

## A Simulation Study on Multicomponent Lipid Bilayer

Srilekha Banerjee<sup>1</sup>, Jayashree Saha<sup>2</sup>

<sup>1</sup>S N Bose National Centre for Basic Sciences,Block JD, Salt Lake,Kolkata 700098,India

<sup>2</sup>Department of Physics,Visva Bharati University,Santiniketan 731235,India

### Abstract

Simulation of a multicomponent lipid bilayer having a fixed percentage of cholesterol is done to study phase transition leading to domain formation. The concept of random lattice has been used in simulation to account for the coupling between the internal and translational degrees of freedom of lipid molecules. Considering a canonical ensemble, dissimilar lipid molecules are allowed to exchange their positions in the lattice subject to standard metropolis algorithm. The steps involved in the process effectively takes into account for the movement of sphingolipids and cholesterol molecules helping formation of cholesterol rich domains of saturated lipids as found in natural membranes.

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*Key Words:* lipid-cholesterol bilayer, simulation, domain formation, phase diagram.

## 1 Introduction

The semipermeable plasma membrane, composed of bilayers of amphiphilic molecules, provides the basic form and structure to the cell and allows transportation of essential materials. These are highly flexible surfaces and are considered to be fluid in the sense that the constituent lipid molecules can diffuse rapidly within the membrane. Morphologically distinct regions or domains are found on the surface membrane of the cells. Each of these domains are specialized for a particular function, e.g. nutrient absorption, cell-cell communication, endocytosis etc. Lipid domains that include caveolae or rafts are high in cholesterol and sphingolipids. The lipid rafts serve as a platform for both integral and peripheral proteins as raft lipids and associated proteins diffuse together laterally on the membrane surface.

A vast literature is found on the numerous work done on lipid bilayers. The interest has intensified in recent years mainly because of role of sphingomyelin in lipid raft formation.

Fig.1 shows a schematic picture of a lipid bilayer and the interdigitated hexagonal lattice used in our model system. Structure and function of membrane rafts have received much attention in recent times from many workers both theoretical and experimental [1, 2, 3, 4, 5].

Proteins found in the membranes either partially penetrate the hydrophobic core or fully span the bilayer. A third possibility is getting adsorbed to the lipid head group region. Extensive work has been done on lipid-protein interaction by various workers [6, 7, 8, 9, 10] considering different angles. Anderson and Jacobson [11] have proposed that the molecular

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<sup>1</sup>To correspond: srilekha@bosen.bose.res.in

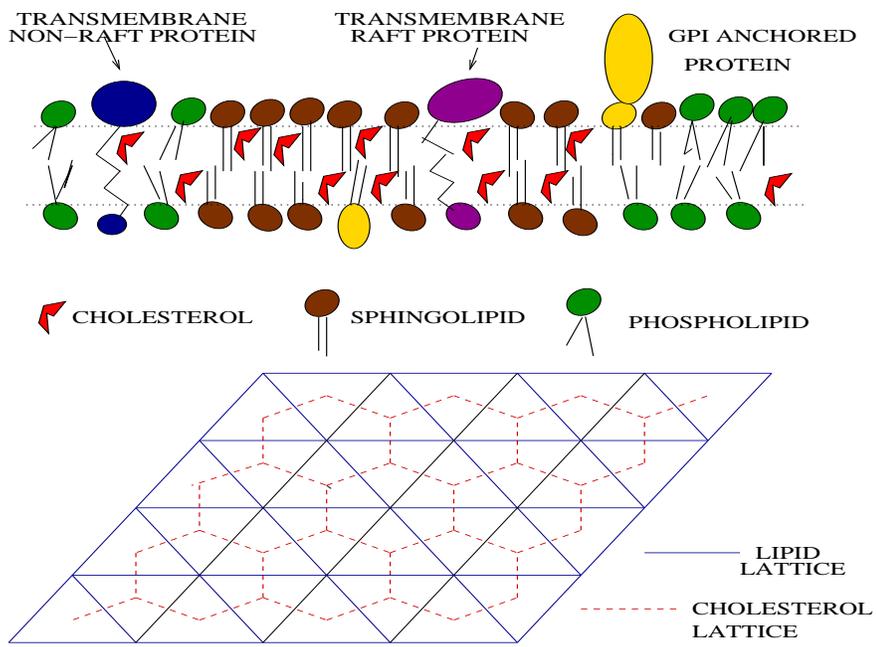


Figure 1: Lipid-cholesterol bilayer and the model hexagonal lattice

address for proteins targeted to lipid domains or rafts is a lipid shell while Simons and Ikonen [12] have pointed out the importance of cholesterol in their formation.

Domain formation in model membranes composed of ternary mixtures of unsaturated and saturated phosphatidylcholines and cholesterol have been studied experimentally by Scherfeld, Kahya and Schwille [13]. They investigated the effect of cholesterol on mixtures of dioleoyl-phosphatidylcholine(DOPC) & dipalmitoyl-phosphatidylcholine(DPPC) and compared it to that on mixtures of DOPC & sphingomyelin. Giant unilamellar vesicles prepared from ternary mixtures of various lipid compositions were imaged by confocal fluorescence microscopy and within certain range of sterol content, domain formation was observed. They also found evidence of a weaker interaction of cholesterol with phosphatidylcholines than with sphingomyelin.

