Eur Biophys J (1985) 12:75–86

European Biophysics Journal

Softening of lipid bilayers

O. G. Mouritsen* **

Department of Chemistry, Aarhus University, DK-8000 Aarhus C, Denmark

M. J. Zuckermann***

Department of Physics, McGill University, Montreal, Quebec, Canada H3A 2T8

Received December 10, 1984/Accepted February 28, 1985

Abstract. The softening of wet lipid bilayer membranes during their gel-to-fluid first-order phase transition is studied by computer simulation of a family of two-dimensional microscopic interaction models. The models include a variable number, q, of lipid chain conformational states, where $2 \leqq q \leqq 10$. Results are presented as functions of q and temperature for a number of bulk properties, such as internal energy, specific heat, and lateral compressibility. A quantitative account is given of the statistics of the lipid clusters which are found to form in the neighborhood of the transition. The occurrence of these clusters is related to the softening and the strong thermal density fluctuations which dominate the specific heat and the lateral compressibility for the high-q models. The cluster distributions and the fluctuations behave in a manner reminiscent of critical phenomena and percolation. The findings of long-lived metastable states and extremely slow relaxational behavior in the transition region are shown to be caused by the presence of intermediate lipid chain conformational states which kinetically stabilize the cluster distribution and the effective phase coexistence. This has as its macroscopic consequence that the first-order transition appears as a "continuous" transition, as invariably observed in all experiments on uncharged lecithin bilayer membranes. The results also suggest an explanation of the non-horizontal isotherms of lipid monolayers. Possible implications of lipid bilayer softening and enhanced passive permeability for the functioning of biological membranes are discussed.

Key words: Lipid bilayer, phase transition, clusters, metastability, compressibility

Introduction

Lipid bilayer membranes, which are model systems of biological membranes (Israelachvili et al. 1981; Quinn and Chapman 1980), undergo a thermally induced endothermic first-order phase transition referred to as the main transition or the gel-to-fluid chain melting transition. In a recent article, one of the authors (Mouritsen 1983) presented a study of the lack of cooperativity of this melting process. The study was based on computer simulations on a family of two-dimensional microscopic interaction models which involve between two and ten conformational states of the lipid chains. The results suggested that the "continuous" melting observed experimentally for pure wet lipid bilayers is explicable in terms of metastable states involving large statistical clusters of lipid molecules. These clusters are stabilized kinetically by the presence of intermediate single-chain conformational states. In a second article, Mouritsen et al. (1983) presented a detailed analysis of the two-state and ten-state models. The same methods of numerical simulation were used and the results were compared to the predictions of mean-field theory. It was found that the melting transition is strongly influenced by thermal fluctuations.

The previous work on the multi-state models clearly indicated in a qualitative manner that the occurrence of clusters in the transition region is closely related to the presence of strong thermal density fluctuations (for a recent review, see e.g. Mouritsen 1984 Chap. 5). With a view to estab-

* To whom reprint requests should be sent

** Supported by the Danish Natural Science Research Council and A/S De Danske Spritfabrikkers Jubilæumslegat

*** Supported in part by the NSERC of Canada and Le FCAC du Quebec

Abbreviations: PC, phosphatidylcholine; DMPC, dimyristoyl PC; DPPC, dipalmitoyl PC; ac, alternating current; DSC, differential scanning calorimetry; T_m, lipid gel-to-fluid phase transition temperature; TEMPO, 2,2,6,6-tetramethylpiperidine-*N*-oxyl

lishing this connection more quantitatively, we report in the present paper on a detailed cluster analysis for q-state models. The analysis is carried out systematically as a function of q, $2 \leqq q \leqq 10$, in an attempt to determine the relative importance of intermediate conformational states. In addition to the cluster analysis, comparative simulation data are presented of the internal energy, the specific heat, and the lateral compressibility. Comparison with mean-field theory is included when appropriate.

Our composite results are discussed under the common theme: softening of lipid bilayers. The term softening is used throughout this paper to signify that the bilayer becomes compliant due to the presence of strong lateral density fluctuations. The softening in the transition region is shown to lead to pseudo-critical behavior of the compressibility and the cluster distribution function. The results are compared to a variety of experimental measurements on lecithin bilayer membranes. Finally, we discuss possible biological implications of our model calculations.

Models and methods of calculation

Multi-state models of lipid bilayers

The q-state models are two-dimensional microscopic interaction models specifically designed to describe the gel-to-fluid phase transition of pure one-component lipid bilayers (Caillé et al. 1980). Within the scope of these models, the bilayer is considered as two monolayers which are assumed to be independent of each other. Since translational degrees of freedom are of little importance for the main transition, each monolayer is represented by a two-dimensional triangular lattice with $N = L \times L$ lattice sites. Every site of the lattice is occupied by a single saturated hydrocarbon chain which can be in one of q distinct conformational states. Each state is characterized by an internal energy, E_p, a cross-sectional area, A_p, and a degeneracy, D_p, where $1 \leqq p \leqq q$. All q states may be derived from the all-*trans* state by rotational isomerism. In a condensed system, only a limited number of the possible conformational states of a chain will be accessed. The basic idea underlying the present family of models is therefore that q can be chosen to be a small number by selecting only the most important states.

Two states are always included in a q-state model, namely the non-degenerate gel-like ground state ($p = 1$) representing the all-*trans* conformation and a highly degenerate excited state ($p = q$) characteristic of the "melted" fluid state. The q-state model is completed by including $q - 2$ intermediate gel-like states which contain kink and jog excitations. These intermediate states are selected subject to the requirement of low conformational energy and optimal packing. For increasing q, the intermediate states are chosen according to decreased probability of occurrence in the $q = 10$-state model (Mouritsen et al. 1983). The values of E_p are determined on the basis that a gauche rotation requires about 0.45×10^{-13} erg relative to the *trans* conformation. The values of D_p are obtained from combinatorial considerations and A_p is calculated via a geometrical construction which assumes that all chain conformations have the same volume (Caillé et al. 1980).

The chains interact via anisotropic forces which model both van der Waals and steric interactions. The lattice approximation automatically takes account of the excluded volume interactions. Finally, the interfacial forces required for the stability of the bilayer are modelled by an internal lateral pressure (Marcelja 1974).

Models with $q = 2$ and $q = 10$ have been used extensively to analyse a variety of experimental data for PC bilayers, both pure and reconstituted with intrinsic molecules. The reader is referred to Caillé et al. (1980) and Mouritsen et al. (1983) and articles cited therein for further details of the models and their applicability to bilayer membranes. In the present paper we report on a systematic study of the phase transition in q-state models with $2 \leqq q \leqq 10$. Rather than optimizing the parameters of each individual model to bring its thermodynamic properties into the closest possible accordance with experiment, we have found it useful for the purpose of a systematic comparative study to derive the models from a particular $q = 10$-state model. Models with $q = 2$, 3, 4, 5, and 6 are then obtained from this model by simply deleting an appropriate number of intermediate single-chain states, but keeping the total number of states constant (i.e. $\sum_p D_p$). Two different three-state models are considered, namely $q = 3_j$, and $q = 3_k$, corresponding to inclusion of jog and kink defects. Models with $q \geqq 4$ include both kinks and jogs.

