

Curvature induced nanoscale rafts in lipid membranes

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1. Introduction to lipid rafts

1.1. The raft hypothesis

Until the 1980s, the most accepted model of cellular membranes was the fluid mosaic model, formulated in 1972 by Singer and Nicolson[117]. In this model, a biological membrane is regarded as a two dimensional liquid of phospholipids, with intra- and trans-membrane proteins randomly distributed along the bilayer (figure 1.1). However, during the 1970s, there was a growing amount of experimental discoveries, which could not be explained satisfactorily in terms of a laterally homogeneous membrane. For example: Anomalous diffusion of lipids and proteins[61, 68, 133]; a discontinuity in the phase separation of microsomal lipids[134]; the results of differential calorimetric measurements[10]; and even direct observation (using electron microscopy) of domains in model membranes [48].

As a consequence, the idea that the membrane lipids themselves may form microscopic domains within the membrane plane[58], became the widely accepted model of the plasma membrane. With accumulating experimental evidence the concept became more refined in the following decades, and these microdomains were coined *lipid rafts*. The general image of rafts were microscopic domains of lipids in a liquid state, but with higher order, called the *liquid ordered (lo) phase*. This phase has to be distinguished from the liquid-crystalline phase usually found in lipid bilayers, which is called the *liquid disordered (ld) phase*, while the gel phase is called *solid ordered (so)* within this terminology[52]. There is also the functional definition that lipid rafts are detergent resistant domains in the membrane, as it is possible to extract cholesterol enriched domains from cold membranes using Triton X-100[15, 96]. However, there are results which suggest, that the addition of Triton X-100 might be the driving force behind the domain formation observed in those experiments[44].

If they existed in vivo, lipid rafts would play an important role in cellular biology. Functional intra-membrane proteins would react to the differences in lipid order and membrane thickness (*hydrophobic mismatch*) and would favor the inside, outside or border of rafts, thus creating reaction centers or, in more complicated models, the rafts might also regulate the function of these proteins[27, 56, 96, 97]. They could also change the local properties of a membrane, such as permeability[38, 56]. The study of lipid rafts would lead to understanding of important aspects of signal transduction on a cellular level, especially T-cell activation[18, 41, 55, 114]; endocytic transport[41, 50]; amyloid- β peptide¹ production[20]; viral shedding[74, 93] and other aspects of viral, bacterial and toxicological activities [16, 17, 57]; and the pathogenesis of several other

¹Extracellular accumulations of the $A\beta$ peptide are hypothesized to be the cause of Alzheimer's disease.

1. Introduction to lipid rafts

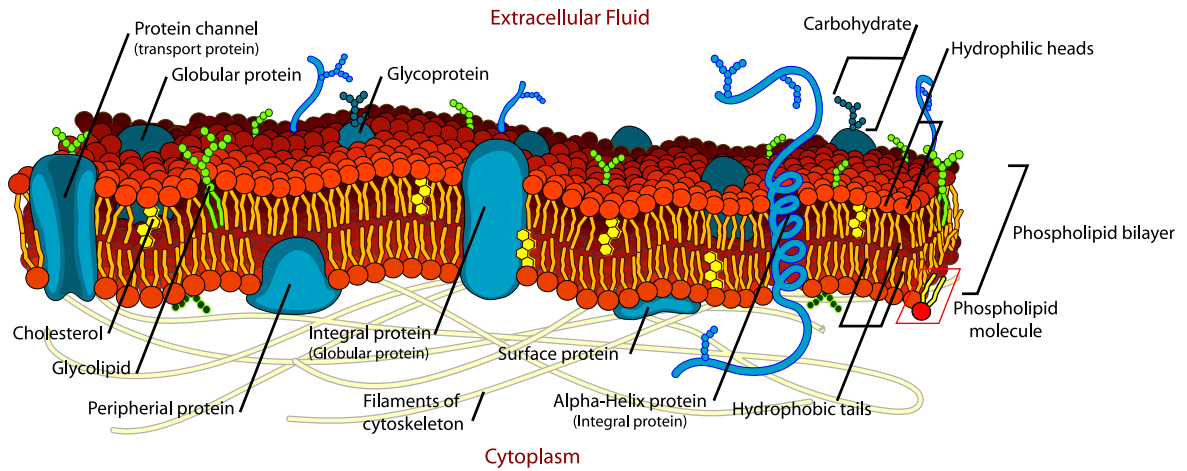


Figure 1.1.: A biological membrane as conceived in the fluid mosaic model[132].

disorders[31, 62, 113].

While micrometer sized raft-like structures can be readily observed in model membranes, there is no direct evidence yet, that lipid rafts exist in living cells. This suggests that lipid raft formation may be driven solely by lipid-lipid interactions but that there exists a mechanism in living cells that limits their size to below levels directly observable by light microscopy. Although they were once believed to have a diameter of several hundred nanometers [54, 112, 116], later studies suggest a size below $50nm$, even as small as $5nm$ [28, 99, 111]. Other studies propose that lipid rafts may not be stable and instead exist in a transient state[60, 98]. There are also arguments, that lipid rafts may not exist *in vivo* at all. Although demixing phase transitions have been observed in lipid mixtures extracted from living cells[126], these transitions were seen way below physiological temperatures, but there are other experiments which indirectly observe domain coexistence *in vivo*[82]. Lipid rafts also need to exist on long enough time scales ($\approx \mu s$) in order to be biologically significant[87]. Although the raft hypothesis is widely accepted in cell biology, the uncertainty concerning many aspects of the hypothesis have always led to criticism of the whole concept or parts of it[59, 73, 89].

This leaves, amongst others, several important open questions concerning the nature of lipid rafts:

- If they exist *in vivo*, what is the physical mechanism behind lipid rafts?
- If they exist *in vivo*, why are rafts so small?
- If they are small, transient structures, how can they still fulfill the biological functions ascribed to them?

In this work, we present a coarse grained model for lipid mixtures, that enables us to simulate systems of up to several ten thousand chain molecules with a resolution that retains information about the order, structure and orientation of single molecules.

We observe that even a two component system can separate into rafts of finite size, and we study these rafts and other membrane structures in details. We look at the characteristics of our model that enable these phenomena and how they may relate to lipid-cholesterol or lipid-lipid mixtures. We propose an explanation for our findings using elastic theory to describe a possible mechanism of raft stabilization via curvature differences between coexisting lipid phases and we investigate whether this theory can be used to explain the results of our computer simulations.

1.2. Possible mechanisms for lipid raft formation

Most models of lipid rafts assume that lipid-lipid interactions are the key factor in their formation. The traditional idea is that membranes exist in a state of coexistence of a *ld* phase and a *lo* phase, but instead of separating, there is some mechanism that restricts the *lo* domains to finite rafts within a “sea” of *ld* lipids. One such mechanism could be the influence of a line active² agent[115], reducing the interfacial tension between domains, impeding macroscopic phase separation. The resulting mixture would be an microemulsion of one phase within the other. Proteins[42] and certain types of lipids[14, 135] have been proposed as candidates for such line active agents, but as Schick objects[108], these models cannot explain why nanoscopic domains can be seen in model mixtures that contain no apparent components that could serve as line active agents [33]. Cholesterol, which is widely regarded as a key component in the lipid raft hypothesis (although even this is disputed[59]), cannot fulfill the dual role of line active agent and stabilizer of one of the phases, as these functions contradict each other. Instead, Schick argues that the heterogeneities may be the result of a coupling between composition and curvature of a bilayer, and that rafts could arise as droplets of a curvature-induced microemulsion[40, 108]. Others propose that the rafts may simply be critical fluctuations near critical points of a miscibility phase transition[47, 126]. This, of course, requires a critical point nearby.

Another class of models focuses on lipid-protein interactions. Intra-membrane proteins would form a shell of cholesterol and sphingolipids around themselves which might then combine with other shells, forming larger rafts[2]. A problem with this model is the necessary strong interaction of proteins and lipids to bring about the shell structure and the fact that, if the lipids interact in a fashion that allows the occurrence of lipid shells, then this interaction should also be able to explain raft formation without the proteins[115].

Apart from these models that focus on lipid-lipid and lipid-protein interactions in equilibrium, there is also the suggestion, that lipid rafts may be non-equilibrium structures[79, 122].

In this work we will consider lipid-lipid interactions as origin of raft structures. Experiments on model membranes indicate, that at least three components might be needed to bring about raft domains, one of which is cholesterol, often paired with a saturated and an unsaturated kind of lipid. Such mixtures undergo a separation into two phases in

²The membrane and rafts are regarded as two-dimensional. Thus, a “surfactant” is active on a line.

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model membranes, one rich in cholesterol and saturated lipids, the other rich in unsaturated lipids [127]. The small size of lipid rafts in vivo could then be explained by one of the mechanisms mentioned in the last passage that is not present in the experiment, such as the effects of the cytoskeleton[136]. There is, however, evidence that certain types of mixtures may already form a microscopic domain structure by themselves. Feigen-son introduces the terms “Type I” and “Type II” mixtures in order to categorize these lipid mixtures by their miscibility behavior[32]. Type II mixtures exhibit macroscopic phase coexistence between ordered and unordered phases, whereas type I mixtures show the same coexistence, but with nanoscopic domain sizes. There is evidence that binary mixtures of a lipid and cholesterol may be type I mixtures. Experiments probing local membrane properties, such as ESR, NMR and diffusivity studies have affirmed the existence of immiscible liquid phases in lipid-cholesterol mixtures[52, 107, 127]. On the other hand, fluorescence microscopic studies only find one homogeneous phase[127]. This would suggest that these mixtures phase separate, but on a nanoscale, beyond the ability of the microscopic techniques to identify different domains. If binary lipid-cholesterol mixtures are type I mixtures, they already feature many of the properties ascribed to lipid-rafts. Of course, this does not explain why they show this behavior, but a study of binary mixtures may contribute to the understanding of lipid raft formation. Experiments published shortly before the completion of this work further establish this idea, as they find direct evidence for nanoscopic domain formation in such mixtures[4, 100]. These experiments can be seen as a breakthrough on this topic and are in excellent agreement with our results in this work.

1.3. Raft Stabilization by Curvature

1.3.1. Preliminary remarks

We would like to start our discussion of a possible mechanism for raft formation with some remarks:

First, we would like to anticipate that we will indeed see finite, cholesterol enriched domains in our simulation of two-component lipid mixtures. The cholesterol enriched domains can be identified as an ordered state of the lipids and has a much greater spontaneous curvature than the surrounding disordered lipids (section 8.3.2). We also observe a tendency of these rafts to appear at opposite sides of a bilayer (section 8.1).

Second, we would like to remind the reader that rafts are not the only nanometer sized structure in lipid membranes. In the region of the main transition between (tilted) gel phase ($L_{\beta'}$) and the fluid phase (L_{α}) there appears a modulated phase, the ripple phase, $P_{\beta'}$. This phase only appears when the lipid gel phase exhibits a tilt, which has been confirmed in both experiments[64] and computer simulations[23, 24, 65, 72], although the exact microscopic details of the ripple structure are still controversial.

As the typical length scales ascribed to lipid rafts and the ripple phase are the same, one could suspect that the two phenomena could have similar origins. Indeed, the theory we present here also gives an explanation for modulated structures within the

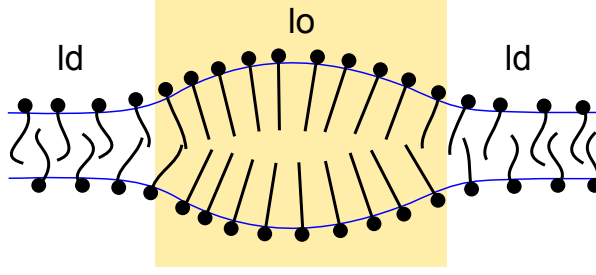


Figure 1.2.: Raft stabilization by curvature. The positions of *lo* and *ld* domains in the two bilayer leaflets are correlated. *lo* and *ld* domains have different curvatures. Reproduced from [83].

same framework that gives a possible mechanism for finite raft domains.

1.3.2. Theory

Here, we present a mechanism in which rafts could be stabilized due to the spontaneous curvature of the monolayers. The elastic energy would then promote the formation of finite domains, but could also be used to explain modulated phases like the ripple phase.

This mechanism can be formulated in the framework of elastic theory in which we suppress the molecular details of the bilayer and regard each monolayer as a plane with a certain thickness and curvature. Each remaining degree of freedom (i.e. bending, compression) is associated with a certain modulus which depends on the microscopic details of the membrane. This not only involves a dependence on the composition but can also encompass many other quantities which can change the microscopic structure, including bending and compression themselves. Furthermore, there is a spontaneous curvature inherent to each monolayer and, in case of a bilayer, a possible coupling between the monolayers. Both of these properties can potentially depend on the microscopic details, same as the moduli.

Here, we use several simplifications: First, we assume that the curvature only depends on composition. Furthermore, the (local) composition in each monolayer is considered to be equal and induces no bending of the bilayer. This model and the restrictions correspond to a picture of rafts similar to the one in figure 1.2.

For the monolayer elastic energy F_{el} , we use a combination of a model by Leibler and Andelman[69]; and Dan, Pincus et. al. [3, 21, 90]:

$$F_{\text{el}} = \int d^2r \left\{ \frac{k_c}{2} (\nabla^2 u)^2 + \frac{k_A}{2t_0^2} u^2 + 2k_c \left(c_0 + \frac{\zeta}{t_0} u \right) \nabla^2 u + k_G \det(\partial_{ij} u) \right\} \quad (1.3.1)$$

Here, $u(\vec{r})$ is the local deviation from the mean monolayer thickness t_0 . Thus, the local monolayer curvature is given by $\nabla^2 u$. The k 's are the aforementioned material properties. More precisely, k_c is the bending modulus, k_A the compressibility modulus and k_G the Gaussian rigidity. c_0 is the spontaneous curvature of the monolayers and ζ is another material parameter associated with the perturbation energy due to local curvature[3].

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As was explained above, all these parameters could have a complex dependence on the microscopic structure, but as a result of the assumptions made above, only the spontaneous curvature c_0 is a non-constant.

Strong segregation limit

We will now assume, that the system undergoes a lateral phase separation into two different phases and that the boundaries between the two phases are thin enough to be treated as a line. As a result, c_0 only depends on the phase. Inside the bulk of the domains, c_0 is constant and the difference of c_0 between the two different phases is denoted by δc_0 . In order to describe the resulting lateral structure, we have to minimize F_{el} . Thus, using the generalized Euler-Lagrange equation, we get the condition

$$\begin{aligned} 0 &= \frac{k_A}{t_0^2} u + \frac{2k_c \zeta}{t_0} \nabla^2 u + k_c \nabla^4 u \\ &= u + 2ba^2 \nabla^2 u + a^4 \nabla^4 u \end{aligned} \quad (1.3.2)$$

Here, we identify $a = \sqrt[4]{\frac{k_c t_0^2}{k_A}}$ as a system inherent length scale, the in-plane correlation length; and $b = \frac{2\zeta a^2}{t_0}$ as a dimensionless characteristic membrane parameter. If we insert actual numbers for DPPC from both experiments and simulations, we find $a \sim (0.9 - 1.4) \text{ nm}$ and $b \approx 0.7$ [83, 130]. We can rescale all lengths in equation 1.3.1 by a . If we consider the topology of the membrane fixed, we can also drop the contribution of the Gaussian rigidity because, according to the Gauss-Bonnet theorem, the integral over the curvature depends only on the Euler characteristic, which is a topological invariant. We obtain:

$$F_{\text{el}} = \int d^2 \hat{r}^2 \left\{ \frac{1}{2} (\hat{\Delta} \hat{u})^2 + \frac{1}{2} \hat{u}^2 + (2ac_0 + b\hat{u}) \hat{\Delta} \hat{u} \right\} \quad (1.3.3)$$

In order to solve this fourth order equation, we need four sets of boundary conditions for u and its derivatives. These boundary conditions must be chosen in a way to minimize the free energy. We can already deduce from equation 1.3.1 that u and $\vec{n} \nabla u$ must be continuous, or otherwise the integrals over Δu would diverge (with $\vec{n}$ the normal vector at a boundary). In order to obtain the remaining conditions, we carry out a variation of F_{el} . We obtain:

$$\begin{aligned} \delta F_{\text{el}} &= \int d^2 r \left\{ k_c \Delta u \delta(\Delta u) + \frac{k_A}{t_0} u \delta u + 2k_c \left(c_0 + \frac{\zeta}{t_0} u \right) \delta(\Delta u) + 2k_c \frac{\zeta}{t_0} \Delta u \delta u \right\} \\ &= \int d^2 r \delta u \left\{ k_c \Delta^2 u + \frac{2k_c \zeta}{t_0} \Delta u + \frac{k_A}{t_0^2} u \right\} \\ &\quad + \int d\vec{\sigma} \vec{n} \delta u \left\{ k_c \nabla \Delta u + 2k_c \frac{\zeta}{t_0} \nabla u \right\} \\ &\quad + \int d\vec{\sigma} \vec{n} \delta(\nabla u) \left\{ k_c \Delta u + 2k_c \left(\frac{\zeta}{t_0} u + c_0 \right) \right\} \\ &\stackrel{!}{=} 0 \end{aligned}$$

The surface integrals $d\vec{s}$ run over all boundaries of all domains. As the membrane is completely filled with domains, each of these surface integrals will appear twice, once from each side. The first term gives us a condition identical to the Euler-Lagrange equation. As we already know that u and $\vec{n}\nabla u$ need to be continuous, we can see from the second and third expressions that $\nabla\Delta u$ and $(\Delta u + 2c_0)$ need to be continuous, too, which gives us the four boundary conditions we need. The last condition is identical to a jump of Δu by $2\delta c_0$ at the boundary.

We can insert these conditions into equation 1.3.1. The bulk terms vanish due to the condition obtained from the Euler-Lagrange equation. Of the remaining surface terms, all but one vanish due to the condition that u , ∇u and $\nabla\Delta u$ need to be continuous: As each boundary element appears twice, once from each direction, many of these contributions cancel each other. The one remaining term is the one originating from Δu . As Δu jumps by $2\delta c_0$ at the boundary, we remain – after partial integration – with the following expression for F_{el} :

$$F_{\text{el}} = k_c \delta c_0 \int dl \vec{n} \cdot \nabla u \quad (1.3.4)$$

where the integral runs over all boundaries and u is a solution of the Euler Lagrange equations, satisfying the boundary conditions.

We will now consider some simple topologies: For a single stripe domain of width D (in units of a) normal to the x -direction, we can give an exact solution for u , satisfying all of the conditions derived above:

$$u(x) = -\frac{2a^2\delta c_0}{\sqrt{1-b^2}} \begin{cases} \Re [i \exp(i\alpha D/2) \cos(\alpha x)] & \text{if } 0 < x < D/2 \\ \Re [\sin(-\alpha D/2) \exp(i\alpha x)] & \text{if } x > D/2 \end{cases}$$

with

$$\alpha = \sqrt{\frac{1}{2}} \left(\sqrt{1+b} + i\sqrt{1-b} \right)$$

and $u(-x) = u(x)$. The boundary conditions can be checked easily by inserting this equation into the conditions.

If L is the length (perpendicular to the x -direction) of such a stripe, then the elastic free energy F_{el} of the stripe can be calculated by inserting the derivative of u at $x = D/2$

$$u'(D/2) = -\frac{a^2\delta c_0}{\sqrt{1-b^2}} \Re (\alpha(e^{i\alpha D} - 1))$$

into equation 1.3.4, which gives us the result $F_{\text{el}} = 2L\lambda_{\text{el}}$ with

$$\lambda_{\text{el}}^{\text{stripe}}(D) = -\frac{ak_c\delta c_0^2}{\sqrt{1-b^2}} \Re [\alpha (1 - e^{i\alpha D})].$$

The total free energy F of the stripe domain includes the bare tension λ_0 , $F = 2L(\lambda_0 + \lambda_{\text{el}}^{\text{stripe}})$. In the limit $D \rightarrow \infty$ we recover the line tension $\lambda = \lambda_0 + \lambda_{\text{el}}^\infty$ with

$$\lambda_{\text{el}}^\infty = -\frac{ak_c\delta c_0^2}{\sqrt{2(1-b)}}.$$

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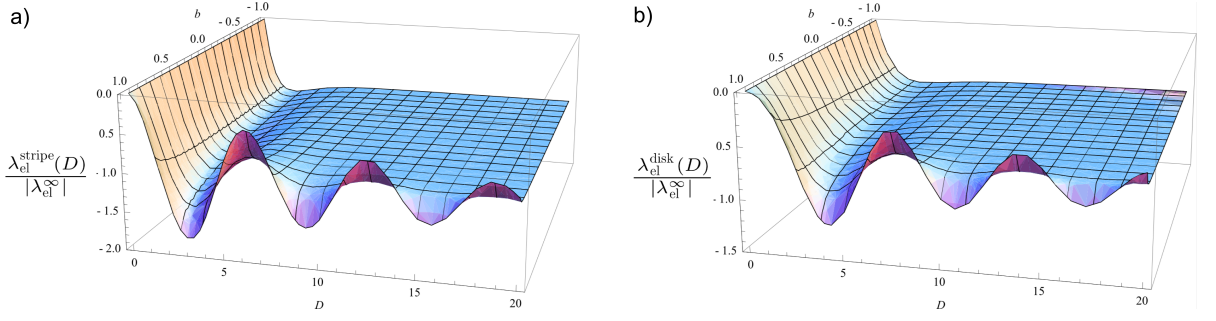


Figure 1.3.: Elastic contribution λ_{el} to the line tension for stripe (a) and disk (b) geometries. D is the thickness/diameter of the rafts in units of the in-plane correlation length a . b is the dimensionless membrane parameter. Reproduced from [83].

As $\lambda_{\text{el}}^{\text{stripe}}$ (and $\lambda_{\text{el}}^{\infty}$) is always negative, the line tension is reduced by the elastic contribution and can even become negative for sufficiently large δc_0 . This is in agreement with Kuzmin and coworkers, who made similar calculations, also using an elastic model[66]. Figure 1.3 a) shows $\lambda_{\text{el}}^{\text{stripe}}$ over D and b . Interestingly, $\lambda_{\text{el}}^{\text{stripe}}$ oscillates as a function of D , with the first minimum representing an optimal value for the stripe width. For our values of b , one obtains an optimal D of $3 - 5a$. In SI-units this corresponds to stripes of several *nm* width.

We can do a similar calculation for a disk shaped domain. For a disk with diameter D and r as the distance from the center, we obtain the following solution $u(r)$ of the variation problem³:

$$u(r) = \frac{\delta c_0}{\sqrt{1-b^2}} \frac{\pi D a}{2} \begin{cases} \Re \left[a \alpha J_0(\alpha r) H_1^{(1)}(\alpha D/2) \right] & \text{if } r < D/2 \\ \Re \left[a \alpha J_1(\alpha D/2) H_0^{(1)}(\alpha r) \right] & \text{if } r > D/2 \end{cases},$$

where J_n and $H_n^{(1)}$ are the Bessel and Hankel functions of the first kind. In a similar fashion to the calculations above we obtain the elastic contribution to the line tension

$$\lambda_{\text{el}}^{\text{disk}}(D) = -\frac{\pi D k_c \delta c_0^2}{2\sqrt{1-b^2}} \Re \left[a^2 \alpha^2 J_1(\alpha D/2) H_1^{(1)}(\alpha D/2) \right].$$

This function is shown in figure 1.3 b). Again, there is an optimal diameter, which is also about $3 - 5a$ for typical values of b .

Weak segregation limit

We can also approach the problem from the other side, where the system is close to a phase transition instead of already completely separated. In this case, we would have a homogeneous phase and local deviations from this state would be small. Then we

³The condition that Δu needs to jump by $2\delta c_0$ at $r = D/2$ is fulfilled because of the identity $J_1(x)(H_0^{(1)}(x) - H_2^{(1)}(x)) - H_1^{(1)}(x)(J_0(x) - J_2(x)) = \frac{4i}{\pi x}$

can describe a phase transition in the membrane by using a Ginzburg-Landau ansatz, taking some order parameter Φ (which is also assumed to be identical on both sides of the bilayer) and the Ginzburg-Landau energy

$$F_\Phi = \int d^2r \left\{ \frac{g}{2} (\nabla \Phi)^2 + f(\Phi) \right\},$$

where $f(\Phi)$ is a functional with two minima which describes the local tendency to phase separate. The total free energy is then given by

$$F = F_{\text{el}} + F_\Phi.$$

The assumption that the system is in a homogeneous phase with only small deviations translates to the assumption that Φ is small for all r . Using the in-plane correlation length a , we can again rescale this equations and obtain:

$$F_\Phi = \int d^2\hat{r} \left\{ \frac{g}{2} (\hat{\nabla} \Phi)^2 + \hat{f}(\Phi) \right\}, \quad (1.3.5)$$

with $\hat{f}(\Phi) = a^2 f(\Phi)$. There is also a coupling between u and Φ as both depend on the internal degrees of freedom of the membrane[69]. Once again, we assume that c_0 is the only non-constant amongst the elastic moduli. Here, we will assume that c_0 depends linearly on Φ .

Once again, we apply the Euler-Lagrange condition. This time, the indirect dependency of c_0 on r through Φ cannot be ignored, as there is no longer a bulk phase with a constant Φ . We obtain the Euler-Lagrange equation

$$\hat{\Delta}^2 \hat{u} + 2b\hat{\Delta} \hat{u} + \hat{u} + 2\hat{c}\hat{\Delta} \Phi = 0,$$

with $\hat{c} = ac_0(\Phi)/\Phi$. We can solve this in Fourier space (with q as wave vectors):

$$\hat{u}_q = \frac{2\hat{c}q^2}{q^4 - 2bq^2 + 1} \Phi_q$$

Thus, we obtain the total free energy (ignoring boundary terms):

$$F = F_{\text{el}} + F_\Phi = \frac{1}{2} \sum_q |\Phi_q|^2 q^2 g_{\text{eff}}(q^2) + \int d^2\hat{r} \hat{f}(\Phi)$$

with

$$g_{\text{eff}}(q^2) = g - 4k_c \hat{c}^2 \frac{q^2}{q^4 - 2bq^2 + 1}.$$

We can see that g_{eff} has a minimum at $q^* = 1$. If $g \leq g^* = 2k_c \hat{c}^2 / (1 - b)$ the expression becomes even negative. This leads to an instability of the homogeneous phase ($\Phi = \text{const.}$, i.e., $\Phi_q = 0$ for $q \neq 0$) at these wavelengths. Close to a phase transition,

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fluctuations with this wavelength will be predominant. We can expand the free energy density up to fourth power in Φ

$$\hat{f}(\Phi) = \frac{\alpha}{2}\Phi^2 - \frac{\gamma}{3!}\Phi^3 + \frac{\lambda}{4!}\Phi^4$$

and $g_{\text{eff}}(q^2)$ up to second power in $q^2 - q^{*2}$:

$$g_{\text{eff}}(q^2) = (g - g^*) + (g - g^*)(q^2 - q^{*2}) + \frac{g^*}{2(1 - b)}(q^2 - q^{*2})^2$$

Introducing the new identifiers

$$\begin{aligned} q_0^2 &= (q^*)^2 (1 + (1 - b)(1 - g/g^*)) \\ \frac{\xi_0^2}{4q_0^2} &= \frac{g^*}{2(1 - b)} \\ \tau &= \alpha - \frac{1}{2}(g - g^*)(1 + q_0^2) \end{aligned}$$

we can put everything together and transform back into real space. We obtain

$$F = \int d^2\hat{r} \left\{ \frac{\xi_0^2}{8q_0^2} \left(\hat{\Delta} + q_0^2 \right)^2 \Phi^2 + \frac{\tau}{2}\Phi^2 - \frac{\gamma}{3!}\Phi^3 + \frac{\lambda}{4!}\Phi^4 \right\},$$

which is the Landau-Brazovskii model[11]. ξ_0 is the bare correlation length; q_0 is the critical wave length; τ is a reduced temperature; and γ and λ are other constants. The Landau-Brazovskii model describes phase transitions driven by short wavelength fluctuations in isotropic or almost isotropic systems. When treated in a mean field way, the model predicts a transition between the disordered phase and several ordered phases.

1.3.3. Interpretation

We have studied an elastic bilayer model composed of two coupled monolayers. We have considered the case where the bilayer undergoes a local phase separation into two phases, both in the limit of strong and in the limit of weak segregation. Furthermore we have assumed that the spontaneous curvature of the monolayers depends on the the local order parameter used to describe the phase transition.

In the strong segregation limit we have seen that there exists a characteristic length scale for domains which is of the order of nanometers. This is the direct result of the curvature difference between the two phases. The curvature of domains induces elastic stress between the two halves of the bilayer (figure 1.2). Intuitively seen, this stress can be released at the boundaries, leading to the reduced line tension we have derived in our calculations. If we consider the total line tension $\lambda = \lambda_0 + \lambda_{\text{el}}^\infty$, we can look at the scaling behavior of λ_0 and $\lambda_{\text{el}}^\infty$ close to the critical temperature T_c of the demixing transition: The bare line tension would scale $\lambda_0 \sim (T_c - T)^\nu$, with the critical Ising exponent $\nu = 1$ (two dimensional system)[47]. On the other hand, when we again make the assumption

that δc_0 depends linearly on the order parameter, then $\delta c_0 \sim (T_c - T)^\beta$ and $\lambda_{\text{el}}^\infty \propto \delta c_0^2$ would scale with the exponent $2\beta = 1/4$. Therefore, close to the transition, $\lambda_{\text{el}}^\infty$ – which we have already established to be negative and to lead to an optimal width on the scale of nanometers for some reasonable topologies – would dominate and the line tension would become negative. This would suppress macroscopic phase transitions near T_c , leading to microemulsions or modulated phases, depending on cholesterol concentration.

In the limit of weak segregation we have shown that our system can be described within the framework of the well known Landau-Brazovskii model. The scale of the characteristic wavelength of phase separated structures within this model is again of the order of nanometers. The Landau-Brazovskii model predicts modulated phases, which can account for structures such as the ripple phase. Close to a order-disorder transition, fluctuations will destroy the long-range order of these structures and change the order of the transition from continuous to first order via the Brazovskii mechanism[11, 12]. Thus, this model can also account for a raft state with a characteristic length scale.

Both of the primary assumptions, the identical composition of the membrane on both sides and the restriction to c_0 as the only material property that depends on the composition, are obviously oversimplifications. The identical composition on both sides can somewhat be motivated by the fact that this kind of behavior can be observed in the case of globally phase separated lipid mixtures[19]. A posteriori we can also remark that the rafts we find in our simulations are positively correlated.

The assumption that most of the material properties are constants is, of course, quite unrealistic. In reality, these properties will at least depend on the local phase. The most obvious candidates would be the mean thickness and the bending modulus, which clearly change between a ordered and a disordered phase. However, we would expect that a more detailed calculation would only result in a more accurate estimate of the length scales involved in the pattern formation and would not qualitatively alter our result, that we have a mechanism for finite domains on a nanometer scale.

2. Developing a cholesterol model

Considering the theory of lipid rafts, one of the most important factors in their formation is cholesterol or, considering other classes of organisms, any sterol. Given that our already existing model lipid model resembles the phospholipid DPPC (chapter 3), we should look at experimental and theoretical data of the effect of sterols on phospholipid membranes, in order to assess which properties of cholesterol might be important features for the general mechanisms of lipid raft formation and how these features could be included in our model system. As the original lipid model was developed *top-down*, we have the freedom to only pick features that might be important to our goal of understanding general principles of lipid raft formation and need not rigorously derive the cholesterol force fields from atomistic considerations.

2.1. Differences between cholesterol and other sterols in experiments

Sterols are a subgroup of steroid organic molecules and important components of biological membranes. They are composed of three cyclohexane and one cyclopentane rings and a hydroxyl head group. Various other compounds or atoms may be added to this structure, forming the different types of sterols. The appearance of higher sterols like cholesterol (animals), sitosterol (plants) or ergosterol (fungi) in living organisms is considered an important step in cellular evolution. These sterols have a distinct effect on biological membranes and are mostly absent in more primitive membranes like prokaryote cells and mitochondria (supporting the endosymbiotic theory). The sterols mentioned above have a significantly different influence on some membrane properties but all have a similar effect on bilayer thickness [95, 124]. While the sterols mentioned above have the ability to stabilize a liquid ordered phase, lanosterol does not [45, 84]. Lanosterol is the precursor molecule from which the other sterols, except certain phytosterols (e.g. sitosterol), are derived [8, 109, 119]. It features additional methyl groups connected to the ring structure compared to other sterols, giving this molecule a less "smooth" profile.

Major differences between the various kinds of sterols include their effect on surface viscosity, which can be interpreted as the ability of cholesterol to cross the bilayer plane and thus couple the monolayers [45], and their effect on the thermal area expansion coefficient [95]. As there is no obvious connection of these effects to lipid rafts and all of these sterols are associated with lipid rafts anyway, despite their differences, we do not expect these phenomena to be important for our model.

2.2. Effects of cholesterol and other sterols on lipid bilayers

The addition of cholesterol or other sterols often lead to a width increase of lipid membranes. The microscopic cause of the membrane swelling is an ordering effect of cholesterol on the phospholipids, which has been confirmed by many independent experimental studies, using NMR, EPR, fluorescence microscopy methods and many others [34, 37, 49, 63, 67, 88, 92, 95, 124], see [105] for a review. We would therefore expect this characteristic of sterols to be an important factor in their biological role. The exact details of the swelling behavior depends on the type of lipid. There are even certain lipid membranes which become thinner, rather than thicker if cholesterol is added [49, 80]. Another effect of the induced order, aside from the swelling of the membrane, is a reduction of the area per lipid, which can also be seen in the experiments mentioned above.

Without any sterols, experimental model membranes show the well known phase behavior of phospholipid membranes: At low temperatures the lipids are in a gel state with an all-trans configuration of the tails, a high local chain order and oftentimes a tilt relative to the normal-direction of the membrane. For lipids with a tilted gel phase, this phase turns into a rippled gel phase at the pretransition temperature. Above the even higher main transition temperature the lipids collectively undergo a transition to a disordered, liquid crystalline phase. Again, the details of these phases and the respective transitions may depend on the exact type of phosphatidylcholines[37]. The addition of sterols changes this phase behavior, the exact shape of the phase diagram depends on the lipid type. A generic phase diagram suitable for many lipid types is shown in figure 2.1. This and similar diagrams are not undisputed, though. In fact, if raft formation is driven by lipid-lipid interactions, *ld* and *lo* phase would *not* phase-separate and instead rafts would appear in the *ld/lo* coexistence region.

Inside the bilayer, the cholesterol is situated inside the monolayers, when they are in the *lo* phase, but can be found between the monolayer planes or crossing between monolayers, when the membrane is in the *ld* phase[45, 104, 107]. Generally, the cholesterol molecules are found in the inside of the membrane with little contact to the surrounding solvent[86, 92].

Martin and Yeagle use geometrical packing arguments, to describe how cholesterol molecules may be arranged within the membrane plane[77]. According to their arguments, cholesterol molecules will prefer to be completely surrounded by lipids in mixtures up to a mole fraction of 22%. Above this concentration, the cholesterol molecules will form dimers and double dimers up to 47% cholesterol, when the whole system becomes unstable and demixes.

2.3. Cholesterol models in other works

There have been many different approaches to cholesterol models in computational physics to this day, which were able to capture some of the experimentally observed

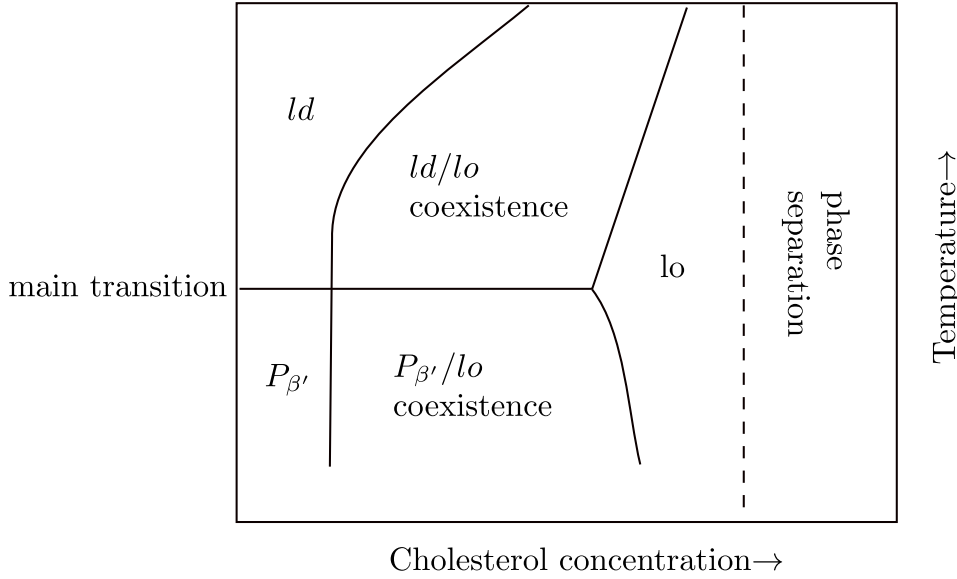


Figure 2.1.: Generic Phase diagram for binary lipid-cholesterol mixtures as originally suggested by Ipsen et. al.[52], reproduced from[100].

properties. While different approaches were used to study different aspects of cholesterol-lipid membranes, all models have again in common the ability to induce order in the lipid bilayer, especially in the liquid phase. Just as in experiments, this ability may vary depending on the exact type of sterol[105].

Approaches using atomic force fields or united atom force fields were able to reproduce the ordering and swelling effects both qualitatively and quantitatively [46, 94, 118]. In simulations using coarse-grained models, the swelling of the bilayer could also be observed[26, 51, 76, 101].

None of these models can be applied directly to the level of course graining we wish to use in this work. The MARTINI force field used by Marrink et. al. comes close, but is not directly compatible with our already established lipid model which we would like to use, as the MARTINI model has difficulty in reproducing tilted gels and rippled phases[91, 129]. Bennun et. al. discuss and compare several approaches to coarse grained lipid and cholesterol models, including our own lipid model [7].

2.4. Conclusions

As we have seen, the exact atomistic details of the sterol molecules have a great impact upon their biological role. On the other hand, the ordering effect of sterols on lipids seems to be pretty universal feature, no matter the details of the model, as long as the sterol can be incorporated into the lipid layer. The microscopic cause of the ordering effect can then be identified as the relative stiffness and smoothness of the sterols, combined with their ability to be associated with the lipids. A top-down approach for a cholesterol model therefore needs to capture these characteristics, but we do not need to account

2. Developing a cholesterol model

for atomistic details such as an exact derivation of the coarse grained force fields.

We have tried a number of different approaches, including some which use a model more similar to a united atom or MARTINI Force field[76] model of cholesterol, but in the end, the most successful models were simple variations of the original lipid model, with a higher stiffness of the bond angle potential and some mechanism to promote a mixing of lipids and cholesterol. Almost all realizations of this "stiff lipid" class of model produce the sought-after swelling of the bilayer, while not demixing from the rest of the lipids. Other classes of models, which we tried, were not able provide both of these characteristics. Another advantage of these models over more complicated approaches is, that the existing program is already optimized for linear chain molecules, which allows a simpler handling of the underlying data structures in some calculations.

When used in large, semi-grand canonical simulation, some¹ of these stiff lipid kind of models did spontaneously form finite, ordered domains, made up of a lipid-cholesterol mixture. We identified these domains as candidates for lipid rafts, making this class of models a prime contender for the models used in this work. All further changes to the model were small alterations of this general class of model cholesterol.

The actual realization of the cholesterol model used in this work is described in section 3.1. Some alterations of the model, which were used in some test cases, are listed in appendix A. The effect of altering the parameters of the model is discussed in detail in chapter 9.

One of the most important changes we made, regarding a correspondence to real cholesterol, is the ratio of in-plane sizes between cholesterol and the phospholipid. Real cholesterol has a smaller and more elliptic shape than typical lipids and does therefore not exactly fit a lipid lattice structure. Our cholesterol model on the other hand, does. Although real cholesterol would induce order in surrounding lipids, it would also disturb the resulting gel structure at the same time. This may be one of the mechanisms that distinguishes the liquid ordered (with cholesterol) and the solid ordered (without cholesterol) phases[115].

¹We have good reason to believe, that *all* realizations of this model class would form rafts, given values of the chemical potential within the correct range. The connection between chemical potential and raft formation will be explored throughout the rest of this work.