Nielsen and co-workers [14, 15] have proposed a set of simple models to study the phase equilibria in two-dimensional condensed systems of particles where both translational and internal degrees of freedom are present and are coupled through microscopic interactions. Based on the concept of random lattice they proposed two models in analogy with spin 1/2 Ising model. In the first, the nearest neighbour particles interact through spatially-dependent spin-spin interactions. In the second model, the two spin states are assigned different degeneracies, one with zero internal (conformational) energy is non-degenerate while the other has a high internal energy corresponding to excitation energy associated with a conformational change and a large degeneracy representing the large number of possible chain conformations having same energy. The second model is similar to Doniach model [16, 17], in which all the possible excited states of the chain are lumped together into a single representative excited state. The effective degeneracy of this state is taken into account. The lowest, rigid (all trans) state of the chain is the ground state. In accordance with Ising notation, ground state may be considered the "spin down" state and the excited state the "spin up" state. Drawing this analogy further, the effect of "spin" state of individual chains, relevant to its specific conformation, on the inter-chain interaction is being incorporated in the Hamiltonian.

In spite of numerous work being done on lipid bilayers, the role of cholesterol in domain formation in a multicomponent system has not been investigated except for experimental studies. We have tried to explore this possibility in the present work, using Monte Carlo simulation technique, in a model system of multicomponent lipid bilayer. The system comprises of a small percentage of sphingolipids having saturated hydrocarbon chains along with phospholipids having unsaturated chains forming the major component. Cholesterol molecules of particular concentration are introduced in the system randomly on an intercalated lattice. Effect of percentage variation of cholesterol has been studied performing a series of simulations at different fixed cholesterol concentrations.

The phase behaviour of lipid-cholesterol bilayer involves two distinct but coupled order-disorder processes in terms of lipid-chain crystalline packing (translational degrees of freedom) and in terms of lipid-chain conformational ordering (internal degrees of freedom). The

concept of random lattice model [14] has been used to account for the coupling between the two degrees of freedom of lipid molecules, since it has been found experimentally that the chain conformational and translational order appear/disappear simultaneously at the main transition temperature. This implies that the main transition involves two distinct coupled transitions. The presence of cholesterol decouples the two ordering processes leading to a liquid ordered (**lo**) phase in which bilayers are liquid having translational disorder while lipid chains are conformationally ordered.

Cluster formation is mainly due to preferential packing of sphingolipid and cholesterol molecules. Sphingolipid head groups occupy larger excluded areas in the plane of the exoplasmic leaflet than do their predominantly saturated hydrocarbon chains. Cholesterol molecule being hydrophobic in nature tries to occupy the voids between associated sphingolipid chains. The hydrogen bonding between OH group of sterol and the amide of sphingolipid helps in the assembly forming a separate liquid ordered phase dispersed in loosely packed liquid disordered phase.

The two types of degrees of freedom are considered to be microscopically coupled through pairwise interaction [15] that include hard core repulsion between nearest neighbours in a hexagonal lattice together with two square well potentials providing an approximation to the intermolecular attraction between any two neighbouring chains. Phase separation has been observed between **so** (solid with translational order and collective ordering in chain conformation) and **lo** (liquid, retaining chain conformational order) phases at low temperature and **ld** (liquid having macroscopic disorder in both translational and chain conformations) and **lo** phases at high temperature. A cholesterol molecule, not assigned with any internal degrees of freedom, tries to secure ordered lipid-chain conformation in **ld** phase, while it has a tendency to break lipid translational order in **so** phase. Thus a cholesterol rich liquid ordered (**lo**) phase is formed. The phase diagrams establish the coexistence of (**so** + **lo**) phases at low temperatures and (**ld** + **lo**) phases at high temperatures. The system has been studied for different cholesterol concentrations but for a single canonical domain formation simulation, the cholesterol number is kept fixed.

A thermodynamic model for condensed complexes of cholesterol and phospholipid in a monolayer mixture was studied by Anderson and McConnell [18], while the chemical activity of cholesterol in this context was explored by Radhakrishnan and McConnell [19]. The phase equilibria of ternary mixtures was also investigated by de Miguel and Telo da Gama [20] using a three dimensional continuum model of amphiphilic mixtures representing water, oil and surfactant. Their findings showed a region of three liquid phase coexistence consisting of water-rich, oil-rich and surfactant-rich phases. Our approach is somewhat different, as has already been discussed, and the model ternary system is composed of a multicomponent lipid bilayer with a fixed percentage of cholesterol.

## 2 Simulation Model

The model bilayer considered in the present work is a multicomponent system comprising of (20%) sphingolipids and (80%) phospholipids. This composition is somewhat similar to that found in some real biomembranes in which the molar ratio of sphingomyelin and phospholipid is approximately 1:4 [21]. Lipid molecules reside at the sites of a 2 dimensional hexagonal lattice, defined by arrays of occupation variable  $la(n2)$  and order parameter  $S(n2)$ ,  $n2 = n*n$  being the number of lattice sites.  $la(i)$  is unity if  $i^{th}$  site is occupied by a lipid having ordered chain conformation, zero otherwise. A cholesterol molecule is allowed to occupy only the center of triangles formed in the lipid lattice. The intercalated lattice so formed by joining the centers of triangles in the main lattice specify cholesterol positions, which may or may not be occupied depending on cholesterol concentration. The cholesterol lattice is defined by an array of occupation variables  $lb(nc2)$ ,  $nc2 = 2*(n-1)*(n-1)$  being the number of interdigitated lattice sites.  $lb(i)$  is unity if  $i^{th}$  cholesterol site is occupied, zero otherwise. The range for percentage cholesterol concentration  $x_c$  is chosen from  $x_c = 0$  to maximum of 20% i.e.  $x_c = 0.2$ . This is justified from the fact that at high ( $\geq 25\%$ ) cholesterol concentration, the gel to liquid crystalline phase transition is eliminated and a stable liquid phase is produced [22].