The parameters of the generating $q = 10$-state model are those pertinent for DPPC bilayers (Mouritsen et al. 1983). These parameters were initially obtained by Caillé et al. (1980) from a fit to both thermodynamic and Raman data for pure saturated PC bilayers. Since no attempt is made here to fit experimental data for $q < 10$, the transition temperature of each model is different. However, our purpose is to investigate the influence of q on the thermodynamic properties as well as on the cluster distributions in the transition region and therefore

our results are presented as functions of reduced temperature, T/T_m, where T_m is the transition temperature.

Computer simulations

At present, computer simulation (Mouritsen 1984) constitutes the only reliable method of calculating the thermodynamic properties of realistic microscopic models of such complicated systems as lipid bilayer membranes. Here, we have used a conventional Monte Carlo importance-sampling procedure to generate the numerically exact solution to the statistical mechanical problem of the q-state models. A computer simulation approach to this problem has a number of unique advantages over conventional theory (Mouritsen 1984). Two of these are particularly striking in the present context. Firstly, the same computational procedure can be applied for any value of q without introducing individual approximations. Secondly, the simulation gives access to the microscopic configurations of the bilayer system thereby allowing a detailed analysis of the cluster distributions which characterize phenomena peculiar to the phase transition. It is noteworthy that such detailed information can only rarely be obtained from experimental studies. A Monte Carlo simulation may be thought of as a computer experiment on a well-defined system carried out under completely controlled conditions, and the numerical results can be considered in the same light as experimental data.

The Monte Carlo calculations are performed on finite lattices subject to toroidal boundary conditions. Effects due to the finite size are estimated by comparing results for a series of different system sizes, $N = 900$, 1600, and 3600. The calculations are carried out for increasing as well as decreasing series of temperatures in order to reveal possible metastable states. The calculated bulk thermodynamic properties include the internal energy, $E(T)$, and the average cross-sectional area per chain, $A(T)$, as well as the corresponding response functions, the specific heat and the lateral compressibility, as calculated from the fluctuation theorem

$$C(T) = (k_B T^2)^{-1} \sigma^2(E(T)), \tag{1}$$

$$\chi(T) = (k_B T)^{-1} \sigma^2(A(T)). \tag{2}$$

It is noted that the compressibility in Eq. (1) is a two-dimensional quantity which should not be confused with the bulk compressibility that measures the response in volume to a three-dimensional hydrostatic pressure. The bulk compressibility is only of minor interest in connection with the melting transition since the volume changes less than four per cent at T_m compared to a twenty per cent change in cross-sectional area.

Cluster analysis

In vizualising microscopically the melting process of the q-state models, the standard overall picture of a first-order phase transformation in a two-phase system applies: when the temperature is increased from below, statistical clusters of melted regions are seen to be formed in the solid gel matrix. The number and size of these clusters increase as T_m is approached. Eventually, at T_m the fluid clusters percolate and form a macroscopic phase in coexistence with the gel phase. Above T_m, the picture is reversed and clusters of gel are formed in the fluid phase.

The presence of clusters of the opposite phase in a one-phase region is a manifestation of local thermal density fluctuations. These fluctuations are directly related to the lateral compressibility of the system through Eq. (2). During a first-order transition, the compressibility stays finite and only clusters of finite size can develop. This is in contrast to a critical phenomenon which is characterized by fluctuations covering all wave-lengths and thus by a diverging compressibility.

The computer simulation provides the microscopic configurations characteristic of thermodynamic equilibrium at the temperature under consideration. The statistics of the cluster distributions may readily be derived from these configurations. A quantitative description of clusters requires definition of a cluster entity as well as characterization of the cluster distribution. We define a cluster by a nearest-neighbor connectivity criterion. The q possible single-chain states are divided into a group of $q-1$ gel-like (g) states characteristic of the low-temperature phase and a group of the one fluid (f) single-chain state characteristic of the high-temperature phase. An α-cluster ($\alpha = g, f$) is then simply defined by the requirement that all chains be in the α-state and that any chain in the α-cluster should connect to any other chain in the cluster by a series of nearest-neighbor bonds of the triangular lattice.

The instantaneous cluster distribution is characterized as follows: The size, l^α, of an α-cluster is taken as the number of chains in the cluster. The number of clusters of size l^α is denoted n_l^α. The probability of occurrence, $P^\alpha(l)$, of a cluster of size l^α is then

$$P^\alpha(l) = n_l^\alpha / \sum_l n_l^\alpha; \quad \alpha = g, f. \tag{3}$$

From the probability distribution a number of interesting quantities can be calculated, e.g. the average

cluster size

$$\overline{l^\alpha} = \sum_{l \geqq 3} l^\alpha P^\alpha(l) . \quad (4)$$

We have restricted the sum in Eq. (4) to include only clusters with more than two chains. The thermal ensemble averages of cluster properties are obtained by averaging $P^\alpha(l)$ over a large number of equilibrium configurations.

Results from model calculations

Bulk properties and response functions: Phase transition and metastability

Figure 1 gives the simulation data for the internal energy per chain, $E(T)$, as a function of reduced temperature, T/T_m. This figure shows that a discontinuous phase transition with a clear hysteresis behavior takes place for $q \leqq 5$, whereas $E(T)$ varies continuously through the transition for $q \geqq 6$. Similar results have previously been reported for the

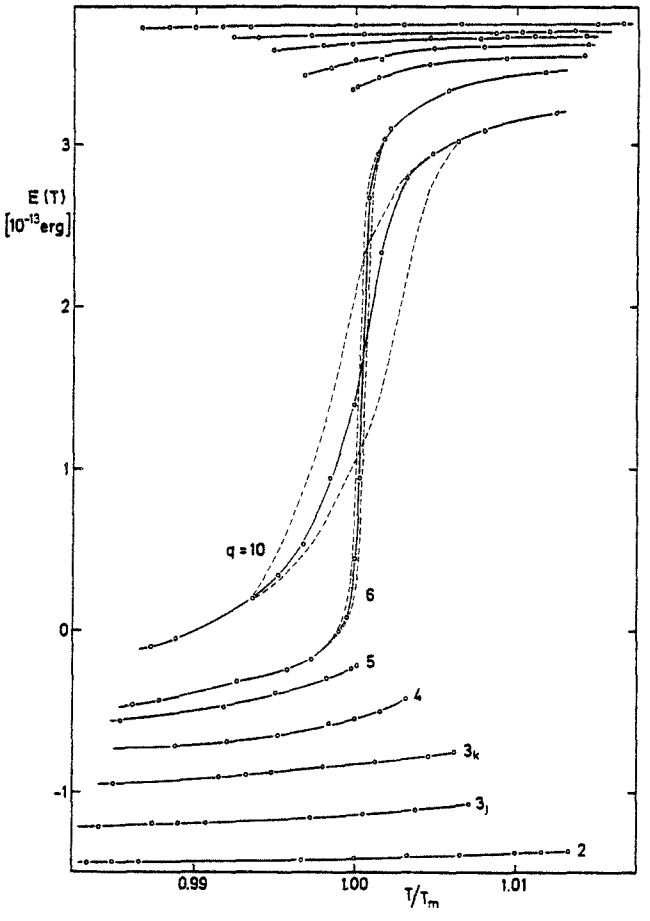

Fig. 1. Internal energy per lipid chain, $E(T)$, as a function of reduced temperature, T/T_m, for q-state models, where T_m is the equilibrium transition temperature. Monte Carlo data are indicated by circles. The dashed loops bound the range over which metastable states have been encountered for the $q = 6$ and $q = 10$-state models

average cross-sectional area, $A(T)$, as a function of temperature (Mouritsen 1983). The temperature is changed in very small steps during the simulation and the system is equilibrated at each temperature for a very long time ($\sim 10^4$ excitations per chain). When shorter equilibration times are used for $q \geqq 6$, metastable states and closed hysteresis loops, indicated by dashed lines in Fig. 1, are observed. That the phase transition is first-order is easily verified for all values of q by calculating the free energy function by a numerical integration of the internal energy within each phase (Mouritsen et al. 1983).