3. Coarse-Grained Model

This chapter describes the model used for the simulations. The saturated phospholipid model (P) was first used by Duchs and Schmid[25]. The phantom solvent was introduced by Lenz and Schmid[71]. The combination of lipid and phantom solvent was studied in several later works; an overview of the results is presented in the section about the lipid model. The cholesterol molecule (C) was modeled by taking the lipid model and generalizing and altering its parameters to match the features of sterols and their effects on lipid membranes. At first, the standard cholesterol model, which was used for most of the simulations, will be described. The rationale of the modeling process and alternative models are discussed in section 3.2.1.

3.1. Membrane model, standard cholesterol model

The membrane components are modeled as linear chain of beads. The first bead of a chain is called the head, the rest of the chain is the tail. Consecutive beads in a chain are bound by a finite extensible nonlinear elastic (FENE) potential

$$U_{\text{FENE}}(r) = -\frac{1}{2}\epsilon_{\text{FENE}}(\Delta r_{\text{max}})^2 \log \left(1 - \left(\frac{r - r_0}{\Delta r_{\text{max}}} \right)^2 \right), \quad (3.1.1)$$

where r is the distance between two interacting beads. Parameters are the bond strength ϵ_{FENE} , the equilibrium distance r_0 and the maximum deviation from this distance Δr_{max} . Additionally, any three consecutive beads interact via a cosine bond angle (BA) potential

$$U_{\text{BA}}(\Theta) = \epsilon_{\text{BA}} (1 - \cos(\Theta)), \quad (3.1.2)$$

which gives the chain a bending stiffness. Θ is the angle between the three beads.

All beads, except direct neighbors in a chain, are also subject to a non-bonded interaction with all other beads and solvents via a Lennard-Jones (LJ) potential, giving the notion of a diameter d to the beads. For the sake of computational efficiency, the LJ potential is cut off at a range r_c and is shifted to make the resulting transition at r_c continuous:

$$U_{\text{LJ}}^{\text{shifted}}(r) = \begin{cases} U_{\text{LJ}}(r/\varsigma) - U_{\text{LJ}}(r_c/\varsigma) & \text{if } r < r_c, \\ 0 & \text{otherwise.} \end{cases} \quad (3.1.3)$$

$$\text{with } U_{\text{LJ}}(r/\varsigma) = \epsilon_{\text{LJ}} \left(\left(\frac{\varsigma}{r} \right)^{12} - 2 \left(\frac{\varsigma}{r} \right)^6 \right) \quad (3.1.4)$$

3. Coarse-Grained Model

Phospholipid:	$\epsilon_{\text{FENE}} = 100\epsilon$	$r_0 = 0.7\sigma$	$\Delta r_{\text{max}} = 0.2\sigma$
Cholesterol:	$\epsilon_{\text{FENE}} = 100\epsilon$	$r_0 = 0.6\sigma$	$\Delta r_{\text{max}} = 0.15\sigma$

Table 3.1.: Parameters of the bond length potentials

Phospholipid (all angles):	$\epsilon_{\text{BA}} = 4.7\epsilon$
Cholesterol (angles 1-4):	$\epsilon_{\text{BA}} = 100\epsilon$
Cholesterol (5th angle):	$\epsilon_{\text{BA}} = 4.7\epsilon$

Table 3.2.: Parameters of the bond angle potentials

The parameter ς is the mean value of the diameters of the interacting beads. In this notation, a cutoff $r_c \leq \varsigma$ indicates a purely repulsive interaction (also known as Weeks-Chandler-Andersen potential), whereas interactions with $r_c > \varsigma$ have an attractive part.

The solvent in this model is also represented by explicit beads. The solvent beads are special as they do not interact with each other, only with the membrane beads. This way, the solvent is computationally cheap as only solvents near the membrane contribute to the computational effort, while still exploring the free volume around the membrane and restricting the movement of the membrane beads to the bilayer. Also, the free gas equation can be used to regulate the pressure of the system by adjusting the volume available to the fixed number of solvent beads. As the solvent layer has no inner structure, even solvent layer that is relatively thin – compared to self-interacting solvent models – is sufficient to prevent interactions between periodic images of the lipid bilayer.

The interaction parameters of the lipid and the most common cholesterol model are listed in tables 3.1, 3.2 and 3.3. A computer rendering of the two models is shown in figure 3.1. The coloring used in that figure will be used throughout this work.

bead type-bead type	$\epsilon_{\text{LJ}}/\epsilon$	ς/σ	r_c/ς
head(any)-head(any)	1.0	1.1	1.0
head(any)-tail(any)	1.0	1.05	1.0
head(any)-solvent	1.0	1.1	1.0
tail(P)-tail(P)	1.0	1.0	2.0
tail(P)-tail(C)	1.0	1.0	2.0
tail(C)-tail(C)	0.9	1.0	2.0
tail(any)-solvent	1.0	1.05	1.0
solvent-solvent	0	-	-

Table 3.3.: Parameters of the LJ-potentials

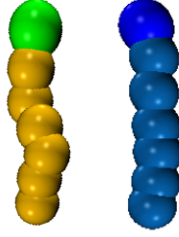


Figure 3.1.: A rendering of the lipid (green/yellow) and the cholesterol (blue) model. The diameter of the spheres representing the beads corresponds to the cutoff radius of the non-bonded interaction. The chains are in a configuration that could be typically found at $T = 1.4$. Note that they both feature approximately the same end to end distance, despite the shorter bead to bead distance of the cholesterol.

3.2. Alternative models

3.2.1. Different cholesterol models

Many different variations of the standard cholesterol model were used, which are described in appendix A. These models have in common, that they can, in principle, form rafts. Other models, trying to closer mimic actual cholesterol rather than slightly altering the existing lipid model, did mostly just demix completely from the lipid and also suffered from very poor acceptance rates for the identity switch move required for simulation in the semi-grand canonical ensemble (section 4.2). Features of these more realistic models were smaller non-bonded interaction ranges, with even smaller beads at the beginning and the end. The number of beads was often smaller than 7. Just as the models above, they were modeled to be very stiff, especially in the slightly thicker middle area.

The particular choice for standard cholesterol model used throughout this work stems from several factors:

- This model formed raft-like structures in the test runs, a necessary prerequisite for using the model.
- The similar size of the beads, especially the head, and the whole chain compared to the lipid model led to higher acceptance rates for the identity switch move.
- The reduced tail-tail interaction between cholesterol chains was deemed more natural than an increased interaction strength between cholesterol and lipids (for example models 2, 3, 6 and 7, described in appendix A).
- The last bead was chosen to be flexible as a means to allow closer contact between bilayer leaflets. Real cholesterol molecules also have a more flexible part at the end of their tail.

3. Coarse-Grained Model

- Both models, lipid and cholesterol, having 7 beads simplified some operations and data structures during data analysis. It also precluded any unforeseen problems, which might stem from different bead counts during the identity switch move, even if the theory in 4.2.2 predicts none.

3.2.2. MD compatible model

The models described thus far all use FENE as bond length potential. When trying to use the lipid model for molecular dynamics simulations, the sudden strong increase near the cutoff proved to be problematic.

An alternative bond length potential suitable for MD-simulation was suggested:

$$V_b(r) = 0.25\epsilon(\Delta r_{\max})^2 \left(\left(1 - \left(\frac{r - r_0}{\Delta r_{\max}} \right) \right)^{-2} - 1 \right)$$

The values for the feather constant ϵ , the cutoff $\Delta r_{\max}$ and the equilibrium length r_0 are identical to those of the FENE-lipid and the respective cholesterol model.

4. Simulation techniques

4.1. General method

Most of the simulations are done using Monte Carlo methods in a constant temperature, constant pressure and constant particle number¹ ensemble. The system uses periodic boundary conditions with a sheared unit cell as described in [70]. The system size and the shear can fluctuate during a simulation run, thus stabilizing the pressure and keeping the membrane tension free. In some simulations, particle identity is not preserved (i.e. particles of type A can become particles of type B). The simulation program itself is not parallelized, only trivial parallelization was used. In order to speed up the simulation, we use an adaptive scheme for the move ranges (both individual bead moves and collective moves) which adjust the ranges during a prerun to find a good compromise between movement range and acceptance rate. We also use a cell lists data structure in order to speed up nearest neighbor calculations. We obtained the best speed up using only a small number of different cell lists, using broad categories such as “head beads”, “tail beads” or “solvent beads” instead of individual cell lists for all bead types. The resulting reduction in computational complexity outweighed the possible benefits of optimizing the cell list size for the interaction range of each bead type. We also tested an approach using a combination of Verlet lists and cell lists but again the possible gains were overcome by the reduced complexity of only using cell lists.

4.1.1. Parallelization

Several different techniques were tried to parallelize the program, first among them classic geometric decomposition, which was already used in previous incarnations of the program[123, 131]. However, efficiency (that is speedup per processor) of all schemes was deemed too low even for large systems ($2 \times 100 \times 100$ chains) and small numbers of processors (≤ 4) to justify usage and the necessary testing. The reason for the low efficiency was tracked down to the unfavorable ratio of local computing time and time for data transfer. Of course, for some very huge systems parallelization would become feasible² but these system sizes would exceed the memory limits of most computers. The technical reason for the even lower efficiency than in [131], which uses a similar system, is due to increased data volumes that need to be transferred (beads are more complex), and an increase in local computing power whereas data transfer rates stagnated.

¹Though particle identity is not necessarily preserved. See section 4.2.

²Time spent for local moves roughly goes with $O(N \log(N))$, whereas communication time goes with $O(N)$.

4.2. Simulation in a semi-grand canonical ensemble

At the scale of our simulation, we expect lipid rafts to be relatively large structures, several dozens chain molecules wide and encompassing large fractions of the simulated membrane. In a canonical (MC-)simulation of mixtures, i.e. with fixed fractions of particle species, long wavelength fluctuations are subject to long relaxation times[6, 9, 36]. Thus, one could not be sure whether large structures appearing in such a simulation are true equilibrium structures or the result of the slow relaxation. A simple solution to this problem is simulation in the grand canonical or semi-grand canonical ensemble[9].

As our simulation model consists of a rather dense system of chain molecules, a grand canonical simulation is impractical. A MC-move to insert a new molecule into the simulation would almost inevitably fail because the new molecule would intersect with some pre-existing molecules leading to a huge or even infinite change in potential energy, an impractically low acceptance rate, and, due to this low rate, large statistical errors in the chemical potential difference³. In contrast, in a semi-grand canonical simulation, i.e. a canonical simulation with fixed particle number but flexible ratio of particle species, a suitable MC-move could replace an old chain by a new one of different type, avoiding this problem.

However, such an identity switch move would still suffer from a unfeasibly low acceptance rate if the new chain were just grown randomly. Thus, a biased Monte Carlo scheme is used in order to grow the new chain into the cavity left by the removed chain, at the same time paying attention to internal and external interactions of the new chain in order to keep the change in potential energy small or even negative. This bias must be accounted for when calculating the acceptance probability of the new configuration. A simple Metropolis criterion, using just the change in energy, can no longer be used.

The algorithm used here is a generalization of a scheme presented in “Understanding Molecular simulation”[35] by Frenkel and Smit. It uses Rosenbluth sampling[103] to grow the new chain and correct the acceptance rate for the bias introduced this way. By adding a new prefactor to the calculation of the Rosenbluth weight one can influence the acceptance rate in favor of one species of chain molecules. This will be shown to be equivalent to adjusting the chemical potential of the simulated system. We will also look at the influence of internal bond potentials and the possibility of unequal bead numbers in different chain types, which were not examined in the original source.

4.2.1. Description of the algorithm

We will look at a move from an old configuration o to a new configuration n by replacing a chain of species A with a chain of species B . When growing a new chain, two factors have to be taken into account:

1. Internal interactions of the new molecule. When growing the new chain, the potential energy due to bonded interactions within the new chain itself, U_B^{bond} , must be compatible with the temperature of the system.

³Both, measuring (section 5.2.2) and when simulating with a fixed potential difference.

2. External interactions with the surrounding, pre-existing molecules. The growth algorithm must avoid placing chain beads into areas with large external energies U_B^{ext} .

Let l_A and l_B be the number of chain segments. The algorithm for the identity switch move is:

- In each MC-sweep, randomly decide whether to do an identity switch. The probability for this type of move stays fixed over the course of the simulation.
- Randomly choose a chain to remove. W.l.o.g. let A be the species of this chain. Randomly choose a different chain type, called B , to insert.
- Remove the A -chain from the system.
- Place a B -head at the position of the former A -head.
- Grow the remaining $l_B - 1$ beads of the B -Chain. Assuming we have already placed $i - 1$ of the l_B beads, then the next segment is added the following way:
 - Generate a set of k trial configurations $\{\vec{b}\}_k$ ⁴. The exact algorithm is given below. The distribution of the trial segments depends on the internal bond potentials $u_{i,B}^{\text{bond}}$. **All** internal bond potentials. In this work that comprises the bond length and the bond angle potentials.
 - For each of the k trial segments $\vec{b}_j$ compute its Boltzmann weight $\exp\left(-\beta u_{i,B}^{\text{ext}}(\vec{b}_j)\right)$, using the external potential energy $u_{i,B}^{\text{ext}}(\vec{b}_j)$. This includes potential non-bonded interactions with already placed beads of the new chain.
 - Randomly pick one of the trial segments ($\vec{b}_{i,\text{new}}$) with probability

$$p_i^{\text{ext}}(\vec{b}_{i,\text{new}}) = \frac{\exp\left(-\beta u_{i,B}^{\text{ext}}(\vec{b}_{i,\text{new}})\right)}{w_{i,\text{new}}^{\text{ext}}\left(\{\vec{b}\}_k\right)}, \quad (4.2.1)$$

with

$$w_{i,\text{new}}^{\text{ext}}\left(\{\vec{b}\}_k\right) = \sum_{j=1}^k \exp\left(-\beta u_{i,B}^{\text{ext}}(\vec{b}_j)\right).$$

That is, trial segments, which would lead to a large external potential energy, are exponentially suppressed.

- The next bead in the chain is inserted at $\vec{b}_{i,\text{new}}$.

⁴In general, k could depend on i and B . Here we will only consider a constant k . The generalization is straightforward.

4. Simulation techniques

- When the whole chain is grown, calculate the Rosenbluth weight of the new chain

$$W_{\text{new}}^{\text{ext}} = \prod_{i=1}^{l_B} w_{i,\text{new}}^{\text{ext}},$$

with the weight for the head at $\vec{r}_h$:

$$w_{1,\text{new}}^{\text{ext}} = k \exp(-\beta u_{1,B}^{\text{ext}}(\vec{r}_h))$$

- Do a similar weight calculation using the positions and potential energies of the old chain:
 - The weight of the old head is

$$w_{1,\text{old}}^{\text{ext}} = k \exp(-\beta u_{1,A}^{\text{ext}}(\vec{r}_h))$$

- For the weight of the i 'th segment, $k - 1$ trial configurations are generated (using the internal potential energies of chain type A). In combination with the actual position of the old bead ($\vec{b}_{i,\text{old}}$), they form a set of k orientations $\{\vec{b}'\}_k = \{\vec{b}_{\text{old}}, \{\vec{b}'\}_{k-1}\}$, equivalent to the set of configurations generated for placing the new segments. Likewise, the weight for the old segment is

$$w_{i,\text{old}}^{\text{ext}}(\{\vec{b}'\}_k) = \sum_{j=1}^k \exp(-\beta u_{i,A}^{\text{ext}}(\vec{b}_j)).$$

- Similarly, the weight for the whole old chain is

$$W_{\text{old}}^{\text{ext}} = \prod_{i=1}^{l_A} w_{i,\text{old}}^{\text{ext}}.$$

- The Rosenbluth weights are multiplied with chain type dependent prefactors S_A and S_B before calculating the acceptance probability of the move:

$$\text{acc}(\text{old} \rightarrow \text{new}) = \min\left(1, \frac{S_B W_{\text{new}}^{\text{ext}}}{S_A W_{\text{old}}^{\text{ext}}}\right) \quad (4.2.2)$$

The trial configurations $\{\vec{b}'\}$ are generated with a distribution according to the Boltzmann weight of the internal bond potentials of the i 'th segment:

$$p_i^{\text{bond}}(\vec{b}) d\vec{b} = \frac{\exp(-\beta u_i^{\text{bond}}(\vec{b})) d\vec{b}}{\int d\vec{b} \exp(-\beta u_i^{\text{bond}}(\vec{b}))} = C_i \exp(-\beta u_i^{\text{bond}}(\vec{b})) d\vec{b} \quad (4.2.3)$$

The value of the normalization constant C_i is unknown. As the bond potentials depend on the chain type A/B , so does C_i .

In addition to the above weights, the trial configurations of the first tail bead are biased to lie in the same half sphere as the old first tail bead (seen from the position of the head). This ensures that the new chain grows into the direction of the membrane, away from the solvent. This greatly enhances the acceptance probability of the identity switch move. As this approach automatically ensures that the old first tail bead direction lies in the same half sphere as the new first tail direction, these biases cancel each other and no further adjustment is needed.

From equation 4.2.3 it follows that, even without knowledge of C_i , trial directions with the correct distribution can be sampled by uniformly distributing trial vectors $\vec{b}$ in the region of possible trial directions (i.e. a spherical shell where the energy of the FENE bond is finite), calculating $u_i^{\text{bond}}(\vec{b})$ and accepting that direction with

$$\text{acc}(\vec{b}) = \exp\left(-\beta u_i^{\text{bond}}(\vec{b})\right).$$

This rejection method works as long as $u_i^{\text{bond}} \geq 0$, which is given in this work. A better approximation of the potentials could lead to a higher acceptance rate but this simple method was found to work well enough with the potentials used in our models.

4.2.2. Justification and relation to chemical potential

The algorithm works correctly if the correct equilibrium distribution is sampled. That means, states should be distributed according to their Boltzmann weight $\exp(-\beta U)$ and the rate for reaching a state must be identical to the rate of leaving that state. The latter condition is usually achieved in MC simulations by enforcing the even stronger detailed balance condition, i.e. the transition rate from one particular state to another is identical to the reverse rate.

In general, the flow K of systems going from a state “old” to a new state “new” is

$$K(\text{old} \rightarrow \text{new}) = \mathcal{N}(\text{old}) \cdot \alpha(\text{old} \rightarrow \text{new}) \cdot \text{acc}(\text{old} \rightarrow \text{new}). \quad (4.2.4)$$

Here, $\mathcal{N}(\text{old})$ is the probability of being in state “old”. Thus, in order to comply with the first condition for correctness of the algorithm,

$$\mathcal{N}(\text{old}) = \exp(-\beta U_{\text{old}}). \quad (4.2.5)$$

$\alpha(\text{old} \rightarrow \text{new})$ is the probability of generating the move from “old” to “new” and $\text{acc}(\text{old} \rightarrow \text{new})$ is the probability of accepting it.

Considering only one segment i , the generation of the move involves the creation of $k - 1$ trial segments b^* plus the one actually accepted, $\vec{b}_{i,\text{new}}$. The calculation of the proposed acceptance criterion requires the generation of the $k - 1$ trial directions b'^* around the old segment. It is essential that the generation of the set b'^* is a part of the generation of the move $\text{old} \rightarrow \text{new}$. The distribution of the $\vec{b}$ depends only on the internal bond potentials. The probability of generating a particular combined set (b^*, b'^*) is $P^{\text{bond}}(b^*, b'^*)$. In combination with the probability of generating the new segment $\vec{b}_{i,\text{new}}$

4. Simulation techniques

(equation 4.2.3) and the probability of accepting this particular trial direction (equation 4.2.1), one can write down the flow K for one particular choice of (b^*, b'^*) as

$$K_i(\text{old} \rightarrow \text{new}, b^*, b'^*) = \exp(-\beta u_{i,A}(\text{old})) \cdot C_{i,B} \cdot \exp\left(-\beta u_{i,B}^{\text{bond}}(\vec{b}_{i,n})\right) \cdot \frac{\exp\left(-\beta u_{i,B}^{\text{ext}}(\vec{b}_{i,n})\right)}{w_{i,B}^{\text{ext}}(\vec{b}_{i,n}, b^*)} \\ \cdot \text{acc}(\text{old} \rightarrow \text{new}, b^*, b'^*) \cdot P^{\text{bond}}(b^*, b'^*)$$

Similarly, for the reverse move, using the same set (b^*, b'^*) :

$$K_i(\text{new} \rightarrow \text{old}, b^*, b'^*) = \exp(-\beta u_{i,B}(\text{new})) \cdot C_{i,A} \cdot \exp\left(-\beta u_{i,A}^{\text{bond}}(\vec{b}_{i,o})\right) \cdot \frac{\exp\left(-\beta u_{i,A}^{\text{ext}}(\vec{b}_{i,o})\right)}{w_{i,A}^{\text{ext}}(\vec{b}_{i,o}, b'^*)} \\ \cdot \text{acc}(n \rightarrow o, b'^*, b^*) \cdot P^{\text{bond}}(b'^*, b^*)$$

In order to achieve detailed balance, the sum over all flows using all possible sets (b^*, b'^*) must be equal. This condition will obviously be satisfied if the stronger condition of so called *super detailed balance* is applied, i.e. the flows for all particular sets (b^*, b'^*) are equal: $K_i(\text{old} \rightarrow \text{new}, b^*, b'^*) = K_i(\text{new} \rightarrow \text{old}, b'^*, b^*)$. Keeping in mind that $P^{\text{bond}}(b^*, b'^*) = P^{\text{bond}}(b'^*, b^*)$ and $u = u^{\text{bond}} + u^{\text{ext}}$, one can derive a condition for the acceptance rates:

$$\frac{\text{acc}(\text{old} \rightarrow \text{new}, b^*, b'^*)}{\text{acc}(\text{new} \rightarrow \text{old}, b'^*, b^*)} = \frac{\exp(-\beta u_{i,B}(\text{new}))}{C_{i,B} \cdot \exp\left(-\beta u_{i,B}^{\text{bond}}(\vec{b}_{i,\text{new}})\right) \exp\left(-\beta u_{i,B}^{\text{ext}}(\vec{b}_{i,\text{new}})\right)} \\ \cdot \frac{C_{i,A} \cdot \exp\left(-\beta u_{i,A}^{\text{bond}}(\vec{b}_{i,\text{old}})\right) \exp\left(-\beta u_{i,A}^{\text{ext}}(\vec{b}_{i,\text{old}})\right)}{\exp(-\beta u_{i,A}(\text{old}))} \\ \cdot \frac{w_{i,B}^{\text{ext}}(\vec{b}_{i,\text{new}}, b^*)}{w_{i,A}^{\text{ext}}(\vec{b}_{i,\text{old}}, b'^*)} \frac{P^{\text{bond}}(b^*, b'^*)}{P^{\text{bond}}(b'^*, b^*)} \\ = \frac{C_{i,A}}{C_{i,B}} \frac{w_{i,B}^{\text{ext}}(\vec{b}_{i,\text{new}}, b^*)}{w_{i,A}^{\text{ext}}(\vec{b}_{i,\text{old}}, b'^*)}$$

Looking at the whole chain, one gets a product of these factors for each segment:

$$\frac{\text{acc}(\text{old} \rightarrow \text{new}, \{b^*\}_{l_B}, \{b'^*\}_{l_A})}{\text{acc}(\text{new} \rightarrow \text{old}, \{b'^*\}_{l_A}, \{b^*\}_{l_B})} = \frac{\prod_{i=1}^{l_B} \frac{1}{C_{i,B}} w_{i,B}^{\text{ext}}(\vec{b}_{i,\text{new}}, b_i^*)}{\prod_{j=1}^{l_A} \frac{1}{C_{j,A}} w_{j,A}^{\text{ext}}(\vec{b}_{j,\text{old}}, b_j'^*)} = \mathfrak{C} \cdot \frac{W_{\text{new}}^{\text{ext}}}{W_{\text{old}}^{\text{ext}}}$$

This would fit the proposed acceptance criterion 4.2.2 if it were not for the factors

$$\mathfrak{C} = \frac{\prod_{i=1}^{l_B} \frac{1}{C_{i,B}}}{\prod_{j=1}^{l_A} \frac{1}{C_{j,A}}} \quad \text{and} \quad \mathfrak{S} = \frac{S_B}{S_A}.$$

However, looking at the flow 4.2.4, the factor $\mathfrak{C} \cdot \mathfrak{S}$ can be absorbed into the distribution of states 4.2.5 as an additional energy contribution $M = -\frac{\log(\mathfrak{C}) + \log(\mathfrak{S})}{\beta}$ when switching states from o to n . This additional change in potential energy when replacing an A -chain by a B -chain can be identified as a chemical potential difference. We will call the contribution of the prefactors $\mu = -\frac{\log(\mathfrak{S})}{\beta}$ and the contribution of the bond potential normalization integrals $\omega = -\frac{\log(\mathfrak{C})}{\beta}$. The factor μ can be directly adjusted by changing the prefactors in equation 4.2.2. The factor ω presents a constant offset to the chemical potential that depends on the temperature and the bond potentials.

This approach can be generalized to any number of chain molecule species. As only two types participate in any particular move, nothing about the algorithm or the acceptance criterion has to be changed.

Number of trial segments

The number of trial segments per bead, k , can be freely chosen by the implementation. It represents a choice between speed (smaller k) and acceptance rate (larger k). In this work, a k of 200 was used a compromise between these two options, leading to an acceptance rate in the order of 0.01 without a notable impact on execution speed. An adaptive scheme, similar to the adaption of move ranges in the prerun (see section 4.1), was also considered but was not used as the fixed value of k was deemed good enough.

4.3. Units, coordinates and simulation setup

Throughout this work, whenever units are used, they are based on two fundamental scales used in the simulation: The unit of length, σ , and the unit of energy, ε . These fundamental scales are associated with the tail-tail interactions of the basic phospholipid model, whose parameters ϵ_{LJ} and ς are set to 1σ and 1ε , respectively. See section 3.1 and table 3.3 for an explanation of these parameters. Lenz, who used the same phospholipid model as we do, has already mapped the simulation units to standard units and found that $1\sigma \approx 6\text{\AA}$ [70]. The conversion factor for the energy unit was estimated to be $\varepsilon \sim 0.36 \cdot 10^{-20} J$ by matching the main transition temperature of the model lipid and DPPC[130]. However, as his work deals primarily with questions concerning the structure of the lipid membrane, the energy scales will be mostly irrelevant and only lengths are converted to SI units where appropriate.

Whenever a temperature is used throughout this work, it is given in terms of a reduced temperature

$$T^* = \frac{k_B T}{\varepsilon}.$$

Unless stated otherwise (see appendix B), all simulations are set up in such a way, that the lipid bilayer is oriented parallel to the x - y -plane and z is the direction normal to the membrane. W.l.o.g. we will call the leaflet with the greater z -coordinate the “top” or “upper” layer of the membrane and the other one the “bottom” or “lower” layer. If

4. *Simulation techniques*

not stated otherwise, the simulations start with a completely ordered bilayer with all chains arranged on a regular, hexagonal lattice. If multiple chain types are present, the different types are randomly distributed throughout the membrane. The initial shift of the periodic images of the simulation box is chosen as to avoid a mismatch of hexagonal lattice at the boundaries.

All simulations within this work are carried out at a fixed pressure of $p = 2\varepsilon\sigma^{-3}$. The necessary collective volume and shear moves were done with a frequency of 1 per 50 MC sweeps (independently for each coordinate direction and shear factor; 1 per 10 in preruns). In semi-grand canonical simulations the frequency of the identity switch moves was subject to experimentation, in the end we settled for a rate of 1 per MC sweep, which is used in all semi-grand canonical simulations throughout this work, although some simulation runs may have begun with a different (usually smaller) rate. The increased rate of identity switch moves greatly accelerated the equilibration process. All these moves were not done at fixed intervals, but randomly with said frequencies.

Appendix D gives an overview of typical equilibration and autocorrelation times.

5. Data analysis

5.1. Finding rafts in simulated membranes

Looking at visualized simulation data, it is easy to see patterns which can be identified as rafts. However, the exact boundaries of these structures are not always obvious and a process similar to visual identification is hard to implement in a computer program.

Therefore, a precise, simple definition of what constitutes a lipid raft or non-raft is required in order to automate the detection of rafts in large sets of simulated data. The following algorithm describes a way for automated detection of high order cholesterol enriched structures and can also serve as a definition of rafts:

In the data analysis sections of this work, *lipid rafts* are structures found by this algorithm.

This algorithm was tuned, using trial and error, until the resulting structures closely resembled the rafts seen by visual analysis:

- First, the two bilayer leaflets are separated by sorting the chain molecules according to their head-tail orientation.
- Each layer of chains is separated into square bins with a side length of 1.5σ . The chains are assigned to these bins using the xy -position of their respective head.
- For each bin, the number density ρ , the cholesterol density ρ_C and the nematic order S^1 are measured.
- At each vertex of the square lattice, the mean value of these quantities in the surrounding bins is calculated.
- If $\rho \cdot \rho_C \cdot S > 0.15\sigma^{-4}$ at a vertex, that membrane position is marked as possibly being part of a raft.
- Apply the DBSCAN ([29] and appendix C) cluster detection algorithm with $Eps = 3\sigma$ and $MinPts = 3$ to the set of vertices previously marked as possible parts of a raft². The resulting clusters are identified as rafts.

¹The nematic order parameter measures, how well aligned the end-to-end vectors of chains are. It is the eigenvalue of the largest eigenvector of the matrix $S_{ij} = \frac{1}{2N_{\text{lipids}}} \sum_{n=1}^{N_{\text{lipids}}} (2x_i^{(n)}x_j^{(n)} - \delta_{ij})$, where $x_i^{(n)}$ is the i 'th component of the n 'th end-to-end vector and δ is the Kronecker symbol.

²The Eps parameter is a cutoff length up to which points are considered neighbors. $MinPts$ is the minimum number of neighboring points required to form a cluster.

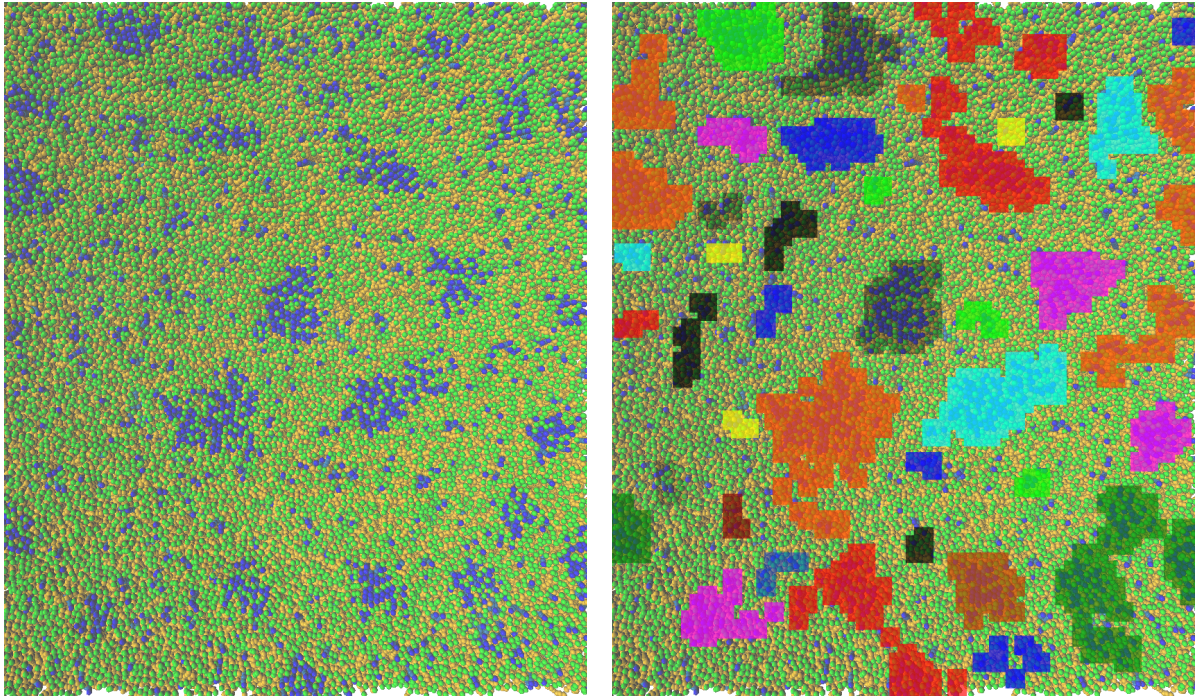


Figure 5.1.: Example usage of the raft detection algorithm. On the left is the original membrane surface, on the right is the same membrane, the 39 rafts found by the raft detection algorithm are marked by different colors. Snapshot taken from a simulation at $T = 1.4$, $\mu = 8.5k_B T$. The points used to depict the rafts are larger than the actual underlying grid, in order to give a better visual impression of cohesion. Note, how the detection algorithm is able to capture the finer structure of the raft borders, but also occasionally connects rafts which are visually distinct (lower right corner). This was a necessary compromise when fine tuning the detection algorithm.

- During this process, periodic boundary conditions are taken into account. Thus, rafts on the edge of the simulation box are identified as one raft, not as two separate rafts.

The same algorithm is used to find non-raft areas. The only difference is the criterion for marking the vertices: Vertices with $\rho_C = 0$ are marked as possible non-raft candidates.

Figure 5.1 shows an exemplary usage of the algorithm.

5.2. Observables

5.2.1. Pair correlation function

In this section we consider a projection of each membrane leaflet onto the z -plane. Thus, all considerations are made for two-dimensional planes.

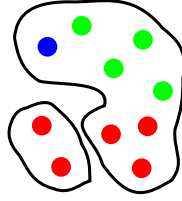


Figure 5.2.: Beads “seen” by the blue bead. Beads in sight are green, beads out of sight are red.

The pair correlation function (PCF) $g(r)$ of a system of particles is, among other applications, a useful quantity for describing the short distance spatial ordering of the particles. The PCF describes how the particle density changes as a function of distance from a particle, normalized by the overall particle density ρ . Given a particle from a homogeneous and isotropic system, the average density at the distance r from this particle is given by $\rho g(r)$. This gives rise to a very simple way of computing $g(r)$ from a given set of particle coordinates: One considers all particle pairs and generates a histogram of their distances. The histogram is normalized with respect to ρ , taking into account the size of each bin of the histogram. For a histogram of equidistant bins, $N(r)$, with width dr one obtains (for two dimensions) the PCF:

$$g(r) = \frac{N(r)}{\rho\pi(dr^2 + 2rdr)}$$

Here, however, we are only interested in the PCF inside or outside a raft. Therefore, one has to make sure to only count particle pairs with a direct and straight connection inside/outside a raft, taking into account the shape of the object (see fig. 5.2). When normalizing the histogram one has to use the correct weight, depending on the area “seen” by a particle. Furthermore, one cannot use the overall particle density ρ of the system but has to calculate the density inside/outside of the rafts.

Finding particle pairs in contact is not trivial nor unambiguous. In essence, the task boils down to a two dimensional field of view (FOV) problem. A significant simplification of the problem can be achieved by approximating the shapes of objects (the rafts) by shapes on a square grid. This process is compatible with the algorithm for detecting rafts from section 5.1, which already uses a square grid.

The problem of FOV on a grid is prevalent in computer games, especially the so called “rogue like” games. Ideas for efficient solutions can be found by studying the algorithms used in these games[102]. As these FOV calculations are usually permissive (that is, they tend to err on the side of making the FOV larger than it would be with an exact calculation) one cannot apply these algorithms to the PCF calculation without adjustments.

In this work we adapted a shadow casting algorithm, using a line of sight (LOS) definition based on the Bresenham line algorithm.

First, one divides the plane into square grid cells. The size of the cells determines the resolution of the FOV algorithm. As the FOV calculation will be restrictive, larger grid

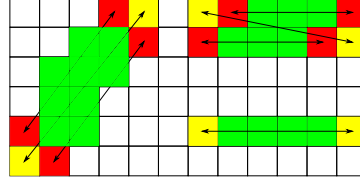


Figure 5.3.: Example LOS definition. Yellow squares are source and target square of a LOS calculation. Red squares are start and end points of the additional lines adjacent to the direct connection. Green squares are squares marked by Bresenham's algorithm, when drawing these lines. Note the omitted adjacent lines in the case of a connection along the grid axis (bottom right).

cells will lead to more particle pairs that are discarded, even if they would be within LOS of each other when using an exact calculation. Each grid cell is marked as either opaque or transparent, depending on the data received from the raft detection algorithm.

A pair of particles is defined to be within LOS of each other if their respective grid cells are within LOS of each other. Two cells of the grid are defined to be in line of sight of each other if one can draw a straight line from one cell to the other without going through an opaque square. To err on the side of caution, the line is made several grid cells wide. This is achieved by drawing two additional lines, one below and one above the direct connection, starting from two cells adjacent to the starting point and ending in cells adjacent to the target quadrant. These additional lines are omitted in the case of a connection along one of the main axes of the grid. The lines are “drawn” using Bresenham's line algorithm, an algorithm commonly used in computer graphics and LOS calculations[13]. Figure 5.3 shows some example lines.

The cell-LOS definition is used to compute a shadow map that associates a cell with all the cells that would be blocked from view if this cell were opaque (i.e. all cells “behind” or “in the shadow” of this cell), seen from the origin. This is done for all cells within a certain cutoff radius around the origin. In order to avoid unnecessary double counting of particle pairs, only half a circle around the origin is considered. These calculations are finished before the main loop of the algorithms starts.

With these preparations, the main algorithm is simple. An example FOV calculation is shown in figure 5.4:

- Loop over all grid cells. For each cell A :
 - Generate a set F of possible cells within the FOV of A (Fig. 5.4 b))
 - Loop over all the cells S in the shadow map (using the cell A as offset). For each of these:
 - * Check the opacity of the cell S
 - * If S is opaque, remove the cells shadowed by S from the set F (Fig. 5.4 c))
 - Loop over all cells B remaining in F (Fig. 5.4 d))
 - * Use the cells A and B in the PCF calculation (see below)

The PCF calculation itself uses a histogram technique similar to the simple algorithm used in the homogeneous, isotropic case (see above) but each histogram bin also records the area “measured” by the bin. For each particle P in cell A the intersection area of cell B and the bins (circular shells) around P is calculated and added to the area measured by the bins³. Then, the distance r of each particle in B to the particle P is calculated and the value of the respective bin $N(r)$ is increased.

The final result is again

$$g(r) = \frac{N(r)}{\rho A(r)},$$

where $A(r)$ is the total area measured by the bin at r . The overall density ρ is approximated

$$\rho = \frac{\sum_r N(r)}{\sum_r A(r)}.$$

5.2.2. Chemical potential difference

The chemical potential difference μ of two different chain species can be gathered from simulation by looking at the statistics of the identity switch move from section 4.2. This process will be described in the first part of this section. Afterward, we will look at the contribution to μ by the entropy of mixing and how it relates to the technicalities of the measurement process.

Measurement

We would like to know the chemical potential difference μ of two chain species A and B . In a semi-grand canonical ensemble, $\mu(C)$ is the chemical potential that keeps the concentration C of the chain species fixed. Looking at the explanation of the identity

³Although it only requires elementary Euclidean geometry, the problem of intersecting circles and squares is surprisingly involved, due to the sheer number of different cases that have to be taken into account. Even after considering all available symmetries, there are still about a dozen possible configurations, each with a different solution.

5. Data analysis

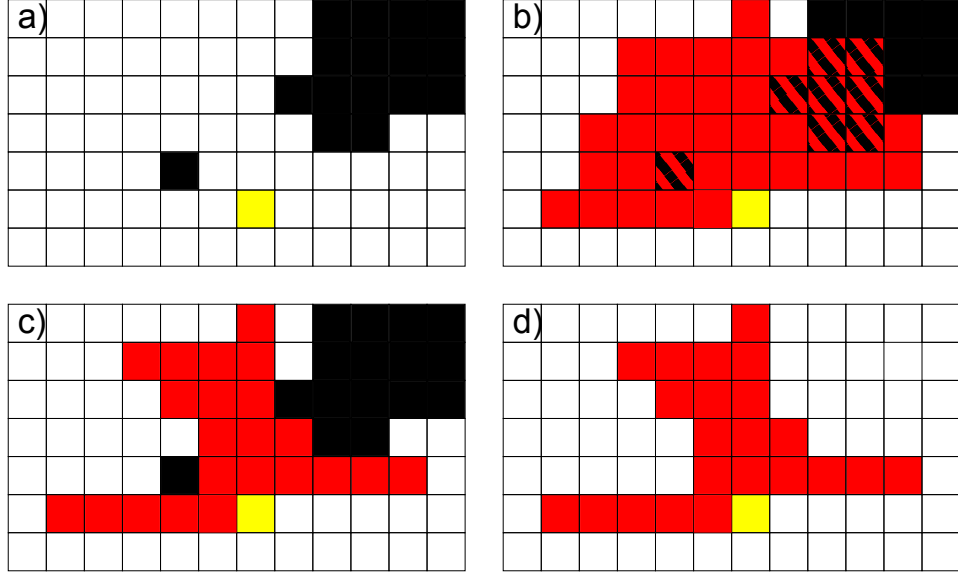


Figure 5.4.: Example field of view creation. Black cells are opaque, white cells are transparent. The yellow cell is the origin (transparent) for this example. a) Initial situation. b) A set of all cells within range (here: 5) and within a half circle is created (red cells). c) Cells shadowed by opaque cells are removed from the set. d) The resulting FOV of the yellow cell.

switch move in section 4.2, this amounts to finding a prefactor $\mathfrak{C}$ so that the flow K of systems with N_B B -chains to systems with $N_B + 1$ B -chains is equal to the reverse flow from $N_B + 1$ B -chains to N_B B -chains. The total number of chains, the temperature and all other model parameters must be equal in both systems.

