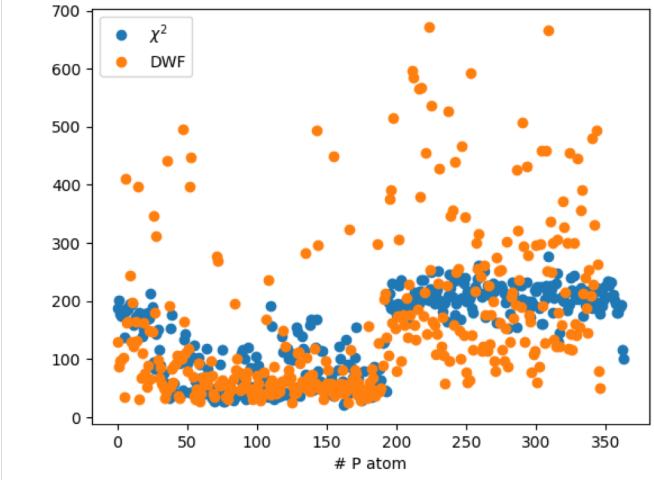

FIGURE S6. Plot showing correlation between Scaled DWF values and χ^2 values for PSM/DOPC/Chol system.

2. COMPARISON OF χ^2 AGAINST S_{cd} AND ϕ_6 ORDER PARAMETERS

To highlight the advantage of using χ^2 over existing characterization parameters to identify molecular-level phase co-existence in the CG and AA systems, we calculate hexatic order parameter (ϕ_6) and deuterium order parameter (S_{cd}) and compare it to phases identified by χ^2 characterization.

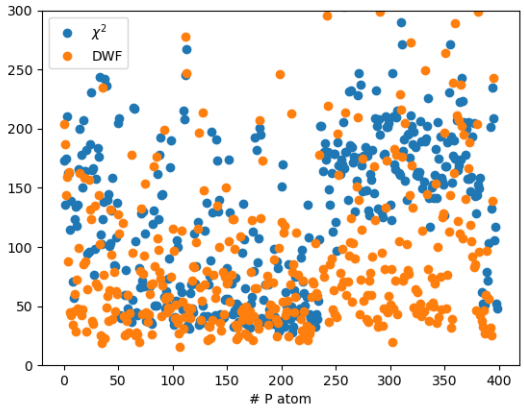

FIGURE S7. Plot showing correlation between Scaled DWF values and χ^2 values for PSM/POPC/Chol system.

ϕ_6 is calculated for the six nearest neighbours of the central atom. Its given as:[2]

$$\Psi(r) = |\frac{1}{6}\sum_{j\in nn(l)} exp(i6\theta_{lj})|^2 \tag{1}$$

here θ_{lj} is the angle between reference axis and a vector connecting particle l to particle j and the summation $j \in nn(l)$ is over the six nearest neighbours of particle l. $\langle\Psi\rangle$ is unity for a perfectly hexagon packing and zero when hexagonal packing is completely absent.
S_{cd}, measure the flaccidity of the lipid alkyl chain tails, is the second order Legendre polynomial $P_2(cos\theta)$:

$$S_{cd} = \frac{\langle 3cos^2\theta - 1\rangle}{2} \tag{2}$$

here θ is the angle between C-H bond vector and the bilayer normal and angular brackets denote ensemble average. S_{cd} values range between 1 (highly ordered) to -0.5 (disordered). Calculation of S_{cd} requires the knowledge of position of all the C-H vectors of the alkyl chain while calculating χ^2 requires the position of any site from each lipid monitored with time. Hence χ^2 has a dynamic nature hidden in its formalism.

Figure S8a shows the comparison between ϕ_6, S_{CC} and χ^2 to identify L_o and L_d phases in DUPC/DPPC/CHOL CG system. The image on the left shows domains as identified by the chemical identity of lipids. While lipids corresponding to different phases are easily identified using χ^2 values, the phases are less apparent using ϕ_6 order parameter. Figure S8b shows a comparison of ϕ_6, S_{cd} and χ^2 to define molecular-level phases in the three AA systems. While ϕ_6 is a good order parameter to distinguish packing in gel and fluid phase, as seen in Figure S8b, it fails to characterize L_o and L_d phases in fluid phase coexisting systems. S_{cd} and χ^2 values are anti-correlated.[3] While both these quantitates can be used to distinguish L_o and L_d phases, as discussed above, calculating χ^2 is computationally less expensive and also has a hidden dynamical nature. Hence we use χ^2 values to mark L_o and L_d lipids and use them as lattice site parameters over the other existing methods to characterize L_o and L_d phases in the simulation and experiment literature.