The behavior of $E(T)$ indicates that the system is relaxing extremely slowly towards equilibrium in the transition region. The larger q is the slower the relaxation becomes, i.e. it is more difficult to equilibrate the system when a large number of intermediate single-chain states are specified in the model. A slow relaxation in the transition region is also signalled by the greatly increased intensity of the fluctuation quantities, $C(T)$ and $\sigma^2(A)$, shown in Figs. 2 and 3. Both quantities have pronounced peaks at T_m and the peak intensities increase with increasing q. Since $\sigma^2(A)$ gives a direct measure of the strength of the lateral density fluctuations (Zuckermann and Pink 1980), Fig. 3 shows explicitly that the fluctuations are considerably enhanced in the transition region as q increases. In addition, fluctuation effects occur over a temperature range which increases with q. Finally, we see from Figs. 2 and 3 that fluctuations are much stronger for the $q = 10$-state model than for the $q = 6$-state model.

A discontinuity in $C(T)$ is resolved in the computer simulations for $q \leqq 4$ whereas the statistics only allow a possible discontinuity in $\chi(T)$ to be revealed for $q = 2$ and $q = 3_j$. In fact, the Monte Carlo data in Figs. 2 and 3 in the immediate vicinity of T_m (and quite clearly at T_m) may for the high-q models be affected by finite-size rounding. In agreement with mean-field calculations we find $\chi^g(T_m) > \chi^f(T_m)$ for $q = 2$ and $\chi^g(T_m) \leqq \chi^f(T_m)$ for $q > 2$ whereas $C^g(T_m) \geqq C^f(T_m)$ for all q. The mean-field results for the lateral compressibility are given in Fig. 4 for all values of q considered. In all cases, mean-field theory predicts a jump-singularity in $\chi(T)$. Figure 4 shows that the mean-field values of $\chi(T)$ hardly exhibit any variation with temperature in the transition region when compared to the corresponding simulation data of Fig. 3. This is a further proof of strong fluctuations being present near T_m, particularly in the high-q models. We note in passing that despite the inadequacy of mean-field theory to account for the fluctuations in the transition region it is found, somewhat surprisingly, that the mean-field values of T_m are extremely accurate.

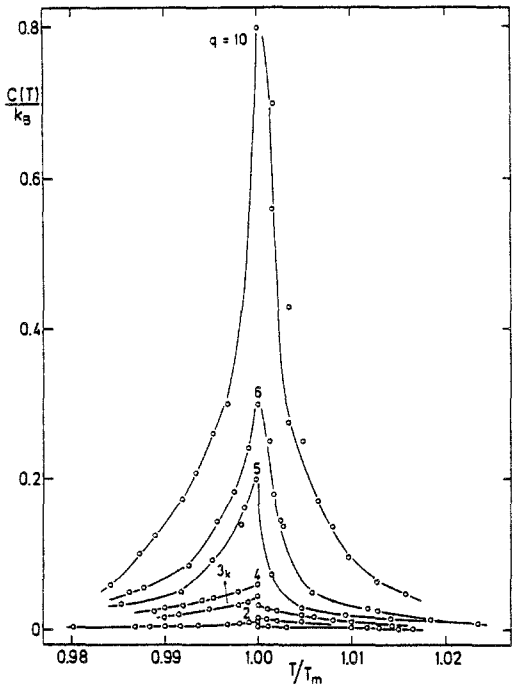

Fig. 2. Specific heat per lipid chain, $C(T)$ Eq. (1), as a function of reduced temperature, T/T_m for q-state models. Monte Carlo data are indicated by circles. For the sake of clarity, metastable branches have been omitted

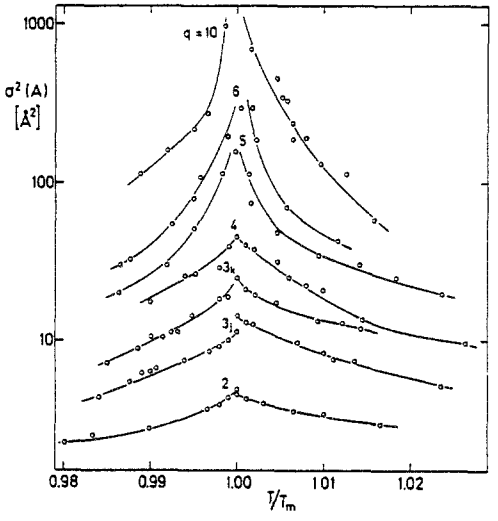

Fig. 3. Lateral compressibility per lipid chain, $\sigma^2(A) = k_B T \chi(T)$, as a function of reduced temperature, T/T_m, for q-state models. Monte Carlo data are given by circles and only equilibrium values are plotted. Note that the vertical axis is logarithmic

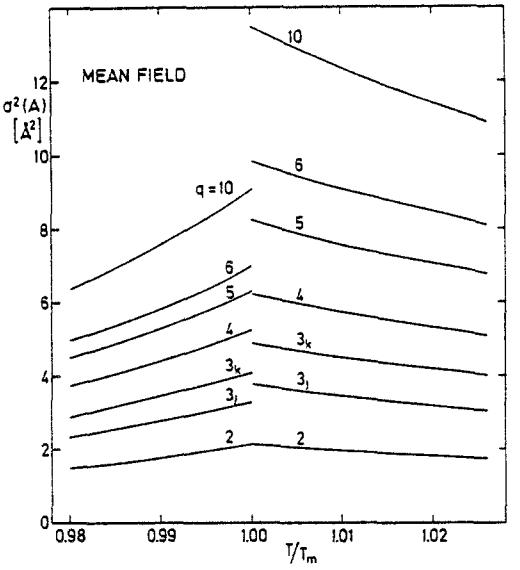

Fig. 4. Lateral compressibility per lipid chain, $\sigma^2(A) = k_B T \chi(T)$, as a function of reduced temperature, T/T_m, for q-state models as predicted by mean-field theory