In our model simulation, initial positions of lipid molecules, considered to be hard core particles, are selected by filling up the main lattice of dimension  $(n \times n)$  randomly in accordance with the percentage of respective lipid components, while positions of cholesterol molecules are chosen at random on an interdigitated lattice, partially occupied only by cholesterol molecules. The system has been studied for four different lattice sizes namely  $n = 10, 20, 30$  &  $40$ .

The acyl-chain conformational order parameter is given by

$$\langle S \rangle = 0.5(3\cos^2\theta - 1) \quad (1)$$

$\theta$  being the tilt angle of lipid molecules with respect to the layer normal [17]. For sphingolipids with saturated straight chains  $\theta = 0$ , so the order parameter is 1. The unsaturated chains of phospholipids are tilted with respect to layer normal. With increase of temperature, thermal fluctuations destroy long range order. The order parameter at a given temperature  $t$  is considered as [23, 24]

$$S_{ud} = S_{uo} \exp(-(t - t_0)^2/12) \quad (2)$$

The reference temperature  $t_0$  corresponds to fully ordered gel phase of unsaturated chains having uniform molecular tilt of  $\pi/6$ . Using Eq.(1) the order parameter at  $t_0$  becomes  $S_{uo} = 0.625$ ,  $S_{ud}$  is set to zero as it reaches a value less than a given threshold at a particular temperature that pertains to the main chain melting transition.

### 3 The Hamiltonian

The lateral mobility of individual lipid molecules strongly depend on chain conformational states. Pairwise interaction potentials couple microscopically the conformational and translational degrees of freedom of lipid molecules. Nearest neighbour interaction depends on the state of the particles and inter-particle distance. It includes a hard core repulsion between nearest neighbours together with two square well potentials providing an approximation to the intermolecular forces between any two neighbouring chains [14, 15]. The short range and the long range interaction potentials are given by -

$$\begin{aligned} V^s(R) &= -V^s \\ V^l(R) &= -V^l \end{aligned} \quad (3)$$

The range for short range interaction is  $d < R \leq R_0$  while for the long range one it is  $d < R \leq d_{max}$ , where  $d$  is the sum of radii of two interacting particles,  $R$  the distance between their centers of mass,  $R_0 = 1.3d_{uo}$  and  $d_{max} = 1.69d_{uo}$ ,  $d_{uo}$  being the diameter of the phospholipid in the ordered state.

In the hexagonal lattice each lipid molecule has six lipid neighbours and is surrounded by six cholesterol sites pertaining to intercalated lattice which may either be occupied or not. Each cholesterol site is surrounded by three other filled/unfilled cholesterol sites and three lipid neighbours. Lipid-lipid interaction is between two lipids either both in ordered state or one ordered and the other in disordered state. Cholesterol interacts with neighbouring lipid chain which may be either in ordered or in disordered configuration and also with neighbouring cholesterol molecules. Periodic boundary condition is considered in each case. The Hamiltonian for the system -

$$H = \sum_i E_d * (1 - la(i)) + H_{ll} + H_{lc} + H_{cc} \quad (4)$$

where  $E_d$  denotes the excitation energy of the disordered state of lipid chains and  $H_{ll}$ ,  $H_{lc}$ ,  $H_{cc}$  are lipid-lipid, lipid-cholesterol, cholesterol-cholesterol interactions.

$$\begin{aligned} H_{ll} &= \sum_{(i<j)} V_{oo}^l S(i)S(j)la(i)la(j) + \sum_{(i<j)} V_{od}^l S(i)S(j)(1 - la(i)la(j)) \\ &+ \sum_{(i<j)} V_{oo}^s S(i)S(j)la(i)la(j) + \sum_{(i<j)} V_{od}^s S(i)S(j)(1 - la(i)la(j)) \end{aligned} \quad (5)$$

$la(i)la(j) = 1$  only if both sites have ordered chains.

$$\begin{aligned} H_{lc} &= \sum_{(i<j)} V_{oc}^l S(i)la(i)lb(j) + \sum_{(i<j)} V_{dc}^l S(i)(1 - la(i)lb(j)) \\ &+ \sum_{(i<j)} V_{oc}^s S(i)la(i)lb(j) + \sum_{(i<j)} V_{dc}^s S(i)(1 - la(i)lb(j)) \end{aligned} \quad (6)$$

$la(i)lb(j) = 1$  if  $i^{th}$  lipid site is ordered and  $j^{th}$  cholesterol site is occupied.

$(1 - la(i))lb(j) = 1$  if  $i^{th}$  lipid site is disordered and  $j^{th}$  cholesterol site is occupied.

$$H_{cc} = \sum_{(i<j)} V_{cc}^l lb(i)lb(j) + \sum_{(i<j)} V_{cc}^s lb(i)lb(j) \quad (7)$$

$lb(i)lb(j) = 0$  if either of the cholesterol sites is not occupied.

All length scales considered in the article are in angstroms. The parameter values used for pairwise interaction potentials are in terms of  $J_0 \equiv V_{oo}^l$ , the strength of long range interaction between chains in ordered state. The dimensionless parameters are  $V_{oo}^l = 1.0$ ,  $V_{oo}^s = 1.55$ ,  $V_{od}^l = 0.5$ ,  $V_{od}^s = -0.5$ ,  $V_{oc}^l = 2.45$ ,  $V_{oc}^s = -1.75$ ,  $V_{dc}^l = 0.35$ ,  $V_{dc}^s = -0.35$ ,  $V_{cc}^l = 0.5$ ,  $V_{cc}^s = -1.0$  and  $E_d = 1.303$ [15].