Additionally, to charecterize the modes that give site to the χ^2 deformations, we calculate the Debye Waller Factor of the P atom sites and find its correlation to the non-affine deformation values. The soft modes of the P atom configurations by calculations of the Debye Waller Factors (DWF). DWF is defined as the variance in particle position during time interval tDW. tDW is taken to be 4.8 ns (20 frames = 20 * 240 ps) for these systems. The structurally soft modes are generally identified by the DWF calculations and play an important role in the dynamics of the system. We observe that DWF in PSM/DOPC/Chol system carries information about the specific lateral rearrangements giving rise to χ^2 in PSM/DOPC/Chol system. This is seen by Fig S4. In PSM/POPC/Chol system where there are the large energy barriers between the metabasins we find least correlation between the non-affine displacements and DWF. In other words, in PSM/DOPC/Chol system, the modes captured by the DWF play an important role in capturing the rearrangements leading to non-affine displacements.

3. Lattice evolution

Figure S10 shows the energy minimization for the DPPC/DOPC/CHOL system for example of how simulated annealing monte carlo algorithm minimizes energy of the lattice configuration.

4. PSM/DOPC/CHOL lattice configurations

Figure S13 shows the final lattice configuration for the PSM/DOPC/CHOL system from different initial configurations. The final configurations are similar to the phase separated L_o/L_d organization in the AA system.

5. Effect of changing the ratio of L_o:L_d lipids in lateral organization patterns

Population distribution of L_o and L_d lipids with varying χ^2 values for DPPC/DOPC/CHOL and PSM/POPC/CHOL system are shown in figure S14 and figure S16 respectively.

PSM/POPC/CHOL: A cut off of 60 is applied on the X2 values to distinguish between the Lo and Ld phases. Ratio of number of lattice sites below and above this cut-off value is varied to study the effect of organization with changing ratios of liquid ordered and liquid disordered lipids. This change in ratio reflects the up-regulation or down-regulation of PSM and POPC lipids in the cell membrane.

DPPC/DOPC/CHOL: A cut off of 50 is applied on the X2 values to distinguish between the Lo and Ld phases. Ratio of number of lattice sites below and above this cut-off value is varied to study the effect of organization with changing ratios of liquid ordered and liquid disordered lipids. This change in ratio reflects the up-regulation or down-regulation of DPPC and DOPC lipids in the cell membrane.

Figure S15 shows the invariance in lattice organization in DPPC/DOPC/CHOL system with different ratio of L_o:L_d equal to 0.33:0.67.

6. Details of Trajectory Movie files

MovieS1.mp4 - Lattice evolution of DPPC/DOPC/Chol system from initial phase separated configuration. MCS and corresponding energies indicated.
MovieS2.mp4 - Lattice evolution of DPPC/DOPC/Chol system from initial random configuration. MCS and corresponding energies indicated.
MovieS3.mp4 - Lattice evolution of PSM/POPC/Chol system from initial phase separated configuration. MCS and corresponding energies indicated.
MovieS4.mp4 - Lattice evolution of PSM/POPC/Chol system from initial random configuration. MCS and corresponding energies indicated.

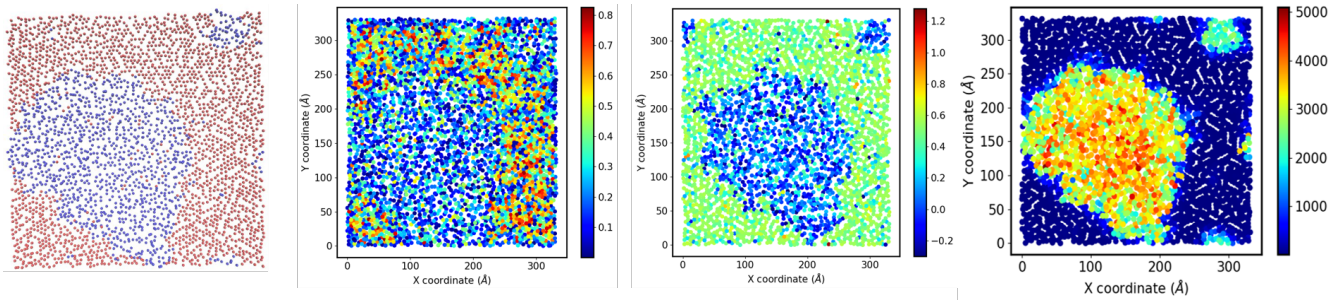

Figure S8a. Comparison of hexatic order parameter, S_{CC} and χ^2 values respectively for coarse grain (CG) DPPC/DUPC/CHOL system. First image from left shows mid-C atoms of the alkyl chains color coded based on their chemical identity (DPPC-red and DUPC-blue).