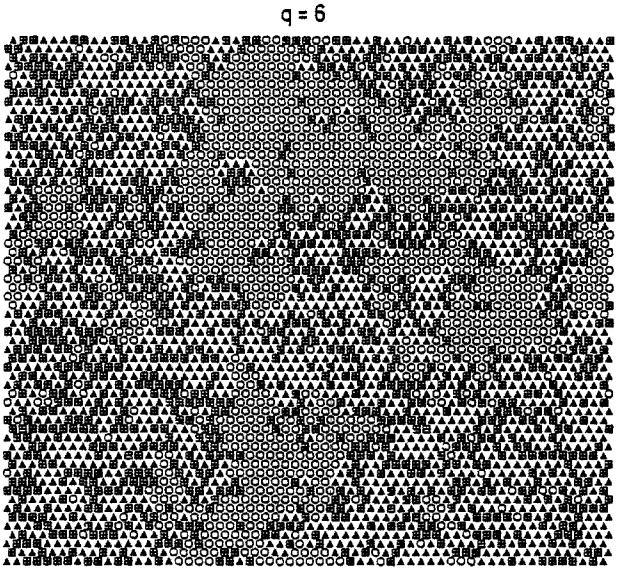

Fig. 5. Snapshot of a microscopic configuration for the $q = 6$-state model. The configuration is typical for a temperature less than 0.05 per cent below the equilibrium transition temperature. The system consists of 3600 lipid chains on a triangular lattice. The single-chain conformational states are denoted by *solid triangles* (all-*trans* state), *squares with a plus sign* (the four intermediate states), and *circles* (the fluid state)

Cluster distributions

The presence of large statistical clusters near T_m can be directly verified from a visual examination of typical microscopic equilibrium configurations such as the one shown in Fig. 5. This figure shows for the $q = 6$-state model immediately below the melting point ($[T_m - T]/T_m < 0.001$) that huge clusters of melted lipids have formed in the solid gel matrix. Clusters with as many as 500 chains have been observed. Obviously, a given cluster fluctuates in size as time elapses. The clusters appear as compact entities and the degree of ramification is fairly low.

The cluster distribution functions, n_l^g and n_l^f, for the $q = 6$-state model in the immediate vicinity of the phase transition are shown in Fig. 6. n_l^g and n_l^f are calculated for a system with 3600 chains at temperatures $0.999\,T_m$ and $1.001\,T_m$, respectively, i.e. precisely at the boundaries of the region in which metastabilities occur in this model (cf. Fig. 1). The results given in Fig. 6 should therefore correspond to equilibrium. As expected for an equilibrium situation at any temperature in a one-phase region, the maximum of n_l^α occurs at $l \to 0$. Within the uncertainty of the cluster statistics, we find the symmetric relation, $n_l^g \cong n_l^f$, close to T_m. For the small clusters, the insert of Fig. 6 gives the probability $P^\alpha(l)$, Eq. (3), for finding an α-cluster of size l. In the following section we shall return to a discussion of the decay of $P^\alpha(l)$. We note that $P^g(l) \cong P^f(l)$ close to T_m.

Figure 7 gives the temperature dependence of the average cluster size $\overline{l^\alpha}$, Eq. (4), for selected models and shows that the average cluster size increases as the transition is approached from either side. This is obviously the microscopic explanation for the enhancement of the lateral compressibility in Fig. 3. Moreover, an increase in q is found to facilitate the formation of larger clusters and lead to a larger dispersion in cluster sizes. It should be noted that the unphysical flattening of the wings in $\overline{l^\alpha}$ for $q = 4$ is due to the omission of very small "clusters" in the averaging, Eq. (4). The curves in Fig. 7 have a distinct asymmetry away from T_m which indicates that at the same relative distance from T_m, gel clusters are more easily formed and grow larger than the corresponding fluid clusters. This is caused by the asymmetry inherent in the multi-state models which have several similar gel states but only a single fluid state. The nucleation of the gel clusters is strongly facilitated by the availability of excited gel-like states which lower the interfacial energy. The consequences of this asymmetry are washed out as T_m is approached, especially for the high-q models.

Pseudo-critical behavior and percolation

The strongly fluctuation-dominated behavior of the q-state models in the transition region as shown in Figs. 2, 3, and 7 suggests that the phase transition may be close to a critical point. In view of the fact that a number of experiments on lipid bilayer melting have been analyzed in terms of pseudo-critical behavior, we shall briefly report on the results of a similar analysis performed on the computer simulation data.

We focus on the more strongly fluctuation-sensitive response function, the lateral compressibility.

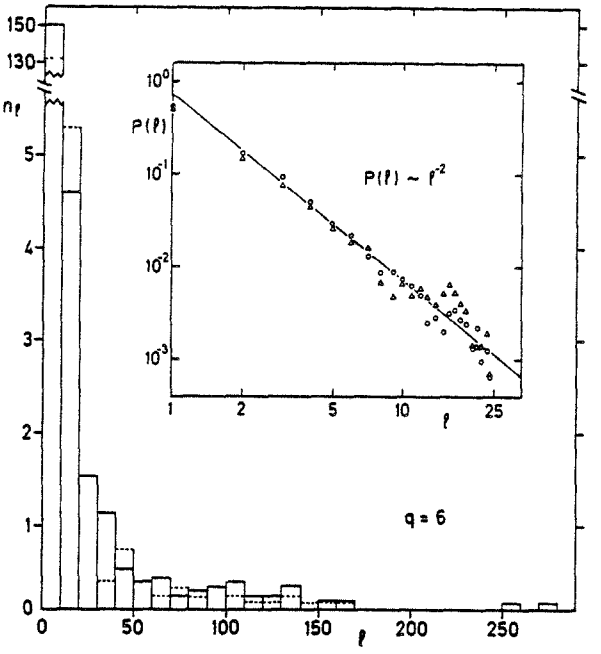

Fig. 6. Cluster distributions, n_l^α, as functions of cluster size, l, for the $q = 6$-state model in the immediate vicinity of the phase transition. $\alpha = g$ (*dashed line*) at $T = 0.999\,T_m$ and $\alpha = f$ (*solid line*) at $T = 1.001\,T_m$. The insert gives a log-log plot of the corresponding probability distributions, $P^\alpha(l)$ Eq. (3). Circles denote fluid clusters and triangles denote gel clusters. The results derive from Monte Carlo calculations on a system with 3600 lipid chains

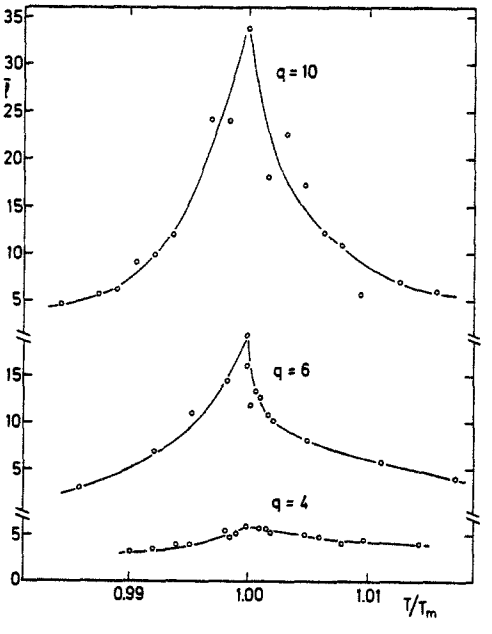

Fig. 7. Average cluster size, $\overline{l^\alpha}$ Eq. (4), as a function of reduced temperature for the $q = 4$, 6 and 10-state models. The results derive from Monte Carlo calculations on systems with up to 3600 lipid chains