## 4 Algorithm and Methodology

We have considered four different system sizes,  $n = 10, 20, 30, 40$ . For each of these, cholesterol concentration  $x_c$  is varied in the range  $0 \leq x_c \leq 0.2$ . Working on a particular system size and a specific cholesterol concentration, Monte Carlo simulation was carried for temperatures in the range  $38^{\circ}C < T \leq 43^{\circ}C$ . The algorithm used is as follows.

The initial system configuration for each simulation run pertains to fully ordered gel phase at  $38^{\circ}C$ , the reference temperature  $t_0$  in Eq.2. The phase space of the system is explored as the configuration evolves through the following four steps, each of which is subject to Metropolis acceptance criterion used in standard Monte Carlo simulation.

Accordingly in a single Monte Carlo (MC) cycle, attempts are made for -

### 1. Transition between conformations (order-disorder) in the lipid chains :

The packing of straight chains of sphingolipids always remain ordered ( $S=1$ ), while for phospholipids the chain conformation varies, so the order parameter  $S$  may change from  $S_{uo}$  to  $S_{ud}$  and vice versa.

### 2. Particle movement in the lipid lattice :

The center of mass of the particle concerned is subject to random displacement  $(dx, dy)$  [14], where

$$\begin{aligned} dx &= (2\zeta_x - 1)r_{max} \\ dy &= (2\zeta_y - 1)r_{max} \end{aligned} \quad (8)$$

$\zeta_x$  and  $\zeta_y$  being random numbers in the range  $0 \leq \zeta_{x(y)} \leq 1$  and  $r_{max} = 0.01 * d_{uo}$ .

### 3. Lateral diffusion of sphingolipids :

Exchange position of a sphingolipid with nearest neighbour dissimilar lipid molecule. This affects interactions at both the exchanging sites.

#### 4. Lateral diffusion of cholesterols :

Move a cholesterol molecule to its nearest neighbour empty site in the interdigitated lattice. Each cholesterol site is surrounded by 3 other cholesterol sites and 3 lipid neighbours. A cholesterol molecule is moved to another neighbouring empty site only if the number of sphingolipid neighbours is more in the new position than in the old one.

The steps 1 and 2 respectively provide for changes in conformational and translational order in lipids while steps 3 and 4 effectively takes into account the movement of sphingolipid and cholesterol molecules.

For each of the steps mentioned above, the system lattice is scanned sequentially. Considering the proposed change at a lattice point, overall impact on the system energy is taken into account using Metropolis algorithm. If the change is favoured, new system configuration is adopted. We move on to the next site to repeat the exercise.

After a sufficiently long period of equilibrium typically around 5,00000 MC cycles, the probability distribution function  $P(E, T, x_c)$  is sampled over next 1,000000 MC cycles . Binning procedure is used to store the histogram of average system energy E. The probability distribution function at the  $i^{th}$  bin is given by

$$P_i(E_i, T, x_c) = \frac{1}{z} N_i e^{-E_i/T} \quad (9)$$

where

$N_i$  = no. of configurations stored in the  $i^{th}$  bin,

T = the temperature,

$x_c$  = the cholesterol concentration and

$z = \sum_i N_i e^{-E_i/T}$  is the partition function.

The transition temperatures were found from peaks of specific heat plots against temperature. It may be noted that since we have considered the multicomponent system as a whole in our simulation, there was no need of taking the melting temperatures of individual components.

The histogram of energy stored can be used to generate data at a temperature close to the actual simulation temperature using reweighting technique of Ferrenberg and Swendsen [25, 26, 27]. The probability distribution function at a temperature  $T'$  is computed as -

$$P'_i(E_i, T, x_c) = \frac{P_i(E_i, T, x_c) e^{E_i(\frac{1}{T'} - \frac{1}{T})}}{\sum_i P_i(E_i, T, x_c) e^{E_i(\frac{1}{T'} - \frac{1}{T})}} \quad (10)$$

The free energy corresponding to the  $i^{th}$  energy bin is given by

$$F_i(E_i, T, x_c) = -\log(P_i(E_i, T, x_c)) \quad (11)$$

## 5 Results and Discussion

The simulation has been carried out for each of the four system sizes, mentioned before, using a range of values of percentage cholesterol concentration  $x_c$ , starting from  $x_c = 0$  (pure system) to  $x_c = 0.2$ . It is found that at very low cholesterol concentration, only the main chain melting transition **so-ld** is present, **so** being the gel phase and **ld** corresponds to liquid crystalline  $L_\alpha$  phase having macroscopic disorder in both translational and chain conformations. At intermediate cholesterol concentration ( $0.05 \leq x_c \leq 0.15$ ), coexisting phases are observed. Fig.2 shows the phase diagram as a function of cholesterol concentration  $x_c$  and reduced temperature  $T/T_M$ ,  $T_M$  being the transition temperature at  $x_c = 0$ . In the absence of cholesterol, there is only gel-fluid transition i.e. solid-ordered to liquid-disordered (**so-ld**) phase giving  $T/T_M = 1$ . For higher cholesterol concentrations, below the main chain melting transition, a transition from fully ordered (**so**) to mixed phase (**so+lo**) is observed and above the main transition at higher temperature a fully disordered phase is found i.e. a transition from mixed (**ld+lo**) to disordered liquid phase (**ld**) occurs. The phase diagram shows these transitions for  $x_c \leq 0.15$ . At high cholesterol concentration ( $x_c \approx 0.25$ ) gel-fluid transition is completely eliminated and a stable liquid phase with relatively high orientational order **lo** is produced.