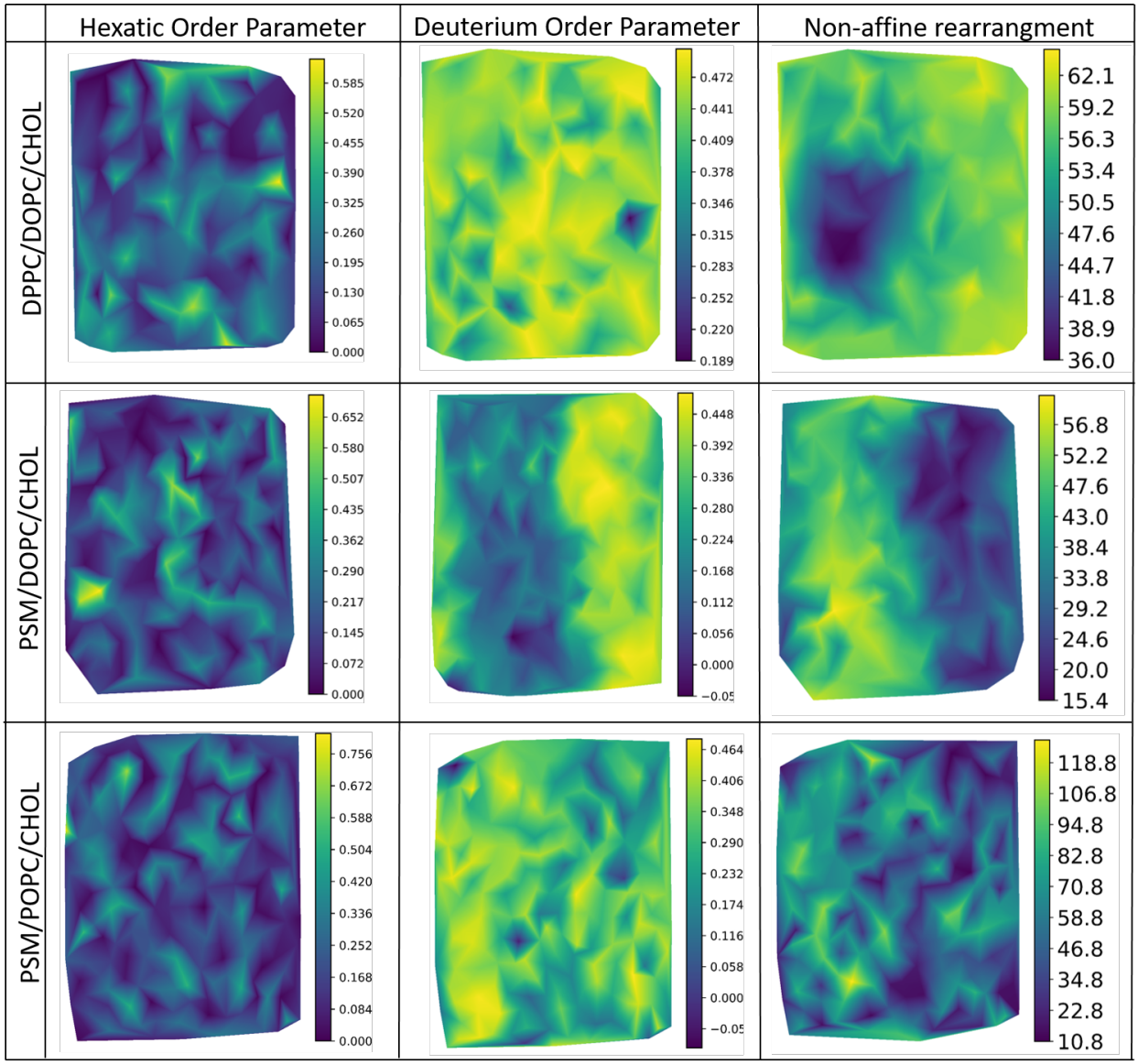

FIGURE S8B. Contour plot of lipid P atom sites coloured based on the (i) Hexatic order parameter (ϕ_6), (ii) Tail order parameter (S_{cd}) and (iii) non-affine parameter (χ^2) for the three AA systems.