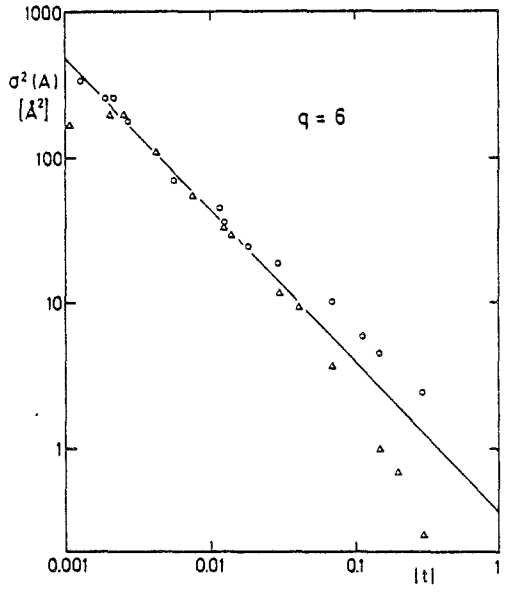

Fig. 8. Log-log plot of the compressibility $\sigma^2(A) = k_B T \chi(T)$ vs reduced temperature, $t = (T - T_m)/T_m$, for the $q = 6$-state model. The data are obtained from Monte Carlo calculations on a system with 3600 lipid chains. Triangles and circles denote data obtained in the gel and fluid phases, respectively. The solid line corresponds to the power law $\chi(T) \sim |t|^{-\gamma}$ with $\gamma \cong 1.05$

The fundamental variable in a critical-point analysis of a thermally induced phase transition is the reduced temperature, $t = (T - T_c)/T_c$, where T_c is the critical temperature. At the critical point, $\chi(T)$ is expected to have a power-law divergence (see e.g. Stanley 1971)

$$\chi(T) \sim A_{\pm} |t|^{-\gamma_{\pm}}, \quad t \to \pm 0. \tag{5}$$

The critical exponents $\gamma_{\pm}$ are believed to be universal numbers which fulfil the symmetric scaling relation $\gamma_+ = \gamma_-$. $\chi(T)$ for the q-state models does not diverge at the equilibrium transition temperature, T_m, but rather at the two spinodal points, T_- and T_+. The spinodal points bound the temperature region beyond which the metastable states go thermodynamically unstable. The values of T_- and T_+ are difficult to estimate accurately. However, for the higher-q models $\delta T_{\pm} = |T_{\mp} - T_m|/T_m$ is very small. Hence it is permissible to use the approximation $T_c \cong T_m$ while neglecting the data closest to T_m where $|t| \lesssim \delta T_{\mp}$. As an example, for $q = 6$ we have $\delta T_{\mp} \cong 0.001$ and one can safely estimate $\gamma_{\pm}$ from the log-log plot in Fig. 8. The striking observation to make from this plot is that the compressibility data are well described in both phases by the simple power law, Eq. (5), over one and a half decades of reduced temperatures. Moreover, the scaling relation holds, $\gamma_+ = \gamma_-$, and it is found that the amplitude ratio, A_+/A_-, is close to one. By subjecting the other models to the same type of analysis it is invariably found that $\gamma_+ \cong \gamma_-$. However, the region of validity of Eq. (5) diminishes as q decreases. Furthermore, the effective exponent value decreases with q, as given by $\gamma_{\pm} \cong 1.2$, 1.05, 0.9, 0.9, 0.7, 0.6, and 0.4 for the $q = 10$, 6, 5, 4, 3_k, 3_j, and 2-state models. There exists no accurate theoretical value of this exponent. A classical phenomenological Landau theory, which is, however, unreliable in predicting exponents, leads to $\gamma_{\pm} = \frac{1}{2}$ (Hatta et al. 1984).

Returning to the cluster statistics in Fig. 6, we note that the probability distribution $P^{\alpha}(l)$ has a form similar to that of the cluster distribution found in the critical region of the Ising model (Stoll et al. 1972). The log-log plot of the distribution function shows that the cluster data, except for the smallest clusters, are well described by the power law

$$P^{\alpha}(l) \sim l^{-\tau_{\alpha}} \tag{6}$$

with $\tau_g \cong \tau_f \cong 2$. The value of the exponent τ is the same as that quoted by Stauffer (1979) for two-dimensional percolation. Of course, the system sizes studied in the present work are too small to provide information in the large-l limit.

Discussion: Softening of lipid bilayers

Interpretation of model results

We have presented evidence in the preceeding sections that the isothermal first-order phase transition in the multi-state models of lipid bilayers is associated with strong thermal density fluctuations. The fluctuations lead to pseudo-critical behavior of the specific heat and of the lateral compressibility (cf. Figs. 2, 3, and 8). Together with the observation of an extremely slow relaxation in the transition region, this suggests that the lipid bilayer *softens* at the transition. Our systematic study of the phase transition as a function of the number of single-chain conformations, q, demonstrates unequivocally that the simultaneous presence of several intermediate conformations is responsible for the softening.

The cluster analysis provides a clear picture of the microscopic phenomena accompanying the softening. Large clusters of correlated chains are formed in the transition region and the average cluster size increases dramatically when the transition is approached from either side (cf. Fig. 7). The clusters are kinetically stabilized by the formation of "soft" domain walls which are composed of lipid chains in intermediate conformational states (Mouritsen 1983). The presence of intermediate states causes, by virtue of the anisotropic steric interactions, a decrease in interface energy relative to the

bulk energy of the clusters and thus impedes at T_m a speedy formation of a uniform bulk phase, simply by decreasing the rate of fusion between the clusters. This is the explanation previously given (Mouritsen 1983) for the slow relaxation to equilibrium, which in turn leads to an apparently continuous melting transition for $q \gtrsim 6$ (cf. Fig. 1). This non-equilibrium phenomenon is usually referred to as being due to a "lack of cooperativity" in the lipid bilayer melting transition (see e.g. Mouritsen 1983 and references therein).

The importance of spatially highly non-uniform configurations (cf. Fig. 5) for the thermodynamics of the lipid bilayer models implies that the mean-field description of the phase transition region is not reliable and at best only qualitatively correct (cf. Figs. 3 and 4). A detailed discussion of this point as well as a comprehensive comparison between computer simulation results and mean-field theory for $q = 2$ and $q = 10$-state models have recently been given by Mouritsen et al. (1983).

The observed lack of cooperativity in the phase transition in models with $q \geqq 6$ suggests that the transition in these models is associated with unusual kinetics. Indeed, a preliminary study of the $q = 6$-state model (Mouritsen 1984 Chap. 5) has shown that the relaxation within the metastability region proceeds as a cascade. Each resolved step in the cascade is characterized by a certain metastable cluster distribution. The average cluster size changes jumpwise when the system is quenched in temperature across the metastability region. Presumably, this cascade of closelying metastable states is responsible for the difficulties encountered in attempts to equilibrate q-state models in the transition region. It is noteworthy that cascading is not found for $q < 5$. This may explain the significant gap between the curves for $q = 4$ and $q = 5$ for the specific heat and the compressibility (Figs. 2 and 3). The case $q = 5$ seems to be marginal: the system relaxes extremely slowly at T_m, metastable states have been detected, but the width of the hysteresis cycle is extremely narrow. A fuller account of the phase transition kinetics will be given elsewhere.