The reweighting technique has been adapted to calculate the distribution function  $P(E, T, x_c)$  using Eq.(10) for a range of temperature very close to the transition region in each of the three cases i.e. so to so+lo, so+lo to ld+lo and ld+lo to ld transitions. From this the free energy  $F(E, T, x_c)$  is calculated by Eq.(11). In the  $F(E, T, x_c)$  vs. E plot, pronounced double minima corresponding to two coexisting phases is found at each transition point. Fig.3 shows the reweighting plots at the main transition temperature,  $\Delta T = \pm 0.005$  for  $x_c = 0.15$  and  $n = 40$ . The middle curve corresponds to the finite-size equilibrium transition temperature.

Fig.4 shows the free energy plot at the main transition temperature  $T_c = 40.5^{\circ}C$  for size-4 ( $n=40$ ) at  $x_c = 0.15$ . The two coexisting phases at  $E = E_1$  and  $E = E_2$  are separated by a barrier  $\Delta F(T, x_c)$ , with a maximum at  $E_{max}$  corresponding to interface between the two phases. The height of the barrier measures the interfacial free energy between the two coexisting phases and is given by

$$\Delta F(T, x_c) = F(E_{max}, T, x_c) - F(E_1, T, x_c) \quad (12)$$

It is found that the double well structure is more prominent with larger system. So the barrier height  $\Delta F_L(T, x_c)$  increases as the system size  $L(= nxn)$  increases. This can be seen in Fig.5. The barrier height is calculated by Eq.12 from the free energy plots at main transition for different system sizes. For  $x_c = 0.15$ , barrier heights are plotted against four lattice sizes used in the simulation.

At low concentrations, cholesterol molecules predominantly influence the conformational degrees of freedom of lipid molecules and tend to promote the domain formation thus

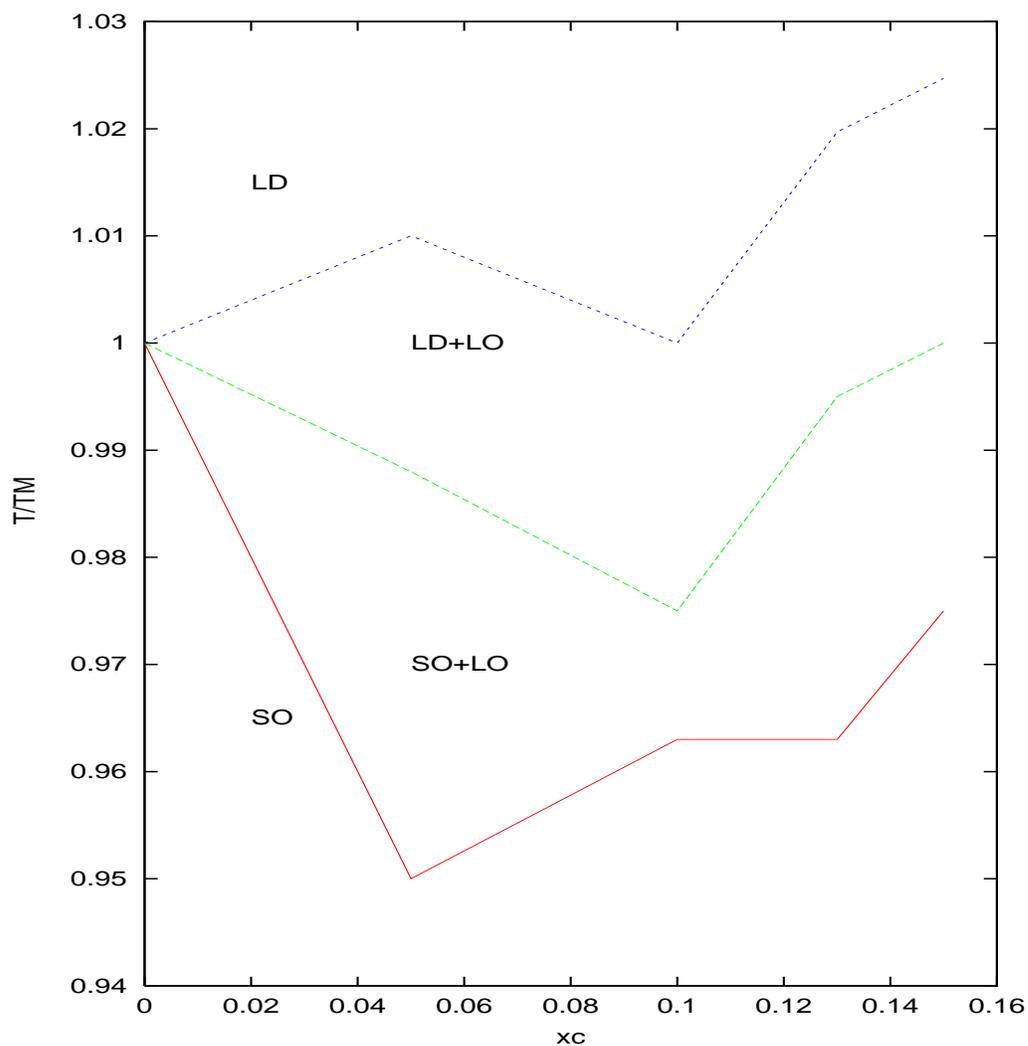


Figure 2: Phase diagram for the lipid-cholesterol model as a function of cholesterol concentration  $x_c$  and reduced temperature  $T/T_M$ ,  $T_M$  being the main transition temperature at  $x_c = 0$ . The different phases labeled are **so**, solid-ordered (gel); **ld**, liquid-disordered (fluid) and **lo**, liquid-ordered, the first letter refers to lateral order of the phase while the second the conformational order.