When we have found such a $\mathfrak{C}_e$ (e for equilibrium), then

$$\begin{aligned}
 K_{N_B+1 \rightarrow N_B} &= K_{N_B \rightarrow N_B+1} && \text{definition of } \mathfrak{C}_e \\
 &= \min \left(1, \mathfrak{C}_e e^{-\beta(U(N_B+1)-U(N_B))} \right) && \text{construction of the acceptance criterion} \\
 &= \min \left(1, e^{-\beta(\Delta E + \mu dN)} \right) && \text{definition of } \mu \\
 \\
 &\Rightarrow \log(\mathfrak{C}_e) = -\beta\mu
 \end{aligned}$$

The factor $\mathfrak{C}_e$ can be determined by simulating systems at N_B and N_{B+1} ⁴, using the identity switch move but never actually performing the switch, just calculating the Rosenbluth weights $\{W_{N_B, \text{old}}, W_{N_B, \text{new}}\}$ and $\{W_{N_B+1, \text{old}}, W_{N_B+1, \text{new}}\}$. When a large number of weights has been gathered from both simulations, one can apply the acceptance criterion 4.2.2⁵ and calculate the flow K for the two; then use a bisection method to

⁴As the monolayers are virtually decoupled concerning the identity switch move, N_B stands for the number of B -chains in each *monolayer*

⁵In the actual implementation, the randomness is removed and instead replaced by the probability of accepting the move. Thus, one gets the same result for a large number of trial moves, but for small sample sizes the results do not depend on the random number generator.

determine a $\mathfrak{C}_e$ so that K is equal for both directions. It is important to note that *all* trial moves are used when calculating the flows, even moves $N_B \rightarrow N_B - 1$ and $N_B + 1 \rightarrow N_B + 2$.

Entropic contribution to μ

As the total flow of systems going from N_B B -chains to $N_B + 1$ B -chains also depends on the probability of trying this move, there is a contribution to μ that depends on the concentration of B -chains. This is nothing else but the manifestation of the entropy of mixing in our simulation. Looking back at section 4.2.2, one sees that for the case of exchanging one chain with another chain with the same potentials and prefactors but just a different name, the factors $\mathfrak{C}$ and $\mathfrak{S}$ are equal to 1 and only the probability of actually choosing this move and the acceptance rate remain. Unfortunately the acceptance rate still depends on the Rosenbluth weights $W_{\text{new}}^{\text{ext}}$ and $W_{\text{old}}^{\text{ext}}$, which even for identical potentials do not cancel each other – not even over many trial moves – as the old chain would usually fit better than the new chain and will therefore usually have a larger weight.

Still, if these factors were to cancel each other perfectly, one would actually measure the probability of choosing this trial move when measuring μ , using the method described above. Thus, for a concentration C_B of B -chains one would get the purely entropic contribution μ_e to the chemical potential

$$\mu_e = -\frac{\log\left(\frac{C_B}{1-C_B}\right)}{\beta} \quad (5.2.1)$$

If one acknowledges the fact that there can only be an integer number N_B of B -chains in a finite system of N chains, this becomes the slightly more complicated

$$\mu_e = -\frac{\log\left(\frac{C_B/N}{1-(C_B+1)/N}\right)}{\beta},$$

which is numerically almost the same as 5.2.1, except for extreme values of C_B or N . Of course, in real simulations, the weight factors will not cancel each other perfectly, but as μ_e goes to infinity for small C_B and to negative infinity for C_B close to 1, the entropic contribution will dominate any other effects from potentials and factors inherent to the algorithm close to these values of C_B .

We can make numerical simulations of the chemical potential measurement process in the case of equal chains, by sampling the Rosenbluth weights $W_{\text{new}}^{\text{ext}}$ and $W_{\text{old}}^{\text{ext}}$ from a fixed distribution and then applying the algorithm from section 5.2.2. If we use uniform distributions with the same maximum and minimum for $W_{\text{new}}^{\text{ext}}$ and $W_{\text{old}}^{\text{ext}}$, we obtain a chemical potential difference that only differs from the prediction 5.2.1 by a constant factor a (figure 5.5):

$$\mu_e = -a\frac{\log\left(\frac{C_B}{1-C_B}\right)}{\beta} \quad (5.2.2)$$

5. Data analysis

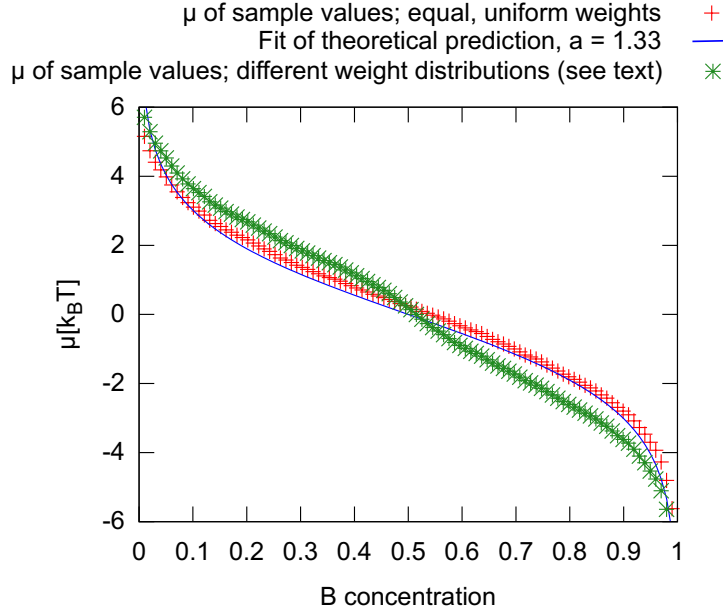


Figure 5.5.: Entropic contribution to μ for artificial data. Red: Rosenbluth weights are equally and uniformly distributed. Blue: A fit of the theoretical prediction 5.2.1. Green: Rosenbluth weights are biased toward keeping the old chain. The weights for the new chain are drawn from a uniform distribution with a maximum half as large as the maximum of the old weight.

Apparently, this parameter can account for the fact, that the probability of accepting

$$\text{acc}(\text{old} \rightarrow \text{new}) = \min \left(1, \frac{PW_{\text{new}}^{\text{ext}}}{W_{\text{old}}^{\text{ext}}} \right)$$

is not P -times larger than

$$\text{acc}(\text{old} \rightarrow \text{new}) = \min \left(1, \frac{W_{\text{new}}^{\text{ext}}}{W_{\text{old}}^{\text{ext}}} \right),$$

as was assumed when deriving the prediction 5.2.1. If we sample the Rosenbluth weights from different distributions, this is no longer the case, as can be seen from the green data points in figure 5.5. Naturally, all curves still have the same behavior close to $C_B = 0$ and $C_B = 1$, no matter the exact distribution of the weights.

Later, we will see that many chemical potential curves are very similar to the red curve, albeit with a constant offset from the potential parameters $\log(\mathfrak{C})$; the difference between the chains themselves; and with different fit parameters a . This suggests a similar behavior of the real acceptance rates as was used for generating red data points.

5.2.3. Shapes

Given a two dimensional set of N points $\vec{r}^i$, such as we obtained in section 5.1, we can define the *radius of gyration tensor*[121]:

$$S_{mn} = \frac{1}{2N^2} \sum_{i=1}^N \sum_{j=1}^N (\vec{r}_m^i - \vec{r}_m^j) (\vec{r}_n^i - \vec{r}_n^j).$$

The ordered eigenvalues $\lambda_x^2 \leq \lambda_y^2$ are the *principal moments* of the shape and describe the extension of the shape along its main axis and in the perpendicular direction. The *radius of gyration*

$$R_g = \sqrt{\lambda_x^2 + \lambda_y^2}$$

is a measure for the overall size of the object. We define the *acircularity*

$$c = \lambda_x^2 - \lambda_y^2$$

as a measure how unbalanced the point distribution is among the main axes. A large value indicates that the shape has a large extension (or density) into one direction, a value of 0 implies a more symmetrical distribution around the center (but not necessarily circular!). Furthermore we define the dimensionless *relative acircularity*

$$\kappa = \frac{c^2}{R_g^4}$$

as a means to compare the acircularity of shapes of different sizes. A value of 1 indicates that all points are distributed along the main axis, whereas a value of 0 again implies a symmetric distribution.

Morphology

A membrane surface with rafts can be interpreted as a 2D pattern of rafts and non-rafts. Image analyzing techniques can be used to characterize the morphology of these patterns. For this purpose the surface is mapped to a grid of $1.5\sigma \times 1.5\sigma$ squares. A raft is represented by a “black” square, non-rafts by “white” squares. Then we can analyze the patterns of the black squares by counting the number of black squares n_s , the number of edges of these squares n_e and the number of vertices next to these squares n_v . We can then compute the three Minkowski functionals

$$\begin{aligned} A &= n_s \\ U &= -4n_s + 2n_e \\ \chi &= n_s - n_e + n_v. \end{aligned}$$

A is the total area covered by black squares. U is the total perimeter of all connected blocks of black squares, where two black squares are connected if they are nearest or

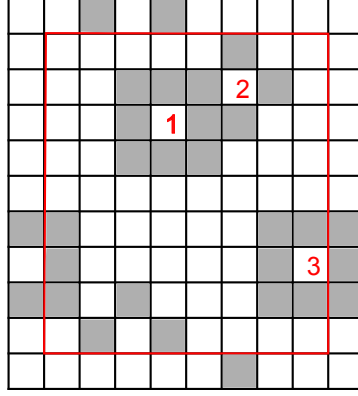


Figure 5.6.: Example calculation of Minkowski functionals with periodic boundary conditions (red). The gray squares form two connected clusters, with three holes (1,2,3). The square 3 only counts as a hole because of the periodic boundary conditions. Only squares, edges and vertices on or within the red boundaries must be counted; edges and vertices on the boundary must only be counted once, disregarding their periodic images. Thus, one arrives at $n_s = 22$, $n_e = 71$ (35 for the upper cluster, 36 for the lower cluster), $n_v = 48$ (23 + 25), $A = 22$ (11 + 11), $U = 54$ (26 + 28) and $\chi = -1$ ($-1 + 0$)

next-nearest neighbors of each other or if they can be connected via a chain of other connected black squares. The last property, χ , is the Euler characteristic, which, in two dimensions, is the number of connected clusters minus the number of white holes within these clusters. All of these properties are additive; χ is also independent of a possible rescaling of the pattern. Periodic boundary conditions can be emulated by surrounding the original pattern with an additional layer of squares whose color is given by the boundary conditions [85]. Figure 5.6 shows an example calculation involving periodic boundary conditions.

Tension and spontaneous curvature

The local pressure can be calculated by using the Irving-Kirkwood method[53], which was already used in the predecessor work to this one[131]. The method is explained in great detail in that work and will not be repeated here. The gist of the method is, that one calculates the forces (not potentials!) F between particles i, j and using these forces one can calculate the contribution to the pressure, $\mathcal{P}$, in a slice n through the membrane (normal to the z -direction, the membrane normal) via the virial theorem:

$$\mathcal{P}_{\alpha\beta}^n = \left\langle \frac{N_n}{V_n} \right\rangle k_B T \delta_{\alpha\beta} + \left\langle \frac{1}{V_n} \sum_{i < j} r_{ij}^\alpha F_{ij}^\beta I_n \right\rangle$$

Here, N_n is the number of particles in slice n , V_n is the volume of slice n , δ is the Kronecker delta, with the indices α and β indicating coordinate directions. r_{ij} is the vector

connecting the two particles i and j and I_n is a weight function, that handles the contribution to the pressure in the case that the particles are situated in different slices. One remaining challenge is the contribution to the pressure in the case of three-particle potentials such as the bond-angle potential. West compares two different conventions, the original Irving-Kirkwood convention and the alternative Götz-Lipowsky convention[39], and concludes that the difference between the two methods is negligible[131]. We only use the Irving-Kirkwood convention. For an explanation of the two conventions we again point to the description in the work by West.

When one has calculated the pressure tensor, one can split it into its normal and tangential components, $\mathcal{P}_N$ and $\mathcal{P}_T$, and use these to calculate the tension profile

$$\gamma(z) = \mathcal{P}_N(z) - \mathcal{P}_T(z).$$

The overall tension $\Gamma = \int_0^\infty \gamma(z)dz$ of a monolayer must vanish if our simulation are correctly equilibrated, but the first moment of the tension profile is connected to the spontaneous curvature c_0 of the monolayer via[106]:

$$k_c c_0 = - \int_0^\infty \gamma(z)z dz, \quad (5.2.3)$$

where the middle of the bilayer is assumed to be at $z = 0$. The factor k_c is the bending rigidity, which will be treated as an unknown material constant throughout this work.

The derivation of equation 5.2.3 in [106] makes the assumption, that both monolayers can be treated as elastic continua. There are some problematic issues with this assumption: We neglect the effect of anisotropies, such as the preferential direction in a tilted gel structure. One might also argue, that a gel itself cannot be well represented by a continuous model. There is also the problem, that we take an integral across the monolayer, over a quantity which is assumed to be continuous but which is actually derived using only one layer of molecules each composed of only a few beads. It is, however, the only method we have to calculate something like the spontaneous curvature of a monolayer. It should still be sufficient to identify qualitative differences of the spontaneous curvatures of different structures, which is one of the main assumptions used in our theory of raft stabilization (section 1.3).

6. Structures and chemical potentials at small system sizes

6.1. Measurement of the chemical potential

Small simulations were run at different system sizes and temperatures. For each system size, several fixed cholesterol numbers were used in pairs of N and $N + 1$ cholesterol chains (per monolayer!). For each of these paired simulation runs, the chemical potential was calculated using the method from section 5.2.2. The data was pre-selected to only use systems in the same state (fluid, tilted gel) for each calculation. In the crossover area between the two states, occasionally a third, interdigitated, structure could be observed, but the data was not sufficient to calculate a chemical potential for this state.

The different values for μ , obtained for a certain set of parameters, are certainly correlated as, given a set of runs with N cholesterol chains and a set of runs with $N + 1$ cholesterol chains, the data is evaluated crosswise. If there are $\#N$ runs with N cholesterol chains and $\#(N + 1)$ runs with $N + 1$ cholesterol chains, one obtains $\#N * \#(N + 1)$ values for μ from only $\#N + \#(N + 1)$ sets of independent data, but the exact nature of the resulting correlation between the μ measurements is hard to grasp. This makes it difficult to give a correct estimate of the statistical error of the resulting sample mean. We can, however, have a look at the distribution of the individual values (figure 6.1): We can see, that the distribution is almost Gaussian. Although this does not tell us anything about the correlation between the values, we can assume that the correct error estimate is somewhere of the order of a naive error estimate (ignoring possible correlations) or at least much smaller than the standard deviation of the distribution. As it turns out, the standard deviation is already very small and would not be representable in the graphs showing the chemical potential and will therefore be mostly disregarded throughout this work.

The chemical potential was measured for systems of $9 \times 9 \times 2$, $12 \times 12 \times 2$, $15 \times 15 \times 2$ and $18 \times 18 \times 2$ chains at temperatures $T = 1.2$, $T = 1.3$ and $T = 1.4$. The result is shown in figure 6.2. The system size has no apparent influence on the measurement of μ . At $T = 1.2$, the chemical potential curve has no notable features. In fact the curve corresponds well to a purely entropic part (equation 5.2.1) plus an offset of about $9.4k_B T$. At higher temperatures a very interesting feature appears: At the cholesterol induced transition from liquid to gel state, the chemical potential has a gap of $\gtrsim 2k_B T$. This gap is absent at $T = 1.2$ as this temperature is below the main transition temperature of even the pure lipid, without any cholesterol.

6. Structures and chemical potentials at small system sizes

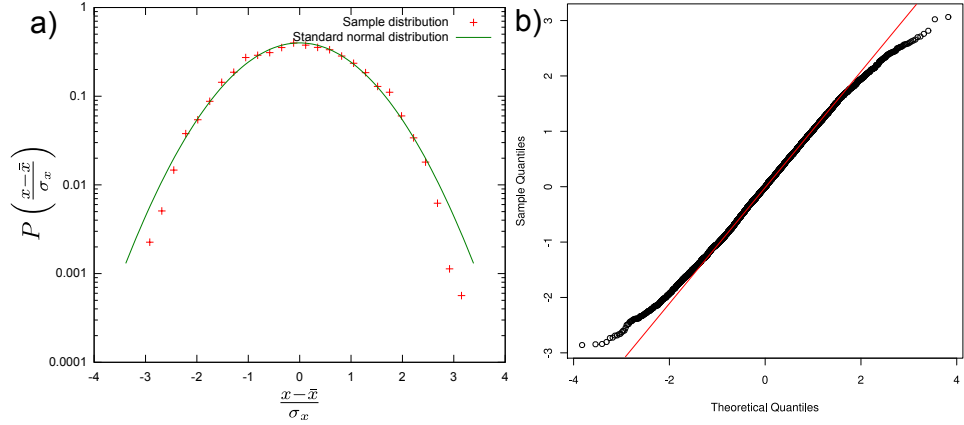


Figure 6.1.: a) Normalized histogram of μ values around their respective means. Note that the standard normal distribution given for comparison was not fitted to the data. b) Q-Q plot of the quantiles of the actual distribution against the quantiles of a standard normal distribution. The red line corresponds to a standard normal distribution. All samples were generated using the standard cholesterol model.

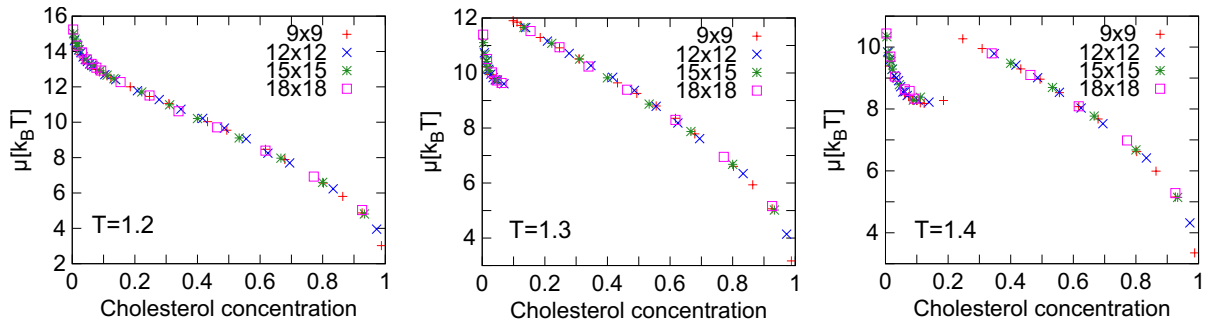


Figure 6.2.: Chemical potential of the standard cholesterol model for various system sizes and temperatures. Error bars would be smaller than the sizes of individual points and are not shown.

6.2. Interpretation of the results

6.2.1. Significance of the gap

The stabilization criterion for the chemical potential μ over the cholesterol concentration C is

$$\frac{\partial \mu}{\partial C} < 0, \quad (6.2.1)$$

as μ is the energy required for *lowering* C . Thus, even if the gap were not a gap but a very steep upward slope, such a configuration would be unstable. Therefore, for μ between the upper and lower bounds of the gap, two different structures are observed: A liquid state with a very low cholesterol concentration and a gel state with a high cholesterol concentration. We note the similarity of these two structures to the liquid ordered and liquid disordered phases in the raft literature (p.e. [125]). Even the concentration of cholesterol where both states appear is of the same magnitude as the concentration where both phases exist in model membranes [1, 5, 81]. The distinguishing property of the liquid ordered phase compared to a gel is its higher lateral diffusion rate; a dynamic property that is not readily accessible in our Monte-Carlo simulations. The ordered structure in our simulations looks identical to what was previously identified as the (solid) gel phase but we would not be able to distinguish, whether the higher cholesterol content in these structures has an impact on lateral diffusion. Still, as the main reason for the appearance of the gap in μ is a structural transition depending on the cholesterol concentration, this feature could also exist in real mixtures of phospholipids and cholesterol, independent of the microscopic details of the structures. Indeed, we will see in section 9.5 that raft structures appear even between gel-gel transitions.

The effects of the chemical potential on simulations of large systems will be discussed throughout the next chapter. We will indeed observe, that systems simulated in the two-state region exhibit the two different structures as described here. However, these structures form finite domains, which may be explained by the mechanism described in section 1.3. For values of μ , where only the liquid structure should be stable, this is also the case in large systems. However, in regions where only the gel structure should appear, the systems retain some small liquid “trenches” between areas in the gel state. The characteristics of these structures will also be discussed throughout the next chapter. A discussion about the trench structures and their physical reality can be found in sections B.3 and 10. One can see typical snapshots of large membranes for these three cases in figures 7.1 and 7.2.

6.2.2. Structural differences between system sizes

Although the measurement of μ gives similar results for different system sizes, there are some effects in larger systems that do not occur in the small systems. At 18×18 and low overall concentrations of cholesterol, the system sometimes forms small ordered clusters with relatively high cholesterol content. But the system is not large enough to form multiple, finite rafts. At higher concentrations, around the (cholesterol induced)

liquid-gel transition, the larger systems will form a ripple structure. The ripple cannot be formed in small systems due to the size of the structure. On the other hand, the smallest systems (9×9) sometimes form an interdigitated gel very close to the liquid-gel transition. Multiple jumps between fluid and interdigitated state were observed during the same simulation runs. These system spanning interdigitated configurations only appear at small system sizes. In the larger systems, the interdigitated configuration appears as a part of the ripple structure, with the lipids at the center of the structure being interdigitated, while the rest of the system exhibits a gel like structure. See [70] for a detailed description of the ripple structure in pure lipid systems.

Overall the existence of the ripple structures in larger systems did not influence the measured values of μ , as was seen when comparing the values for different system sizes. The values for μ in the ripple state lie on the same section of the curve as the values for the gel structure. When one only picks small systems in the interdigitated state, one can also measure μ for these systems. One obtains results similar to the gel state but the interdigitated structure is observed too rarely to make good quantitative statements.

The ripple state in the 18×18 -system appears in the wide range from $\approx 13\%$ to $\approx 25\%$ cholesterol for $T = 1.3$ and in a narrow regime around $\approx 30\%$ at $T = 1.4$. The interdigitated structure was observed in the 9×9 -system around 11% cholesterol and was not seen at $T = 1.4$.

Figure 6.3 shows sample configurations, illustrating the structures characterized above. The interdigitated structure is not fully developed, one can see how the system wants to form a rippled structure, but it does not fit. In the larger system, pictured underneath, the ripple is fully developed.

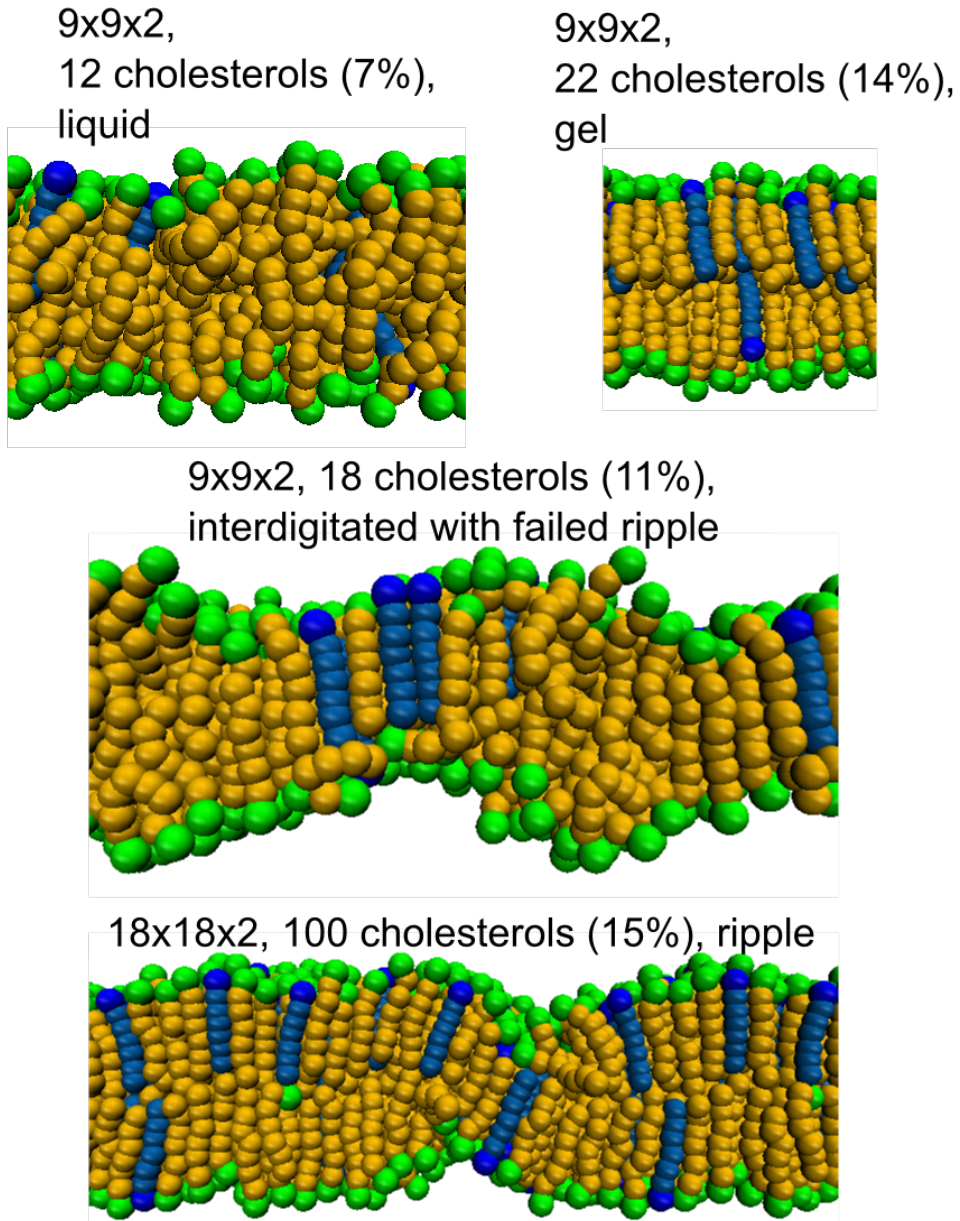


Figure 6.3.: Sample configurations from small systems, rendered with periodic images to the left and to the right. The temperature is $T=1.3$.

7. Global properties of large membranes

Using the results from the previous chapter we can simulate large membranes in the semi-grand canonical ensemble at interesting values for μ . Throughout this chapter we will discuss in detail the properties of these membranes.

7.1. General observations

Based on the results from section 6.1 there are at least three broad categories into which simulations of large membranes can fall:

- Simulations with μ above the two state region
- Simulations within the two state region
- Simulations with μ below the two state region

Furthermore, at lower temperatures, there is no two state region and, at higher temperatures the membrane just disintegrates.

We observe the following (figures 7.1 and 7.2):

- Above the two state region, the simulated membranes are wholly liquid, with very few, if any, cholesterol chains. Very rarely a few cholesterol chains may form a small cluster of higher chain order.
- Within the two state limit, one observes finite, stable regions of high cholesterol content with high local chain order. We identify these regions as rafts and will explore their properties. We will call the upper and lower limits of the two state region the *raft limits*.
- Below the two state region, one does not get a system spanning gel structure. Instead, there appear “trenches” of liquid lipids in the systems. We were never able to make these trenches disappear completely for any values of μ used in the simulations. Although at some point they no longer percolate (see section 8.2), there still remain at least some pockets of liquid lipids. However, there must be a point where these pockets disappear, too, as a pure cholesterol membrane clearly cannot form any lipid containing structures.

- When there is no coexistence, the system is mostly homogeneous, but may include occasional interruptions by ripples.

We therefore suggest the state diagram in figure 7.3 for our model at fixed pressure. In the following chapters, especially section 9.1, we will look at evidence for this diagram. The dotted lines in figure 7.3 denote areas that were not explored in detail. It may be possible that the trenches are completely replaced by rafts at the point of disintegration, or possibly even the rafts replaced by the pure liquid phase. These details would also depend on the exact pressure and microscopic properties of the cholesterol, whereas this phase diagram is a very general suggestion. In this work, we were unable to clearly determine whether the trench structures are true equilibrium structures or simulation artifacts. This is discussed later, in sections B.3 and 10. Should the trench structures turn out to be part of the homogeneous gel phase, our diagram would very much resemble typical proposals for cholesterol-lipid phase diagrams (Figure 2.1 and references [52, 100]), but in the area of *lo/ld* coexistence, we observe the formation of rafts. In our simulations we would be unable to clearly distinguish between solid and liquid ordered structures. As our cholesterol model is tuned to mix well with the lipids, it will not demix at very high concentrations, as real cholesterol does. Our simulations are not suited to reproduce this effect.

7.2. Morphology of the raft pattern

Applying the technique from section 5.2.3 to the 100×100 simulations, one observes three very distinct patterns of the Minkowski functionals for the three regimes of μ above, below and within the raft forming zone. These can be seen in figure 7.4, where the values of the Minkowski functionals are plotted against each other. The systems above the raft-limit (i.e. almost fully liquid membranes) have very few and small structures in them and therefore are found near the origin of each graph. Note that χ is usually positive for these configurations; those few cholesterol rich structures that exist, are too small to have any or many holes in them. Raft forming systems are found at high values of χ (many distinct structures) with a low ratio of area to perimeter (small structures). Finally, systems below the rafts limit have a negative χ (few, but very large (usually system spanning) structures with some islands of liquid lipids within them) that get even more negative if one increases μ toward the raft limit (more lipids are added). In the area-perimeter plane these systems follow a crescent shaped path as μ is increased. While it is obvious that the perimeter would increase for greater μ while the area shrinks as more lipids are added, it is not immediately apparent why the area shrinks for very small μ . This effect likely stems from the fact that the absolute area is measured and not related to the size of the membrane. While all configurations are roughly the same size, for high cholesterol concentrations one would see a decrease of the total area because of the better packing of cholesterol rich systems.

There are two minor details to note:

- The configurations at $T = 1.3$ have slightly smaller areas than the systems at $T =$

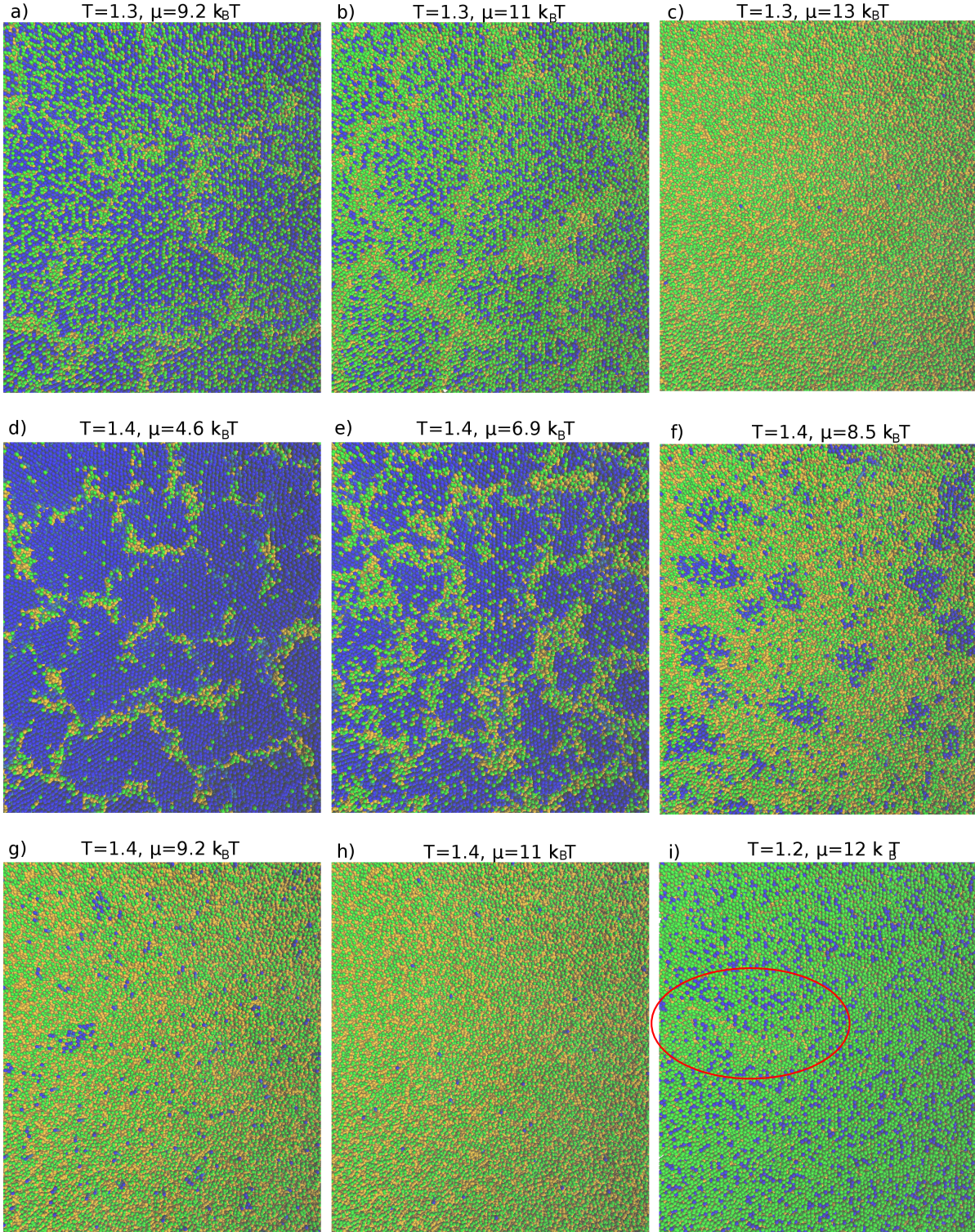
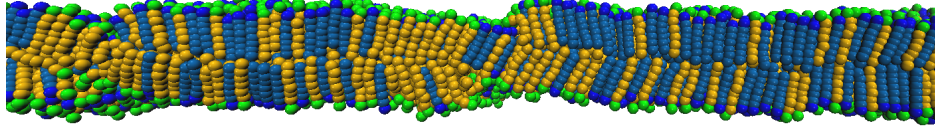
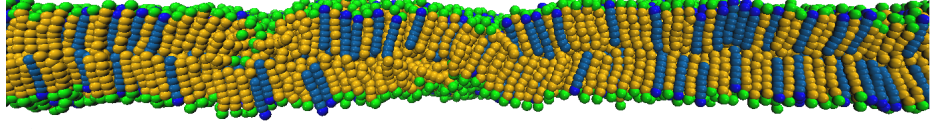


Figure 7.1.: Snapshots from 100×100 systems with different parameters. a+d) Far below the raft limit. b+e) Just below the raft limit. f+g) Within the raft limit. c+h) Above the raft limit. All snapshots cut to equal size and scale; actual configurations may be larger, depending on temperature and cholesterol content. The last snapshot i) is taken from a system without phase coexistence. Note the ripple, marked red.

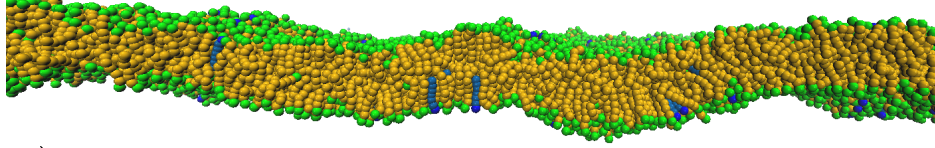
a) $T = 1.3, \mu = 9.2k_B T$



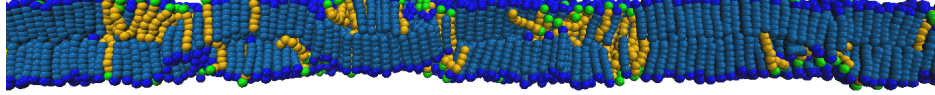
b) $T = 1.3, \mu = 11k_B T$



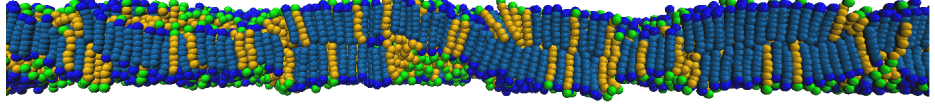
c) $T = 1.3, \mu = 13k_B T$



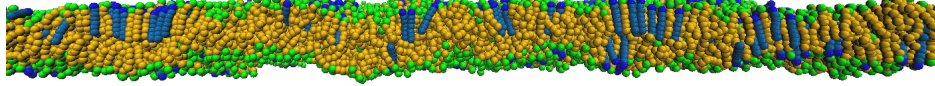
d) $T = 1.4, \mu = 4.6k_B T$



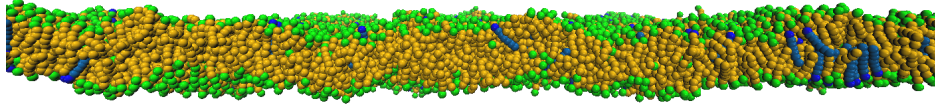
e) $T = 1.4, \mu = 6.9k_B T$



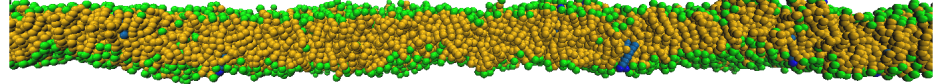
f) $T = 1.4, \mu = 8.5k_B T$



g) $T = 1.4, \mu = 9.2k_B T$



h) $T = 1.4, \mu = 11k_B T$



i) $T = 1.2, \mu = 8.5k_B T$

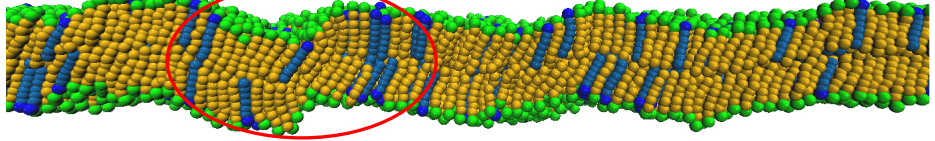


Figure 7.2.: Side views of systems at the same parameters as in figure 7.1. The configuration i) is in fact the same configuration as before and the marked ripple is the same ripple.

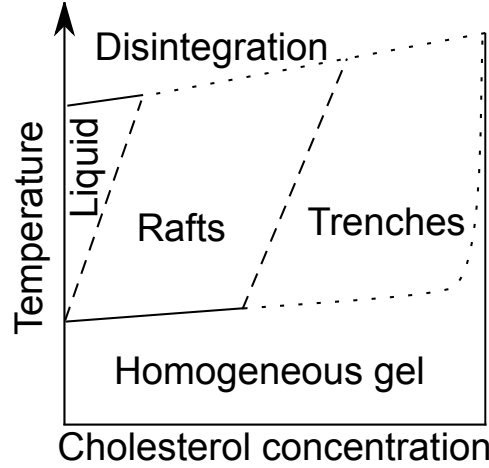


Figure 7.3.: Suggestion for a concentration-temperature state diagram at constant pressure. Though this is not a phase diagram, the continuous lines denote known phase transitions of the model. The dashed lines denote the borders of regions of different states that we found in our simulations. The dotted lines denote borders of these regions that were not explored within our simulations but are reasonable extrapolations using the existing data.

1.4 although otherwise their behavior is qualitatively and quantitatively identical. This is likely the same effect as for small μ as the packing density is slightly higher at lower temperatures.

- There is one set of parameters that is divided between the behavior of systems above and within the raft limit (hollow pink squares). These are systems at $T = 1.4$ and $\mu = 9.2k_B T$ and thus very close to the upper limit for rafts.