Comparison with experiments

A detailed comparison of static properties of multi-state models with the results of experimental studies of lipid bilayers have previously been reported (Caillé et al. 1980, Mouritsen et al. 1983 and references therein). This comparison has mainly focussed on the enthalpies of melting, ^{2}H-NMR order parameters, and Raman band intensities and its successful outcome has provided mounting evidence that the family of multi-state models contains the essential physics for describing a considerable variety of properties of the gel and fluid phases of pure lipid bilayers, including the quantitative characteristics of the main phase transition. We restrict ourselves to a discussion of the applicability of q-state models to describe the softening of lipid bilayers.

It was pointed out earlier (Mouritsen 1983) that a systematic study of q-state models may provide a reason for the appearance of the first-order transition as a continuous transition in every experimental study of uncharged PC bilayers (Albon and Sturtevant 1978; Davis 1979; McKay 1981; Evans and Kwok 1982). As demonstrated in Fig. 1, the first-order transition appears to be smeared when a sufficiently large number of intermediate chain conformational states are specified. Thus, the intrinsic molecular properties of the lipid chains can alone lead to the observed continuous transitions and it is therefore unnecessary to invoke more complicated explanations of the experiments in terms of impurities or lack of experimental resolution.

The cluster analysis provides a picture which on a microscopic level explains the lack of cooperativity in the melting process. The interaction between the large clusters which occur in the transition region is screened due to the formation of soft domain walls. This in turn diminishes the cooperativity of the system. Our finding of large weakly-interacting clusters near T_m is in accordance with the results of standard van't Hoff analysis of calorimetric data (Mabrey and Sturtevant 1976; Freire and Biltonen 1978; Marsh et al. 1976; Black and Dixon 1981). According to this approach, the size of a cooperative unit ($\sim$ isolated cluster) is defined as the ratio between the van't Hoff enthalpy of melting and the calorimetrically determined enthalpy. The numbers derived from this type of analysis lie in the range from 100 to 1000 molecules in a cluster. This is in agreement with cluster sizes detected in the computer simulations of the $q = 6$ and $q = 10$-state models near T_m. Direct experimental evidence for the formation of large clusters during the lipid bilayer melting transition comes from electron microscopic investigations of isolated PC vesicles (Sackmann et al. 1980; see also Hui and Parsons 1975). Large elongated gel clusters are seen to be formed in the fluid bilayer matrix (Gebhardt et al. 1977; E. Sackmann, private communication). Due to a simultaneous formation of ripples in the experimental system we hesitate to make a more detailed comparison with the simulation results.

A number of previous theoretical approaches (Lee 1977; Kanehisa and Tsong 1978; McCammon and Deutch 1975; Tsong et al. 1977; for critical comments on these theories, see Nagle 1980) have also explained the lack of cooperativity on the basis of a

cluster picture. However, in these theories the cluster picture is initially built into the theory and its consequences are then worked out. Conversely, in the present work the cluster picture is *derived* directly from a microscopic interaction model by use of first-principle statistical mechanics. In addition, our purely statistical mechanical approach shows that the clusters are not static domains but rather fluctuating dynamic entities related to the thermal density fluctuations of a bilayer which is softening.

For the high-q models we find near T_m extremely long-lived metastabilities which result in a continuous reversible behavior or closed hysteresis cycles depending on the length of the observation time. This is in close agreement with recent ac calorimetric measurements on DPPC (Black and Dixon 1981) as well as a number of NMR experiments (Davis 1979; McKay 1981).

To the best of our knowledge, Evans and Kwok's (1982) micromechanical measurement of the elastic compliance of giant DMPC vesicles constitutes the only reported systematic study of the temperature dependence of the lateral compressibility of lipid bilayers. The measurements are carried out in a tension-free state of the single-layer vesicles. A comparison should therefore be possible with our model calculations which automatically avoid problems related to edge effects of multilamellar dispersions (Lis et al. 1982). Since we have not optimized our model parameters to describe specifically DMPC, we make no quantitative comparison. A qualitative comparison, however, shows that the experimental results are in accord with Fig. 3 in predicting a pronounced softening in a narrow region about T_m. However, the shape of the experimental compressibility is different in that the wings seem to be suppressed in the one-phase regions. Since the experimentally examined temperature range is very narrow and doubts exist as to whether equilibrium has been attained in the experiment below T_m (E. Evans, private communication), a definite assessment of the ability of q-state models to describe the details of the compressibility function cannot be made until a fuller experimental investigation is completed.

The striking anomalies observed experimentally at lipid bilayer phase transitions have been analyzed in terms of critical behavior for a variety of properties, such as passive permeability (Papahadjopoulos et al. 1973; Doniach 1978), ultrasound velocity (Mitaku et al. 1978, 1983), and specific heat (Hatta et al. 1983, 1984). In a sharp first-order phase transition, the specific heat has a delta-function singularity superimposed on the background specific heat. The area of this anomaly corresponds to the enthalpy of melting. In an experiment as well as in computer simulation, the delta-function will appear as a broadened peak of finite intensity. The background specific heat itself has been shown by recent ac calorimetry (Hatta et al. 1983) on DPPC to have a sharp divergence at T_m. In contrast to DSC, steady-state ac calorimetry does not measure the latent heat of the transition and it is only sensitive to thermal fluctuations. The work by Hatta et al. (1983, 1984) has provided strong evidence that the lipid phase transition is indeed pseudo-critical. Analysis of the experimental ac specific heat at 0.6 Hz in terms of power-law singularities,

$$C(T) = B_{\pm}|t|^{-\alpha_{\pm}} + D_{\pm}|t| + E_{\pm}, \qquad (7)$$

leads to $\alpha_+ \cong \alpha_- \cong 0.5$ and a distance between the spinodal points of only $\delta T_{\pm} \cong 0.0004$. The specific heat results from the computer simulations are not accurate enough to sustain an analysis in terms of Eq. (7), which is severely hampered by the presence of the constant background term, $E_{\pm}$. However, it is gratifying to note that the specific heat peaks of Fig. 2 also signals critical-like behavior. Moreover, the distance between the experimentally determined spinodal points in DPPC is of the same order of magnitude as we find for the $q = 6$-state model.

Relations to lipid monolayer systems

The liquid condensed-to-liquid expanded phase transition in lipid monolayers at the air-water interface is closely related to the main gel-to-fluid phase transition of lipid bilayers (Albrecht et al. 1978; von Tscharner and McConnell 1981; Georgallas et al. 1984). The q-state models are equally good models of this monolayer phase transition (Zuckermann et al. 1982; Caillé et al. 1980; Georgallas and Pink 1982) when the intrinsic lateral pressure of the bilayer is interpreted as the external two-dimensional pressure exerted by the film balance in the monolayer experiment. The isotherms in the pressure-area phase diagram of monolayers are invariably found to be non-horizontal in the liquid expanded-liquid condensed region (Cadenhead et al. 1980). This corresponds to a sharply peaked but broadened lateral compressibility as a function of temperature.

