A simple thermodynamic model of the liquid-ordered state and the interactions between phospholipids and cholesterol

Paulo F. Almeida*
Department of Chemistry and Biochemistry
University of North Carolina Wilmington, Wilmington, NC

SUPPORTING MATERIAL

*Corresponding author. Address: Department of Chemistry and Biochemistry, University of North Carolina Wilmington, Wilmington, NC 28403, USA, Tel: (910) 962-7300, Fax: (910) 962-3013.E-mail: almeidap@uncw.edu

METHODS: MONTE CARLO SIMULATIONS

Monte Carlo simulations were performed on a Linux workstation (Dell Precision T7400) with in-house FORTRAN code programs using the NAG f95 compiler (Numerical Algorithms Groups, Oxford, UK), as described before (1–4). The membrane was represented by a 100×100 triangular lattice with skew-periodic boundary conditions (5). Each lattice site represents one cholesterol molecule or one phospholipid molecule. The latter may exist in one of three possible states: solid, liquid-ordered (L_o), and liquid-disordered (L_d).

Equilibration of the lattice was achieved by two processes. The first process is site exchange using a variation on the method of Kawasaki (6). Rather than exchanging only nearest-neighbors, the two sites for which a switch is attempted are both picked randomly from anywhere in the lattice. This ensures a much faster equilibration of the system. If only exchange of nearest neighbors were allowed, after small domains form a large fraction of the attempted moves leads to no change, because there is a high probability that two neighbors be identical. The second process consists of transitions between the different states of the phospholipids: $L_d \rightleftharpoons$ solid, $L_d \rightleftharpoons L_o$, and $L_o \rightleftharpoons$ solid.

The algorithm is as follows. A Monte Carlo cycle (mcc) is defined as a number of attempted moves equal to the number of lattice sites. For each attempted move, a site on the lattice is picked at random. The random number generator ran2 of Press et al. (7) was used. The decision about which move to try—exchange or state change—is also random. The probability (p) of each move is determined by the Gibbs energy change (δG) in the process, $p = \exp(-\delta G/RT)$, where R is the gas constant and T is temperature. Acceptance of the move is based on the algorithm of Metropolis et al. (8): if $p \geq 1$ the move is accepted; if $p < 1$, a random number ($Ran\# \in]0,1[$) is generated, and the move is accepted if $p > Ran\#$. The calculation of δG includes changes in enthalpy (ΔH) of the phospholipid, if a change of state occurs, as well as changes in lipid-lipid interactions between each site and its neighbors, in the final and initial configurations. The lipid-lipid interactions are represented by unlike nearest-neighbor interaction Gibbs energies, or interaction parameters for short, ω_{AB}, given by

$$\omega_{AB} = g_{AB} - \frac{1}{2}\left(g_{AA} + g_{BB}\right), \tag{1}$$

where g_{AA} and g_{BB} are the Gibbs energies of interaction between two A or two B molecules, and g_{AB} is the Gibbs energy of interaction between one A and one B molecule, which may be cholesterol or phospholipid, in which case ω_{AB} varies depending on the phospholipid state.

Consider for example a system with only two states, A and B, with an unlike interaction ω_{AB}, and a transition $A \rightarrow B$ between them, characterized by enthalpy and entropy differences ΔH and ΔS, where $\Delta S = \Delta H/T_m$ and T_m is the transition temperature. The total excess Gibbs energy is given by

$$\Delta G = n_B(\Delta H - T\Delta S) + n_{AB}\omega_{AB}, \tag{2}$$

where n_B is the number of B lipids and n_{AB} is the number of AB contacts (3). Thus, at each step, the Gibbs energy change is

$$\delta G = \delta n_B(\Delta H - T\Delta S) + \delta n_{AB}\omega_{AB}, \tag{3}$$

where δn_B and δn_{AB} are the changes in the number of state B molecules and unlike AB contacts (3). A simple example where lipids exchange positions, but no change of state occurs, is illustrated in Fig. 1. In this case, counting only the interactions of the lipids that exchange sites,

$$\delta G = 9\omega_{AB} - 3\omega_{AB} = 6\omega_{AB}. \tag{4}$$

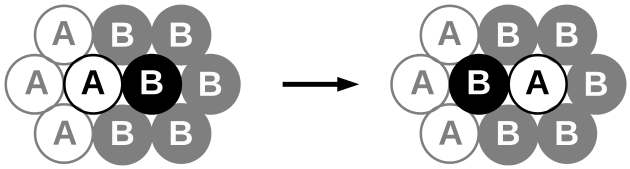

Figure 1: Example of exchange of position between two lipids.