7.3. Interdigitated structures

As was described in section 6.1, there are certain parameter sets, where an interdigitated structure exists between the fluid and the gel (on the cholesterol axis). In larger systems, the interdigitated structure appears as a structural element of the membrane. This is not surprising, as the interdigitated structure is an element of the ripple state in pure lipid membranes[70]. Therefore, we would expect to find this structure near the crossover to gel-like states. The appearance and shape of these domains will be analyzed in the following.

From visual analysis of snapshots and by fine-tuning of thresholds, one can derive a criterion for detecting interdigitated domains in a membrane: An interdigitated area is defined as having a nematic order $S_{\text{total}} = S_{\text{upper monolayer}} + S_{\text{lower monolayer}} > 1.65$ and a thickness (head to head) $d < 6.2\sigma$. Note, that this criterion is rather generous, as the nematic order in a gel-like structure can be close to 1 (for one monolayer) and a fully interdigitated membrane could have a thickness of down to $d \approx 4.5\sigma$. Still, this allows

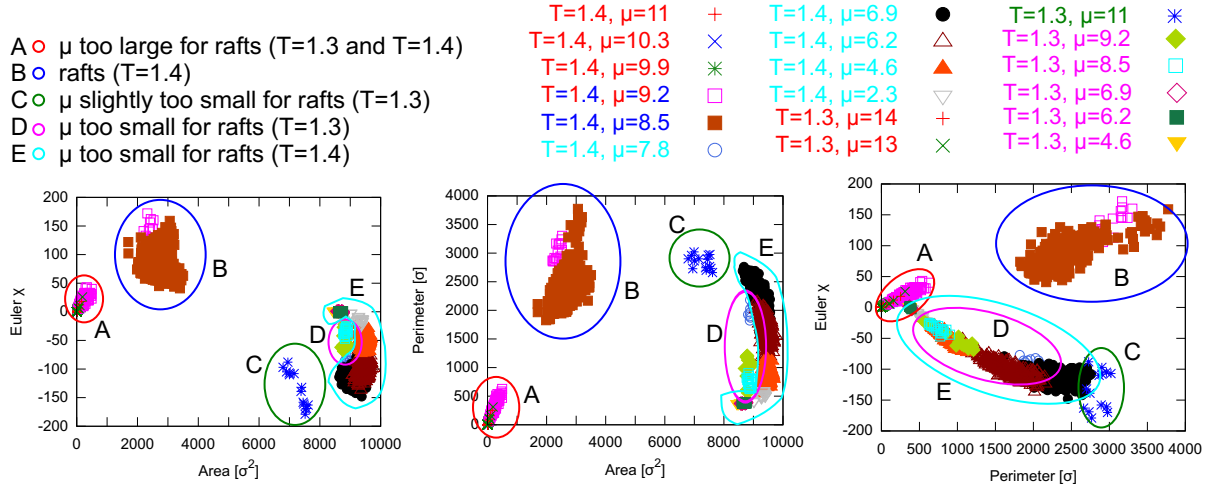


Figure 7.4.: Results of the measurement of Minkowski functionals. Each point represents a single, independent configuration. Different symbols and colors denote different parameter sets. Values for μ given in units of $k_B T$.

a clear differentiation from gels ($d \approx 4.2\sigma$ for one side) and liquids ($S < 0.2$) while still being able to identify small or not fully interdigitated areas.

The result of measuring the area fraction of the bilayer in the interdigitated state is shown in figure 7.5. Above the raft limit, there are no interdigitated domains. Within the raft limit, interdigitated areas begin to emerge, with a maximum appearance just below the raft limit. Towards smaller values of μ , the interdigitated fraction of the membrane decreases again. These effects are developed stronger at $T = 1.4$ than at $T = 1.3$; the fraction of interdigitated areas can be as high as 20 – 25% at $T = 1.4$. None of these effects are surprising: Above the raft limit, there is not enough cholesterol to form the interdigitated structure. On the other hand, at small values of μ almost the whole membrane is a cholesterol rich gel. Just below the raft limit there are many trenches in the large cholesterol enriched gel structures which are just the kind of structure that favor the interdigitated structure. This can be seen in figure 7.6, where trench and interdigitated areas of a sample configuration are plotted against each other. Clearly, the trenches and interdigitation are related. Although the trenches themselves are liquid, the border to the cholesterol enriched structure is interdigitated.

7.3.1. Analysis of interdigitated structures

We apply the DBSCAN algorithm ([29] and appendix C) to the interdigitated areas, in order to analyze their shape. In figures 7.6 and 7.7 b), one can already perceive that the interdigitated-areas mostly form lines within the membrane but make up only a relatively small proportion of the whole bilayer.

The ratio of the radius of gyration of the interdigitated areas and their respective size can tell us about the shape of these areas. If the general shapes are independent of the size, then the radius of gyration can be fitted to the size by a power law, with

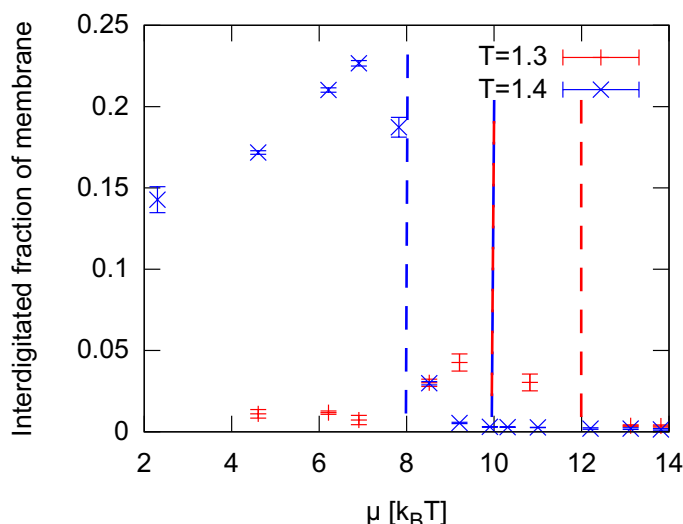


Figure 7.5.: Fraction of interdigitated areas in the bilayer. The dashed lines mark the approximate raft limits.

an exponent between 0.5 (perfect circles) and 1 (straight lines). This is related to the fractional dimension of the object, in particular, as we are working on a grid, it is identical to the Minkowski–Bouligand dimension (box counting dimension), where the radius of gyration corresponds to the side length of the boxes and the size (area) corresponds to the number of boxes[30].

Indeed, using a weighted least square fit on the systems with a large fraction of interdigitated areas (figure 7.5) one finds that a power law fits the data well. An example for one such fit can be seen in figure 7.8 a). The exponents for all the systems are shown in figure 7.8 b). While the exponents are quite spread out with no clear dependence on μ , they are all much larger than 0.5, indicating a preference of line-like shapes of the interdigitated areas. These exponents correspond to a fractional dimension of $D \approx 1.4$. As a reference, the fractal dimension (Hausdorff dimension¹) of a self avoiding walk in two dimensions (i.e. a 2D polymer) is $D = \frac{4}{3}$, the coastline (not the island itself!) of Great Britain has a fractal dimension of $D \approx 1.25$ [75], a diffusion limited aggregation (DLA) of clusters in two dimensions (i.e. a cluster grown of clusters) has $D \approx 1.44$ [128], and for a DLA of single particles $D \approx 1.72$ [128].

The Euler χ parameter (see section 5.2.3) of these clusters is shown in figure 7.9 a). While there is a large spread of χ -values and no clear trend for different values of μ , the main finding is, that the χ -values are relatively close to 1 (1 indicates a structure without any holes and is the maximum possible χ in two dimensions). Even for large clusters, typical χ values are larger than -5 and even 1 is still a common value. This implies that there are very few holes or closed loops in these structures.

¹The Hausdorff dimension is an alternative definition of fractional dimension. The Hausdorff dimension is always lower or equal to the box-counting dimension.

7. Global properties of large membranes

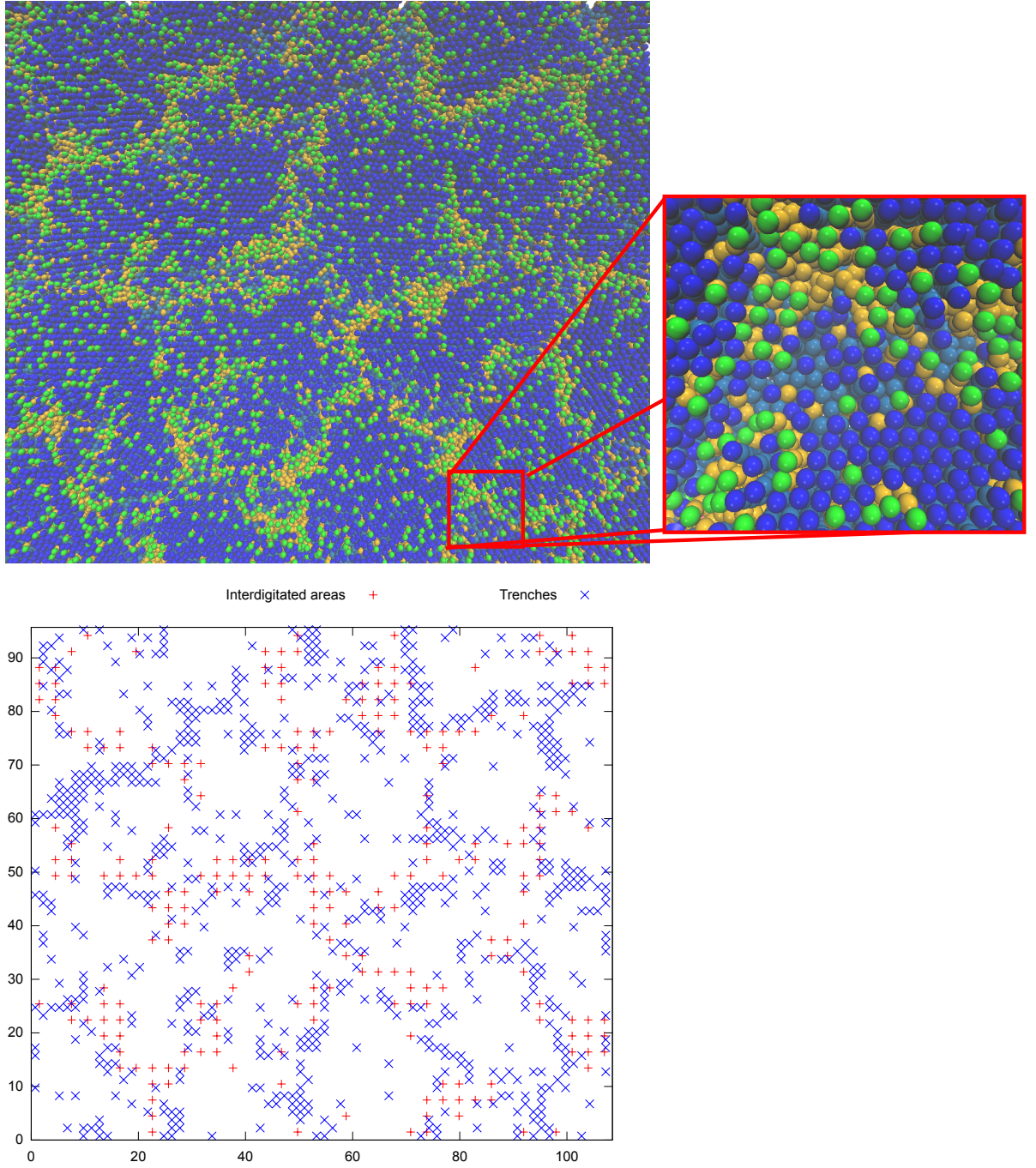


Figure 7.6.: Relationship of trench structures and interdigitated areas. Taken from a simulation at $T = 1.4, \mu = 6.9k_B T$. The trenches were detected by a fine tuned method as described in section 7.6.2. In the close-up, the interdigitated part is where the cholesterol tails from the other side (lighter hue of blue) are visible through the heads.

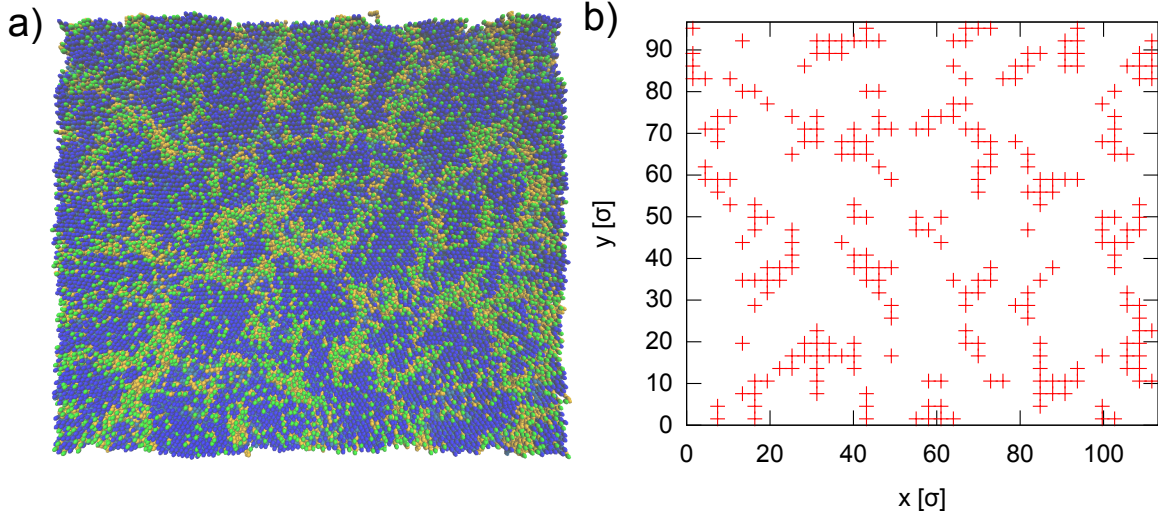


Figure 7.7.: Example for interdigitated structures: a) View onto the membrane from the top. b) Position of lipids in interdigitated structure for the configuration in a). Configuration taken from a simulation at $T = 1.4$, $\mu = 6.9k_B T$.

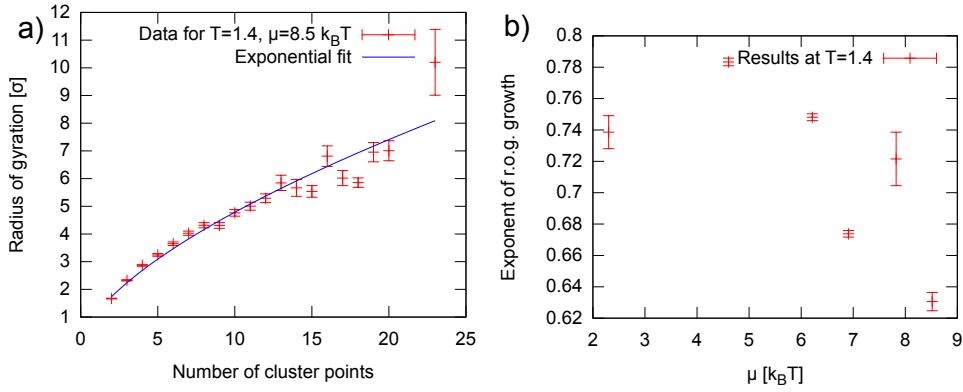


Figure 7.8.: a) Example of power law fit of interdigitated cluster growth for $T = 1.4$ and $\mu = 6.9k_B T$. b) Growth exponents over μ for $T = 1.4$.

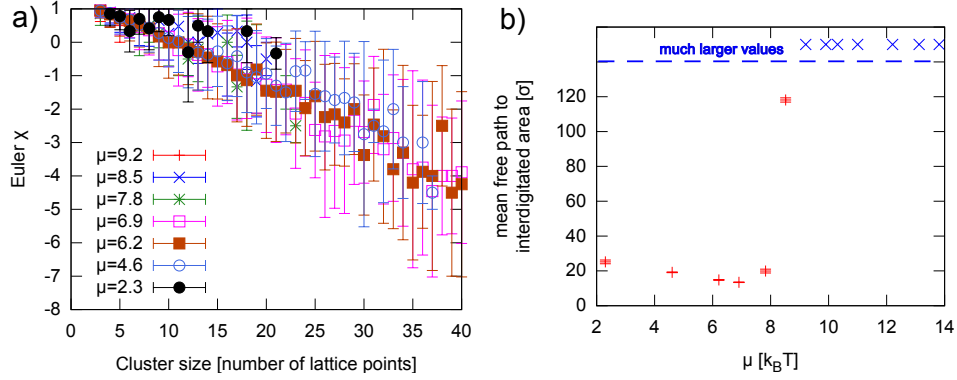


Figure 7.9.: All results for $T = 1.4$, values for μ given in units of $k_B T$: a) Euler χ over size of interdigitated area ($3\sigma \times 3\sigma$ lattice). The bars denote the standard deviation. b) Mean free path until encountering an interdigitated structure in a membrane. Values over 120σ are only shown as “larger” as they exceed the largest typical length scales of the simulated membranes.

We can also simulate, how long it takes from a random starting position, going straight into a random direction, to encounter one of the interdigitated domains. This “mean free path” for a point like particle is $\approx 30\sigma$ in the systems below the raft limit at $T = 1.4$ (figure 7.9 b)). Although this is a static analysis and the dynamics of these structures are unknown, it is probable – because of this short distance – that these interdigitated areas could interact with proteins embedded in the membrane and influence their dynamics.

Together with the result that the general shape of these areas tends to be more open, rather than globule like, this implies that the interdigitated region form mostly stretched lines with no or few pores, making the structure seen in 7.7 c) typical examples. We can further confirm this statement by looking at the relative acircularity of the structures, as defined in section 5.2.3. The resulting histogram (figure 7.10 a)) has some very sharp peaks and is skewed to the right, but this is actually a systematic error due to the finite minimum size of the structures. There are only a few possible shapes that can be formed by a small number of points on a lattice, which results in sharp peaks at values corresponding to these shapes. If we only take into account clusters of 11 or more lattice points, we obtain the histogram shown in figure 7.10 b). The sharp peaks disappear, but the distribution is still clearly skewed towards larger acircularities. This confirms that, indeed, the interdigitated areas mostly assume very elongated shapes.

7.4. Cholesterol concentration

The total cholesterol concentration averaged over the whole membrane is shown in figure 7.11. At $T = 1.3$ the concentration curve almost perfectly fits the prediction that can be made by reversing the graph 6.2. At $T = 1.4$ the measured concentration is a bit smaller than predicted, likely due to the appearance of the trenches, which do not occur in the smaller systems used to generate the prediction. It is notable, that the overall

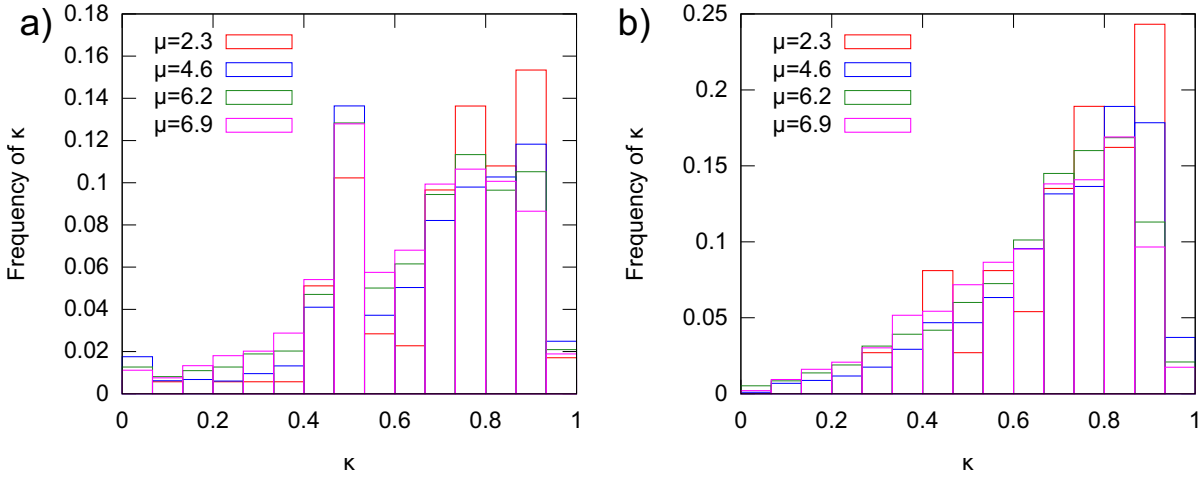


Figure 7.10.: Acircularity of interdigitated areas at $T = 1.4$. a) counting all areas detected as interdigitated. b) Counting only areas composed of > 10 points on the underlying grid.

concentration in the coexistence area seems to be dominated by the concentration in the fluid structure, with only small corrections from the gel-rafts.

We can also measure the concentration of cholesterol only inside the raft structures (or the cholesterol enriched gel structures sans the trenches, when the configuration is taken below the raft limit). The result can be seen in figure 7.12. Below the raft limit we get a result very similar to the whole membrane, as almost the whole bilayer is composed of a single gel sheet. But we are more interested in the concentration within the “real” rafts. From a simple Maxwell construction on the graphs in figure 6.2 we would expect a cholesterol concentration in the rafts of about 30% at $T = 1.3$ and about 45% at $T = 1.4$. We actually see such values within the raft limits. For $T = 1.4$ the concentration is a bit lower than expected but this may be due to the finite size of the grid used to detect the raft structures. As the rafts are already very small at these parameter sets (as shown in section 8.2) the measurement could falsely count the surrounding cholesterol devoid liquid structures as part of the rafts, decreasing the mean cholesterol concentration. Above the raft limit we measure the occasional small cholesterol enriched structures that rarely appear during the simulations. Their cholesterol concentration levels out at about 25% for both temperatures; the remark, that this concentration might be lower than the actual concentration, still applies.

7.5. Pair correlation functions

7.5.1. Pair correlation function of beads

The pair correlation function (PCF) can be measured inside and outside of rafts, by using the technique explained in section 5.2.1. In rafts, one can also distinguish the PCF between lipids and other lipids (ll); lipids and cholesterol (lc); and cholesterol

7. Global properties of large membranes

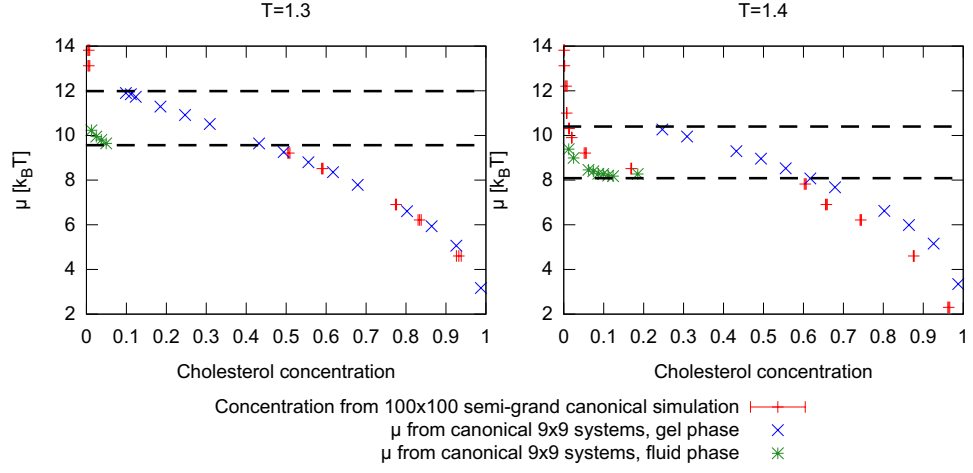


Figure 7.11.: Comparison of cholesterol concentration measured in the semi-grand canonical simulations for certain values of μ , and μ measured in the canonical simulations for certain values of the cholesterol concentration. Between the dotted bars, rafts may appear.

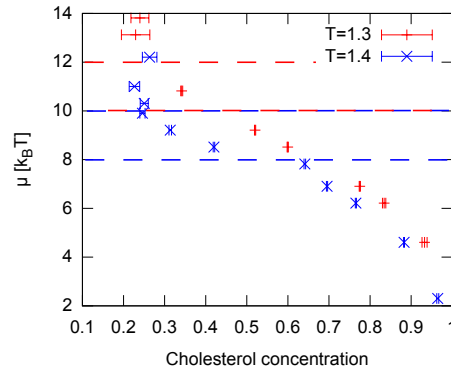


Figure 7.12.: Cholesterol concentration inside rafts. The dotted lines denote the upper and lower raft limits.

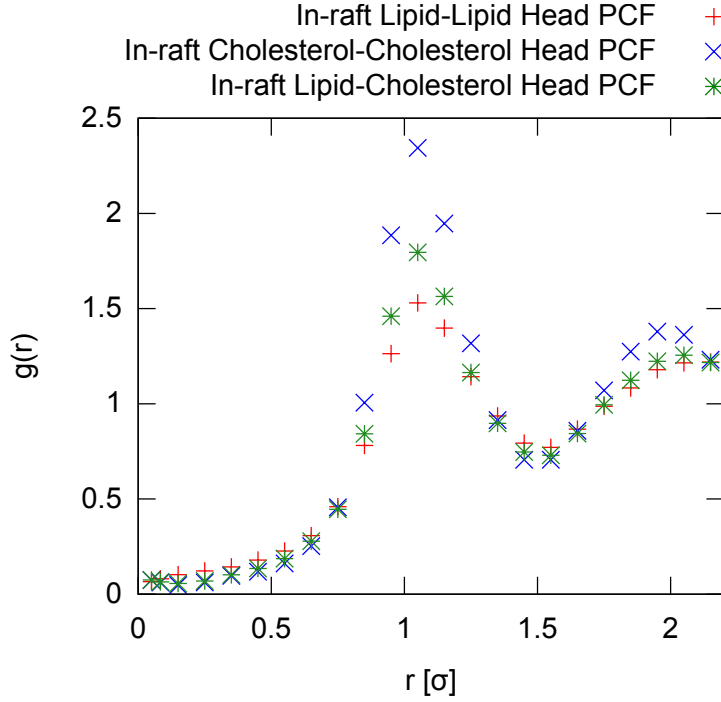


Figure 7.13.: Comparison of PCFs inside rafts. Measurements taken inside the raft limit, at $T = 1.4$ and $= 8.5k_B T$.

and cholesterol (*cc*). Only the PCFs of the head beads are considered in this section. Furthermore, the beads are projected onto the membrane surface, losing information about the 3D-structure.

In rafts, the *ll*-PCF has peaks at positions related to a preferred head-head distance $\approx 1.05\sigma$ (figure 7.13). The cholesterol-cholesterol PCF has the sharpest peaks, but for all these PCFs the statistics of the measurement do not allow for a clear resolution of any peaks beyond the second. This is a problem of statistics, not an actual lack of these peaks. Below the raft limit, the PCF function is very similar (figure 7.14), but the measurement allows for a much clearer result up to large distances. From this measurement we can infer that the peak-peak distance is slightly smaller than in the rafts, i.e. $\approx 0.9\sigma$. In section 7.6.3 we will see, that the underlying structure is actually a hexagonal grid. While the peak distances are compatible with this result, the fine-structure of such a configuration could not be resolved with the PCF-method.

In contrast, the *ll*-PCF outside of the rafts is a typical PCF for a liquid of repulsive circles with even the second peak being barely visible (figure 7.15). Again, the distances between the peak correspond to a radius of about 1.05σ . This result is also confirmed in the section about the structure factor.

7.5.2. Pair correlation function of rafts

The PCF can also be calculated between the geometrical centers of the rafts. The result, $g(r)$, is similar to the PCF of a (non-ideal) gas (figure 7.16). The very weak first and only

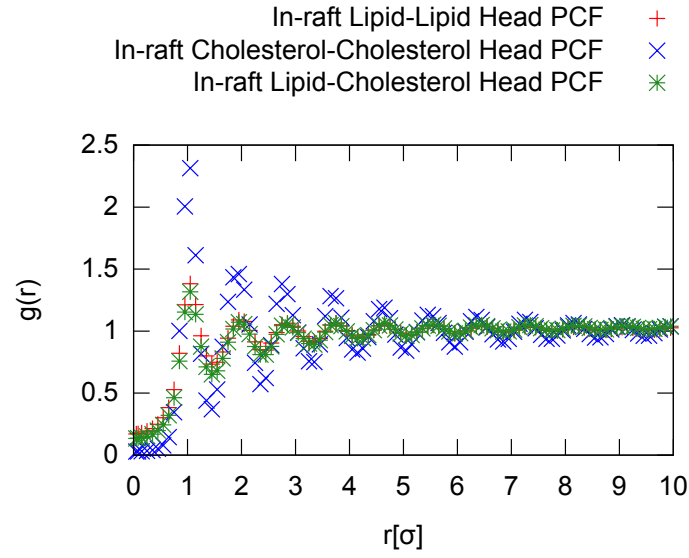


Figure 7.14.: Comparison of PCFs below the raft limit, $T = 1.4$, $\mu = 4.6k_B T$.

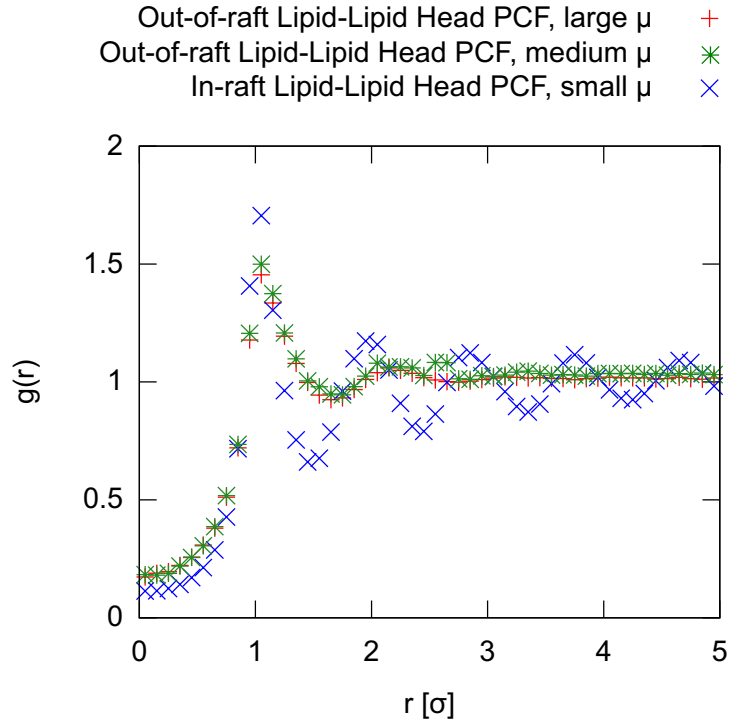


Figure 7.15.: Comparison of PCF inside and outside of rafts ($T = 1.4$), measured above ($\mu = 10k_B T$), within ($\mu = 8.5k_B T$) and below ($\mu = 6.2k_B T$) the raft limit.

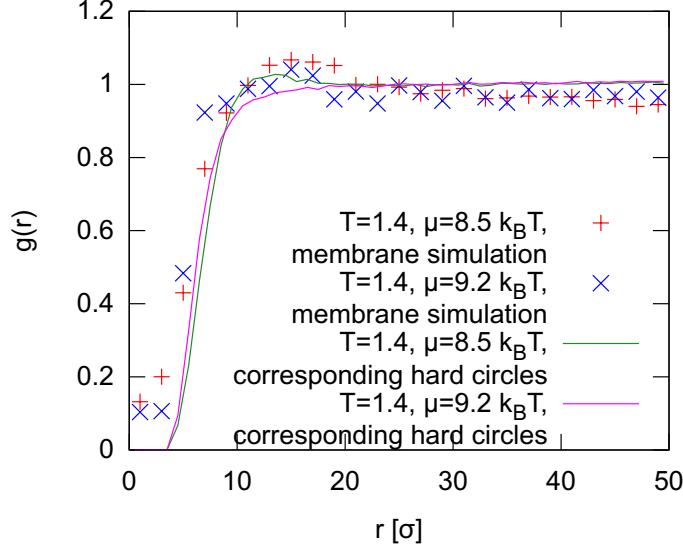


Figure 7.16.: Raft-Raft pair correlation function and comparison to a numerical simulation of hard circles, using the density and size distribution described in section 8.2.

peak of the $\mu = 8.5k_B T$ PCF is at $r = 15\sigma$, which is mostly consistent to a simulation of a gas of hard circles with density and size distribution as measured in section 8.2. This shows that there are no interactions between the rafts other than the fact that two rafts cannot occupy the same space.

7.6. Structure factors

7.6.1. Structure factor of rafts

Using the same grid pattern as in section 5.2.3, one can define two functions, Γ^t and Γ^b , with

$$\Gamma(x, y) = \begin{cases} 1 & \text{if there is a raft at } x, y \\ 0 & \text{otherwise} \end{cases},$$

for both sides of the bilayer. One can then proceed to compute the structure factor $S(q)$ of the raft pattern, by calculating the Fourier transformation $S(\vec{q})$ of the Γ -functions and taking the mean of the absolute value as a function of $q = |\vec{q}|$. The result is shown in figure 7.17. As expected, there is no visible structure in systems far above the raft limit. The same is true for systems far below the limit, as the membrane is almost completely covered by cholesterol rich structures with only occasional small cracks of liquid between them. Close to or within the raft limits, there appear long range structures. Within the raft limit and below, there is even a preferred length scale for these structures which ranges between 20σ and 40σ . Although appearing visually similar, these structure factors **cannot** be fitted to the simple model structure factor for microemulsions proposed by

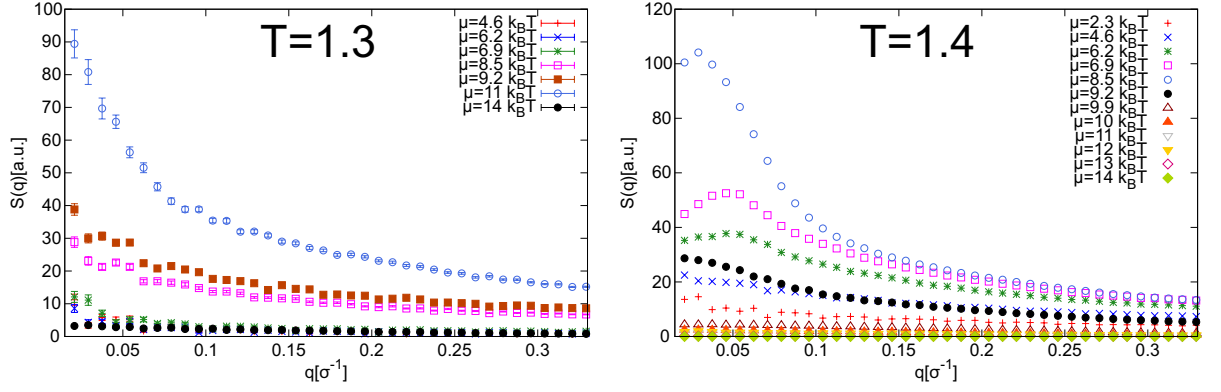


Figure 7.17.: Structure factor of the raft pattern

Teubner and Strey[120]:

$$S(q) = (a + gq^2 + cq^4)^{-1}$$

7.6.2. Structure factor of trenches

We can use the same technique to measure the structure factor of the trenches seen below the raft limit. Unfortunately, the usual technique, for detecting cholesterol enriched structures and liquid areas, fails to capture the essence of these smaller structures. Therefore, different algorithms based on the cholesterol and lipid density had to be used, each one fine tuned to the respective parameter set by feedback from visual comparison of snapshot and detected trench structure. The result may not capture the fine structure of these trenches, such as their width, but should do well for the analysis of the broader structure of the whole trench-system. The algorithms are described in appendix E.

The resulting structure factor is shown in figure 7.18. There is a very clear peak around $q = 0.05/\sigma$, indicating a preferred trench-trench distance of about 20σ . This value is roughly compatible with the ripple repeat distance which was estimated to be $15 - 20\sigma$ [70].

7.6.3. Structure factor of beads

We can also look at the structure factor of head beads. For simplicity, the bead coordinates will again be projected onto the membrane plane, but unlike section 5.2.1, we will calculate the structure factor over the whole membrane, without regard for the raft-structure. As pair correlation function and structure factor are closely related, this approach would not yield a great amount of new information; however, taking into account the whole membrane yields much better statistics and thus better results for the low μ configurations, when almost the whole membrane is in the gel state. Also, the structure factor is readily accessible in scattering experiments, whereas the radial distribution function is usually determined indirectly via its relation with the structure factor.

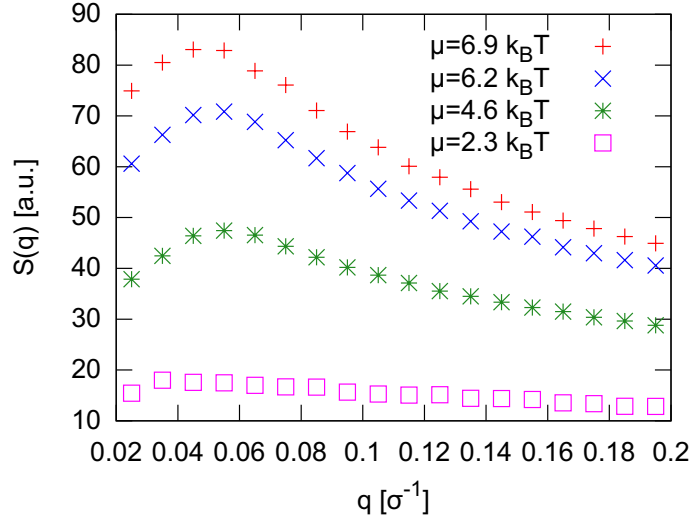


Figure 7.18.: Structure factor of trench patterns

The 1D-structure factor for various values of μ is shown in figure 7.19. In membranes with a large percentage of gels, the structure factor of the head beads, both lipid and cholesterol, is a perfect example of a hexagonal lattice structure. Figures 7.20 and 7.21, which show the actual 2D structure factor, confirm this observation. Likewise, for the largely liquid membranes, we see no preferential orientations but only concentric rings, indicating a preferred distance between the beads. From the 1D structure factor we can see, that these distances are - as one would expect - the repulsive radii of the head-head potentials.

In the case of membranes with rafts, we see a mixture of these two patterns. This raises two questions: How can there be the same preferential orientation of the cholesterol lattice, not only over distant rafts but also over hundreds of different configurations? The latter is most probably an artifact of the simulation technique and also responsible for the uniform preferential orientation of the subraft gel lattice over different configurations. As all simulations are started from a tightly packed hexagonal lattice, there is already a lattice structure in the system, which will be preserved when the whole system remains in an ordered state. There is also the fact that the volume moves only change the system size amongst the x , y and z coordinate directions, introducing another slight anisotropy to the system; there are no rotational moves to compensate. However, this does not explain, why the rafts, which should be free floating in the surrounding liquid, still preserve the initial lattice orientation. This can be explained when we look at the structure factor of the lipid tail beads (figure 7.22). While the lipid heads showed no clearly discernable preferential orientation in the raft case (figure 7.20, $\mu = 8.5k_B T$), the pattern of the cholesterol heads reappears for the middle lipid tail beads, especially the 3rd and 4th tail beads. This suggests that the liquid is not completely disordered and in fact, mediates the lattice orientation between distant rafts.

The preferred distances that can be derived from the structure factor peaks are com-

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patible with the results of the pair correlation function method in section 7.5.1. In the gels, especially below the raft limit, this is about 0.9σ , in the membranes with a larger portion of liquid, it is $\approx 1.05\sigma$.