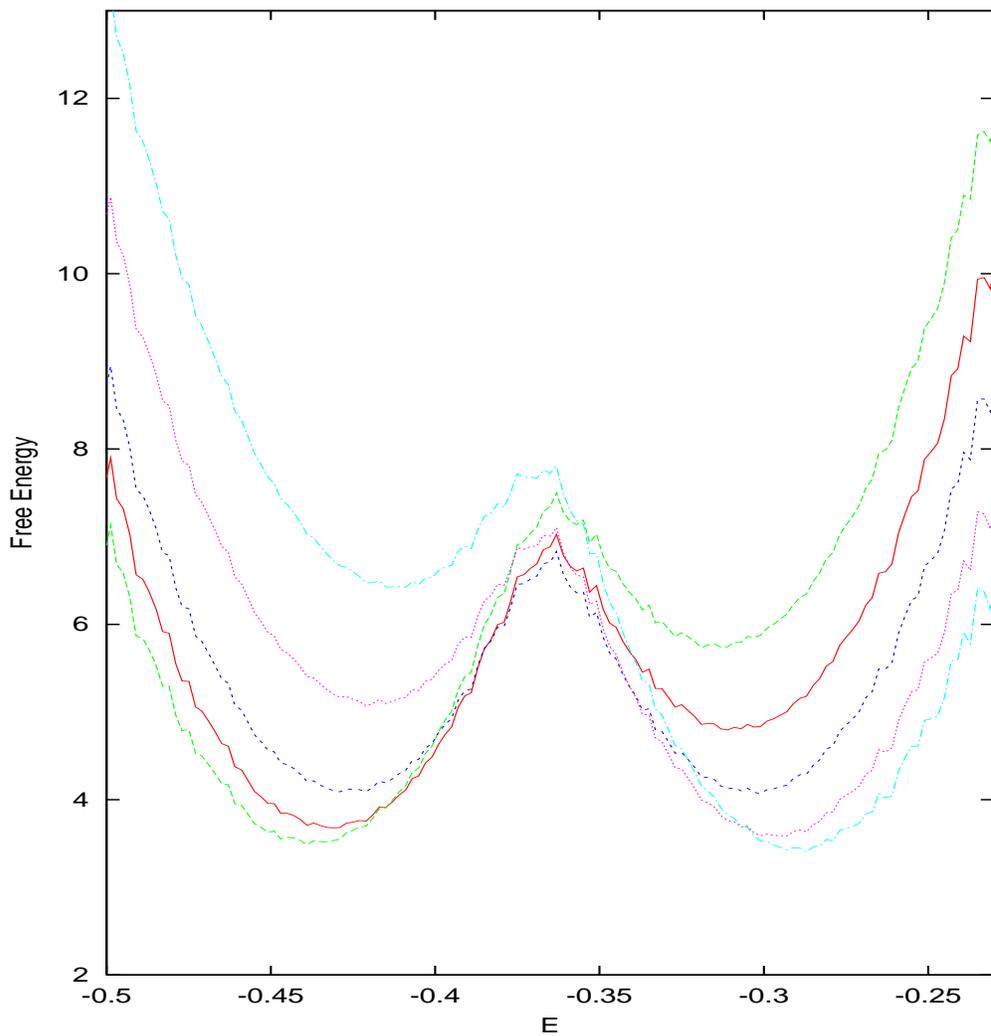


Figure 3: Free energy  $F(T, x_c)$  plots for  $n = 40$  for temperatures close to the equilibrium transition temperature. Using reweighting technique, extrapolations are done for temperature difference of  $\Delta T = \pm 0.005$ .