The enthalpy of the lattice is determined by ΔH between the different phospholipid states, with solid as reference, and by differences in the mutual interactions of lipid neighbors. In the calculation of the enthalpy ω_{AB} were used as enthalpies, except in the case of L_o/Chol interaction, which is the only one for which a an experimental temperature-dependence is available, corresponding to $\Delta H_{AB} = -2.1$ kcal/mol (9). This approximation, however, introduces very little error in the calculation because the direct contribution of the ω_{AB} to the heat capacity is very small. Their essential effect is in determining the cooperativity of the transition, not the heat. If the transition is narrow, the results are actually identical whether ω_{AB} are assumed exclusively enthalpic or entropic (3). ΔH (or the energy) converges rapidly, but it is not the best indicator of convergence of the calculation.

The excess heat capacity function ΔC_p was calculated through the fluctuation-dissipation theorem (10),

$$C_p = \frac{\langle H^2 \rangle - \langle H \rangle^2}{RT^2}, \tag{5}$$

where $\langle H^2 \rangle - \langle H \rangle^2$ denotes the fluctuations in enthalpy. A second derivative of the free energy and a correlation function, ΔC_p is a very good indicator of whether the Monte Carlo simulation has been carried for sufficient time to ensure that the calculated values correspond to equilibrium. Thus, an approximately constant value of ΔC_p was the criterion adopted to set the simulation length. In addition, simulations started from different initial states led to the same results.

The values of the parameters used are given in Table 1 in the main paper, which is reproduced here for convenience.

Table 1: Lipid-lipid interaction parameters, transition enthalpies, and transition entropies used in the Monte Carlo simulations.

Lipid pair (A/B)	ω_{AB} (cal/mol)	ΔH (kcal/mol)	ΔS (cal/mol/K)
solid/L_d	+360	8.7	27.65
solid/L_o	+330	3.5	10.15
L_o/L_d	+330	5.2	17.5
Chol/L_d	+20	–	–
Chol/L_o	-340^a	–	–
Chol/solid	+350	–	–

[a]Value at 20^{o}C. This parameter is temperature-dependent, with $\omega_{AB} = -2120 + 6.07T$ cal/mol (T in K).

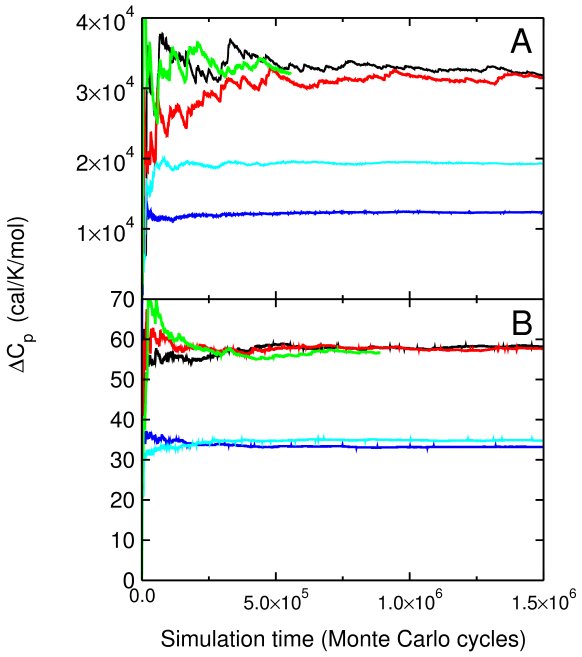

Figure 2: The excess heat capacity, ΔC_p, as a function of the number of Monte Carlo cycles (mcc). (A) Pure DPPC at T_m for lattices of different sizes: 20×20 (blue), 30×30 (cyan), 100×100 (black), 200×200 (red), and 300×300 (green). (B) DPPC/Chol 70:30 at 22^oC for the same set of lattices (same colors).