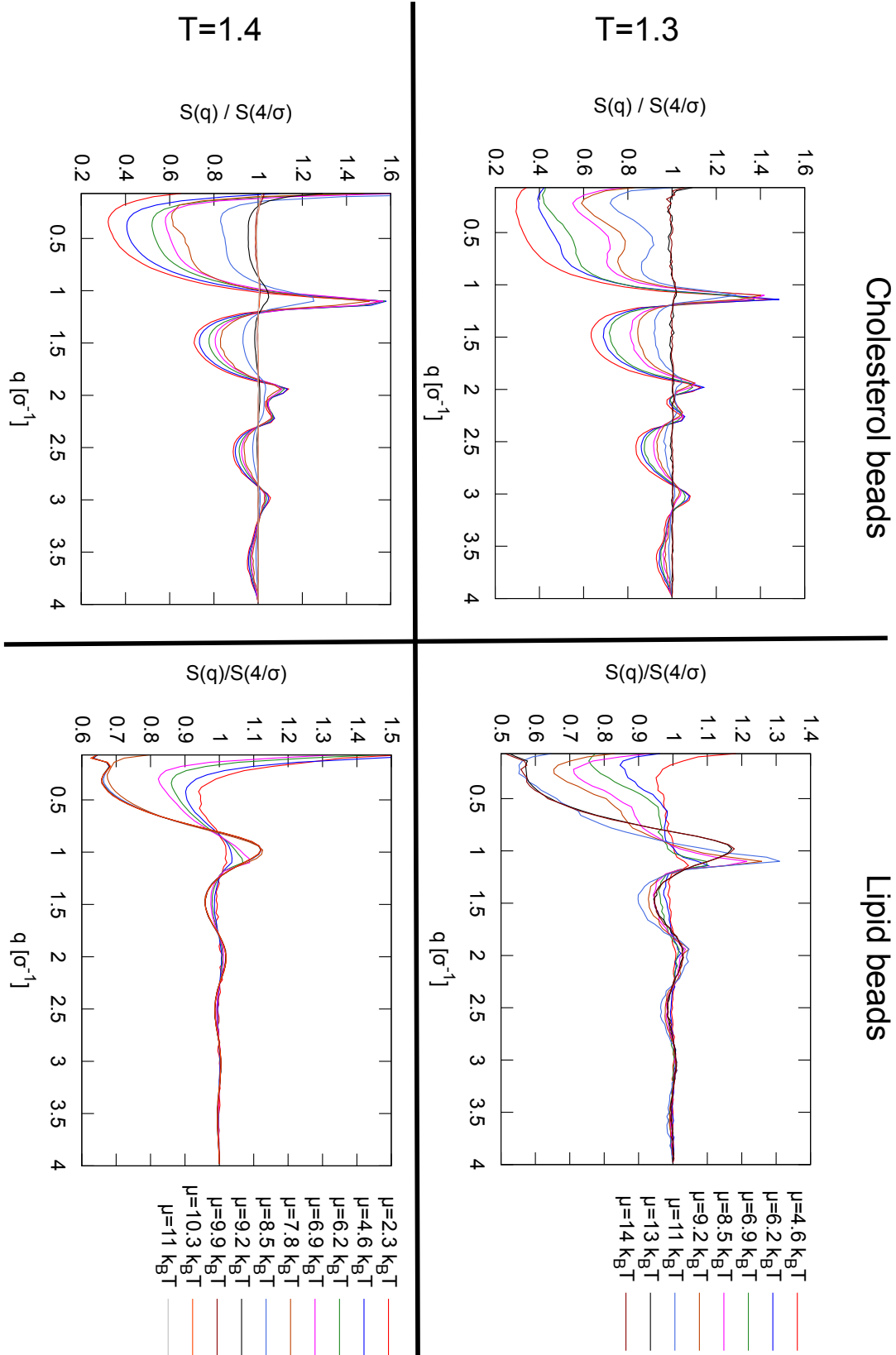


Figure 7.19.: Structure factor of head beads. The curves are normalized by their right-most values. This normalization factor is related to the overall number of head beads of the respective kind and thus the “intensity” of the pattern.

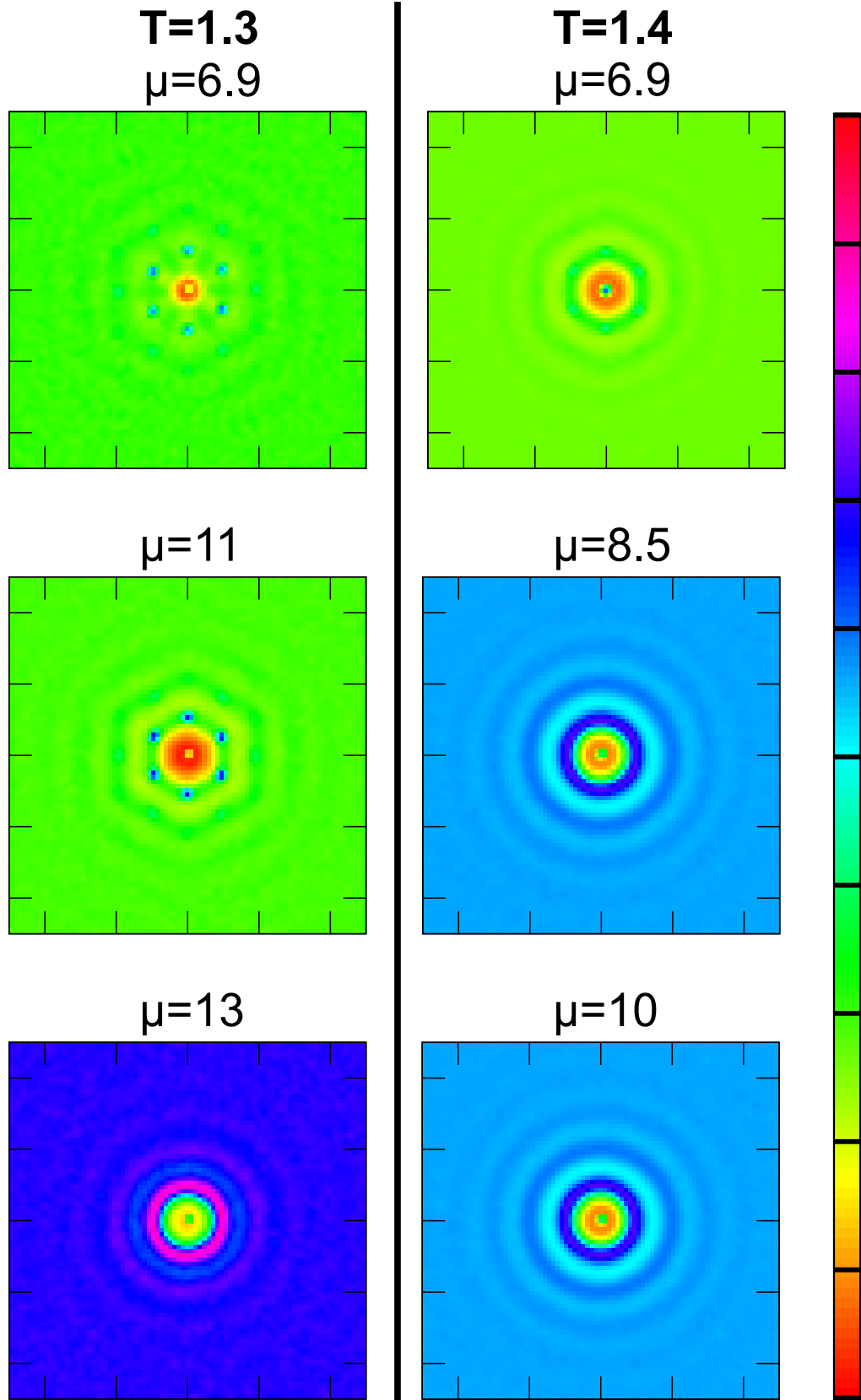


Figure 7.20.: 2D structure factor of lipid head beads. All colors relative to their respective plots; color values from different plots cannot be compared! Plot axes range from $-5/\sigma$ to $5/\sigma$ for all graphs. The middle point, $S(0)$, is not shown to full intensity.

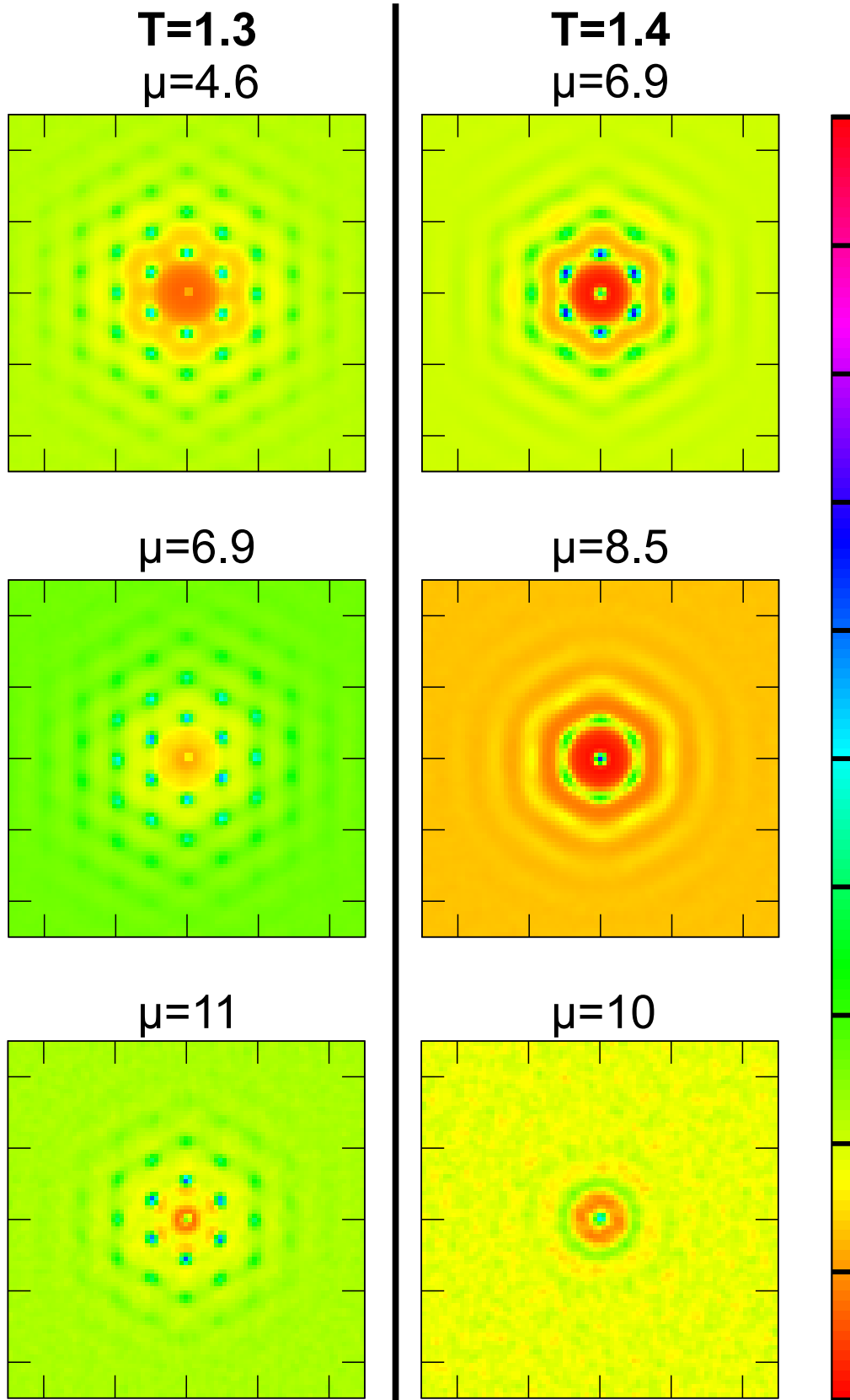


Figure 7.21.: 2D structure factor of cholesterol head beads. All colors relative to their respective plots; color values from different plots cannot be compared! Plot axes range from $-5/\sigma$ to $5/\sigma$ for all graphs. The middle point, $S(0)$, is not shown to full intensity.

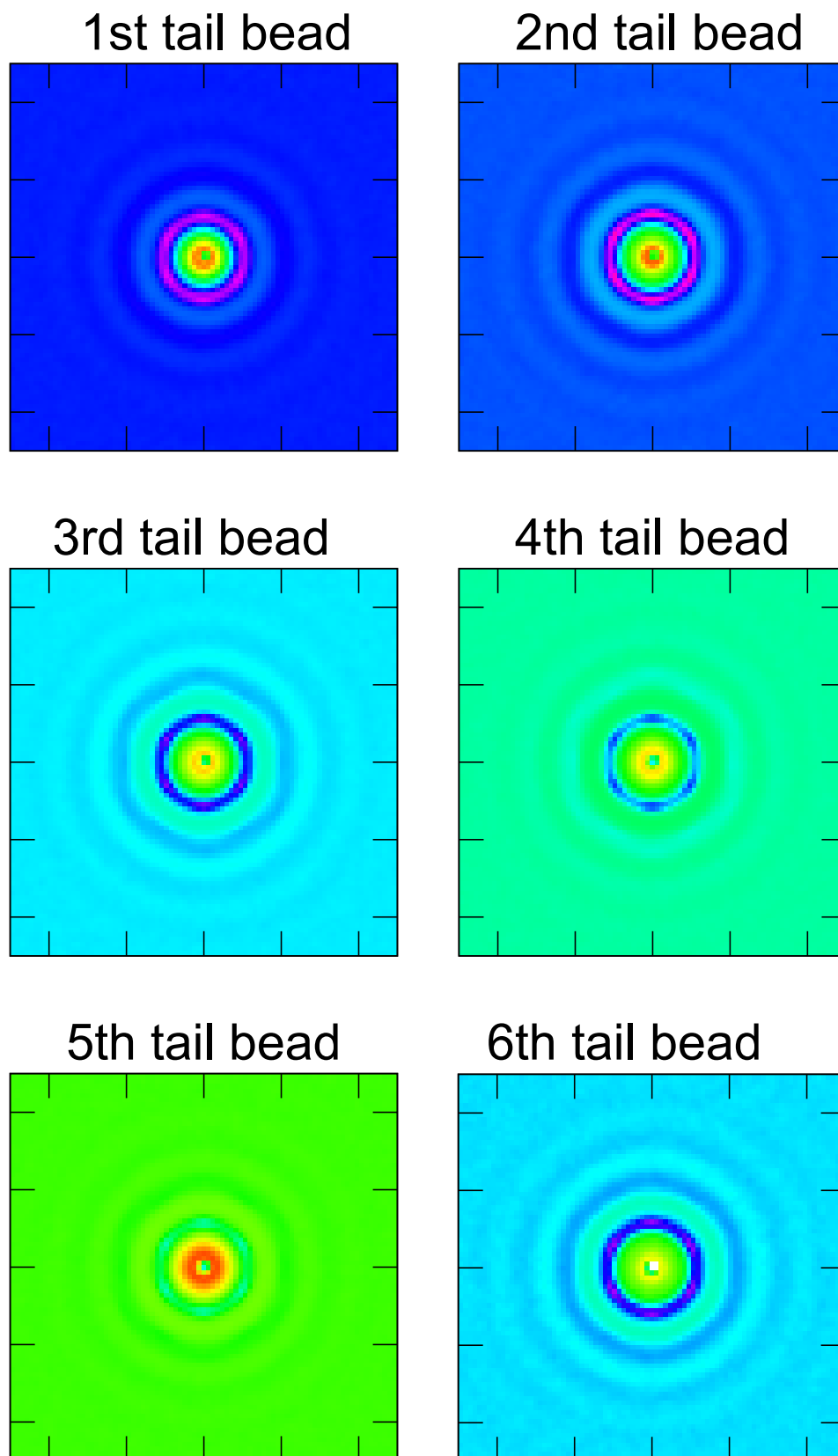


Figure 7.22.: 2D structure factor of lipid tail beads. All colors relative to their respective plots; color values from different plots cannot be compared! Plot axes range from $-5/\sigma$ to $5/\sigma$ for all graphs. The middle point, $S(0)$, is not shown to full intensity.

8. Characteristics of rafts

8.1. Correlations between rafts

Given the pattern functions Γ^t and Γ^b from section 7.6.1, one can calculate the normalized cross-correlation function

$$C = \frac{1}{N} \sum_{x,y} (\Gamma^t(x,y) - \bar{\Gamma}^t) (\Gamma^b(x,y) - \bar{\Gamma}^b) \frac{1}{\sigma_t \sigma_b},$$

where $\bar{\Gamma}$ is the mean value of Γ over all grid points x, y of the respective monolayer and σ is the standard deviation of Γ over the whole monolayer. N is the number of grid squares $x \cdot y$. The normalization of the cross correlation function accounts for different coverage of the monolayers with cholesterol/non-cholesterol structures. Thus, two monolayers, covered completely by a cholesterol enriched structure or by a liquid, will not have a huge cross correlation. Instead, the positions of the minority structures need to be correlated or anti-correlated in order to have an impact on C .

Furthermore, given a cross-correlation C^i for a pair of monolayers i , one can introduce a random lateral offset between the monolayers and recalculate the correlation function for the monolayers displaced by that offset. Given a large number of random displacements and their corresponding cross-correlations, one can compare the non-displaced cross-correlation to the displaced cross-correlations. The percentile of displaced cross-correlations, whose absolute value is greater than the absolute value of the non-displaced cross-correlation, is a measure for the significance of this particular cross-correlation value. A low percentile indicates that this value is special as it only appears in the original system, indicating some kind of coupling between the monolayers; a large percentile of larger cross-correlations of the displaced systems indicates that the overall geometry of the structures on each side of the bilayer is the crucial factor, not any kind of coupling between the bilayer leaflets.

One finds four different classes of results, which again can be linked to μ being above, within oder below the raft limit (figure 8.1). Above the raft limit, cross-correlations are very small (note the values on the x-axis) and the percentiles are almost evenly distributed between 0 and 1 (figure 8.1 a)). The mean cross-correlation is slightly, but significantly, negative. At this value of μ , most, or all, of the bilayer is occupied by lipids in the liquid state with very few, if any, cholesterol induced structures. If a structure appears, it is very likely to be on the opposite side of a liquid area just because the other leaflet has most likely no cholesterol-enriched structures of its own. This leads to a slightly negative cross-correlation with a large percentile, explaining the observed result. There is one outlier with a positive correlation and a small percentile. This is

likely caused by two rafts appearing on opposite sides by random chance. As they are the only (or two of very few) rafts in the bilayer, they have a huge contribution to the normalized cross-correlation function and a small percentile.

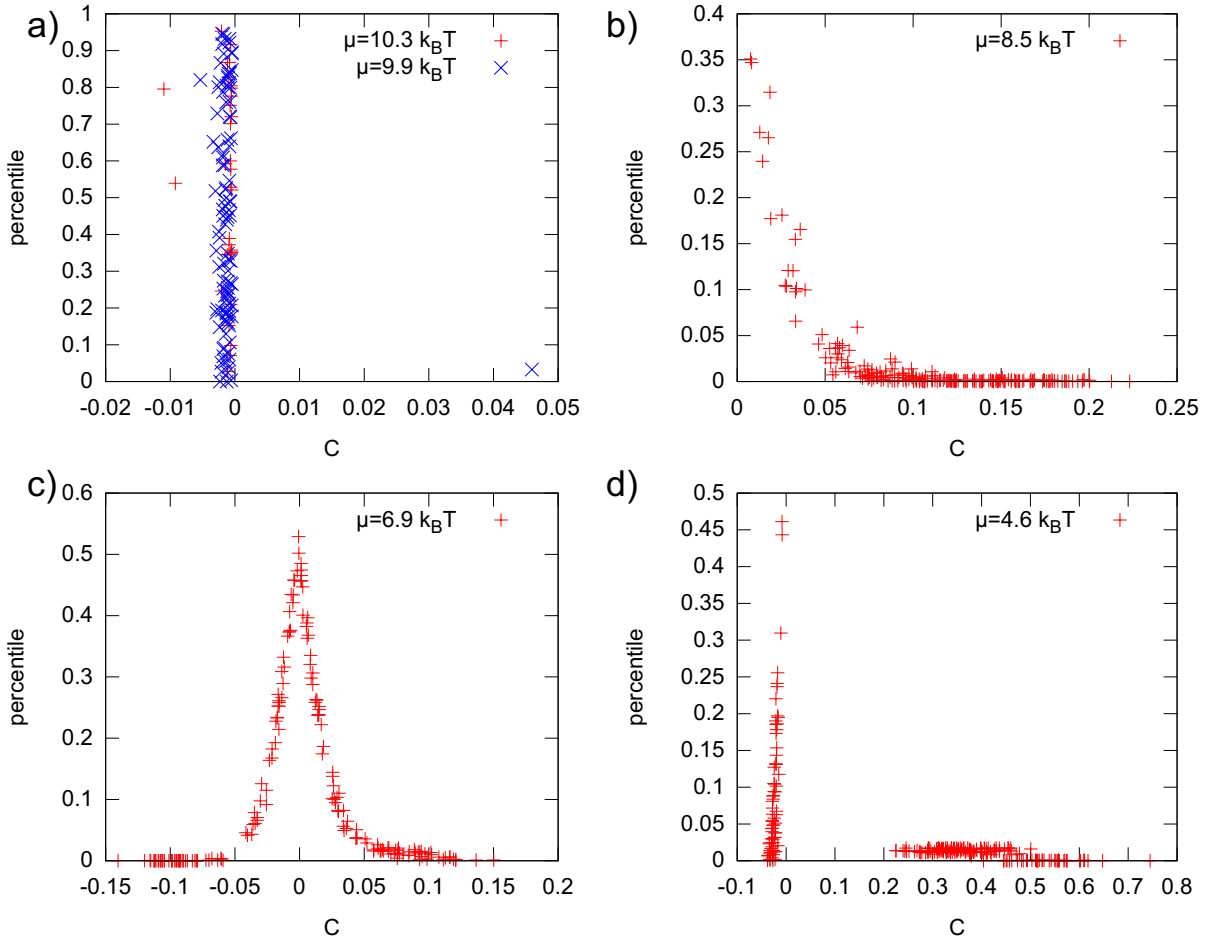
When μ lies within the raft limit, the picture is completely different. There are strong positive correlations with small or even zero percentiles. No anti-correlations whatsoever are observed. This result suggests that there is a positive coupling between rafts on the both sides of the bilayer. (figure 8.1 b)).

Slightly below the raft limit, the correlations disappear. The correlations form a symmetric curve around $C = 0$. This indicates no coupling at all between the leaflets at this μ . The cross-correlation shown in 8.1 for $\mu = 6.9k_B T$ corresponds to systems such as 7.1 e), with an approximately even distribution of rafts and non-rafts. The reason for this observation might be a weakness in our method: The normalized cross-correlation is especially well suited to detect correlations (or anti-correlations) of the minority structures. In this case however, there is no clear minority structure. It is of course possible, that there genuinely is no coupling for this parameter set, but if there is a coupling, our method might not be able to clearly detect it. Considering the clear correlations between the rafts and the correlations between the trench structures, we suppose that these larger trenches and larger rafts should show a similar coupling and that it might indeed be a failure of our method to not detect such a fact. We also note that we found about 20% of the membrane to be in an interdigitated state in chapter 7.3. At least these structures must be correlated due to their nature.

At even smaller μ , a mixture of the correlated and uncorrelated conformation appears, with some positive, low percentile correlations remaining in some systems and the slightly negative, large percentile correlations reappearing. The positive correlations are very large, much larger than the correlations in the raft forming systems. The part with the small anticorrelation can be interpreted as a case similar to the anti-correlation at high μ values, but here almost the whole system is in a state of high cholesterol gel, with a few trenches as they are seen below the raft limit. If these structures are too small, then only a few of them are detected as non-rafts. Those that are detected, are then often found in random places and thus result in a slight anti-correlation. On the other hand, if a trench is still large enough to be clearly detected by the algorithms described in section 5.1, then they are strongly correlated. A similar correlation pattern can be observed at $T = 1.4$ and $\mu = 9.2k_B T$, when the cholesterol structures begin to be too small to be detected. Those that are found are still positively correlated, though, just as they are at $\mu = 8.5k_B T$.

8.2. Shape of rafts

The size distribution of rafts can be seen in figure 8.2. There is no preferred size of cholesterol-enriched clusters, instead the frequency of larger sizes decays roughly exponentially. Note that the apparent maximum of the frequency distributions at a radius of gyration of 3σ is systematic, as there is a cutoff minimal raft size in the detection algorithm. Unsurprisingly, the mean raft size increases as μ decreases. The largest

Figure 8.1.: Normalized cross-correlation of bilayer leaflets at $T = 1.4$

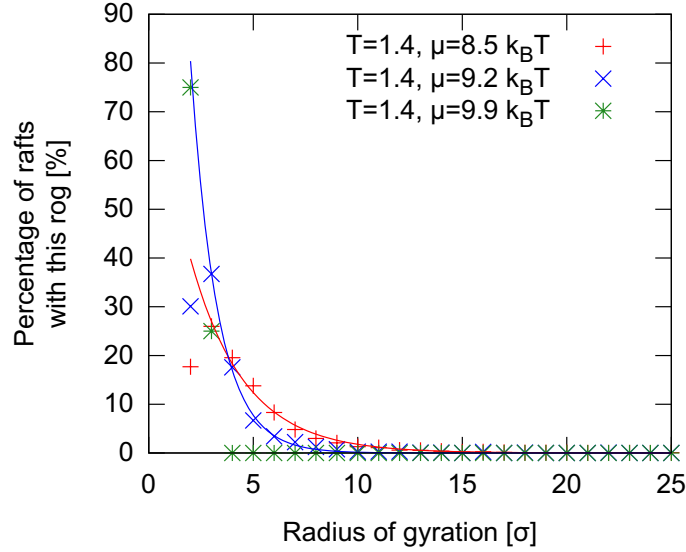


Figure 8.2.: Normalized distribution of raft sizes. Portions missing to 100% are larger than 25σ and are not shown in this graph. The continuous line is an exponential fit, excluding the value at 2σ . At smaller μ , (almost) all rafts are of the order of the system size. At larger μ all of the few existing rafts have a very small radius of gyration.

rafts seen at $T = 1.4$ and $\mu = 8.5k_B T$ have a radius of gyration of no more than 15σ . For comparison: Typical sizes of the simulated membranes are about $10,000\sigma^2$. The overall coverage of the membrane surface with rafts is 35% for $T = 1.4, \mu = 8.5k_B T$ and 20% for $T = 1.4, \mu = 9.2k_B T$ ¹. Overall, there are on average 54.5(6) rafts on each side of a $100 \times 100 \times 2$ -chains membrane at $T = 1.4, \mu = 8.5k_B T$ and 21.7(13) rafts at $T = 1.4, \mu = 9.2k_B T$.

Below the raft limit, sizes of the cholesterol-clusters grow almost immediately up to percolation size (figure 8.3 a)) and the whole membrane is covered by one large structure, which is crisscrossed by the trench structures seen in the snapshots in figure 7.1.

The reversed case, when the trenches between the rafts percolate, is much harder to grasp with an algorithm, as the smaller trenches are difficult to find and connect. Therefore, visual inspection was used. As the number of independent configurations is much larger than a human could check in a feasible timeframe, only a sample of all configurations was examined. The percolation of trenches can be observed up to several $k_B T$ below the lower raft-limit (figure 8.3 b)). The crossover is less abrupt as in the case of the rafts, though this might be a systematic error due to the poor technique used for determining trench-percolation.

Using the radius of gyration tensor described in section 5.2.3 we can make more statements about the shape of the rafts than just analyzing their size. Looking at the

¹As a comparison: Experimental estimates of raft coverage vary between 13% and 50%[43, 78, 110]. The great variation may depend on the cell type[96].

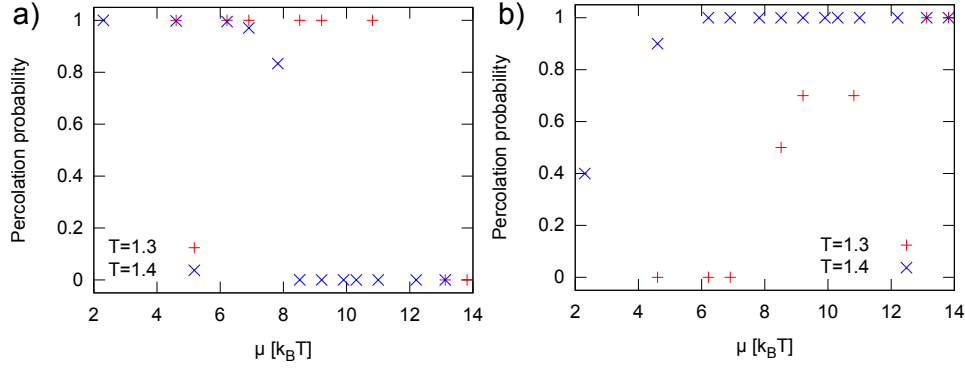


Figure 8.3.: Percolation probability of a) membrane areas in the gel state (measured from all configurations) and b) membrane areas in the liquid state (estimated by visual inspection of a random subset of configurations).

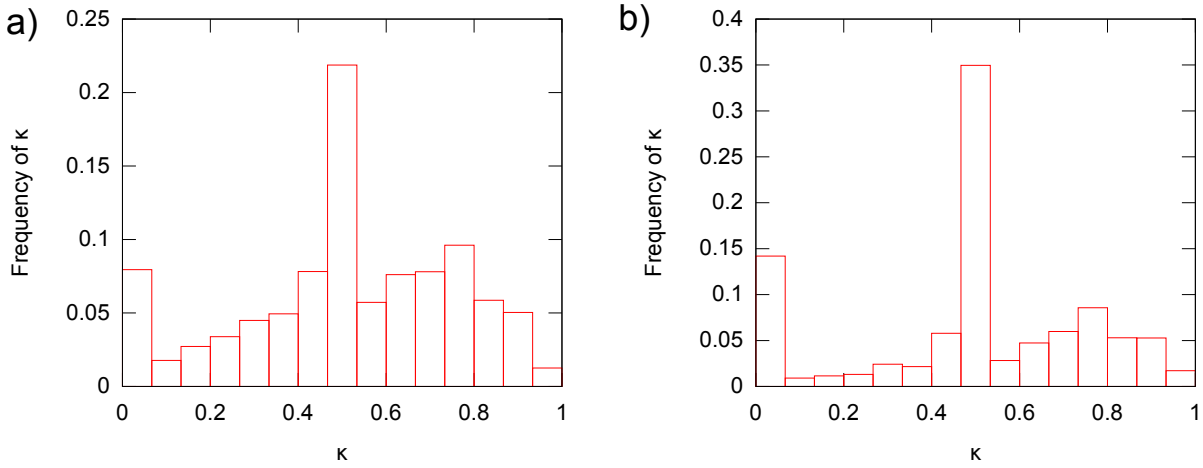


Figure 8.4.: Relative acircularity of rafts. a) $T = 1.4, \mu = 8.5k_B T$. b) $T = 1.4, \mu = 9.2k_B T$.

relative acircularity (figure 8.4) we see some sharp peaks around certain values like 0, 0.5, 0.75 and 1. Overall the distribution seems to be skewed towards values > 0.5 . However, as was already explained in section 7.3.1, this is a systematic artifact of the underlying raft detection method, that uses a grid to describe the rafts. If we plot a large number of κ -values, we see that the peaks of the histogram are even sharper than the histogram resolution suggests (figure 8.5). These special values stem from rafts that are made up of only a few points on the grid. There are not many possible arrangements for these points, therefore they all have one of the few values that can be seen as sharp lines in the plot. As small rafts are pretty common, they hugely contribute to the histogram of κ -values.

If we exclude small rafts and only look at rafts with 11 or more points, we see a completely different picture (figure 8.6): There are no more sharp peaks. Overall the values are distributed around 0.5, which indicates a slightly elongated shape of the rafts. But the distribution is also very broad, meaning there are many different aspect ratios

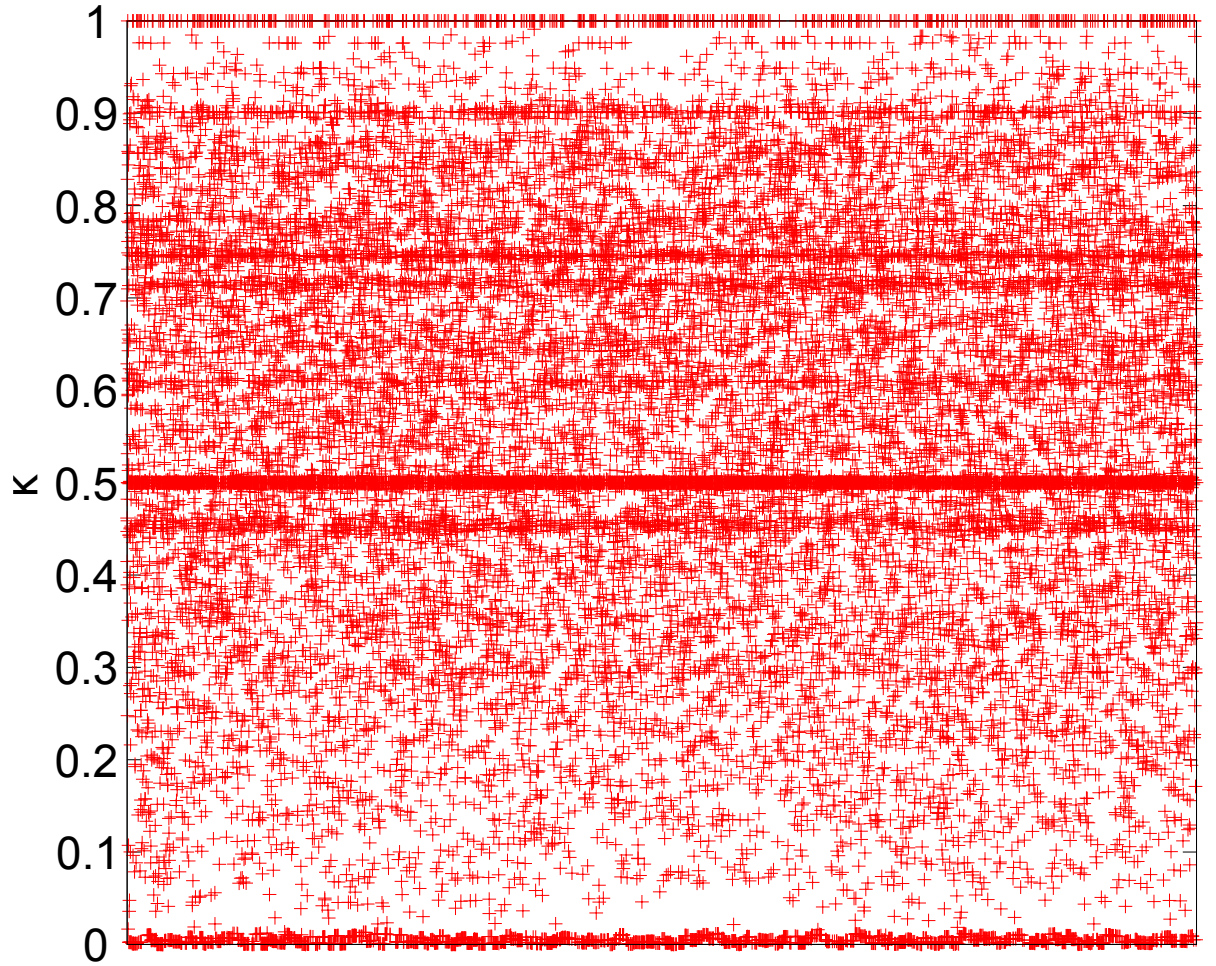


Figure 8.5.: A large number of κ values at $T = 1.4, \mu = 8.5k_B T$. Each point indicates the acircularity of an individual raft.

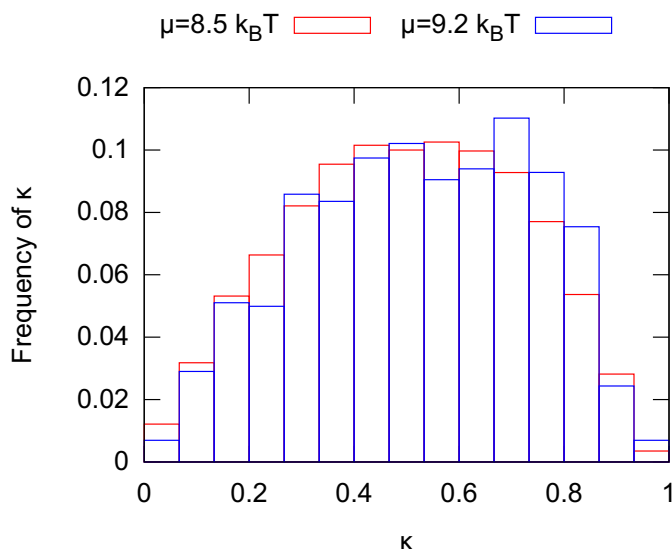


Figure 8.6.: Relative acircularity of large rafts.

which are about equally frequent. The perfect circular shape is somewhat rare, though.

Using the radius of gyration and the area of the rafts, we can make a similar analysis as in section 7.3.1 to find the fractal dimension of the rafts. We obtain exponents of 0.64(2) for $T = 1.4, \mu = 8.5k_B T$ and 0.67(2) for $T = 1.4, \mu = 9.2k_B T$. These correspond to fractional dimensions of 1.56(5) and 1.49(5), respectively.

8.3. Profiles across the bilayer

We use again the grid projection of rafts and non-rafts described in section 7.6.1. Using this $1.5\sigma \times 1.5\sigma$ grid, we search for $4.5\sigma \times 4.5\sigma$ areas that are completely inside or outside a raft and have another such area on the opposite side of the bilayer. Thus, local properties measured in these areas can be classified as belonging to an area with either rafts on both sides, non-rafts on both sides or a raft on one side but not the other.

8.3.1. Thickness and vertical composition

One can calculate a density profile of the membrane. A measure of the thickness of the membrane can be taken from the distance between the maximums of the lipid heads. With a raft on both sides, this distance is $8.4(3)\sigma$, without rafts it is $6.5(3)\sigma$, with one of each it is $7.8(3)\sigma$. These results are consistent to results in the (untilted) gel (corresponds to the raft/raft case) and liquid state (corresponds to the non-raft/non-raft case) measured previously for the pure lipid model measured by Lenz[70]. The distance for the mixed state is a bit larger than just the arithmetic mean, but the error margins of both values still overlap. However, there is one large difference to the density profiles measured by Lenz: There is a nonzero chance of a head inside the bilayer (figure 8.7 a)).

8. Characteristics of rafts

For the liquid state, this can be explained by the fact that previous measurements were done at $T = 1.3$ and less. Indeed, a pure lipid bilayer at $T = 1.4$ shows exactly the same features, as we determined by calculating the density profiles of small systems at this temperature. We expect that the high temperature also accounts for the same effect in the gel-like rafts, but as there is no pure lipid gel at this temperature and pressure, we can only compare it to lipid-cholesterol gels in small systems (too small to form rafts). We observe a similar distribution of heads in these systems (figure 8.7 b)).

The distribution of cholesterol heads - in those systems with at least one raft - is on average slightly deeper inside the membrane than the lipid heads. The shorter length of the cholesterol does not alter the distribution of the last tail bead in the center of the bilayer but instead pulls the cholesterol a short distance inside the membrane. Otherwise the distribution of tail beads does not produce any characteristics that can not be seen from the distribution of head beads discussed in the previous passage (figure 8.7 c)).

8.3.2. Pressure, tension and curvature

Using the method described in section 5.2.3, one can calculate a pressure and tension profile of the membrane. Parts of the membrane with a raft on one side and a non-raft on the other side show a tension profile that is approximately a mixture of the tension profiles in the raft/raft and the non-raft/non-raft cases (figure 8.7 d)). Otherwise these tension profiles are qualitatively the same as those of gels and liquids in small, non-raft forming systems (figure 8.7 e)). From these profiles we can measure a spontaneous curvature of $k_c c_0 = 7(1)\epsilon/\sigma$ in the raft/raft case and $k_c c_0 = 3.6(1)\epsilon/\sigma$ in the non-raft/non-raft case. The curvature in the raft forming systems is significantly smaller than in the usual gel structure, where it is around $11\epsilon/\sigma$. This effect disappears below the raft limit, when the spontaneous curvature of the gel structure once again goes up to values of about $12\epsilon/\sigma$ (at $T = 1.4, \mu = 6.9k_B T$) or even higher ($15\epsilon/\sigma$ at $T = 1.4, \mu = 4.6k_B T$ and $19\epsilon/\sigma$ at $T = 1.3, \mu = 6.9k_B T$), due to the increased cholesterol content (The pure cholesterol model membrane has a very high spontaneous curvature, see section 9.5.1). The spontaneous curvature of the non-raft/non-raft system is much larger than the close to zero result of the liquid state by West (which was measured at $T = 1.3$ in contrast to our $T = 1.4$), but close to our own results in section 9.5.1. But as was already found by West and confirmed in section 9.5.1, the measurement of the tension in the liquid state is prone to systematic errors, we cannot be sure whether our result in this section signifies an actual deviation of the near curvaturelessness of the liquid structure.

There is also a more detailed discussion of the effects of cholesterol on the gel structure in section 9.3.1. We would also like to return to mind, that the method for calculating the spontaneous curvature has some caveats, as described in section 5.2.3, and the exact numbers should not be taken at face value. However, as we will see in the next section, 8.4, at least inside the rafts the gel structure is mostly untilted, so that one of the difficulties with this methods does not apply as strongly.