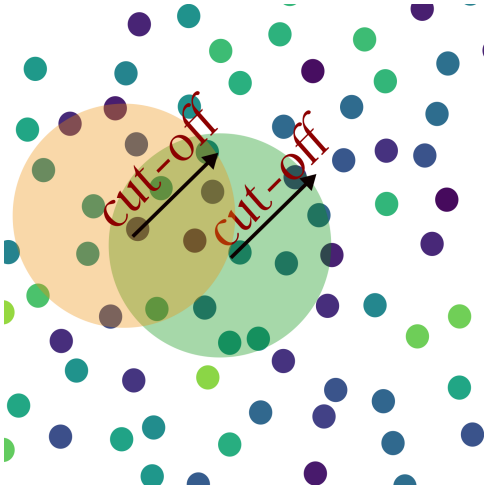

FIGURE S9. Pictorial representation of calculating interactions for AA trajectories. Circles demote the regions within which van der waal and coulombic interaction of the central lipid is calculated.

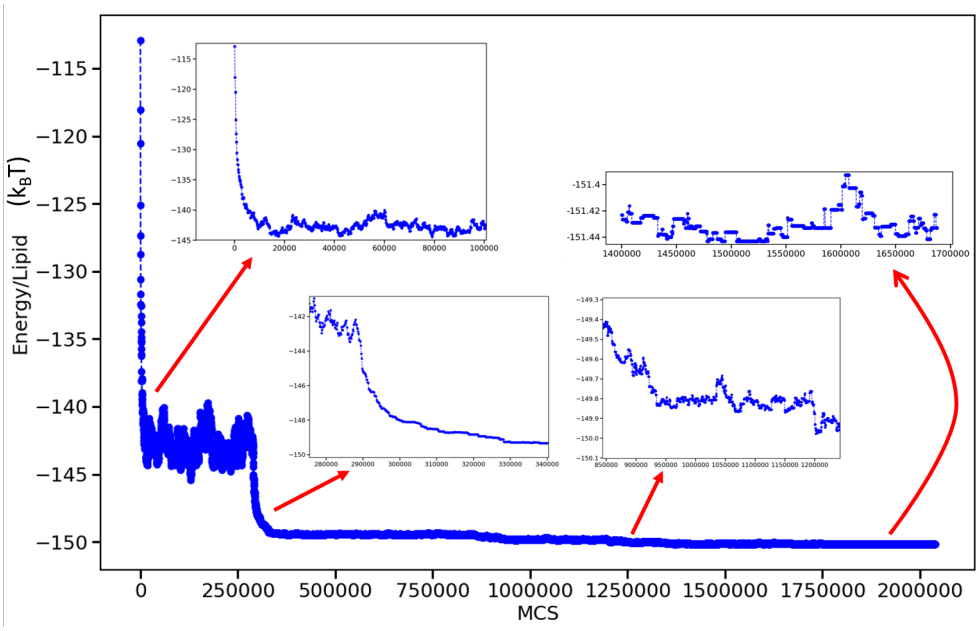

FIGURE S10. Energy decrease in simulated annealing monte carlo run for DPPC/DOPC/CHOL AA system.

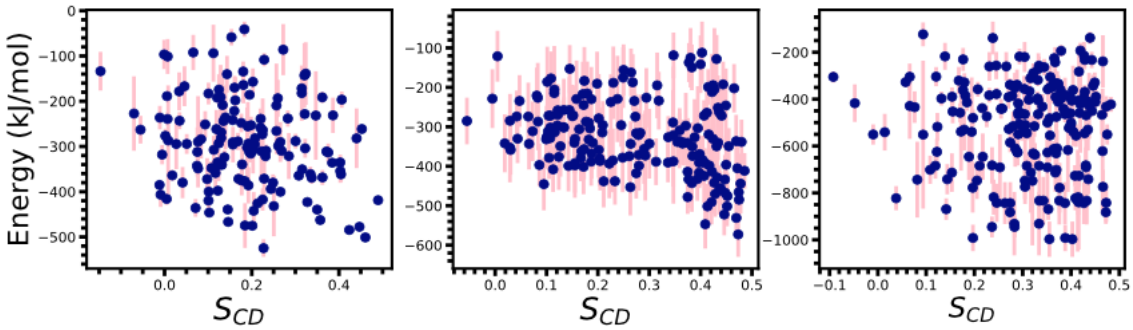

FIGURE S11. nteraction energy plotted as a function of SCD values for (A) DPPC/DOPC/CHol, (B) PSM/DOPC/Chol and (C) PSM/POPC/CHol system.