The monolayer results have been interpreted in the same fashions as the bilayer results in terms of restricted cooperativity of the transition and effective fluid-solid coexistence over a finite range of pressures (Albrecht et al. 1978; Georgallas and Pink 1982). The size of the cooperative unit has been estimated to be of the order of a few hundred molecules. Very convincing evidence from transmission electron microscopy and electron diffraction has recently been given for the presence of large clusters of solid phase in the melted fluid monolayer at the

phase transition (Fischer and Sackmann 1984; Fischer et al. 1984). The coherence length of the solid domains is found to be about 100 Å corresponding to clusters comprising 100–200 lipid molecules, in accordance with the previous experiments.

The finding in the present work of a smeared first-order phase transition in the q-state models is in accordance with the monolayer experiments. In particular, our cluster picture is strongly supported by the electron microscopy work. The sizes of the clusters are even in quantitative agreement. The correspondence between our model calculations and the monolayer experiments suggests that the lack of cooperativity in monolayer systems is also caused by the very nature of the lipid chains whose intermediate states in the transition region kinetically stabilize a dynamic fluctuating cluster distribution.

Implications for biological membranes

Lipid bilayers are model systems of biological membranes (Quinn and Chapman 1980). In contrast to one-component pure lipid bilayers, however, real biological membranes are very heterogeneous systems consisting of a very large number of different lipid species in addition to polypeptides, proteins, and other biologically important "impurity" molecules. Being many-component mixtures, membranes do not display phase transitions in a strict sense. Rather, they have a diverse phase diagram characterized by regions of phase coexistence. Nevertheless, a number of real membranes do exhibit rather abrupt phase changes associated with thermal anomalies. A well-known example is the bacterial plasma membrane of *Acholeplasma Laidlawii* (see e.g. McElhaney 1984). Therefore we expect that the softening of lipid bilayers discussed in the present work has some important bearings on the functioning and structure of biological membranes.

We do not want to go into the ongoing dispute of whether or not lipid phase transitions directly influence the physiological functions of membranes (see e.g. Sandermann 1978; McElhaney 1982). What seems to be commonly agreed upon is that the thermodynamic state of the membrane influences its function. Specifically it is found that a) most plasma and nuclear membranes as well as the membranes of organelles require the lipids to be in their fluid state in order to support biological activity and b) phase separation leading to biologically differentiated regions in the membrane is important for biological viability.

The lateral compressibility of a bilayer is directly related to the passive transmembrane ionic permeability (Nagle and Scott 1978; Doniach 1978; Zuckermann and Pink 1980). Our finding of strong density fluctuations in the transition region is in accordance with experiments on the Na^+ diffusion in PC vesicles (Papahadjopoulos et al. 1973), which shows a pronounced peak at T_m. The electrolytic leakage near T_m is counteracted in eucariotic cell membranes by the incorporation of cholesterol which seals the membranes and decreases the passive permeability (de Gier et al. 1968). The movement of water molecules through lipid bilayers and biological membranes is remarkably fast. It has been demonstrated experimentally that the water permeability of protein-free biological membranes in general depends on the physical state of the lipid matrix and specifically increases dramatically when the membrane is taken through the phase transition (Carruthers and Melchior 1983). Although the water permeability is not directly related to the lateral density fluctuations, it seems obvious that the increased probability for pore formation in the transition region must be accompagnied by a corresponding enhancement of water permeation. It has been suggested in a theory due to Träuble (1971) that the free volume created by thermally excited kink defects may serve as passive carriers of water molecules and small neutral lipophilic molecules. The increased density of kink defects in the transition region (Träuble 1971; Mouritsen et al. 1983) then implies faster permeation of water molecules across the membrane, in accordance with the experimental observations.

In general, we expect an enhancement of permeability of matter at T_m mediated by the presence of long-lived domains with soft walls. The soft walls are characterized by a less effective packing of the lipid acyl chains. Therefore, the walls present a leakage to the passage of small molecules. Such an effect has been demonstrated for the permeability of TEMPO-choline into DMPC vesicles (Marsh et al. 1976). It was suggested by Marsh et al. that the biological significance of the enhanced permeability near T_m (or in a phase separation region) is that it may provide a mechanism by which chemical transmitters or enzymes could be released from secretory granules.

The present computer simulation study shows that the reasons for the decreased barrier to passive permeation in the transition region of lipid membranes may be more complex than previously anticipated (Marcelja and Wolfe 1979). Not only is there an occurrence of strong density fluctuations which increase the probability for instantaneous pore formation, but the density fluctuations are also accompanied by a long-lived cluster distribution. The occurrence of well-defined clusters implies the presence of a substantial fraction of interfacial

regions which facilitate passive permeation of matter.

Furthermore, and in analogy with the observed attraction between impurities and defect structures in lipid bilayers (Rüppel and Sackmann 1983), the soft walls will offer an appropriate environment for the insertion of other membrane components, such as intrinsic polypeptides and proteins. The presence of impurity molecules in the walls will lead to a pinning of the domain pattern (Mouritsen 1983). This, in turn, will slow down the relaxation rate and further strengthen the thermal density fluctuations. The resulting enhancement of the passive permeability is observed in a variety of experiments (see e.g. Jänig 1981 for a list of references). It is perhaps more important from a biological point of view that the presence of soft walls to conveniently accomodate proteins may be significant for supporting enzymatic activity which requires conformational changes in the hydrophobic core of the membrane protein.

Acknowledgements. One of us (MJZ) wishes to thank his colleagues Alexandros Georgallas and David Pink for stimulating discussions and NORDITA for a travel grant. The authors are very grateful to Turab Lookman for pointing out the possibility of pseudo-critical behavior for the cluster distribution. Professor Erich Sackmann is sincerely thanked for useful correspondence and for sending us electron micrographs from his vesicle work.

References

Albon N, Sturtevant JM (1978) Nature of the gel to liquid crystal transition of synthetic phosphatidylcholines. Proc Natl Acad Sci USA 75:2258–2260

Albrecht O, Gruler H, Sackmann E (1978) Polymorphism of phospholipid monolayers. J Phys (Paris) 39:301–313

Black SG, Dixon GS (1981) Ac calorimetry of dimyristoylphosphatidylcholine multilayers: Hysteresis and annealing near the gel to liquid-crystal transition. Biochemistry 20: 6740–6744

Cadenhead DA, Müller-Landau F, Kellner BMJ (1980) Phase transition in insoluble one and two-component films at the air/water interface. In: Sinha K (ed) Ordering in two dimensions. North-Holland, New York, pp 73–81

Caillé A, Pink DA, de Verteuil F, Zuckermann MJ (1980) Theoretical models for quasi-two-dimensional mesomorphic monolayers and membrane bilayers. Can J Phys 58:581–611

Carruthers A, Melchior DL (1983) Studies of the relationship between water permeability and bilayer physical state. Biochemistry 22:5797–5807

Davis JM (1979) Deuterium magnetic resonance study of the gel and liquid crystalline phases of dipalmitoyl phosphatidylcholine. Biophys J 27:339–358

de Gier J, Mandersloot JG, van Deenen LLM (1968) Lipid composition and permeability of liposomes. Biochim Biophys Acta 150:666–675