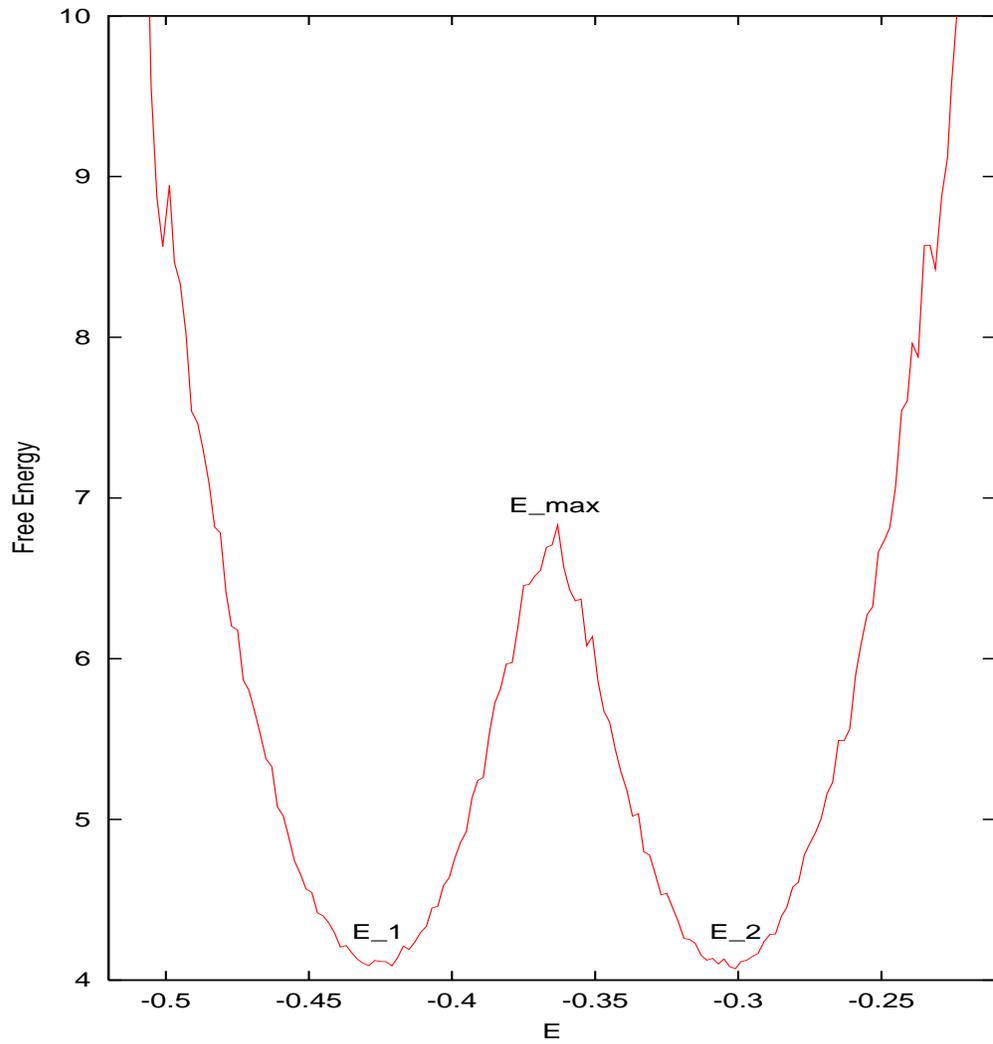


Figure 4: Free energy plot at the main transition for  $n=40$  and  $x_c = 0.15$ . Double well structure confirm the presence of coexisting phases.

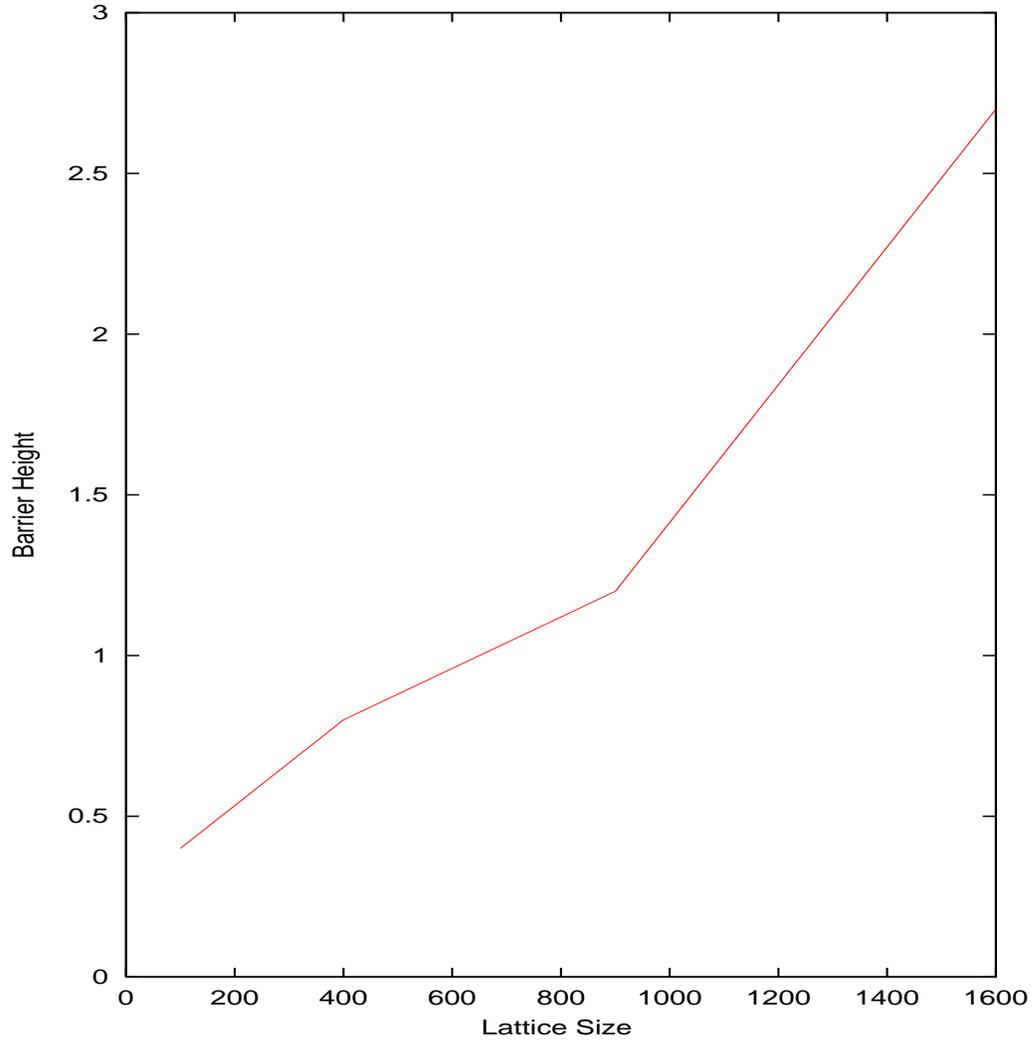


Figure 5: Barrier heights as a function of lattice size  $L$ . The plot shows increase with system size in the 'interfacial energy'  $\Delta F_L(x_c)$ , defined as the height of the maximum relative to the two minima of spectral free energy function

enhancing the dynamic membrane heterogeneity. This effect is illustrated in Fig. 6 for a range of cholesterol concentration. The cholesterol molecules tend to accumulate at the domain interfaces as can be seen in the plots.