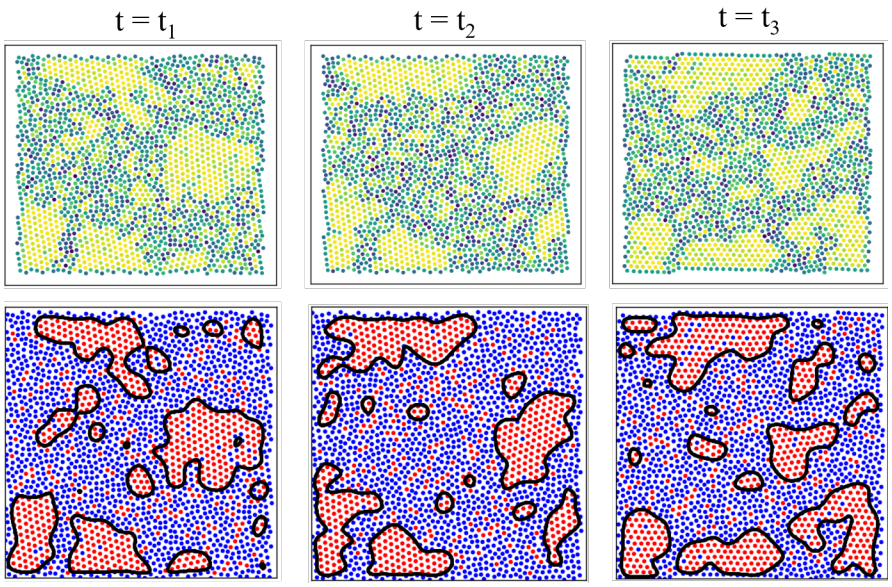

FIGURE S12A. (Top) Scatter plot of 2D binary Lennard Jones particles (with Kob- Andersen potential) at T = 0.9. Particles are colored according to the hexatic order parameter (HOP) values. (Bottom) Particles with HOP larger than 0.6 are marked as a single cluster. Boundaries are marked using support vector machine algorithm. The columns correspond to systems at different time stamps: $t_1 < t_2 < t_3$. Time evolution images of the system shows that at T = 0.9 the system does not undergo large changes in the lateral organization as the system evolves. The thermal energy in the system at this temperature is not sufficient to cross the energy barrier and sample a different metabasin.