Doniach S (1978) Thermodynamic fluctuations in phospholipid bilayers. J Chem Phys 68:4912–4916

Evans E, Kwok R (1982) Mechanical calorimetry of large dimyristoylphosphatidylcholine vesicles in the phase transition region. Biochemistry 21:4874–4879

Fischer A, Sackmann E (1984) Electron microscopy and diffraction study of phospholipid monolayers transferred from water to solid substrates. J Phys (Paris) 45:517–527

Fischer A, Lösche M, Möhwald H, Sackmann E (1984) On the nature of the lipid monolayer phase transition. J Phys Lett (Paris) 45:L785–L791

Freire E, Biltonen R (1978) Estimation of molecular averages and equilibrium fluctuations in lipid bilayer systems from excess heat capacity function. Biochim Biophys Acta 514: 54–68

Gebhardt C, Gruler H, Sackmann E (1977) On domain structure and local curvature in lipid bilayers and biological membranes. Z. Naturforsch 32c:581–596

Georgallas A, Hunter DL, Lookman T, Zuckermann MJ, Pink DA (1984) Interaction between two sheets of a bilayer membrane and its internal lateral pressure. Eur Biophys J 11:79–86

Georgallas A, Pink DA (1982) A new theory of the liquid condensed-liquid expanded phase transition in lipid monolayers. Can J Phys 60:1678–1681

Hatta I, Suzuki K, Imaizumi S (1983) Pseudo-critical heat capacity of single lipid bilayers. J Phys Soc Jpn 52: 2790–2797

Hatta I, Imaizumi S, Akutsu Y (1984) Evidence for weak first-order nature of lipid bilayer phase transition from the analysis of pseudo-critical specific heat. J Phys Soc Jpn 53:882–888

Hui SW, Parsons DF (1975) Direct observation of domains in wet lipid bilayers. Science 190:383–384

Israelachvili JN, Marcelja S, Horn RG (1981) Physical principles of membrane organization. Q Rev Biophys 13: 121–200

Jänig F (1981) Critical effects from lipid-protein interaction in membranes. II. Interpretation of experimental results. Biophys J 36:347–357

Kanehisa MI, Tsong TY (1978) Cluster model of lipid phase transitions with application to passive permeation of molecules and structure relaxation in lipid bilayers. J Am Chem Soc 100:424–432

Lee AG (1977) Lipid phase transitions and phase diagrams I. Lipid phase transitions. Biochim Biophys Acta 472: 237–281

Lis LJ, McAlister M, Fuller N, Rand RP, Parsegian VA (1982) Measurements of the lateral compressibility of several phospholipid bilayers. Biophys J 37:667–672

Mabrey S, Sturtevant JM (1976) Investigation of phase transitions of lipids and lipid mixtures by high sensitivity differential scanning calorimetry. Proc Natl Acad Sci USA 73:3862–3866

Marcelja S (1974) Chain ordering in liquid crystals II. Structure of bilayer membranes. Biochim Biophys Acta 367: 165–176

Marcelja S, Wolfe J (1979) Properties of bilayer membranes in the phase transition or phase separation region. Biochim Biophys Acta 557:24–31

Marsh D, Watts A, Knowles PF (1976) Evidence for phase boundary lipid. Permeability of Tempo-choline into dimyristoylphosphatidylcholine vesicles at the phase transition. Biochemistry 15:3570–3578

McCammon JA, Deutch JM (1975) Semiempirical models for biomembrane phase transitions and phase separations. J Am Chem Soc 97:6675–6681

McElhaney RN (1982) Effects of membrane lipids on transport and enzymatic activities. Curr Topics Membr Transport 17: 317–380

McElhaney RN (1984) The structure and function of the *Acholeplasma Laidlawii* plasma membrane. Biochim Biophys Acta 779:1–42

McKay AL (1981) A proton NMR moment study of the gel and liquid-crystalline phases of dipalmitoyl phosphatidylcholine. Biophys J 35:301–313

Mitaku S, Ikegami A, Sakanishi A (1978) Ultrasonic studies of lipid bilayer. Phase transition in synthetic phosphatidylcholine liposomes. Biophys Chem 8:295–304

Mitaku S, Jippo T, Kataoka R (1983) Thermodynamic properties of the lipid bilayer transition. Pseudocritical behavior. Biophys J 42:137–144

Mouritsen OG (1983) Studies on the lack of cooperativity in the melting of lipid bilayers. Biochim Biophys Acta 731: 217–221

Mouritsen OG (1984) Computer studies of phase transitions and critical phenomena. Springer, Berlin Heidelberg New York

Mouritsen OG, Boothroyd D, Harris R, Jan N, Lookman T, MacDonald L, Pink DA, Zuckermann MJ (1983) Computer simulation of the main gel-fluid phase transition of lipid bilayers. J Chem Phys 79:2027–2041

Nagle JF (1980) Theory of the main lipid bilayer phase transition. Annu Rev Phys Chem 31:157–195

Nagle JF, Scott HL (1978) Lateral compressibility of lipid mono- and bilayers. Theory of membrane permeability. Biochim Biophys Acta 513:236–243

Papahadjopoulos D, Jacobsen K, Nir S, Isac T (1973) Phase transitions in phospholipid vesicles. Fluorescence polarization and permeability measurements concerning the effect of temperature and cholesterol. Biochim Biophys Acta 311: 330–348

Quinn PJ, Chapman D (1980) The dynamics of membrane structure. CRC Crit Rev Biochem 8:1–117

Rüppel D, Sackmann E (1983) On defects in different phases of two-dimensional lipid bilayers. J Phys (Paris) 44: 1025–1034

Sackmann E, Rüppel D, Gebhardt C (1980) Defect structure and texture of isolated bilayers of phospholipids and phospholipid mixtures. In: Helfrich W, Heppke G (eds) Liquid crystals of one- and two-dimensional order. Springer, Berlin Heidelberg New York (Springer Series in Chemical Physics, vol 11, pp 309–326)

Sandermann H (1978) Regulation of membrane enzymes by lipids. Biochim Biophys Acta 515:209–237

Stanley HE (1971) Introduction to phase transitions and critical phenomena. Clarendon Press, Oxford

Stauffer D (1979) Scaling theory of percolating clusters. Phys Rep 54:1–74

Stoll E, Binder K, Schneider T (1972) Evidence for Fisher's droplet model in simulated two-dimensional cluster distributions. Phys Rev B6:2777–2780

Träuble H (1971) The movement of molecules across lipid membranes: A molecular theory. J Membr Biol 4:193–208

Tsong TY, Geenberg M, Kanehisa MI (1977) Anesthetic action on membrane lipids. Biochemistry 16:3115–3121

von Tscharner V, McConnell HM (1981) An alternative view of phospholipid phase behavior at the air-water interface. Biophys J 36:409–419

Zuckermann MJ, Pink DA (1980) The correlation length and lateral compressibility of phospholipid bilayers in the presence of thermodynamic density fluctuations. J Chem Phys 73:2919–2926

Zuckermann MJ, Pink DA, Costas M, Sanctuary BC (1982) A theoretical model for phase transitions in lipid monolayers. J Chem Phys 76:4206–4216