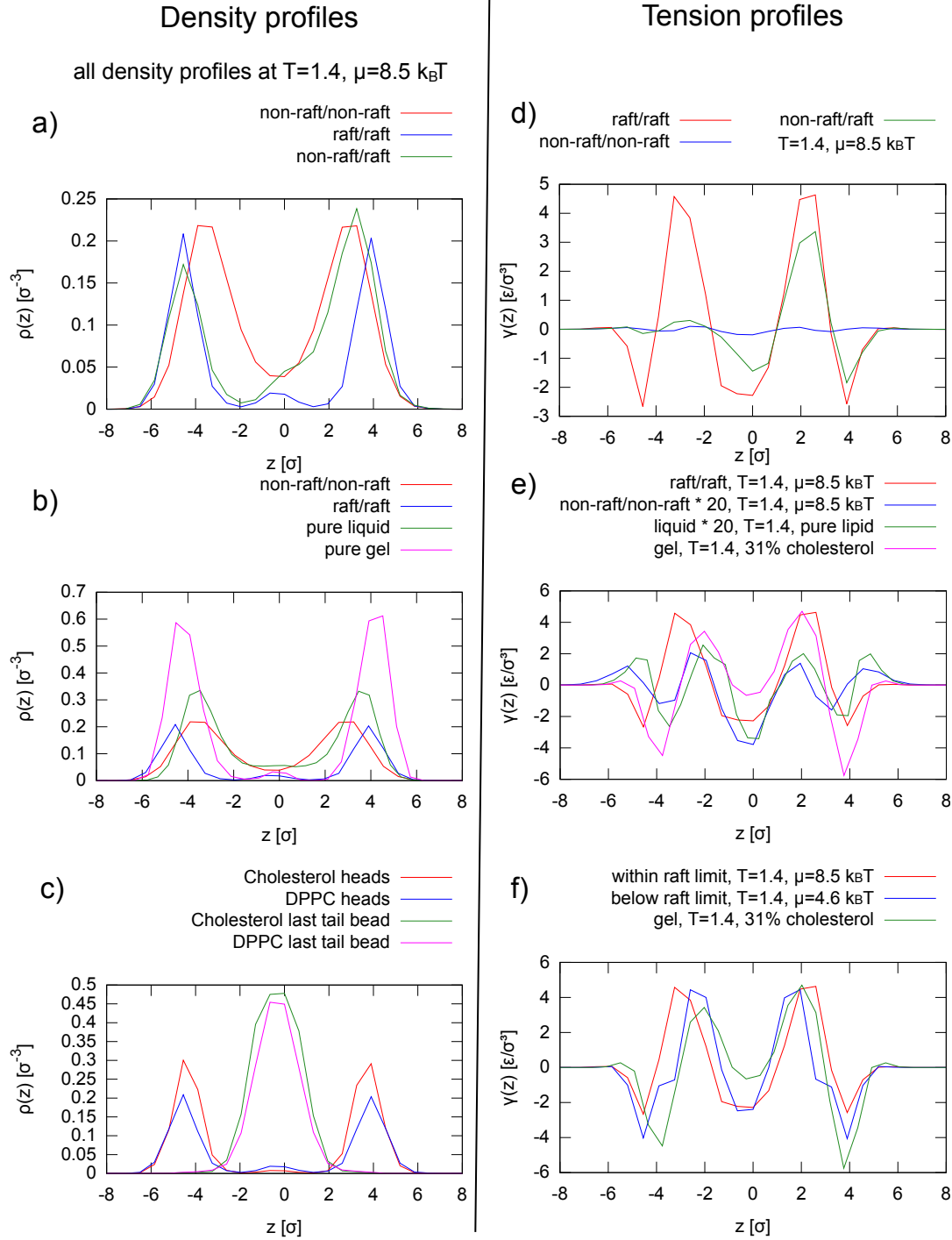


Figure 8.7.: Density and tension profiles. Density profiles: Note that differences in quantity in the density profiles result from different lipid/cholesterol ratios. a) Density profiles of lipid heads in raft-forming systems. b) Comparison of lipid head density profiles in raft-forming and single-state systems. c) Density profile of cholesterol heads and tails in raft-forming systems. Tension profiles: d) Tension profiles from raft-forming systems. e) Comparison of tension profiles in raft-forming and single-state systems. f) Tension in large systems below the raft limit (trench configurations), compared to the raft/raft configuration and a pure liquid gel. The asymmetry of the pure gel tension profile is likely the result of the much poorer statistics of this particular profile.

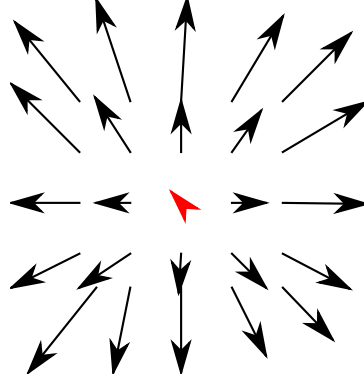


Figure 8.8.: Rationale for alignment criterion: The whole pattern is aligned, we do not want to exclude the central, red lattice point

8.4. Microscopic orientation

The simple picture of the raft structure in figure 1.2 can be tested by looking at the end-to-end vectors of the chains. Given a normalized end-to-end vector $\vec{e} = e_x\vec{x} + e_y\vec{y} + e_z\vec{z}$ (tail to head), we define the *tilt orientation* $\vec{t}$ as the x and y components of $\vec{e}$, where $\vec{z}$ is the normal direction of the membrane surface (global surface, ignoring local deformations). The *tilt strength* is $t = |\vec{t}|$.

We can then try to find areas with aligned tilts: A bilayer leaflet is mapped to a square lattice. For each lattice square the mean tilt of the chains in this square is calculated. This gives us a map of the tilt “flow” in the monolayer. The tilts of two squares are defined to be aligned, if the directions of the tilts do not differ by more than 30° **or** if the tilt strength in both squares is less than 0.2 (i.e. the absolute tilt is less than $\approx 11.5^\circ$). The latter criterium prevents the exclusion of the central areas of star-like patterns (see figure 8.8). We will see that this kind of pattern is common in rafts, whereas almost no false positives are found due to this criterium in otherwise random formations.

A lattice square is considered part of a flow if its tilt is aligned with 6 or more of its 8 nearest neighbours on the lattice. The set of aligned squares is then processed by the DBSCAN algorithm ([29] and appendix C), similar to the algorithm for detecting rafts (section 5.1). For a lattice of $3\sigma \times 3\sigma$ squares the cutoff is chosen to be $\text{eps} = 6\sigma$ and the minimum number of points $\text{minpts} = 9$. These parameters are much stricter than the parameters used in section 5.1, as it was more important to detect finer structural properties of the rafts. As a result, the detected areas of aligned tilt flow are much more rounded than the ragged rafts.

We can measure the strength and orientation relative to the center of the clusters of aligned flow. In order to account for different sizes of these clusters, the distances are scaled by their respective radii of gyration. Only clusters of 13 or more lattice points are used in this analysis. Thus, one can measure the absolute tilt strength depending on the distance to the centerpoint, the dot product of the normalized tilt orientation and the vector pointing outward from the center, and the dot product of the non-normalized tilt orientation and the outward pointing vector. One can also look at the strength of

T	μ	$m_m/0.001$	$m_f/0.001$	$\sigma_{m_f}/0.1$
1.3	4.6	3(115))	5(155)	3(2)
1.3	6.2	4(141)	5(128)	10(11)
1.3	6.9	3(105)	4(176)	4(4)
1.3	9.2	9(64)	12(70)	4(4)
1.3	11	5(12)	5(122)	8(3)
1.3	13	1(1)	125(122)	44(5)
1.4	2.3	7(6)	17(13)	8(4)
1.4	4.6	0(1)	1(13)	12(5)
1.4	8.5	1(1)	12(68)	57(17)
1.4	9.2	1(1)	— ¹	—
1.4	9.9	1(1)	—	—
1.4	10	1(4)	—	—
1.4	11	0(1)	—	—
1.4	12	1(1)	—	—

¹ No flow-centers large enough to be evaluated

Table 8.1.: Strength of the mean tilt of the whole membrane compared to only the flow clusters

the mean tilt $m = | \langle \vec{t} \rangle |$ of the whole membrane (m_m) compared a flow cluster (m_f) (table 8.1). One finds that m_m is overall very small and mostly compatible with 0, meaning that there is no overall tilt in a monolayer – as expected. The same is true for m_f . The variation of m_f on the other hand is very large. That means, individual clusters have a preferred tilt direction, but the clusters of a whole monolayer have no such preference. The standard deviation of the tilt of the individual monolayers is not shown in the table. It is of the same magnitude as m_m and also mostly compatible with 0.

The tilt strength over the distance from the center is shown in figure 8.9. All examined properties increase with the distance from the flow center and we can also pick out some different regimes depending on the temperatures. Our main interest lies with the true rafts, mostly at $T = 1.4, \mu = 8.5k_B T$. We can see that, although the absolute tilt is not as large as in other systems (a)), this tilt is better aligned with the outward direction away from the flow center (b) and c)) than in most other configurations. This gives credence to the simple concept of rafts as shown in figure 1.2.

We can also look at some other quantities related to the microscopic orientation of chains: Let $\vec{n}_2$ be the normalized mean tilt orientation and $\vec{n}_3$ the normalized mean end-to-end vector. Then we define the *splay* $s = \nabla \cdot \vec{n}_2$, the *twist* $t = \vec{n}_3 \cdot (\nabla \times \vec{n}_3)$ and the *bend* $b = (\vec{n}_3 \times \nabla \times \vec{n}_3)^2$. A demonstration of these quantities is given in figure 8.10.

Twist, splay and bend were calculated inside and outside of raft-regions. Mostly, these properties are 0 with no dependency on μ . The twist does not even have a different value

8. Characteristics of rafts

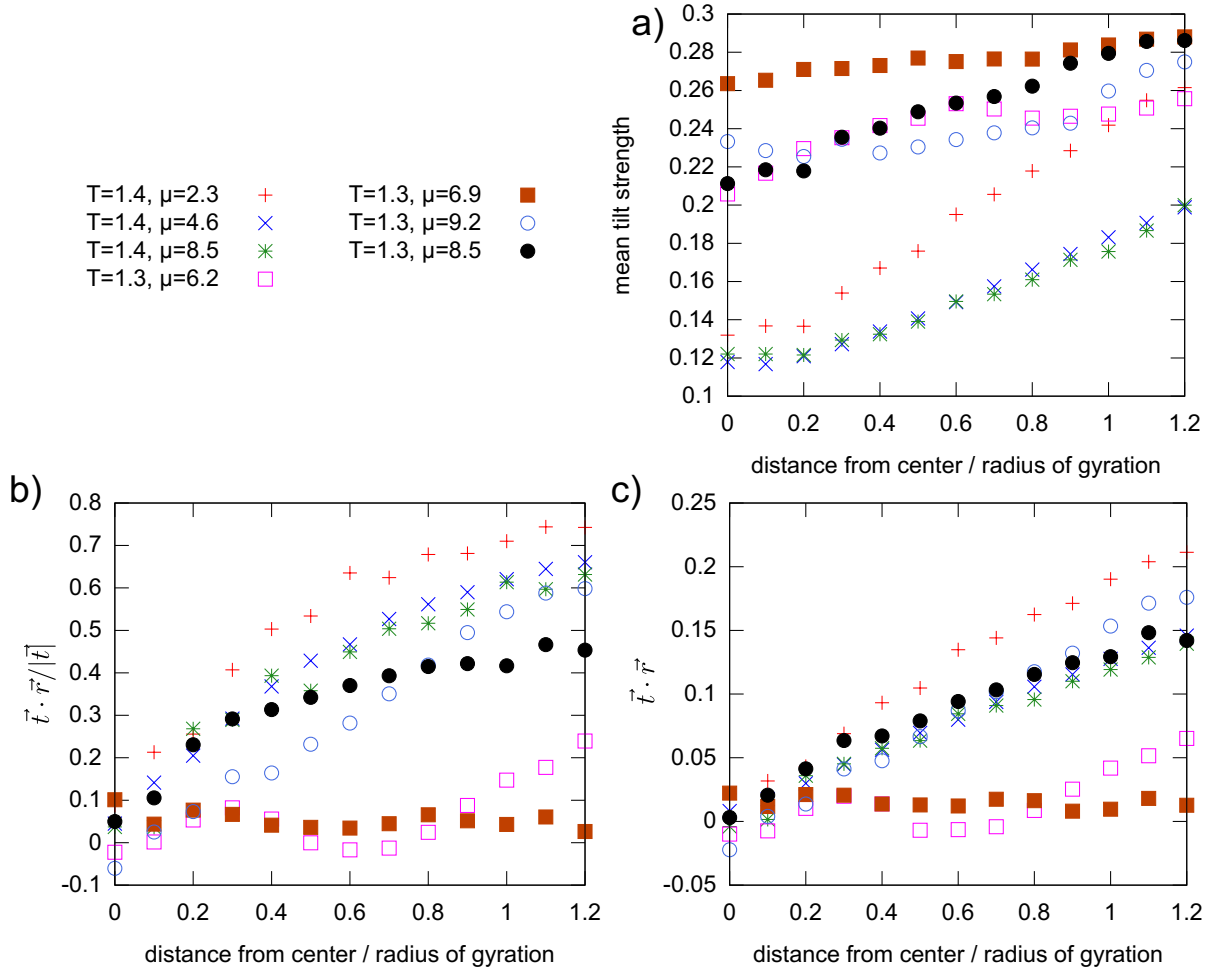


Figure 8.9.: Tilt strength in flow cluster versus distance from center.

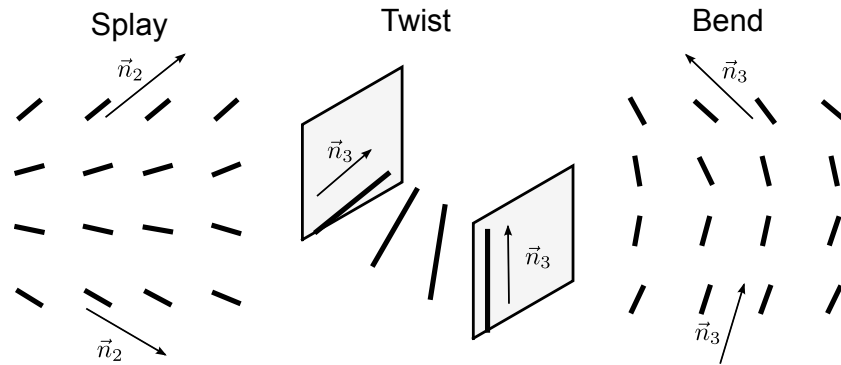


Figure 8.10.: Explanation of splay, twist and bend. Note that twist and bend are computed from the 3D tilt-vector, whereas the splay uses only the 2D-projection.

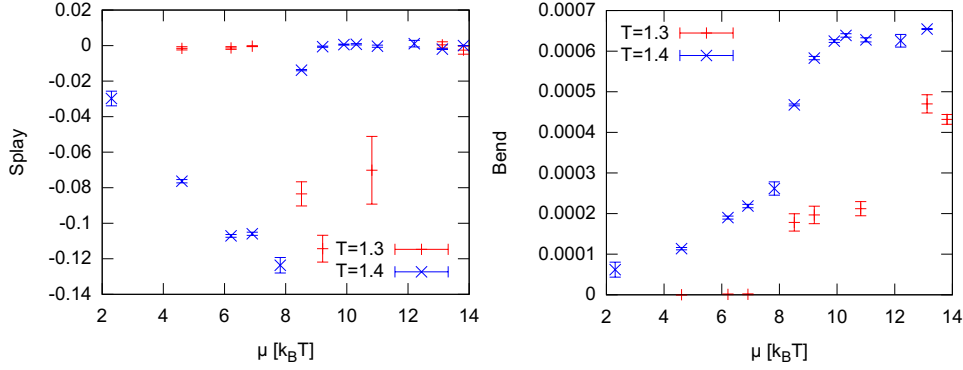


Figure 8.11.: Splay and bend measured outside of rafts

inside or outside of the raft regions. There are, however, some interesting findings for the splay and bend in the areas outside the rafts, shown in figure 8.11. While there is no splay in the large liquid areas at high μ , the splay suddenly increases (to a large *negative* value) when the trench structures appear below the raft limit. This negative splay then declines again, as μ decreases.

The bend shows almost the inverse behavior, with a small increase as μ is increased and a sudden large increase at the lower raft limit.

The decrease of the splay at small μ may be the result of the trench width. Inside the raft, the splay is close to 0, in very narrow trenches the measurement program might still measure parts of a cholesterol rich structure. These bend effects on the other hand are not artifacts of the division into rafts and non-rafts. The bend inside the rafts is also mostly 0, but increases for large μ in a way similar to figure 8.11, but with a much larger variance, indicating that this is likely a result of imperfect detection of the raft regions. In both scenarios, it is the result for the minority structure, which is likely contaminated. This is because even few false positives in the raft/liquid detection can have a large impact, due to the smaller sample size.

We can conclude, that the microscopic structure of the trenches is very different from the typical liquid. Section 7.3 and 7.6.2 confirm, that these structures seem to be connected to the interdigitated/ripple structure.

Figure 8.12 shows that the tilt flow centers described in this section can indeed be identified with the rafts on the membrane. If there are rafts and flow centers in a monolayer, there is a strong, positive spatial correlation between them. On the other hand, the correlation between flow structures on either side of the bilayer is less pronounced (figure 8.13). When the cholesterol enriched structures encompass almost the entire membrane (i.e. below the raft limit), there is still a strong correlation (left plot). But within the raft limit, the correlation graph looks more akin to the raft-raft correlation in the systems far below the raft limit, with a high correlation, low percentile part and a slightly negative correlation, high-percentile part (right plot). This suggests a similar interpretation of the pattern, that the flow centers at this set of parameters are generally positively correlated, when they exist, but many configurations exhibit no or few flow centers that can be detected by the above method (which is rather strict, as it needs

8. Characteristics of rafts

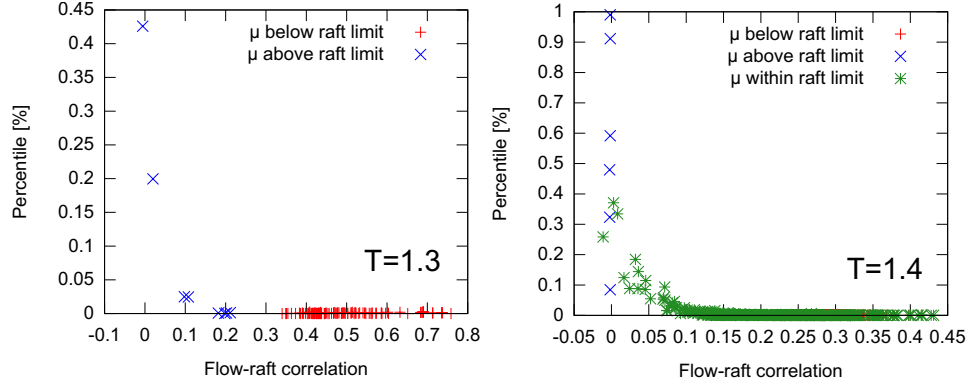


Figure 8.12.: Correlation of tilt “flows” and rafts. Each point represents one single, independent membrane configuration. Only configurations which exhibit at least one raft and at least one flow center according to the criteria in this section are used, hence the few points above the raft limit. At $T = 1.4$ there are many points below the raft limit (red crosses) with a high correlation and a zero-percentile which are concealed behind the points from within the raft limit (green stars). The way to interpret this kind of plot is described in section 8.1

large areas with an aligned tilt, for which many rafts are not large enough), leading to a small anti-correlation when none or few centers are found.

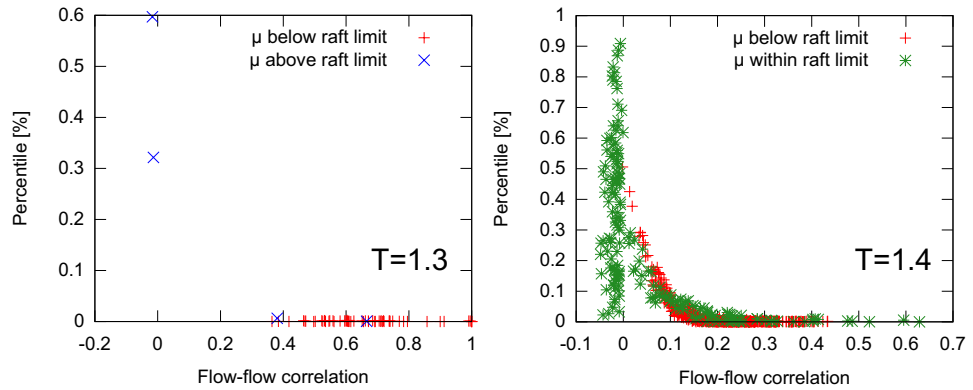


Figure 8.13.: Correlation of flows on both sides of a bilayer

9. Influence of model parameters

We will look at the influence of various model parameters in order to evaluate the general applicability of our model. The model should be robust against most changes. We will also see, which differences between the lipid and the cholesterol model are essential for raft formation, giving us an estimate which real-life systems can be described by our model. These need not necessarily be cholesterol-phospholipid mixtures, as our cholesterol model was inspired by real-life cholesterol but was not rigorously constructed from an atomic model. Our whole system could also well be applied to any binary mixture of lipids with similar differences between the components.

In this chapter we will mostly consider small systems, usually $9 \times 9 \times 2$ chain molecules, as this considerably shortens the required simulation time. In the previous chapters we have already seen, that the chemical potential curve measured in small systems already tells us a lot about the behavior of large membranes. Therefore, we will predominantly study the changes of the chemical potential curve and we expect that models with a similar chemical potential curve also show similar behavior when simulated as a large membrane. When we encounter a particularly interesting chemical potential curve, we actually carry out these larger simulations and check our predictions derived from the results for smaller systems.

9.1. Temperature

The appearance of rafts depends on the coexistence liquid ordered and liquid disordered states. Thus, the existence of a liquid disordered state is necessary for the existence of rafts. The pure lipid has its main transition at a temperature between $T = 1.23$ and $T = 1.24$. The influence of cholesterol on this transition was tested in 9×9 systems at temperatures between $T = 1.21$ and $T = 1.29$ in steps of 0.01.

As the smallest possible amount of cholesterol is one chain, this means the minimum concentration is $\approx 1.2\%$. This amount did not influence the main transition in a measurable way. It still occurs between $T = 1.23$ and $T = 1.24$. However, even the next highest concentration of cholesterol in the simulation ($\approx 2.5\%$) is enough to stabilize the gel at temperatures up to $T = 1.25$. 6.2% cholesterol stabilizes the gel up to $T = 1.27$. At $T = 1.29$ 12% cholesterol was required to stabilize the gel (the next smaller concentration in the simulation was 7.4% cholesterol). The results are summarized in table 9.1.

The typical gap in the chemical potential at the transition from liquid to gel still exists in all simulations with $T > 1.24$, it just moves toward lower cholesterol concentrations as the temperature decreases.

9. Influence of model parameters

% cholesterol	main transition
0.0	1.24(1)
1.2	1.24(1)
2.5	1.25(1)
6.2	1.28(1)
7.4	1.29(1)
12	>1.29
>12	>1.29

Table 9.1.: Influence of cholesterol on liquid-gel transition temperature in $9 \times 9 \times 2$ systems

Overall, values of μ in the gel state at a small fixed cholesterol concentration are almost independent of temperature when measured in units of $k_B T$, with a slight trend to decrease with temperature at low concentrations and a slight trend to increase at higher concentrations (figure 9.1 a)). This was already seen in the detailed measurements of μ in section 6.1, but only for the three temperatures $T = 1.2$, $T = 1.3$ and $T = 1.4$. Unfortunately, the measurements in this section cannot provide more insight than just confirming this behavior, as the measurement errors of the individual data points are relatively large compared to the differences in μ , making it impossible to discern the exact form of the temperature dependence. Measuring μ not in units $k_B T$ but in the absolute unit of simulation energy, increases the trend towards larger values for μ at larger temperatures. A comparison of μ for different temperatures and cholesterol concentrations is shown in figure 9.1.

9.2. Bead distance

Again looking at 9×9 systems, the equilibrium bond length r_0 and the maximum displacement $\Delta r_{\max}$ of the cholesterol model were increased to match the bond potential in the lipid model by varying a parameter ζ with $r_0(\zeta) = 0.6 + 0.1\zeta$ and $\Delta r_{\max}(\zeta) = 0.15 + 0.05\zeta$ and $0 \leq \zeta \leq 1$. This did not result in any major qualitative changes of the μ -curve, but did substantially decrease the absolute value of μ , see figure 9.2. The difference between these curves seems to be independent of the temperature when expressed in units of the simulation energy ϵ , but depends on the state and concentration (figure 9.3). Thus, at $T = 1.3$ and $T = 1.4$, the curves are identical within the error margins. For $T = 1.2$ there is a different behavior at low concentrations, when the $T = 1.2$ systems are in the gel state, whereas the systems at higher temperatures are liquid.

9.3. Head size

Once again looking at 9×9 systems, the effect of cholesterol headsize was studied (where “size” means the cutoff radius of WCA-potential). In the standard model, the cholesterol

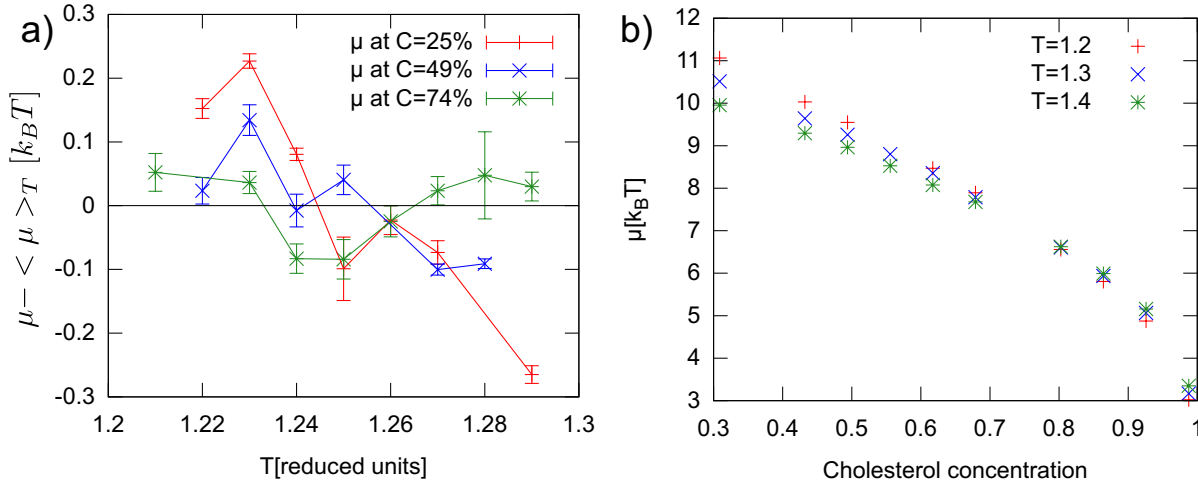


Figure 9.1.: Temperature dependence of μ . a) shows the slight trends of μ for different temperatures described in section 9.1. The lines are given as visual guidance. The mean $\langle \mu \rangle_T$ is taken over all temperatures shown in the plot at the respective cholesterol concentration. That means, at $C = 25\%$, μ is larger than the average μ at this concentration, whereas at higher temperatures it is smaller. b) shows the results for the gel state of the 9×9 systems from section 6.1 plotted alongside each other. The values are taken from fewer temperatures than a), but with a wider range and much better statistics. One can again see that at small cholesterol concentrations, μ is largest for the smallest temperature, $T = 1.2$, whereas at high concentrations, μ is smallest at this temperature. This is the same observation that is shown in greater detail in a).

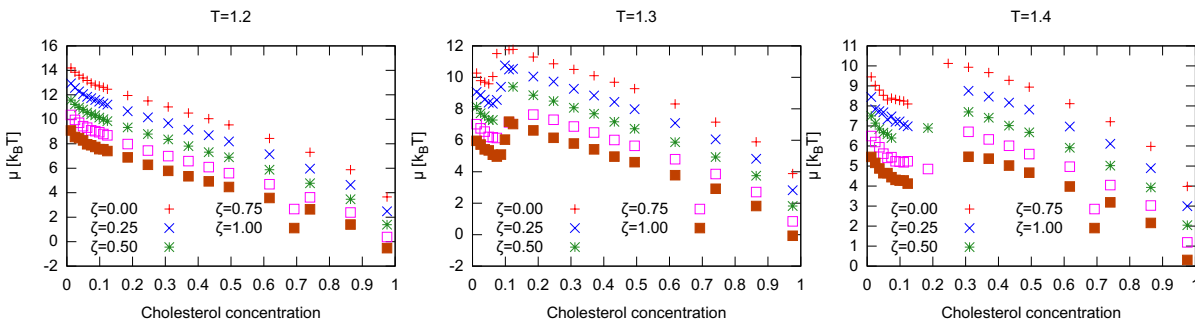


Figure 9.2.: μ curves for different equilibrium lengths of the "cholesterol" bond potential

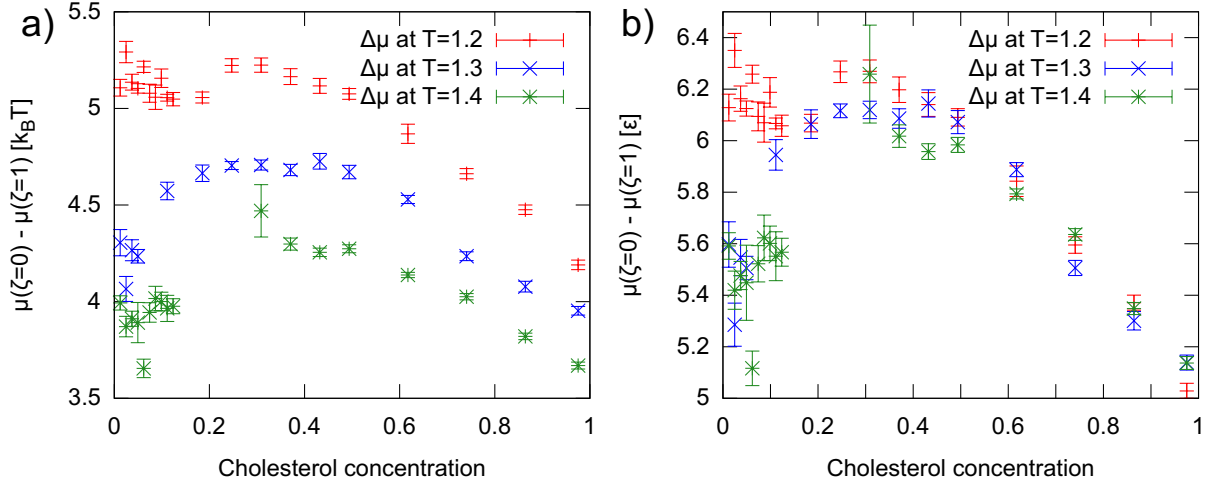


Figure 9.3.: Difference between the μ values for the long and the short “cholesterol”. a) in units of $k_B T$, b) in absolute simulation energy units.

head is the same size as the lipid head. Real world cholesterol would have a considerably smaller headgroup.

When decreasing the headsize, at $T = 1.2$ the chemical potential μ increases significantly, while the μ -curve does not change its general form. This is to be expected as a mismatch in headsize leads to a lower acceptance probability of the semi-grand canonical move from cholesterol to lipid, which then leads to an increase in μ . At a headsize of 0.9σ , the increase in μ is $\approx 1.15k_B T$, constant over a large range of temperatures and concentrations (figure 9.4).

There are, however, two major differences between the small and the large heads: The gel structure is much more stable and is no longer tilted. For a headsize of 0.9σ , even at $T = 1.4$, the gel is stable at 6.2% cholesterol, the smallest concentration simulated in this test series (for a headsize of 0.95σ the system is still liquid at this concentration).

9.3.1. Effect of cholesterol on tension, spontaneous curvature and tilt

The study of the head size variation also yielded detailed data about the effects of cholesterol in general on the gel state. The tension is plotted in figures 9.5, 9.6 and 9.7 for various sets of parameters. As seen above, the membrane is liquid at $T=1.4$ for low cholesterol concentrations, when the cholesterol heads are large. More specifically we can observe the following effects:

- The tension decreases when decreasing the head size from 1.05σ to 0.9σ . This magnitude of this effect, of course, depends on the cholesterol concentration. At 74% cholesterol this can be a 40% decrease of the peak tension values.
- The tension in the bilayer middle is strongly negative for small heads, whereas for the usual, larger heads, it is about 0. The total tension is still approximately 0.

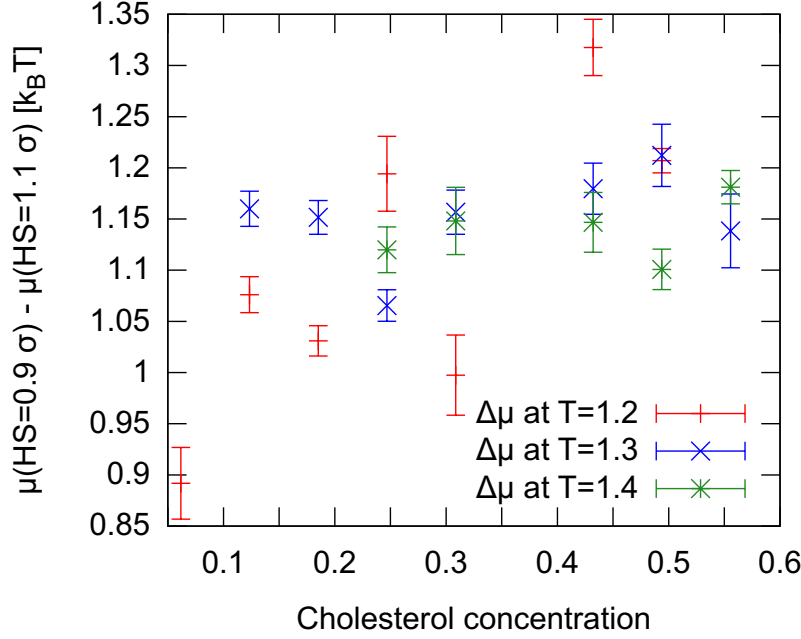


Figure 9.4.: Difference between μ at a cholesterol head size of 0.9σ and the standard head size of 1.1σ , in the gel state.

- The addition of cholesterol generally increases the absolute values of the tension, whatever the head size.
- Temperature, on the other hand, has almost no impact, except triggering the liquid state.

The resulting spontaneous curvature is shown in figures 9.8 and 9.9. We can see that the cholesterol with the decreased head size does not increase the spontaneous curvature of the membrane when found in high concentrations. Although the smallest heads (0.9σ) are actually smaller than the tail beads, the spontaneous curvature only stays constant with a growing cholesterol concentration, but does not decrease. We note, that in every case the curvature of the gel is different from the curvature of the liquid, enabling the raft formation mechanism described in chapter 1.3.

At last, we can have a look at the tilt of the gel structure (figure 9.10). Unsurprisingly, smaller heads decrease the tilt angle. For larger cholesterol concentrations the tilt will almost vanish completely. But even for larger heads, the mean tilt angle decreases for larger cholesterol concentrations. This is in agreement with experimental and other simulated results[22].

9.3.2. Conclusion

As the model cholesterol does not actually reproduce many real world properties of real cholesterol and was never intended to do so, the results in this section are rather an

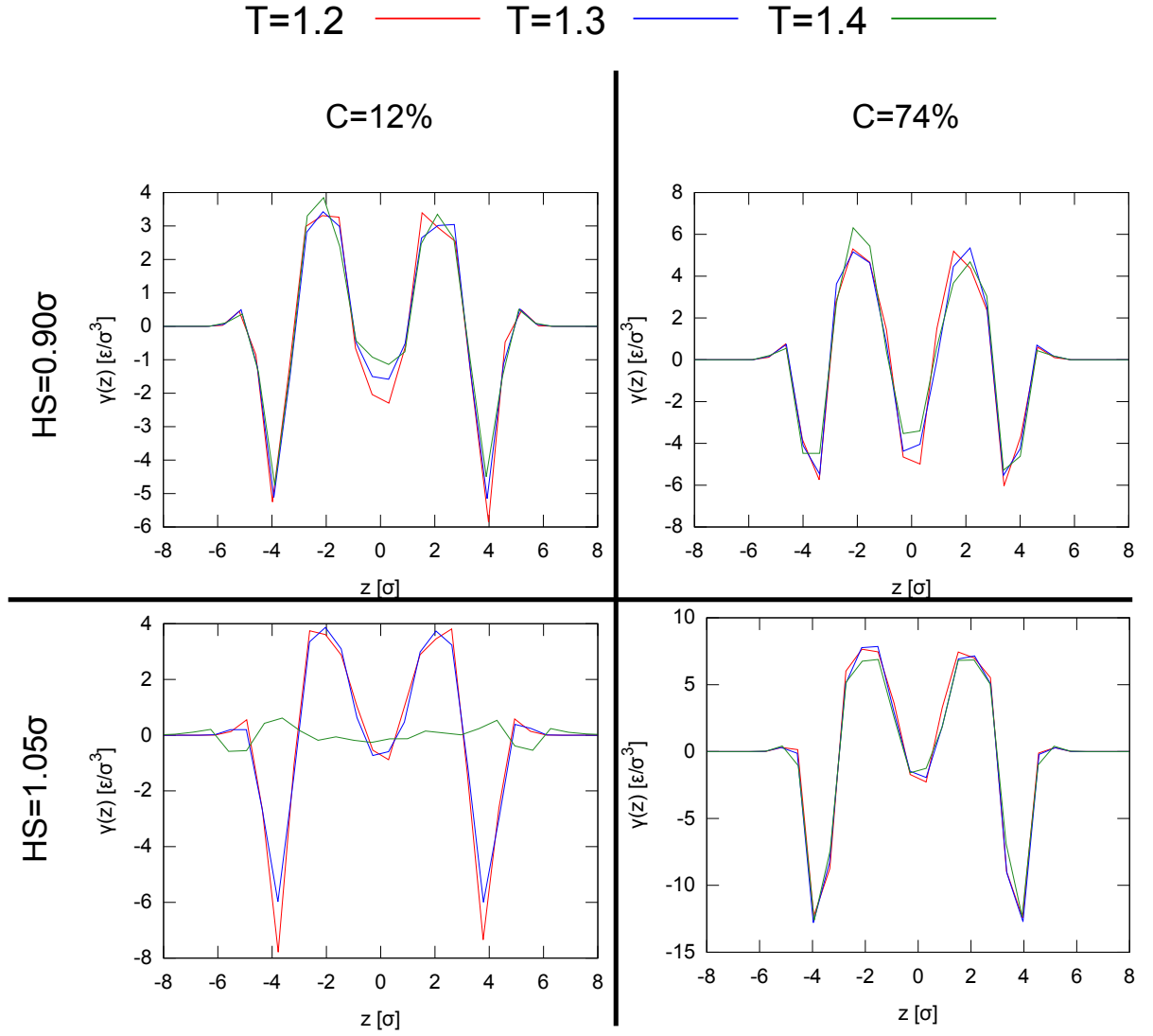
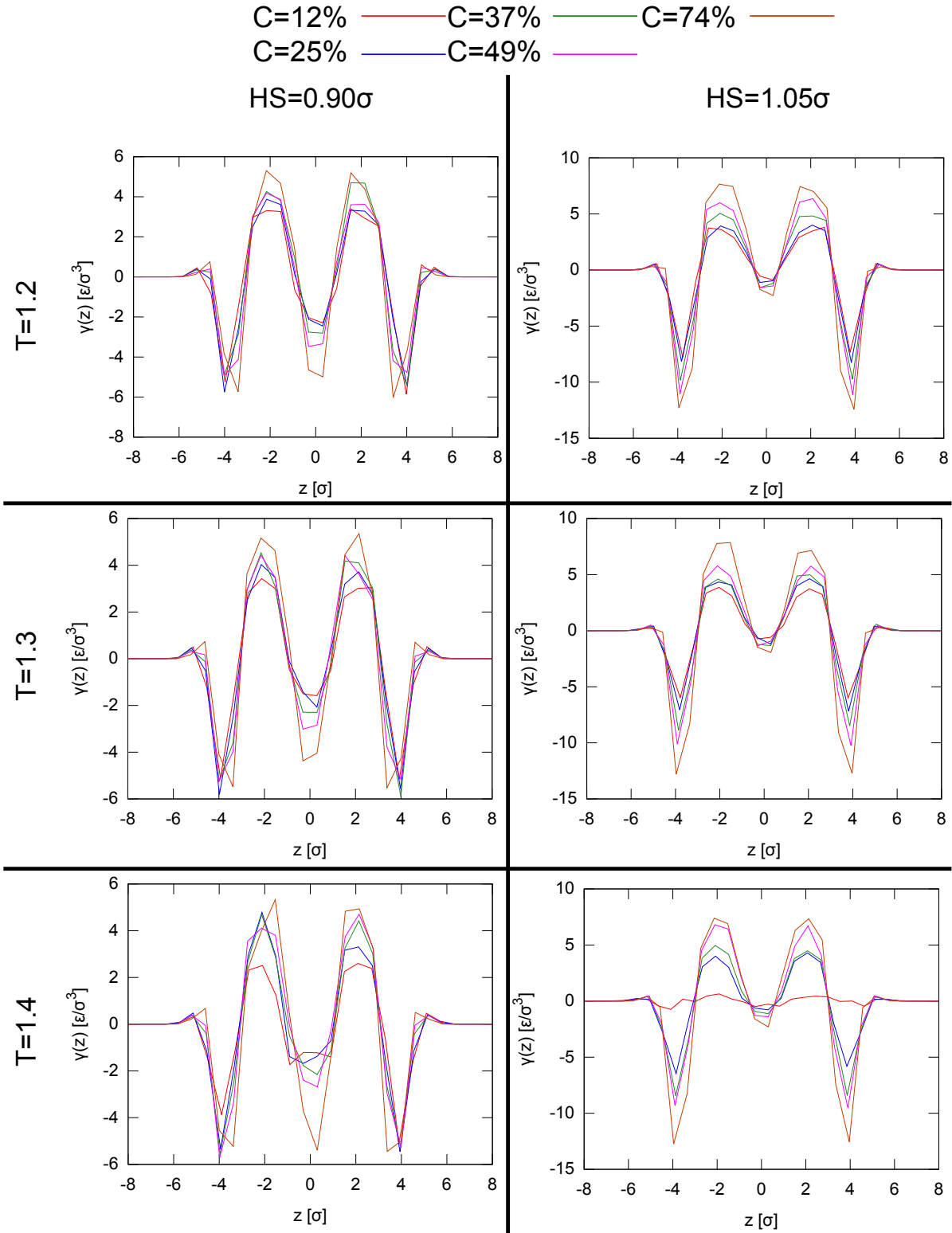


Figure 9.5.: Comparison of tension curves for different temperatures (HS=head size). In the lower left we can see that the gel structure is less stable at high temperatures for larger heads.