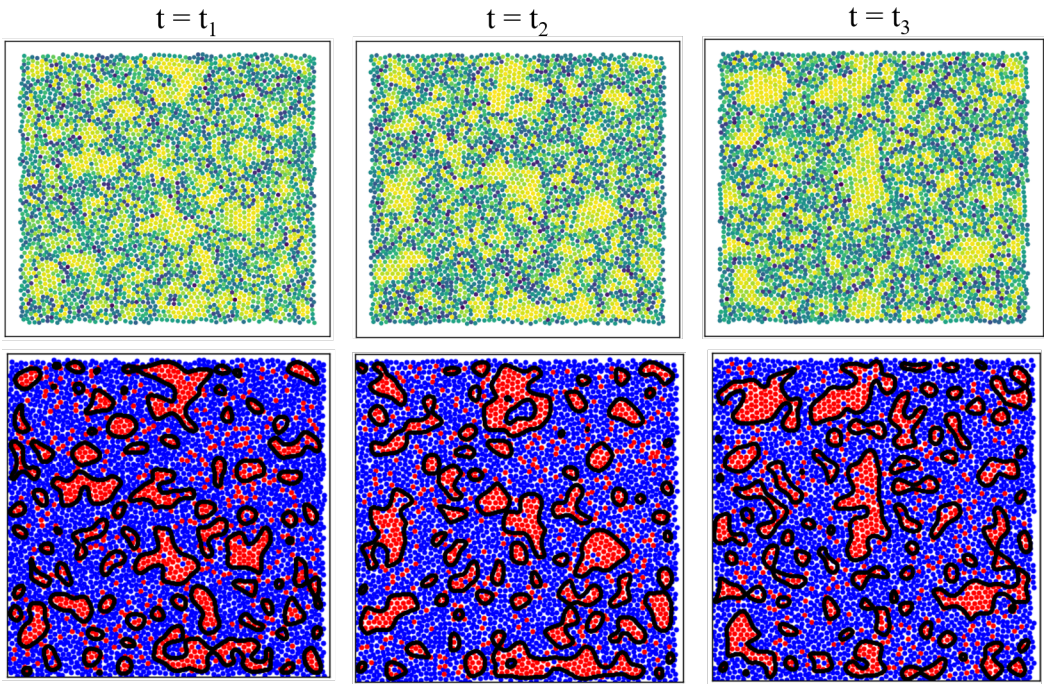

FIGURE S12B. (Top) Scatter plot of 2D binary Lennard Jones particles at T = 1.2. . Time evolution images of the system shows at T = 1.2, the system begins to undergo changes in the lateral organization as the system evolves. Since the organizations observed similar, they correspond to configurations in the same metabasin but perhaps different local minima in the PES.

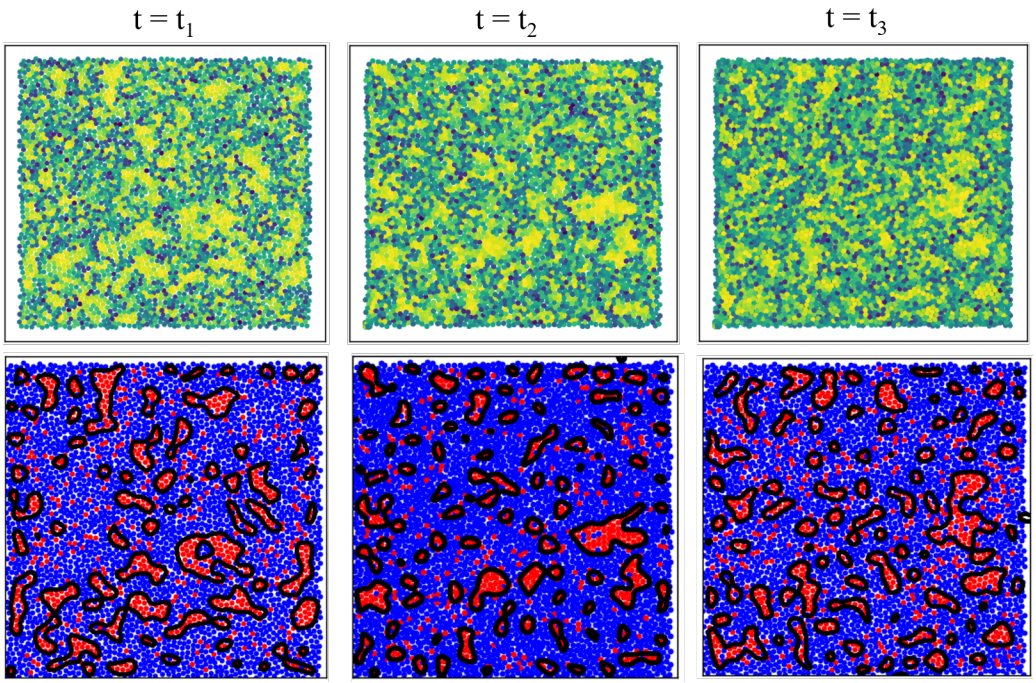

FIGURE S12C. (Top) Scatter plot of 2D binary Lennard Jones particles at T = 1.6. Time evolution images of the system shows at T = 1.6, the system begins to undergo larger changes in the lateral organization as the system evolves. The organizations observed could correspond to different metabasins in the PES. A temperature T = 1.6 results in large thermal fluctuations in the system, with sufficient energy to overcome barriers and jump between the metabasins.