To verify that the size of the lattice (10^4 sites) is not a limitation, and that the results apply to larger systems, a few simulations were performed on 200×200 and 300×300 lattices, as done previously in the study of ternary mixtures (2). Indeed, there are no significant differences between 100×100, 200×200, and 300×300 lattices. For all intents and purposes of this study, the results are identical. Simulations in small lattices (20×20 and 30×30) were also performed; the size does matter if the systems are this small.

The simulations were typically run for a pre-equilibration period of $\approx 10^4$ mcc, followed by 5×10^5 to 10^6 mcc to acquire data. (Occasional longer runs were performed that showed no variation in any property beyond that point, even after a period $10\times$ longer.) In DPPC/Chol mixtures this was sufficient to ensure that equilibrium values were obtained. However, in pure DPPC close to T_m the fluctuations are very large and, to be sure, the simulations were run for 5×10^6 cycles, though this is not strictly necessary. Fig. 2 shows the evolution of ΔC_p as a function of "time" (number of mcc) for pure DPPC (A) and DPPC/Chol 70:30 (B), for several lattice sizes. It is apparent that pure DPPC at T_m requires longer runs than DPPC/Chol 70:30 to obtain the equilibrium values. It is also apparent that the small lattices (20×20 (blue) and 30×30 (cyan)) behave quite differently from the large ones, but there is no significant difference between 100×100 (black), 200×200 (red), and 300×300 (green) lattices.

Snapshots of DPPC for various lattice sizes are shown in Fig. 3. Very large fluctuations in the content of solid (white) and L_d (black) lipids are observed in small lattices (20×20 and 30×30) at different simulation times, but for a 100×100 lattice the snapshots look essentially identical to those of 200×200 lattice. The picture is identical for a 300×300 lattice (not shown). Fig. 4 shows snapshots for the same set of lattices in DPPC/Chol 70:30. Again, it is clear that 20×20 and 30×30 lattices do not yield the same results as the large lattices. But a 100×100 lattice yields results identical to those obtained with a 200×200 lattice and with a 300×300 lattice (not shown).