Figure 9.6.: Comparison of tension curves for different cholesterol concentrations (C)

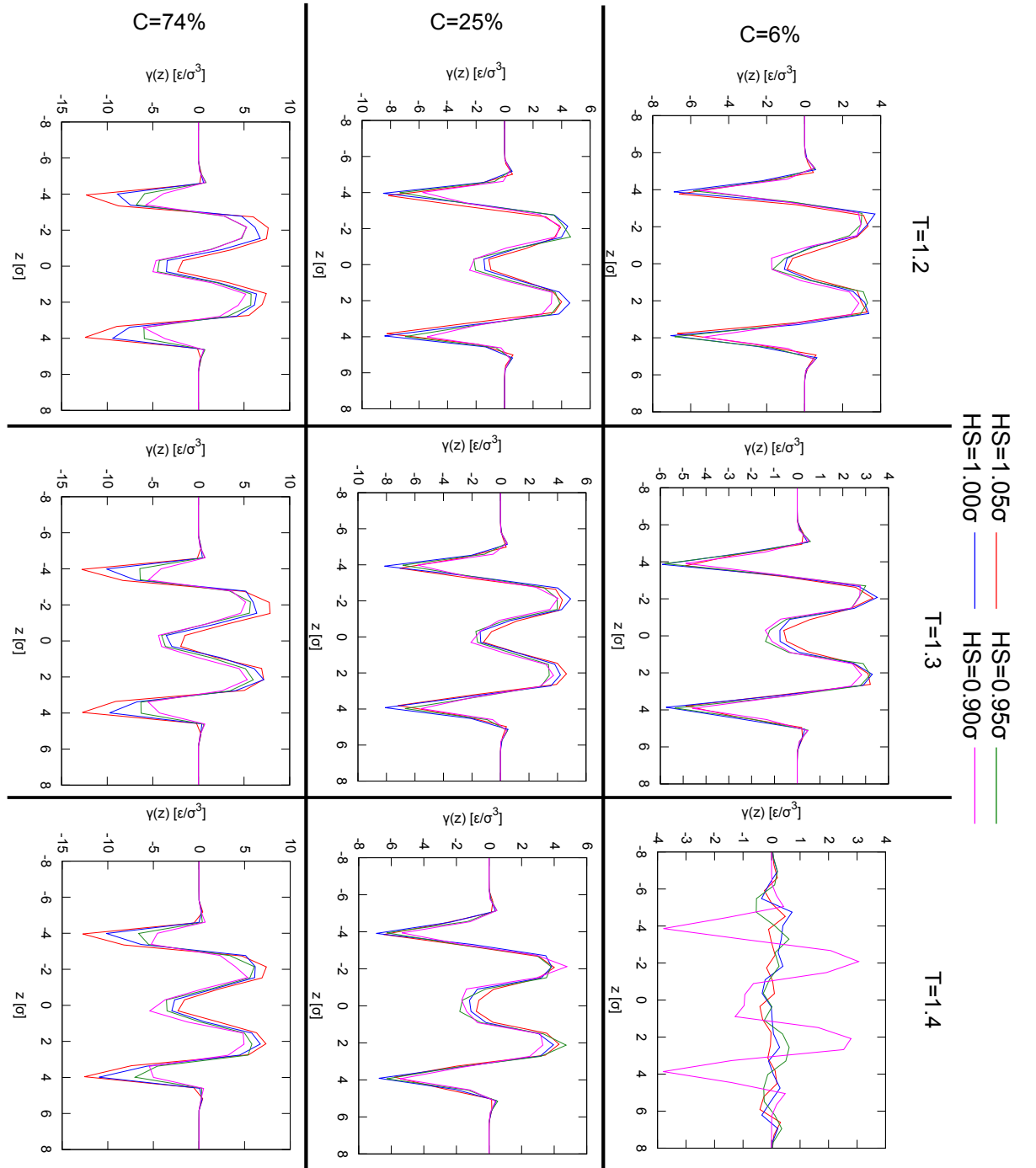


Figure 9.7.: Comparison of tension curves for different cholesterol head sizes

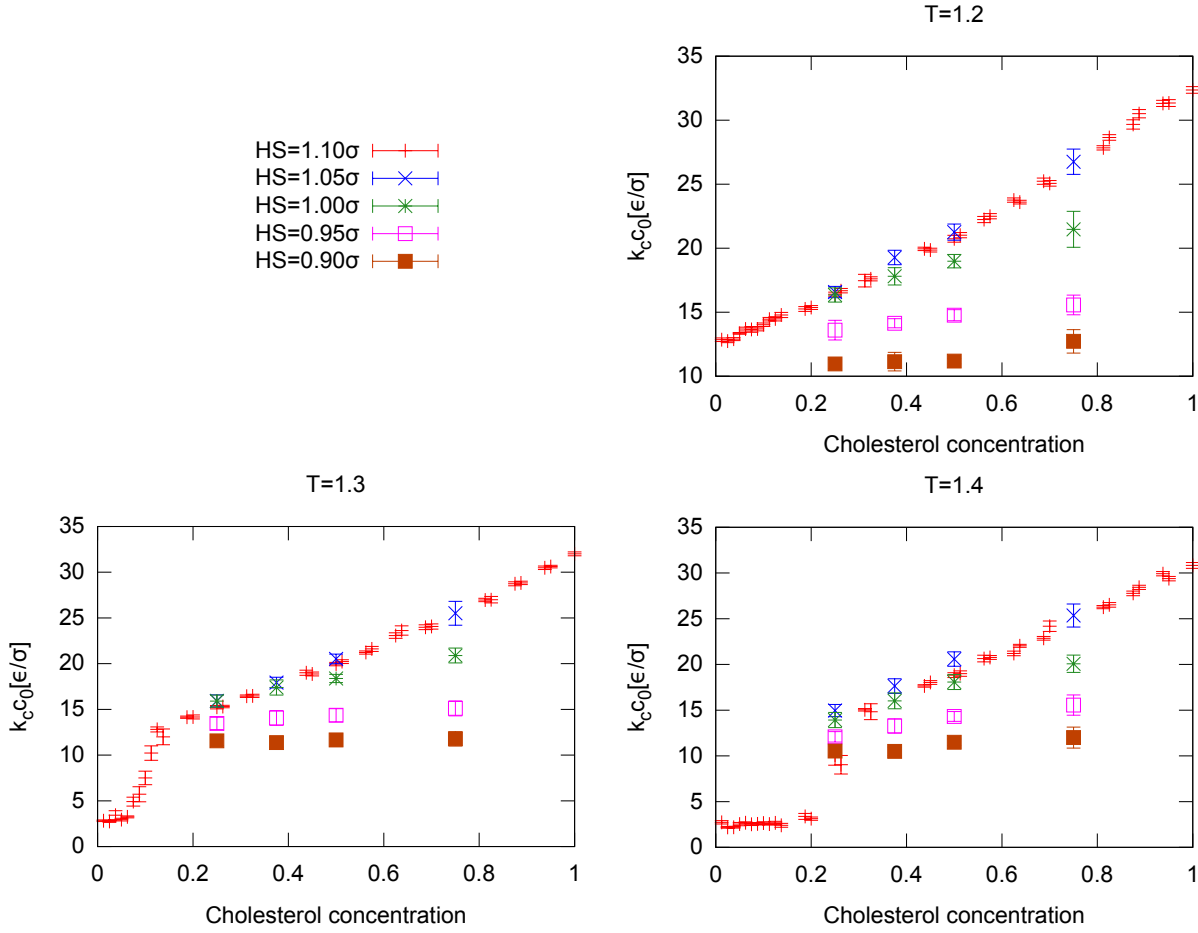


Figure 9.8.: Spontaneous curvature over cholesterol concentration.

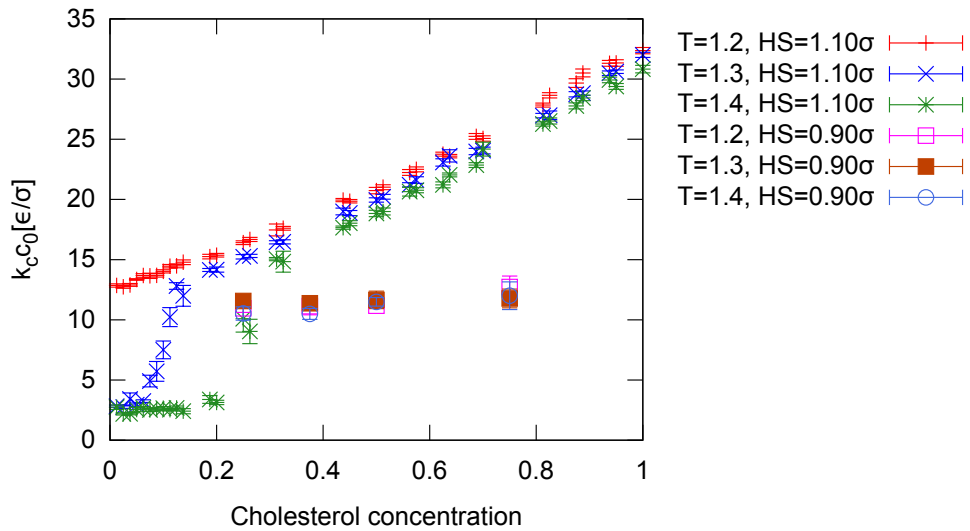


Figure 9.9.: Comparison of spontaneous curvatures for different temperatures

9. Influence of model parameters

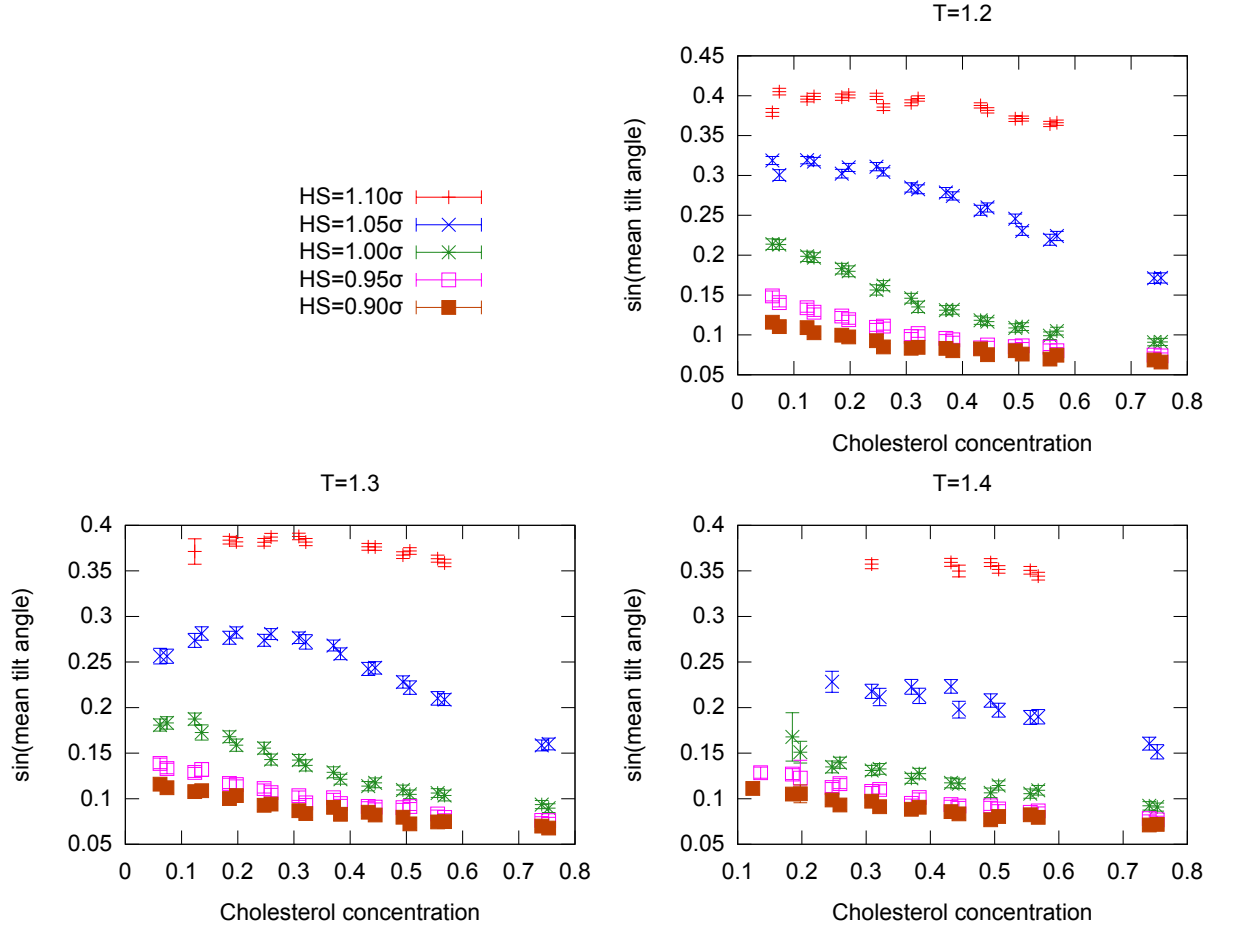


Figure 9.10.: Tilt angle over cholesterol concentration for various cholesterol head sizes. Mean tilt angle of the whole membrane. Only systems in the gel state are shown.

interesting observation on the properties of the model rather than a refutation of the model. Real world cholesterol usually aggregates between the tails of lipids and does not reach (far) into the membrane boundary. The presence of such a large headgroup and its consequences on the microscopic structure of the gel state are results of the necessity to have the secondary membrane component not too dissimilar to the primary lipid in order to enable the semi-grand canonical moves in the simulation, see section 3.2.1.

9.3.3. Large membranes

We also simulated large systems with smaller cholesterol heads (0.9σ). While the resulting configurations at $T = 1.4$ are similar to the regular model (trenches, rafts, system wide liquid), where μ is shifted by the values from figure 9.4, at $T = 1.3$ we see the complete disappearance of the trench structures. When μ is smaller than $\sim 9k_B T$ the systems evolve directly from the homogeneous, lipid rich starting configuration into a homogeneous, cholesterol rich gel, without any large scale structures. When μ is larger, for example $\mu = 11k_B T$ in figure 9.11, the system first forms a (non-percolating) trench structure, but these trenches are closed in the further course of the simulation run (see aforementioned figure). From this observation we can conclude that, in principle, the trench structures can be closed during the process of a simulation run, at least in case of the model with the smaller cholesterol heads. But it also raises several new questions: Does this mean that the trench structure is an unphysical artifact of the simulation technique (akin to grain boundaries in the growth of crystal structures) and only in the case of the small head model can the trenches be “overgrown” by the simulation? But at $T = 1.4$ we observe a regular trench structure that never disappears. Furthermore, we see the same behavior of the small and the large head model at $T = 1.4$, but at $T = 1.3$ we see clear differences, as the large head model exhibits a trench structure below the raft limit, the small head model does not. Is the behavior of the small head model just different than the behavior of the regular model? That would not be unexpected as we changed an important part of our model. A smaller headsize decreases the disposition of the cholesterol rich domains to exhibit a tilt or rippled structures which seem to be connected to the trenches (more on that in section 9.5). But we did not see any major differences between the chemical potential curves of the two models, just a shift. On the other hand, such a difference between the models would not necessarily have an impact on the chemical potential curve.

Therefore, the exact conclusion from this observation remains unclear, apart from the fact that we did observe the disappearance of a trench structure in simulation, proving that such an evolution of a system is possible.

9.4. Bond angle potential

A change of the bond angle potential ε_{BA} between the first six cholesterol beads results in the expected behavior for μ (figure 9.12): The act of decreasing ε_{BA} also decreases μ , as the cholesterol and lipid chains become more similar to each other. Simultane-

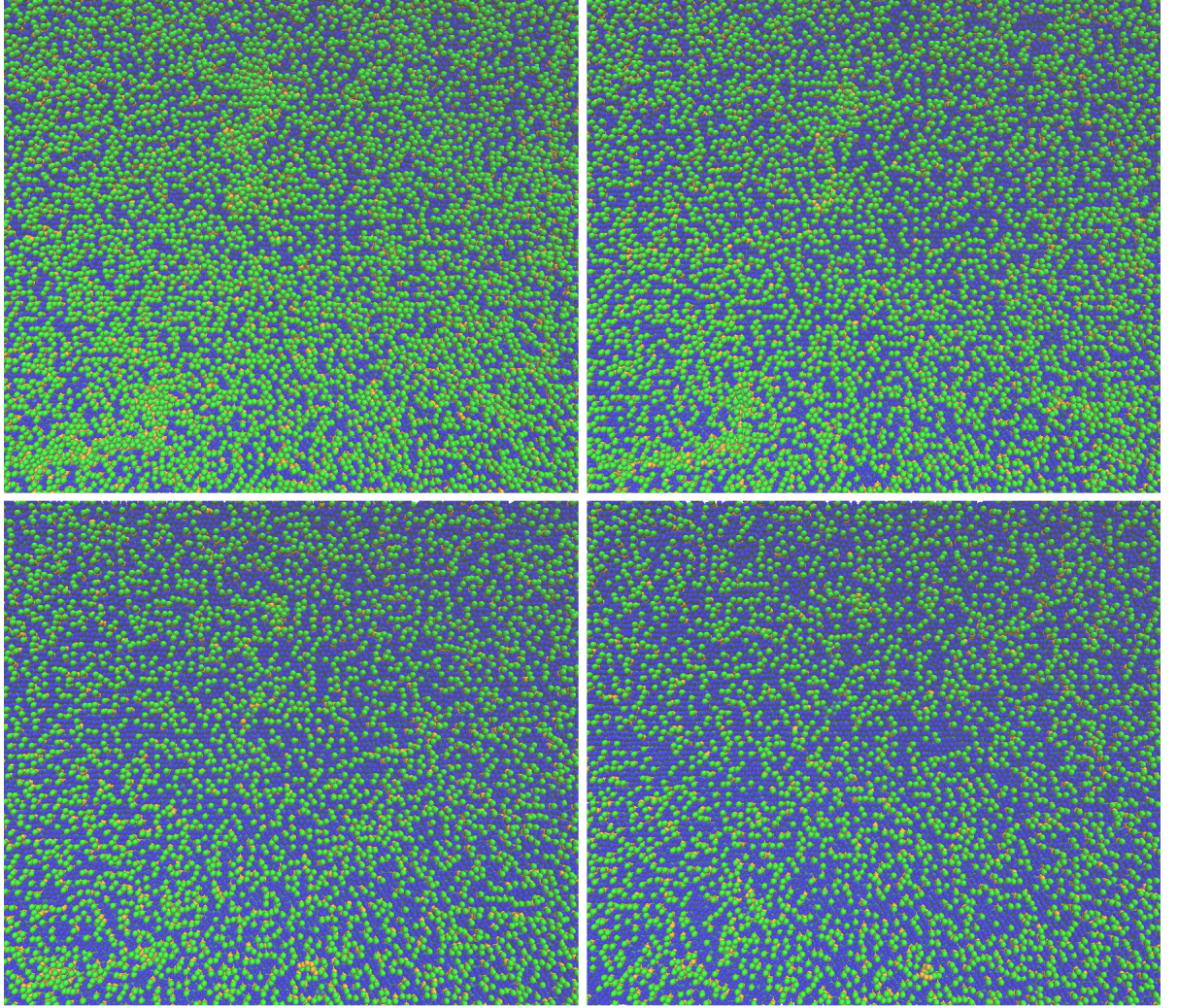


Figure 9.11.: Evolution of a large simulation of the cholesterol model with headsize 0.9σ at $T = 1.3$ and $\mu = 11k_B T$. Starting from a homogeneous, lipid rich gel (not shown), the trench structure appears after a few hundred thousand sweeps (top left) and disappears completely after an additional few hundred thousands sweeps (bottom right).

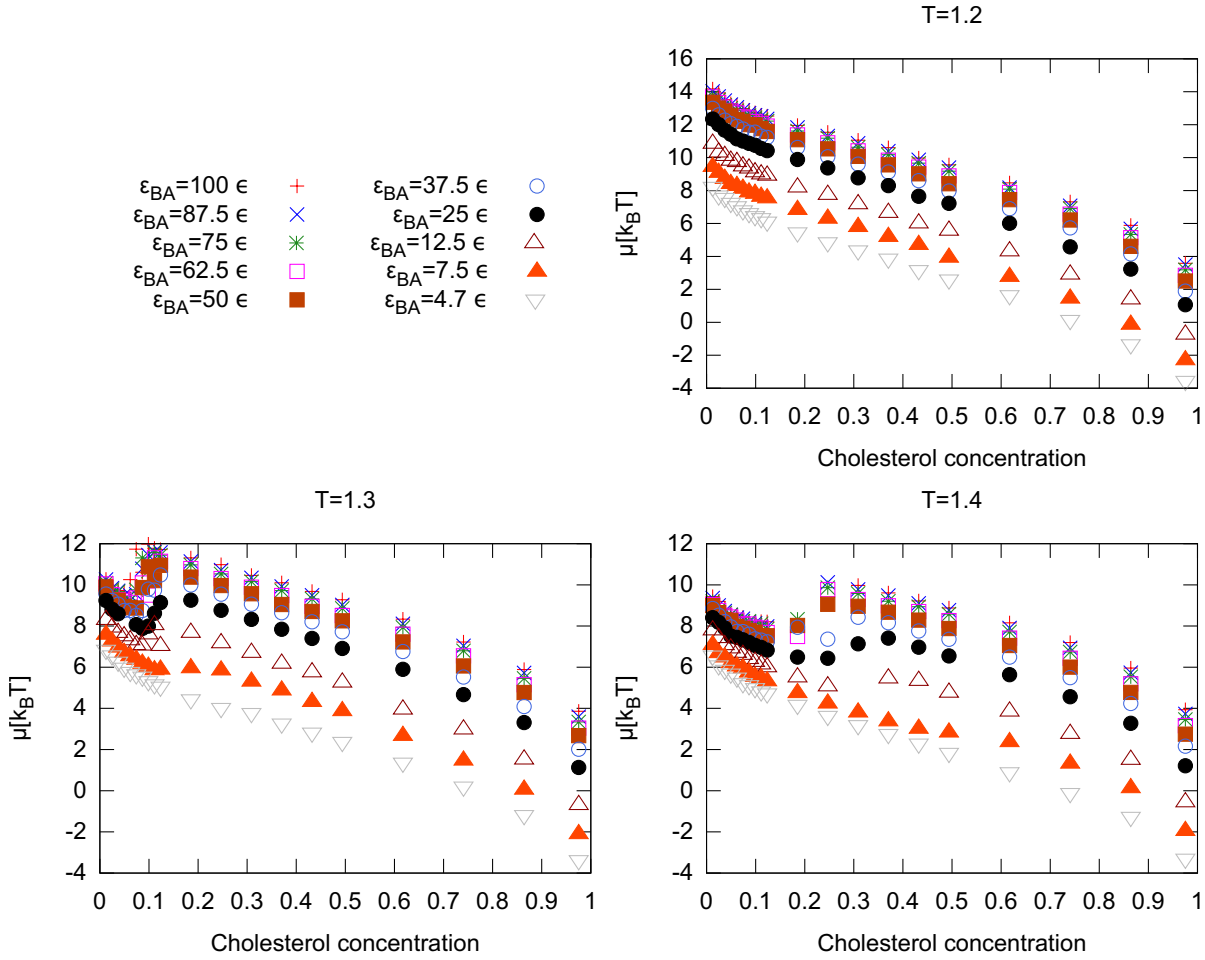


Figure 9.12.: μ at various temperatures and concentrations for different strength of the bond angle potential in 9×9 systems

ously, the gap between fluid and gel state becomes smaller and moves towards higher concentrations. Below a certain value (12.5ϵ at $T = 1.4$ and 7.5ϵ at $T = 1.3$), the gap disappears completely as the cholesterol induced transition to a gel structure disappears.

This presents a good opportunity to test the consistency of the μ measurement. Large systems ($100 \times 100 \times 2$ chains) were simulated at $T = 1.4$ and $\epsilon_{BA} = 7.5\epsilon$. As predicted by the μ measurement there is only one state, a homogeneous mixture of lipids and the secondary component. The cholesterol concentration matches the prediction from the small systems very well, albeit not perfectly. Both results can be seen in figure 9.13.

9.5. Cholesterol to cholesterol non-bonded interaction strength

In the standard cholesterol model, the strength of the tail-to-tail attractive Lennard-Jones interaction of cholesterol was reduced compared to cholesterol-lipid attraction and

9. Influence of model parameters

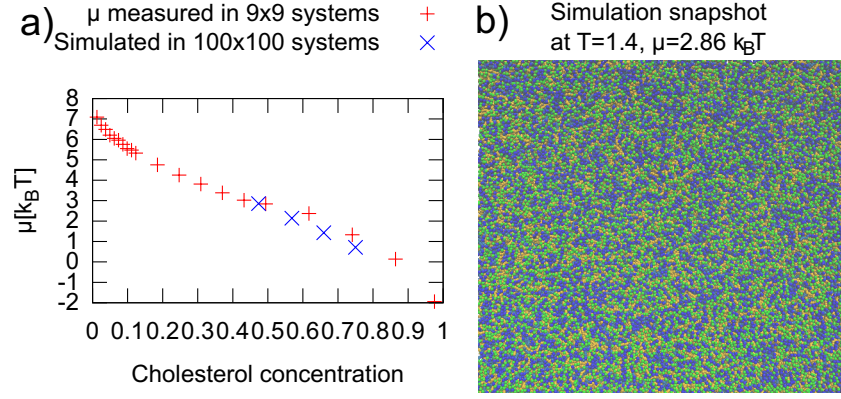


Figure 9.13.: Large system simulation of lowered bond angle potential. a) Prediction of concentration from small systems compared to actual concentration in large systems ($T = 1.4$). b) Snapshot from one such simulation. There are no detectable large scale structures.

lipid-lipid interaction. This difference was introduced in order to promote mixtures of lipid and cholesterol compared to clusters of pure cholesterol, which are already favored because of the better fitting geometry between the tail groups (straightness due to the stiff bond angle potential). Once again looking at 9×9 systems, one notices a huge qualitative difference in the μ -curve at larger values of the cholesterol-cholesterol tail potential strength ε_{CC} (figure 9.14). There is still the usual gap between fluid and gel at $T = 1.3$ and $T = 1.4$, but beyond this gap there is a new phenomenon for $\varepsilon_{CC} \gtrsim 1.025\epsilon^1$: A region of instability ranging from the onset of the gel state up to very high cholesterol concentrations $\gtrsim 90\%$. This region is different from the usual gap as the slope of μ as a function of the cholesterol concentration is truly positive, not just jumping at a certain concentration, meaning that it is impossible to stabilize mixtures with these concentrations. This phenomenon is a consequence of the penalty for mixing the two different chain types with these new, changed potentials, as it is more favourable to completely (or almost completely) demix the two chain species.

This behavior can also be observed at $T = 1.2$, giving us the opportunity to study a system with two stable states which are both gels. This should result in complete separation of these states, if the second assumption for the formation of rafts, a difference in spontaneous curvature, is not met. Unfortunately there is a difference between the spontaneous curvatures of the pure lipid gel and a pure cholesterol gel (with both potentials, the original and the modified one); the absolute difference of the curvatures in units of ϵ/σ of cholesterol rich gels and cholesterol poor gels is even larger than the difference between a cholesterol deprived liquid structure and a cholesterol poor gel. See section 9.5.1 for details.

Regrettably, only four large membranes using this modified cholesterol model could be equilibrated successfully until the time of this writing, the equilibration time of these

¹Note, that this value is greater than the potential of the lipid-lipid interaction and the lipid-cholesterol interaction, which is 1ϵ

9.5. Cholesterol to cholesterol non-bonded interaction strength

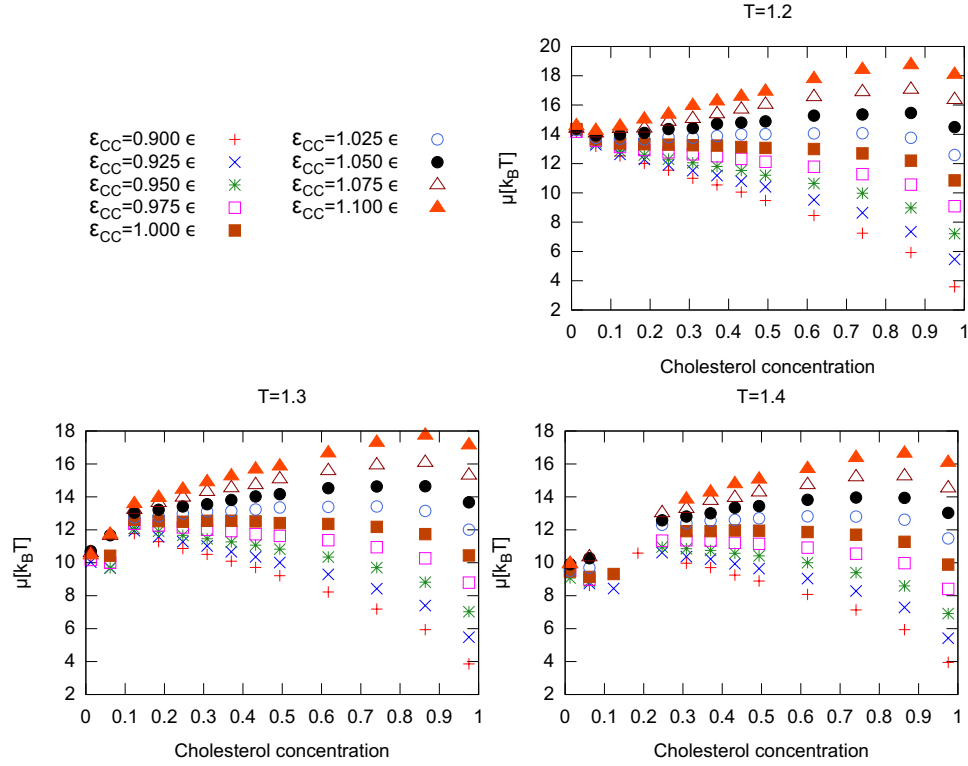


Figure 9.14.: μ for different values of cholesterol tail-tail attraction ϵ_{CC} at different temperatures and cholesterol concentrations.

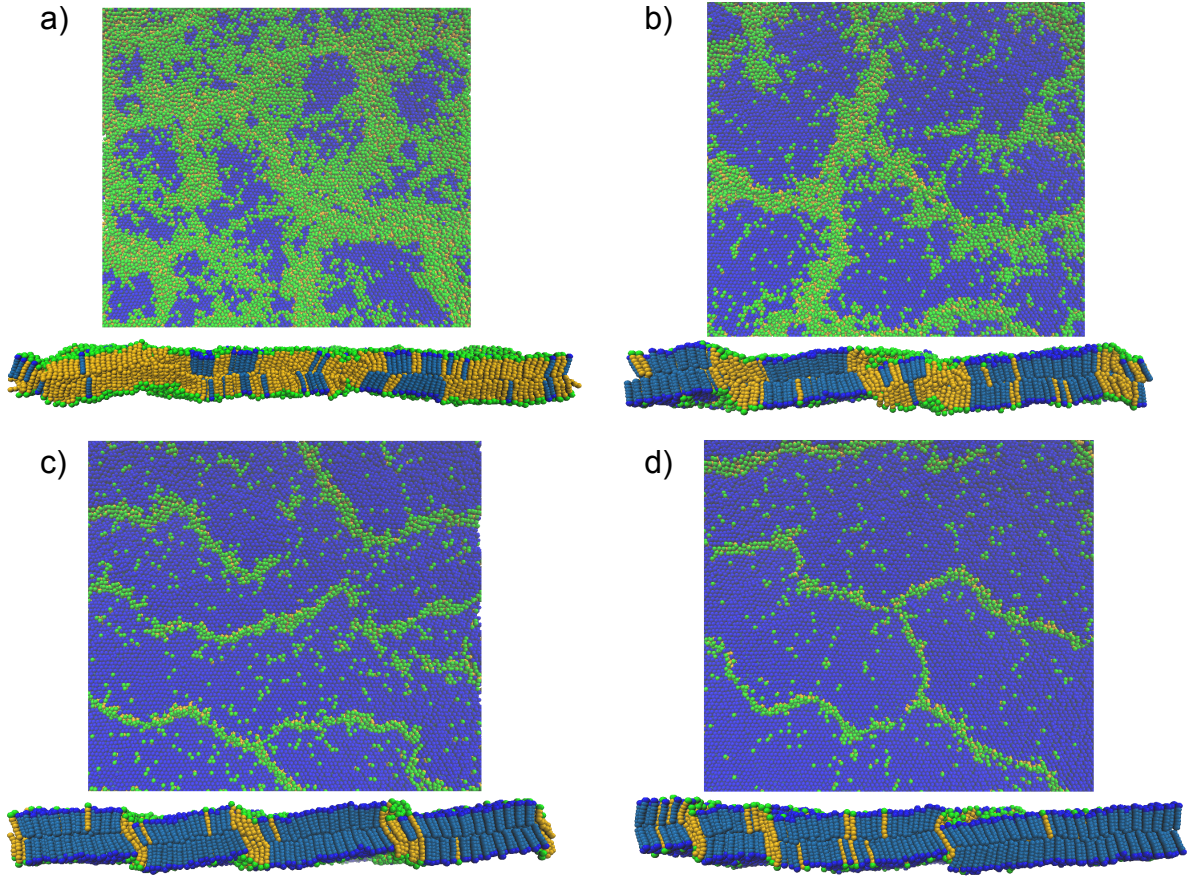


Figure 9.15.: Snapshots of large membranes using the modified cholesterol tail-tail attraction $\varepsilon_{CC} = 1.025\epsilon$ and $T = 1.2$ (Corresponds to blue circles in figure 9.14). a) $\mu = 14.2k_BT$. b) $\mu = 13.9k_BT$. c) $\mu = 13.6k_BT$. d) $\mu = 13.3k_BT$.

systems apparently being much larger than in the raft forming systems. Although we do not have enough data for an in-depth analysis, these configurations still offer some insights into the raft formation process (figure 9.15): The difference in spontaneous curvature is indeed enough to stabilize rafts, as can be seen in 9.15 a). Moreover, below the raft limit of this model, we can again see the trench structure that we already know from our main model, but here we can clearly identify these trenches as a ripple structure (Sideviews, especially 9.15 b) and c). In fact, the small- μ -configurations look “striped” from above, akin to the ripple structure in the pure lipid system. The large rafts in 9.15 b) (which look similar to the patterns in figure 7.1 e), but with a higher contrast between the two structures) are the flat parts of the ripple structure, whereas the surrounding lipids form the actual ripple. The rafts in 9.15 a) (similar to 7.1 f), but larger) seem to be a diluted version of these structures.

9.5.1. Spontaneous curvature

In order to better understand the above results, the spontaneous curvature c_0 of pure lipid and pure cholesterol membranes was measured via the tension profile of 9×9 systems. With these profiles we measured $k_c c_0 = 11.5(4)\epsilon/\sigma$ in the liquid gel state ($T = 1.2$), successfully reproducing the result $k_c c_0 = 11.1(1)\epsilon/\sigma$ by West[131], using only a few quick simulations. k_c is the bending rigidity. In our 182 lipid systems, we measured $k_c c_0 = 2.2(8)\epsilon/\sigma$, much larger than the $k_c c_0 = -0.3(1)\epsilon/\sigma$ measured by West in systems with 200 lipids at the lower temperature of $T = 1.3$. This could be due to the temperature difference (we used $T = 1.4$), but we note that figure 9.8 also indicates a value $\sim 2.5\epsilon/\sigma$, although no actual measurement was done at zero cholesterol. West herself has found difficulties when measuring the tension and curvature in the liquid state; it is possible that our own, less thorough, measurements are subject to these errors.

Nonetheless, the results for the gel structure are consistent over different system sizes and temperatures and the cholesterol membranes are in this state. We measure $k_c c_0 = 33.3(5)\epsilon/\sigma$ for the usual cholesterol model (independent of temperature, as it should be) and an even larger $k_c c_0 = 37.5(4)\epsilon/\sigma$ for the model with the modified tail-tail interaction $\epsilon_{CC} = 1.025\epsilon$.

9.6. Variation of multiple model parameters

9.6.1. Bond angle and non-bonded potential

Setting the cholesterol bond angle potential strength to $\epsilon_{BA} = (100 - 95.3\zeta)\epsilon$ and the strength of the tail-tail non-bonded potential to $\epsilon_{CC} = (0.9 + 0.1\zeta)\epsilon$ and varying zeta (i.e. $\zeta = 0$ is the original cholesterol model and $\zeta = 1$ is a model similar to the lipid, except for the bond length potential), one can observe that the effect of the bond angle potential quickly overcomes the effect of the non-bonded potential - at least when compared in this particular fashion. At $T = 1.2$, the region of instability disappears completely. At higher temperatures there is still an unstable region just beyond the fluid-gel gap, but only over a range of a few percent cholesterol concentration difference ($\approx 5\%$ at $T = 1.3$ and $\approx 10\%$ at $T = 1.4$).

At $\zeta = 1$, the shape of the μ -curve is rather similar to the theoretical predictions for equal chains (equation 5.2.1 and figure 5.5), but there is still an offset of about 5 to $7k_B T$, which is close - but not identical - to the downshift in μ by variation of the bond length alone (section 9.2).