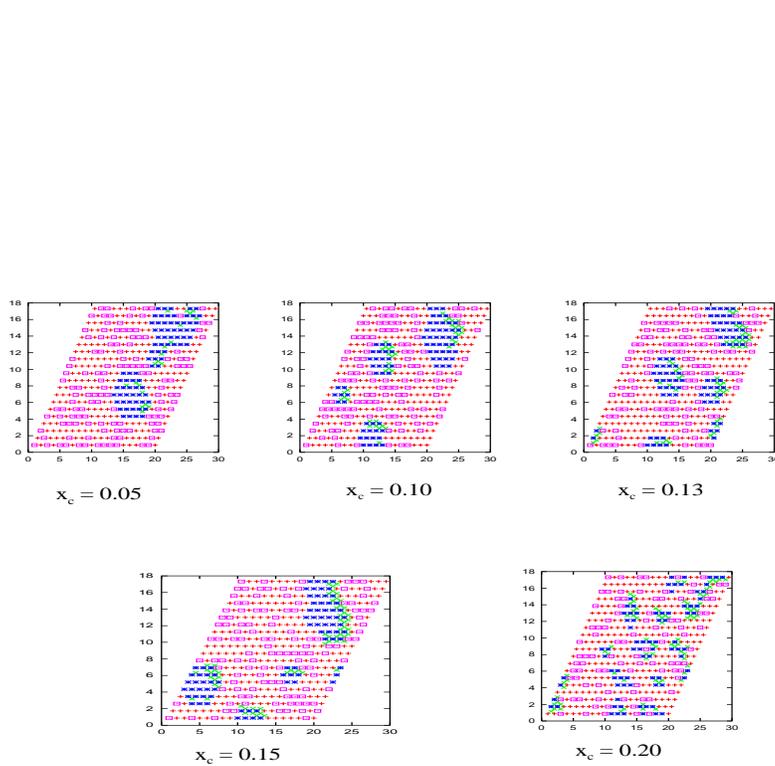


Figure 6: Simulation results showing formation of clusters of sphingolipids (\*) & cholesterol(x) at different cholesterol concentrations. The membrane configurations demonstrate formation of domains in the cholesterol range  $0 < x_c \leq 0.2$ .

## 6 Concluding Remarks

In the present work the phase coexistence has been located by computing the free energy using distribution functions or histograms of internal energy, reweighting of distribution functions very close to coexistence or phase transition and then a subsequent analysis of size dependence by finite-size scaling theory. In the process we have explored the role of cholesterol at different concentration in the formation of domains. It establishes experimentally observed facts to a large extent.

The lipid bilayers that are locally ordered and dynamically organized due to fluctuations and cooperative modes controlled by the underlying phase equilibria are sensitive to molecular agents, active at lipid domain interfaces, that can lower the interfacial tension. Cholesterol is one such agent which can alter the lateral structure of the lipid bilayer. In order to understand the full phase diagram of lipid-cholesterol mixtures, cholesterol's coupling to the translational degrees of freedom has to be taken into account. This has been done in the present work by using a model similar to Doniach model on a random lattice. The cholesterol with its smooth sterol skeleton, is able to break the lipid translational order and at the same time stabilizes the ordered lipid-chain conformation. Hence the effect of cholesterol is to decouple the translational and conformational degrees of freedom.

As discussed by Scherfeld and coworkers[13], the intermolecular interactions between sphingolipid and cholesterol is further strengthened by the hydrogen bonds induced by the amide group at the polar-apolar interface, which can act both as hydrogen bond-donating and -accepting group. This makes the sphingolipid-cholesterol interaction stronger than the phospholipid-cholesterol interaction. As a result, cholesterol intercalates more tightly in sphingomyelin bilayers than in glycerophospholipid bilayers.

An important feature of our work is that we have used a multicomponent lipid system having lipids with saturated as well as unsaturated hydrocarbon chains. This is essential for raft formation. Sphingolipids containing long largely saturated acyl chains readily pack tightly together making enough room in between for cholesterol occupation. Secondly, though the concept of random lattice has been used to ensure coupling of translational and internal degrees of freedom of the lipid molecules, we have worked throughout with constant lattice size. This is because we are interested only in the formation of clusters of sphingolipids and cholesterol molecules. The exchange of dissimilar lipid sites and movement of cholesterol molecules in the intercalated lattice subject to conditions mentioned earlier suffices our requirement.

We further need to focus on the affinity of certain kinds of proteins towards these condensed-complexes of sphingolipids and cholesterol molecules. The molecular packing created by cholesterol and sphingolipids, as has been found in the present simulation, may as well act as the first step to simulate formation of rafts. Considering all these aspects, we intend to study a multicomponent bilayer, comprising of phospholipids, sphingolipids, cholesterol and proteins in appropriate percentages, in aqueous environment to understand the mech-

anism of raft formation.

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