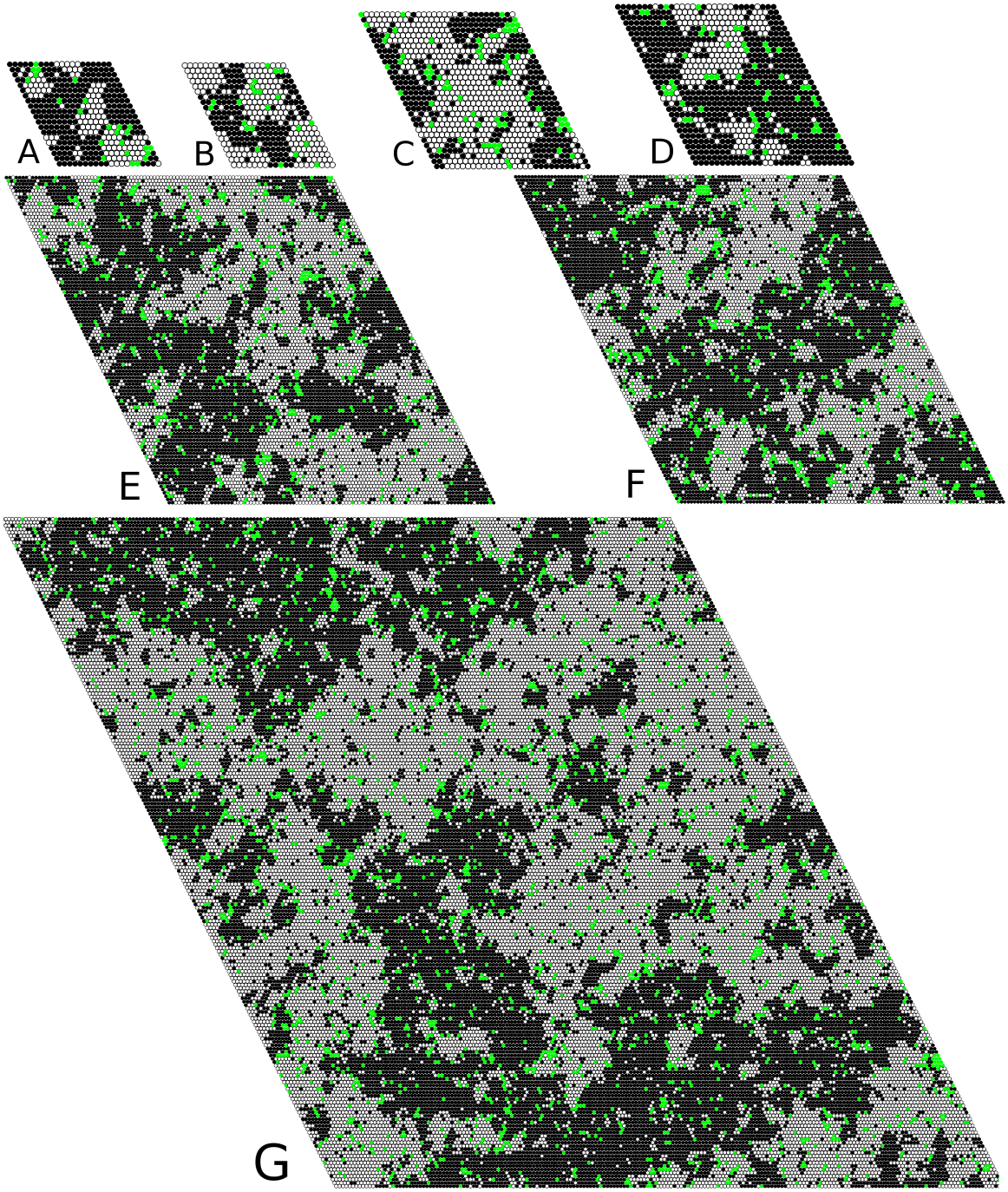

Figure 3: Snapshots of pure DPPC at T_m (41.5^{o}C) for lattices of different sizes, (A,B) 20×20, (C,D) 30×30, (E,F) 100×100 (all at 2.5×10^6 and 5×10^6 mcc), and (G) 200×200 sites, (1×10^6 mcc). Black is L_d, green is L_o, and white is solid.