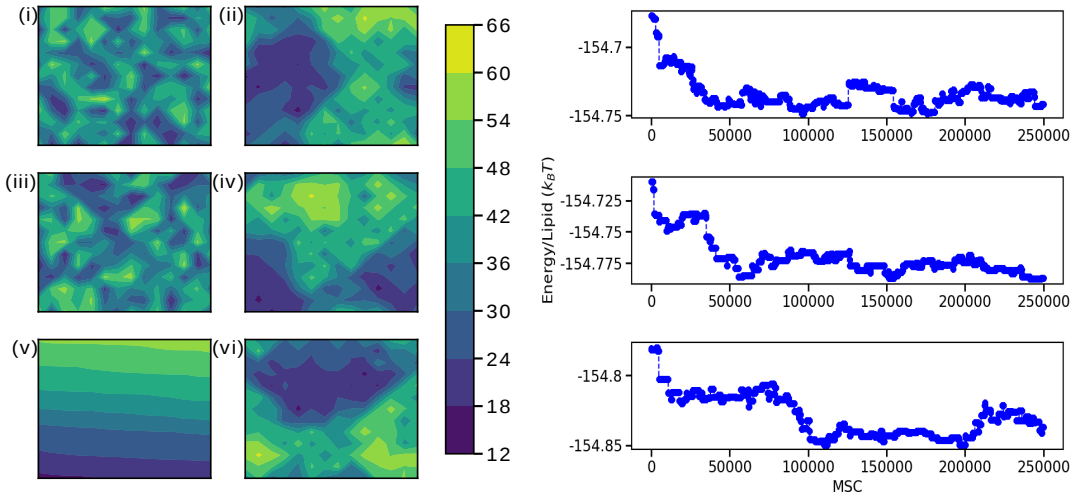

FIGURE S13. Initial (i,iii,v) χ^2 organization and final (ii,iv,vi) χ^2 organization for PSM/DOPC/CHOL mixture. (i)-(ii) and (iii)-(iv) represent lattice evolutions starting from random lattice arrangements. (v)-(vi) represent lattice evolution from a phase separated arrangement of χ^2 values. The sites have been color coded according to the χ^2 values. The Energy/lipid values for the last $2.5 * 10^5$ MCS evolution corresponding to evolutions is shown in the right. Similar energies show that these states are degenerate.

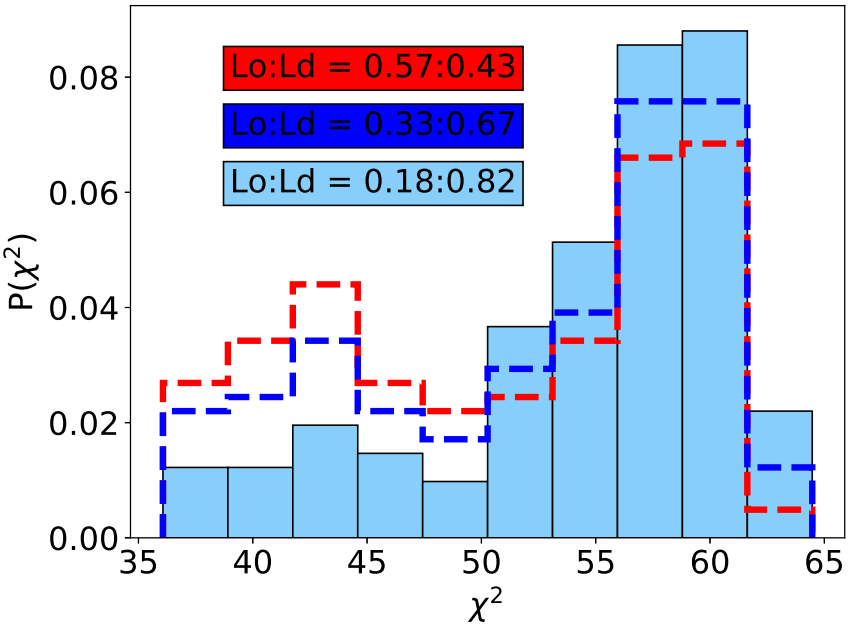

FIGURE S14. Histogram of χ^2 values with varying ratios of Lo:L_d lipids for DPPC/DOPC/CHOL system.

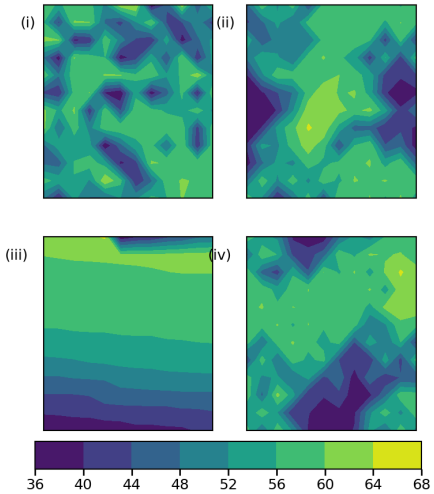

FIGURE S15. Initial (i,iii) χ^2 organization and final (ii,iv) χ^2 organization for DPPC/DOPC/CHOL mixture. (i-ii) represent lattice evolutions starting from random lattice arrangements and (iii-iv) evolution from phase separated lattice arrangement for L_o:L_o ratio of 0.33:0.67. The sites have been color coded according to the χ^2 values.

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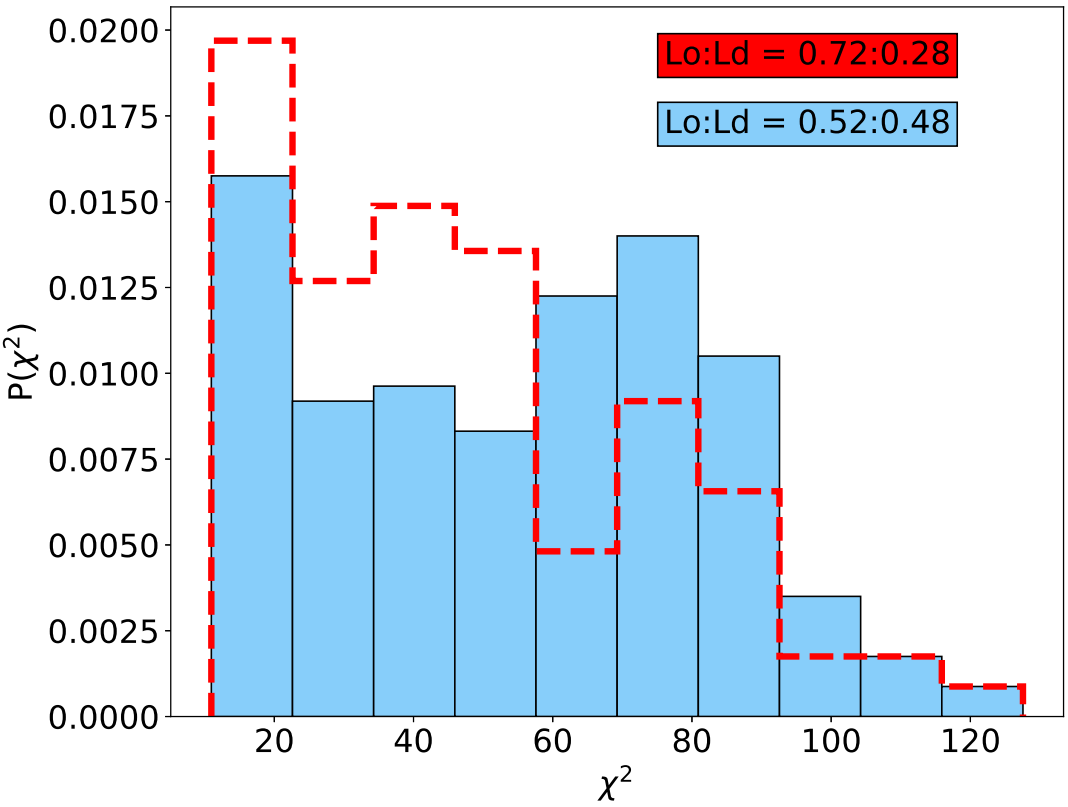

FIGURE S16. Histogram of χ^2 values with varying ratios of Lo:L_d lipids for PSM/POPC/CHOL system.

References

[1] Caroline Ross, Bilal Nizami, Michael Glenister, Olivier Sheik Amamuddy, Ali Rana Atilgan, Canan Atilgan, and Özlem Tastan Bishop. Mode-task: large-scale protein motion tools. *Bioinformatics*, 34(21):3759–3763, 2018.

[2] BI Halperin and David R Nelson. Theory of two-dimensional melting. *Physical Review Letters*, 41(2):121, 1978.

[3] Sahithya S Iyer, Madhusmita Tripathy, and Anand Srivastava. Fluid phase coexistence in biological membrane: Insights from local nonaffine deformation of lipids. *Biophysical journal*, 115(1):117–128, 2018.