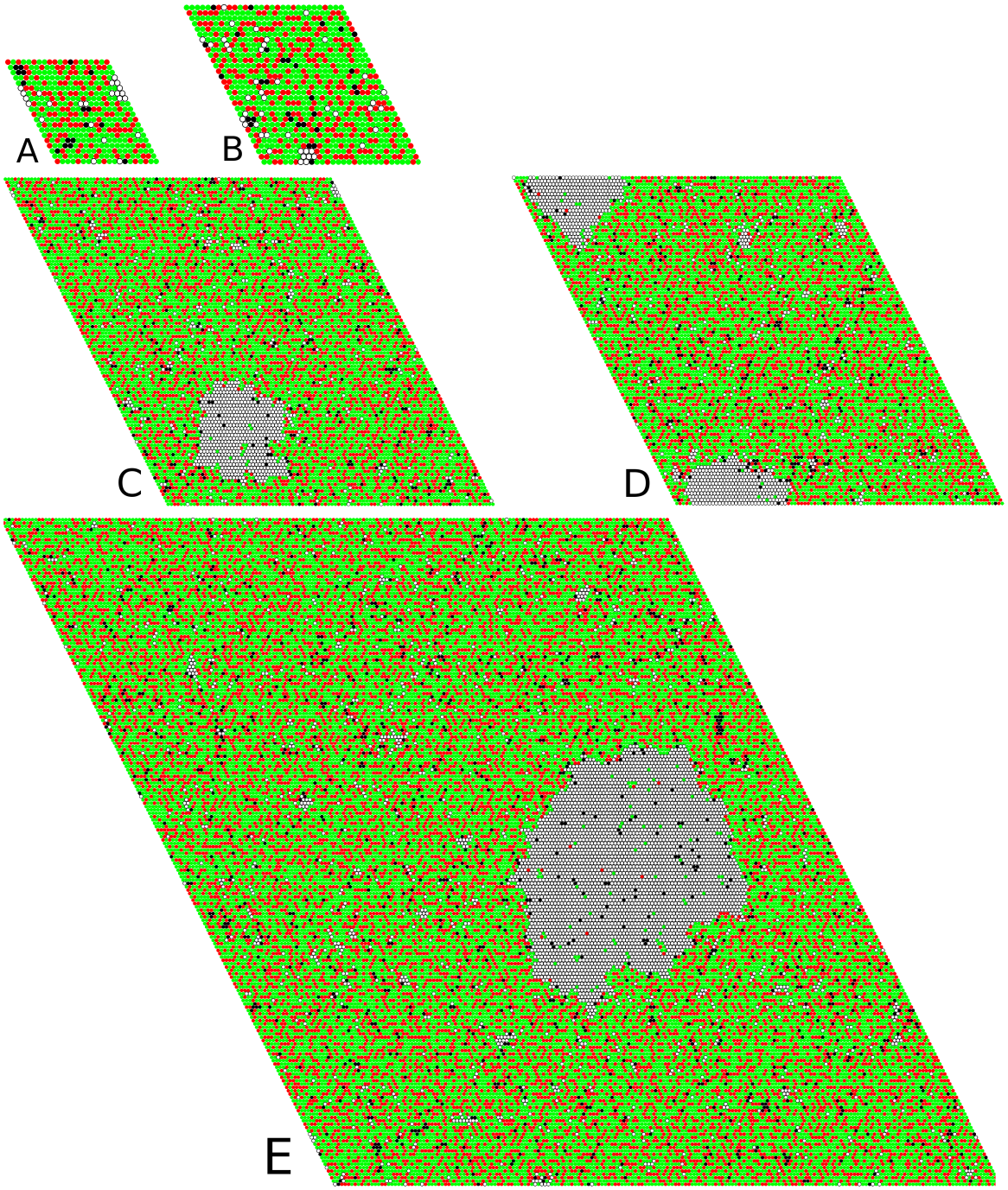

Figure 4: Snapshots of DPPC/Chol 70:30 at 22^{o}C for lattices of different sizes, (A) 20×20 and (B) 30×30 (at 5×10^6 mcc), (C,D) 100×100 (5×10^5 and 5×10^6 mcc), and (E) 200×200 sites (1×10^6 mcc). Black is L_d, green is L_o, white is solid, and red is Chol.

References

1. Sugar, I.P., T.E. Thompson, and R.L. Biltonen. 1999. Monte Carlo simulation of two-component bilayers: DMPC/DSPC mixtures. *Biophys. J.* 76:2099-2100.

2. Frazier, M.L., J.R. Wright, A. Pokorny, and P.F.F. Almeida. 2007. Investigation of domain formation in sphingomyelin/cholesterol/POPC mixtures by fluorescence resonance energy transfer and Monte Carlo simulations. *Biophys. J.* 92:2422-2433.

3. Jerala, R., P.F.F. Almeida, R.L. Biltonen. 1996. Simulation of the gel-fluid transition in a membrane composed of lipids with two connected acyl chains: application of a dimer-move step. *Biophys. J.* 71:609-615.

4. Sugar, I.P., R.L. Biltonen, and N. Mitchard. 1994. Monte Carlo simulation of membranes: phase transition of small unilamellar dipalmitoylphosphatidylcholine vesicles. *Meth. Enzymol.* 240:569-593.

5. Binder, K., and D.W. Heermann. 1997. Monte Carlo Simulation in Statistical Physics. 3rd Edition, Springer, New York.

6. Kawasaki, K. 1972. Kinetics of Ising models. In Phase Transitions and Critical Phenomena. C. Domb and M.S. Green, editors. Academic Press, New York. Vol.2:443-501.

7. Press, W.H., S.A. Teukolsky, W.T. Vetterling, and B.P. Flannery. 1994. Numerical Recipes in FORTRAN: The Art of Scientific Computing. 2nd Edition. Cambridge University Press, Cambridge.

8. Metropolis, N., A.W. Rosenbluth, W.N. Rosenbluth, A.H. Teller, and E. Teller. 1953. Equation of state calculations by fast computing machines. *J. Chem. Phys.* 21:1087-1092.

9. Zhang, J., H. Cao, B. Jing, P.F. Almeida, and S.L. Regen. 2006. Cholesterol-phospholipid association in fluid bilayers: a thermodynamic analysis from nearest-neighbor recognition measurements. *Biophys. J.* 91:1402-1406.

10. Hill, T.L. 1985. An Introduction to Statistical Thermodynamics, Dover, New York.