9.6.2. Matching cholesterol and lipid: Varying all parameters

The last series of simulations in this chapter will vary all parameters that differentiate the model cholesterol from the model lipid: r_0 , $\Delta r_{\max}$, ϵ_{BA} and ϵ_{CC} . All these parameters are varied at the same time as a function of the coordinate ζ , in a fashion similar to sections 9.6.1 and 9.2. The result is very similar to the simulations that only changed

9. Influence of model parameters

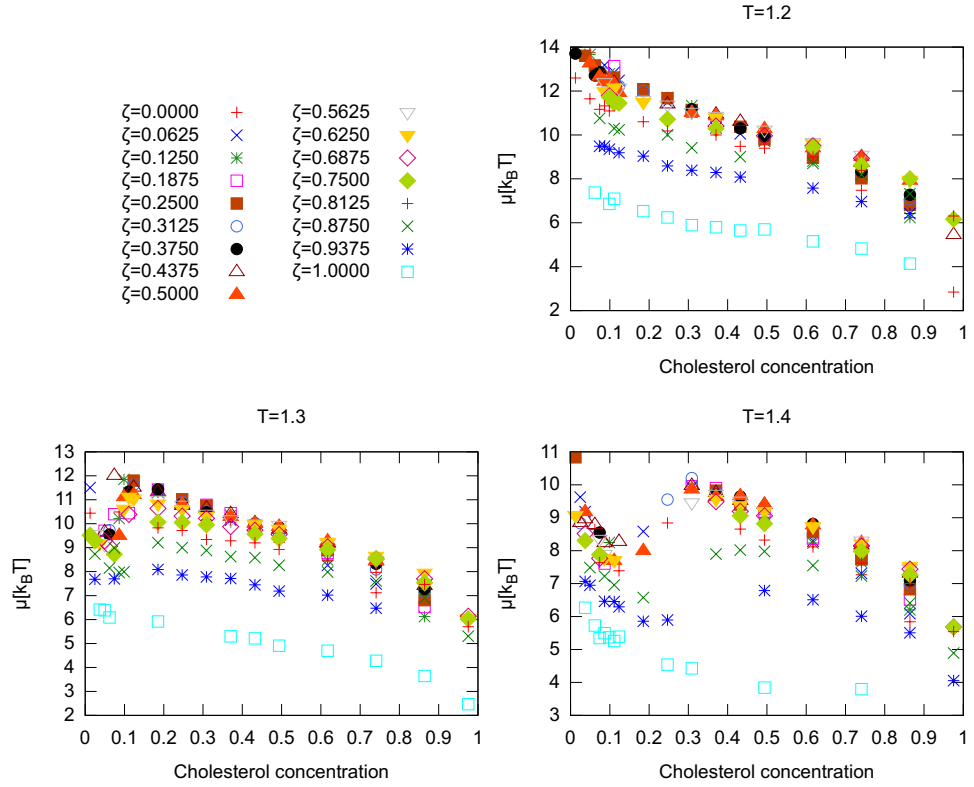


Figure 9.16.: μ for different values of cholesterol tail-tail attraction ε_{CC} and bond angle potential strength ε_{BA} at different temperatures and cholesterol concentrations.

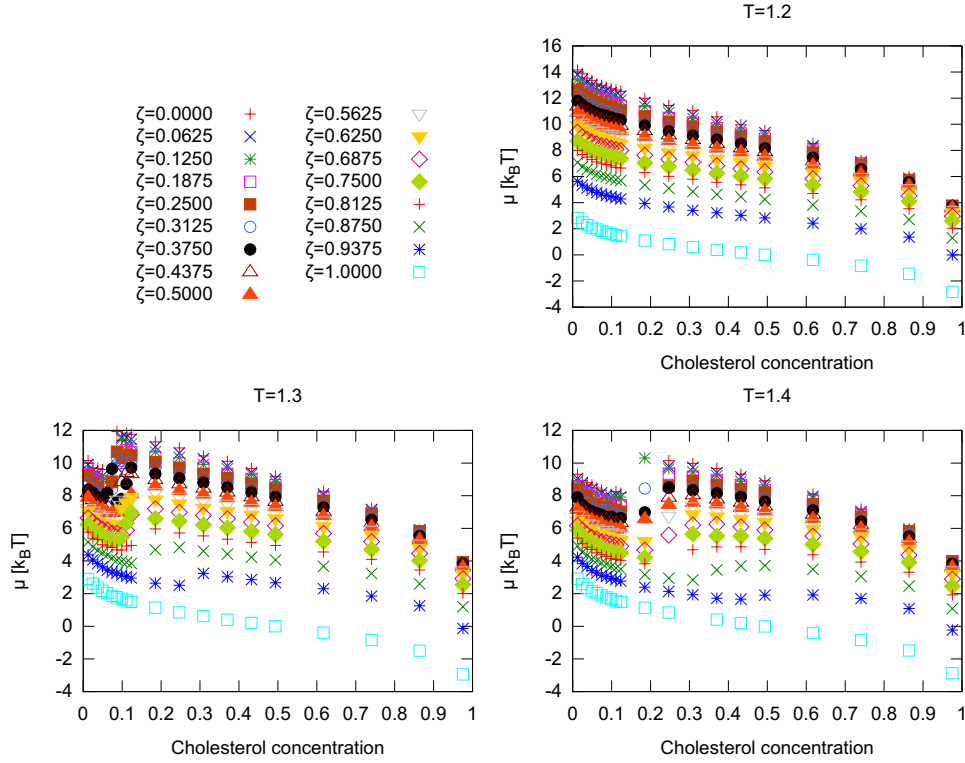


Figure 9.17.: μ for different values of cholesterol parameters, from the usual cholesterol model ($\zeta = 0$) to a cholesterol identical to the lipid model ($\zeta = 1$).

the bond angle and non-bonded potentials, with a large decrease of μ as one approaches the parameters of the lipid model. The instable region seen in section 9.5 is once again quickly suppressed by the complete disappearance of the two-state region because of the smaller bond angle stiffness (figure 9.17).

At $\zeta = 1$, one actually arrives at a μ -curve that is point symmetric around 50% cholesterol and $\mu = 0k_B T$, as it should be for identical chains (section 5.2.2. These curves are identical for different temperatures and can be fitted very well with the theoretical model 5.2.2, see figure 9.18.

9.6.3. MD compatible model

The models with the alternative bond length potential from section 3.2.2 were tested for their feasibility for future MD-simulations. A necessary prerequisite is, that these models still show the raft structures seen in this work. These were just short proof-of-concept studies – the runs were not fully equilibrated. But even from these short runs of 500k-1M MC steps, it was clear, that these models actually work as intended (figure 9.19).

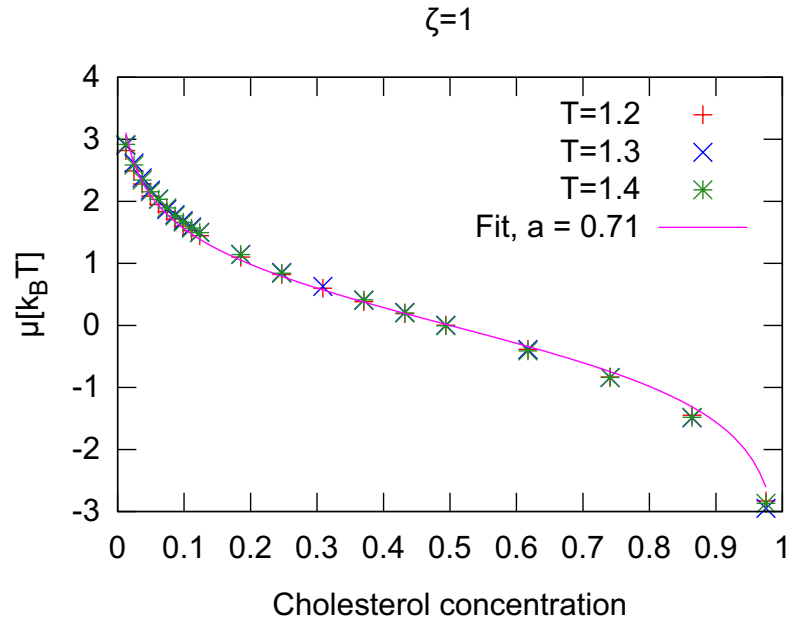


Figure 9.18.: Fit of equation 5.2.2 to the simulated data at $\zeta = 1$.

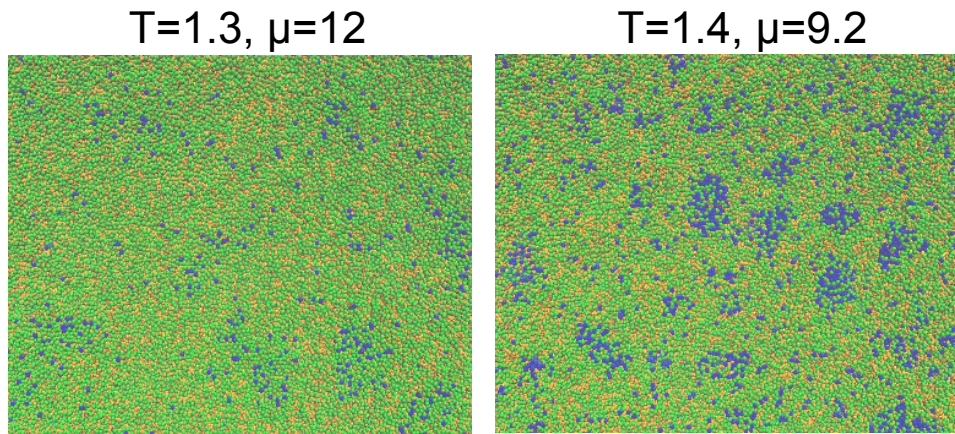


Figure 9.19.: Snapshots from simulation with MD-compatible model. These snapshots are not fully equilibrated, but the evolution of these simulation runs is similar to the runs with the original model. The end result should look similar to the snapshots in figure 7.1.

10. Conclusions

We have developed a model system with two components that represents mixtures of lipids and cholesterol. By simulating this model we have seen raft-like structures of nanometer scale, which can be explained by a theoretical mechanism using curvature difference between ordered and disordered structures as the main reason to prevent macroscopic phase separation. Our rafts are at the lower expected size limit for lipid rafts. If rafts are of similar size in vivo, this could explain why they were not seen yet directly in experiments. Apart from this, our findings confirm that lipid-lipid interactions alone are a sufficient driving force for raft-formation and that even two-component lipid mixtures are capable of showing this behavior. Our results agree with recent experimental findings of nanoscopic domain structures in binary mixtures of cholesterol and lipids.

Our results are also mostly compatible with the theoretical model described in chapter 1.3. We see similar raft sizes as predicted and a positive correlation between rafts in different leaflets of the bilayer. We also see the disappearance of (large) raft formation outside the two state coexistence region. Unfortunately, our model cannot provide a suitable set of parameters where we could test, whether rafts appear within a two-state region without a difference of spontaneous curvatures.

Below the raft limit we saw a trench structure of the membrane, whose nature is still unclear. The trenches somewhat resemble grain boundaries in polycrystalline materials. This could either mean that the membrane in this state actually behaves as such a material but it could also be a simulation artifact as most of our simulations start from a low cholesterol homogeneous mixture and the raft/trench system is “grown” by the simulation. Incompatible microscopic orientation of different domains could prevent a merging of these domains, although the natural state of the system would be homogeneous. We tried to resolve this issue in the sections 9.3.1 and B.3 but with inconclusive results.

Our model is quite general and is not limited to a description of DPPC/cholesterol mixtures. Indeed, some characteristics of our cholesterol model can be better matched to a phospholipid than to the traits of real cholesterol and the whole model system might, after some tweaking, also represent a mixture of two different kinds of lipids.

Two obvious future additions to the model system would be the inclusion of proteins and/or a third lipid component. The interaction of proteins and our lipid model were already subject of past studies but was no part of this work. Taking those protein models as a starting point one could study the interaction of these proteins and the lipid rafts, which would be of great relevance for studying the biological function of rafts, which is the motivation of the whole raft hypothesis. The development of a third lipid component was already attempted during this work but abandoned when two component mixtures

10. Conclusions

already (and at that time surprisingly) showed the sought-after raft structure. The goal for a third-component during our work was to either develop a line-active component or to somehow disturb the liquid ordered phase in order to bring about a separation into ordered and unordered domains. Both these effects are no longer needed for the purpose of studying rafts, but the addition of a third component would enable research regarding the (large scale) demixing of cholesterol rich and the cholesterol poor phases that is observed in three component model membranes. Apparently the third component mitigates the effects of the raft forming mechanism that we observed in binary mixtures.

A. Alternative cholesterol models

All models are linear chains of beads. Beads are numbered 0-(N-1), whereas bead 0 is the head bead and N the number of beads in the chain. Bond length potentials are numbered 1-(N-1), whereas Bond potential i is the potential between the i -1'th and the i 'th bead. Bond angle potentials are numbered 1-(N-2), whereas angle potential i is the angle potential between bead $i-1$ and $i+1$, centered around bead i . Then the commonly used cholesterol models are (highlighted values indicate values different from the standard cholesterol model, which is shown first):

Model 1, standard cholesterol model

Beads: 7

Bond lengths (all bonds): $\epsilon_{\text{FENE}} = 100\epsilon$ $r_0 = 0.6\sigma$ $\Delta r_{\text{max}} = 0.15\sigma$

Bond angles (1-4): $\epsilon_{\text{BA}} = 100\epsilon$

Bond angle (5): $\epsilon_{\text{BA}} = 4.7\epsilon$

bead type-bead type	$\epsilon_{\text{LJ}}/\epsilon$	ς/σ	r_c/ς
head(P)-head(C)	1.0	1.1	1.0
head(C)-head(C)	1.0	1.1	1.0
head(P)-tail(C)	1.0	1.05	1.0
head(C)-tail(C)	1.0	1.05	1.0
head(C)-solvent	1.0	1.1	1.0
tail(P)-tail(C)	1.0	1.0	2.0
tail(C)-tail(C)	0.9	1.0	2.0
tail(C)-solvent	1.0	1.05	1.0

Model 2

Beads: 7

Bond lengths (all bonds): $\epsilon_{\text{FENE}} = 100\epsilon$ $r_0 = 0.6\sigma$ $\Delta r_{\text{max}} = 0.15\sigma$

Bond angles (**all** angles): $\epsilon_{\text{BA}} = 100\epsilon$

bead type-bead type	$\epsilon_{\text{LJ}}/\epsilon$	ς/σ	r_c/ς
head(P)-head(C)	1.0	1.1	1.0
head(C)-head(C)	1.0	1.1	1.0
head(P)-tail(C)	1.0	1.05	1.0
head(C)-tail(C)	1.0	1.05	1.0
head(C)-solvent	1.0	1.1	1.0
tail(P)-tail(C)	1.2	1.0	2.0
tail(C)-tail(C)	1.0	1.0	2.0
tail(C)-solvent	1.0	1.05	1.0

A. Alternative cholesterol models

Model 3

Beads: 7

Bond lengths (all bonds): $\epsilon_{\text{FENE}} = 100\epsilon$ $r_0 = 0.6\sigma$ $\Delta r_{\text{max}} = 0.15\sigma$

Bond angles (**all** angles): $\epsilon_{\text{BA}} = 100\epsilon$

bead type-bead type	$\epsilon_{\text{LJ}}/\epsilon$	ς/σ	r_c/ς
head(P)-head(C)	1.0	1.1	1.0
head(C)-head(C)	1.0	1.1	1.0
head(P)-tail(C)	1.0	1.05	1.0
head(C)-tail(C)	1.0	1.05	1.0
head(C)-solvent	1.0	1.1	1.0
tail(P)-tail(C)	1.1	1.0	2.0
tail(C)-tail(C)	1.0	1.0	2.0
tail(C)-solvent	1.0	1.05	1.0

Model 4

Beads: 7

Bond lengths (all bonds): $\epsilon_{\text{FENE}} = 100\epsilon$ $r_0 = 0.6\sigma$ $\Delta r_{\text{max}} = 0.15\sigma$

Bond angles (**all** angles): $\epsilon_{\text{BA}} = 100\epsilon$

bead type-bead type	$\epsilon_{\text{LJ}}/\epsilon$	ς/σ	r_c/ς
head(P)-head(C)	1.0	1.1	1.0
head(C)-head(C)	1.0	1.1	1.0
head(P)-tail(C)	1.0	1.05	1.0
head(C)-tail(C)	1.0	1.05	1.0
head(C)-solvent	1.0	1.1	1.0
tail(P)-tail(C)	1.0	1.0	2.0
tail(C)-tail(C)	0.9	1.0	2.0
tail(C)-solvent	1.0	1.05	1.0

Model 5

Beads: 7

Bond lengths (all bonds): $\epsilon_{\text{FENE}} = 100\epsilon$ $r_0 = 0.6\sigma$ $\Delta r_{\text{max}} = 0.15\sigma$

Bond angles (**all** angles): $\epsilon_{\text{BA}} = 100\epsilon$

bead type-bead type	$\epsilon_{\text{LJ}}/\epsilon$	ς/σ	r_c/ς
head(P)-head(C)	1.0	1.1	1.0
head(C)-head(C)	1.0	1.1	1.0
head(P)-tail(C)	1.0	1.05	1.0
head(C)-tail(C)	1.0	1.05	1.0
head(C)-solvent	1.0	1.1	1.0
tail(P)-tail(C)	1.0	1.0	2.0
tail(C)-tail(C)	0.8	1.0	2.0
tail(C)-solvent	1.0	1.05	1.0

Model 6

Beads: 7

Bond lengths (all bonds): $\epsilon_{\text{FENE}} = 100\epsilon$ $r_0 = 0.6\sigma$ $\Delta r_{\text{max}} = 0.15\sigma$

Bond angles (1-4): $\epsilon_{\text{BA}} = 100\epsilon$

Bond angle (5): $\epsilon_{\text{BA}} = 4.7\epsilon$

bead type-bead type	$\epsilon_{\text{LJ}}/\epsilon$	ς/σ	r_c/ς
head(<i>P</i>)-head(<i>C</i>)	1.0	1.1	1.0
head(<i>C</i>)-head(<i>C</i>)	1.0	1.1	1.0
head(<i>P</i>)-tail(<i>C</i>)	1.0	1.05	1.0
head(<i>C</i>)-tail(<i>C</i>)	1.0	1.05	1.0
head(<i>C</i>)-solvent	1.0	1.1	1.0
tail(<i>P</i>)-tail(<i>C</i>)	1.2	1.0	2.0
tail(<i>C</i>)-tail(<i>C</i>)	1.0	1.0	2.0
tail(<i>C</i>)-solvent	1.0	1.05	1.0

Model 7

Beads: 7

Bond lengths (all bonds): $\epsilon_{\text{FENE}} = 100\epsilon$ $r_0 = 0.6\sigma$ $\Delta r_{\text{max}} = 0.15\sigma$

Bond angles (1-4): $\epsilon_{\text{BA}} = 100\epsilon$

Bond angle (5): $\epsilon_{\text{BA}} = 4.7\epsilon$

bead type-bead type	$\epsilon_{\text{LJ}}/\epsilon$	ς/σ	r_c/ς
head(<i>P</i>)-head(<i>C</i>)	1.0	1.1	1.0
head(<i>C</i>)-head(<i>C</i>)	1.0	1.1	1.0
head(<i>P</i>)-tail(<i>C</i>)	1.0	1.05	1.0
head(<i>C</i>)-tail(<i>C</i>)	1.0	1.05	1.0
head(<i>C</i>)-solvent	1.0	1.1	1.0
tail(<i>P</i>)-tail(<i>C</i>)	1.1	1.0	2.0
tail(<i>C</i>)-tail(<i>C</i>)	1.0	1.0	2.0
tail(<i>C</i>)-solvent	1.0	1.05	1.0

Model 8

Beads: 7

Bond lengths (all bonds): $\epsilon_{\text{FENE}} = 100\epsilon$ $r_0 = 0.6\sigma$ $\Delta r_{\text{max}} = 0.15\sigma$

Bond angles (1-4): $\epsilon_{\text{BA}} = 100\epsilon$

Bond angle (5): $\epsilon_{\text{BA}} = 4.7\epsilon$

A. Alternative cholesterol models

bead type-bead type	$\epsilon_{\text{LJ}}/\epsilon$	ς/σ	r_c/ς
head(P)-head(C)	1.0	1.1	1.0
head(C)-head(C)	1.0	1.1	1.0
head(P)-tail(C)	1.0	1.05	1.0
head(C)-tail(C)	1.0	1.05	1.0
head(C)-solvent	1.0	1.1	1.0
tail(P)-tail(C)	1.0	1.0	2.0
tail(C)-tail(C)	0.8	1.0	2.0
tail(C)-solvent	1.0	1.05	1.0

Model 9

Beads: 7

Bond lengths (all bonds): $\epsilon_{\text{FENE}} = 100\epsilon$ $r_0 = 0.6\sigma$ $\Delta r_{\text{max}} = 0.15\sigma$

Bond angles (**1-3**): $\epsilon_{\text{BA}} = 100\epsilon$

Bond angles (**4-5**): $\epsilon_{\text{BA}} = 4.7\epsilon$

bead type-bead type	$\epsilon_{\text{LJ}}/\epsilon$	ς/σ	r_c/ς
head(P)-head(C)	1.0	1.1	1.0
head(C)-head(C)	1.0	1.1	1.0
head(P)-tail(C)	1.0	1.05	1.0
head(C)-tail(C)	1.0	1.05	1.0
head(C)-solvent	1.0	1.1	1.0
tail(P)-tail(C)	1.0	1.0	2.0
tail(C)-tail(C)	0.9	1.0	2.0
tail(C)-solvent	1.0	1.05	1.0

Model 10

Beads: 7

Bond lengths (all bonds): $\epsilon_{\text{FENE}} = 100\epsilon$ $r_0 = \mathbf{0.5}\sigma$ $\Delta r_{\text{max}} = 0.15\sigma$

Bond angles (1-4): $\epsilon_{\text{BA}} = 100\epsilon$

Bond angle (5): $\epsilon_{\text{BA}} = 4.7\epsilon$

bead type-bead type	$\epsilon_{\text{LJ}}/\epsilon$	ς/σ	r_c/ς
head(P)-head(C)	1.0	1.1	1.0
head(C)-head(C)	1.0	1.1	1.0
head(P)-tail(C)	1.0	1.05	1.0
head(C)-tail(C)	1.0	1.05	1.0
head(C)-solvent	1.0	1.1	1.0
tail(P)-tail(C)	1.0	1.0	2.0
tail(C)-tail(C)	0.9	1.0	2.0
tail(C)-solvent	1.0	1.05	1.0

Model 11

Beads: 7

Bond lengths (all bonds): $\epsilon_{\text{FENE}} = 100\epsilon$ $r_0 = \mathbf{0.5}\sigma$ $\Delta r_{\text{max}} = 0.15\sigma$

Bond angles (**1-3**): $\epsilon_{\text{BA}} = 100\epsilon$

Bond angle (**4-5**): $\epsilon_{\text{BA}} = 4.7\epsilon$

bead type-bead type	$\epsilon_{\text{LJ}}/\epsilon$	ς/σ	r_c/ς
head(<i>P</i>)-head(<i>C</i>)	1.0	1.05	1.0
head(<i>C</i>)-head(<i>C</i>)	1.0	1.0	1.0
head(<i>P</i>)-tail(<i>C</i>)	1.0	1.0	1.0
head(<i>C</i>)-tail(<i>C</i>)	1.0	1.0	1.0
head(<i>C</i>)-solvent	1.0	1.05	1.0
tail(<i>P</i>)-tail(<i>C</i>)	1.0	1.0	2.0
tail(<i>C</i>)-tail(<i>C</i>)	0.9	1.0	2.0
tail(<i>C</i>)-solvent	1.0	1.05	1.0

Model 12

Beads: 7

Bond lengths (all bonds): $\epsilon_{\text{FENE}} = 100\epsilon$ $r_0 = 0.6\sigma$ $\Delta r_{\text{max}} = 0.15\sigma$

Bond angles (**all** angles): $\epsilon_{\text{BA}} = 100\epsilon$

bead type-bead type	$\epsilon_{\text{LJ}}/\epsilon$	ς/σ	r_c/ς
head(<i>P</i>)-head(<i>C</i>)	1.0	1.1	1.0
head(<i>C</i>)-head(<i>C</i>)	1.0	1.1	1.0
head(<i>P</i>)-tail(<i>C</i>)	1.0	1.05	1.0
head(<i>C</i>)-tail(<i>C</i>)	1.0	1.05	1.0
head(<i>C</i>)-solvent	1.0	1.1	1.0
tail(<i>P</i> , bead 1-2)-tail(<i>C</i> , bead 1-2)	1.0	1.0	1.0
tail(<i>P</i> , bead 3-6)-tail(<i>C</i> , bead 3-6)	1.0	1.0	2.0
tail(<i>C</i>)-tail(<i>C</i>)	0.9	1.0	2.0
tail(<i>C</i>)-solvent	1.0	1.05	1.0

B. Different initial conditions

In order to confirm that the rafts found in the simulations are real and not just artifacts of the simulation technique, the parameter space that showed raft formation was approached from different directions. In the usual simulation runs, the system was set up with a randomly mixed bilayer, with each chain perfectly straight and untilted on a hexagonal lattice. The cholesterol concentration was already at a level, which would be expected from the equilibrated system. The system would then be simulated in the semi-grand canonical ensemble, using the desired temperature, pressure and chemical potential.

In contrast, several simulation runs were done where this procedure was changed. The results should be the same, nonetheless.

B.1. Canonical continuation

The system was simulated in the semi-grand canonical ensemble. When an equilibrated configuration, featuring rafts, was found, the identity switch moves were turned off and the simulation was continued in the NPT-ensemble. This was done for all temperatures and chemical potential differences with no apparent change of the configurations for several million MC-sweeps. However, we should note that this number of steps is still on the same scale as the autocorrelation time of the large scale structures (with identity-switch move) and the deactivation of this move might have further frozen the MC “dynamics”.

B.2. Heating

The system is first equilibrated at $T = 1.2$, where no rafts appear because the chemical potential curve has no local maximum. Then the temperature is increased up to a value where should appear. While this works reasonably well for small systems (figure B.1, resulting in a separation of the two states), systems large enough to form rafts take prohibitively long to equilibrate using these techniques. However, the most progressed simulation runs show the onset of the typical raft formation process: Once a patch of liquid lipids occurs, it will quickly (in Monte Carlo “dynamics”) grow to a system spanning trench, that spawns more trenches, which will grow until balanced by the rafts or until they fill the whole system (figure B.2).

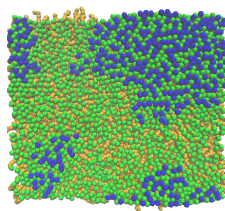


Figure B.1.: Snapshot of a small system at $T = 1.3$ and $\mu = 11.7k_B T$, heated from a homogeneous mixture at $T = 1.2$. The simulation uses cholesterol model 6 (see appendix A), not the standard cholesterol model. Thus, the value for μ is not comparable to most of the other simulations in this work.

B.3. Diluting

Systems were set up with a pure or almost pure cholesterol bilayer. The systems were then simulated in the semi-grand ensemble, using the desired parameters. The simulations behave very similar to the heated simulations, but take even longer to equilibrate. At first, more and more lipids are inserted into the membrane. However, the distribution remains mostly homogenous, just the concentration of lipids and cholesterol changes. Only after the concentration reaches the levels akin to the starting concentrations of normal simulations (i.e. somewhere between the concentration found within rafts and outside of rafts) do the systems start to form rafts. As it already takes millions of MC-steps to reach this point, and experience from the normal simulations tells us that it will take a few more million steps to equilibrate the raft configurations, none of the systems simulated with a high cholesterol starting concentration could be fully equilibrated (figure B.3) but they were clearly showing the same patterns as the systems with a low cholesterol starting concentrations during equilibration.

We also tried a similar process far below the raft limit ($\mu = 4.6k_B T$) but the trench structures that we observed when starting from lower cholesterol concentration did not appear for several million MC steps. This might indicate that the trench structures are simulation artifacts, but might also stem from the high barrier the system would have to take to form a trench structure. If the trenches develop in a similar fashion as in the raft-case shown in figure B.3, then a lipid nucleus of a certain size and concentration is required to start the process. Due to the high cholesterol concentration (about 95% at this value of μ) it is extremely unlikely to ever produce such a nucleus.

Similar simulations at $\mu = 6.9k_B T$ could not be finished in time for this work.

We would like to note that we have observed a complete merging of domains, when simulating with a μ far below the raft limit. These simulations were done “by accident” when simulating the cholesterol model with the smaller head (section 9.3).

The issue of the trench structures will be further discussed in the conclusions, chapter 10.

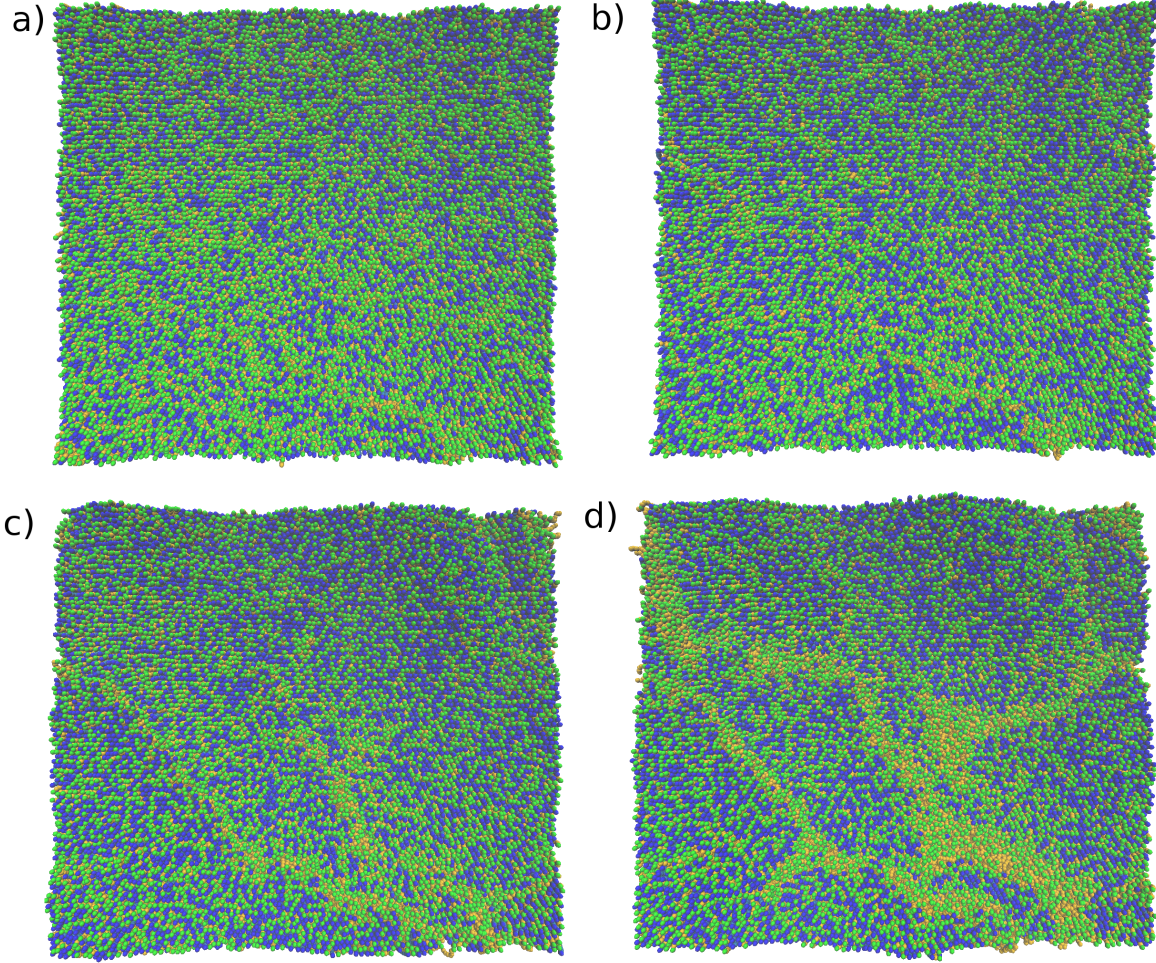


Figure B.2.: Snapshots from heating a large membrane at $\mu = 9.2k_B T$. a) a few thousand MC-sweeps after raising the temperature from $T = 1.2$ to $T = 1.4$. There is a small density fluctuation in the lower right corner. b) At the higher temperature, the fluctuation can grow instead of disappearing again. c) System spanning trenches are spawned from the fluctuation. d) Latest snapshot after several million sweeps (not yet equilibrated!): More trenches appear, their width grows.

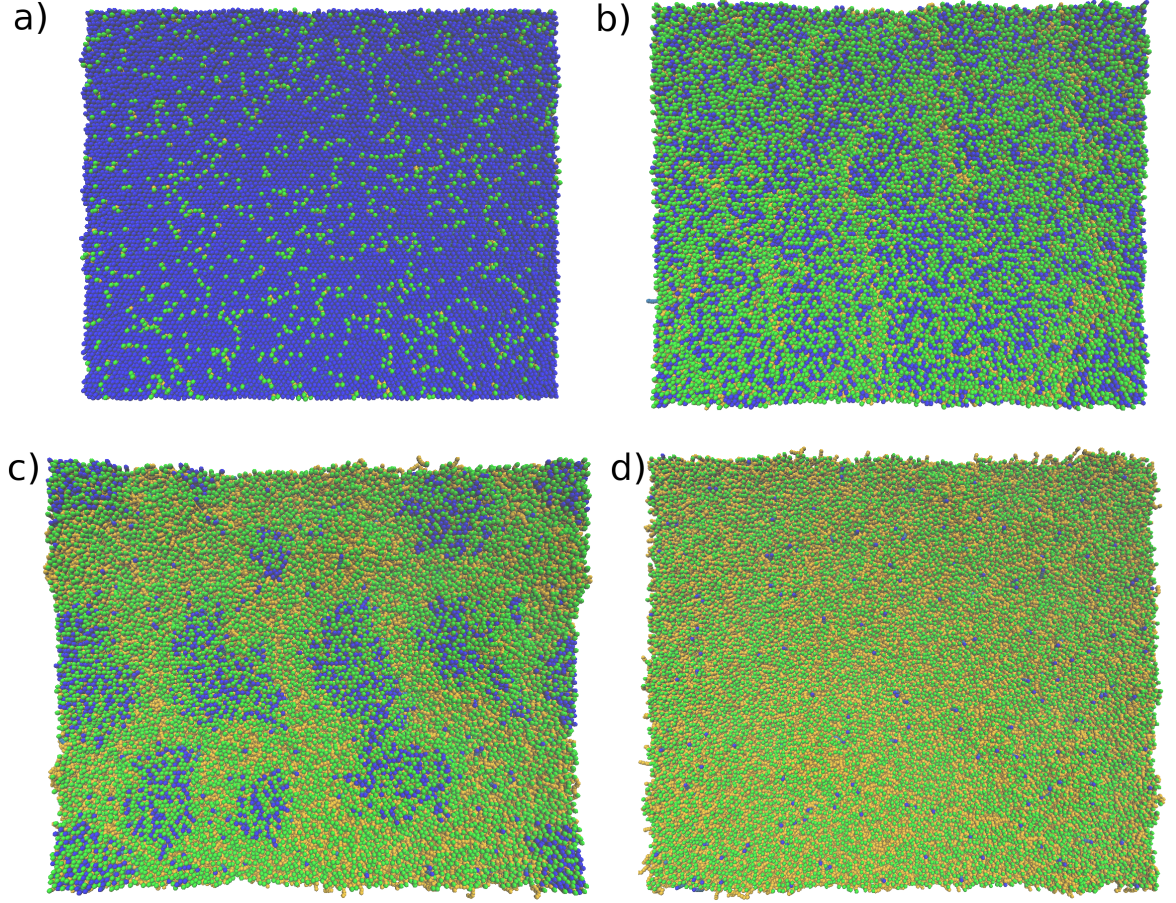


Figure B.3.: Snapshot of a system at $T = 1.4$ and $\mu = 10k_B T$ (i.e. *above* the raft limit), starting with a very high cholesterol concentration (a)). After a few million MC-sweeps the cholesterol concentration has approached usual starting concentrations but is still mostly homogeneous (b)). After another 1.5 million MC-steps the system looks like other simulations with these parameters after the first few million steps (c)). The configuration c) was the furthest progressed simulation of this kind, but did not yet reach an equilibrium configuration similar to d), which was taken from a simulation with a low starting concentration after many million mc-sweeps. The rafts in c) are not stable and still visible shrink as the simulation progresses.

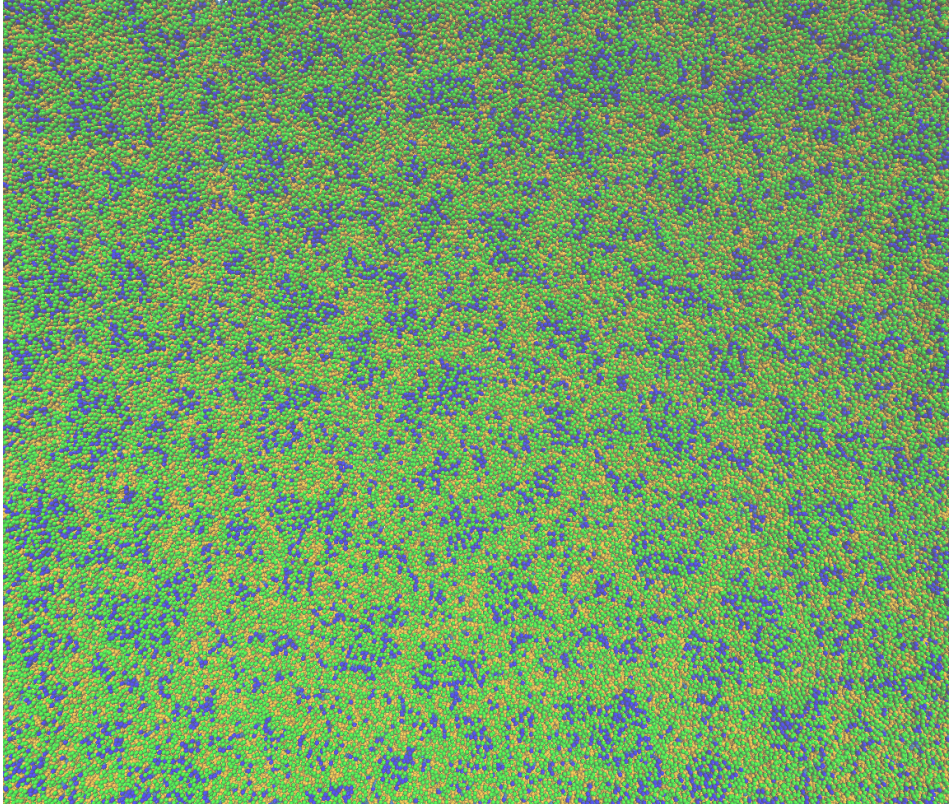


Figure B.4.: Snapshot from a system of 80,000 chain molecules. $T = 1.4$, $\mu = 10k_B T$.

B.4. Larger systems

In order to study the effect of the finite system size we simulated systems of 80,000 chain molecules, four times the size of our usual “large” systems. However, we ran into three kinds of limitations: The amount of data required for the simulations exceeded the main memory capacity of many computers at our disposal; the place required to store and evaluate the obtained trajectories severely taxed the available hard disk space until the introduction of a new file server a few months before the finishing of this work; and lastly the amount of (wall clock) time required to equilibrate these systems far exceeded even that of the already slow systems of 20,000 chains. As a result we only could produce four configurations, which are not even fully equilibrated. Figure B.4 shows such a configuration. It looks similar to the large systems seen within the raft limit, but with a much larger number of rafts and is actually simulated at $\mu = 10k_B T$, at the upper limit of raft formation, a value where almost all of the raft domains should vanish according to the simulations in smaller systems. Indeed, these domains are still in the process of shrinking, when looking at the “dynamics” of the simulation. A 20,000 chain system at this chemical potential undergoes a similar evolution during the equilibration stage.

C. A description of the DBSCAN algorithm

The “Density-based spatial clustering of applications with noise” (DBSCAN) algorithm is used several times throughout this work for the detection of connected structures from a set of spatially distributed points. It is a density-based clustering algorithm proposed in 1996 by Ester and coworkers[29]. Although we use it mostly for points on two dimensional lattices, the algorithm can be applied to any set of points with some notion of a *distance*. Using this distance one obtains the notion of a *density*, which is then used to define clusters via the concept of *density reachability*. The reason for choosing DBSCAN in this work is the ability to discover an arbitrary number of clusters of arbitrary shape and can cope with noise, i.e. points that do not belong to any clusters. In contrast, the downsides of DBSCAN, that is the inflexibility of the density criterion for data with varying density and the difficulty of using distance functions in high dimensional spaces, did not apply here. Another minor advantage of DBSCAN is, that it only requires two parameters, *Eps* and *MinPts*, with a rather intuitive interpretation (*Eps* is a cutoff radius and *MinPts* is the minimal number of points that have to be within this radius) and does not require intensive fine-tuning of these parameters for a new class of data sets. We will now give a description of the algorithm:

Definition: A point p is *directly density reachable* from a point q if the distance from p to q is smaller than the parameter *Eps* **and** if there are at least *MinPts* points within the *Eps*-neighborhood of q (this number includes the point p if the first half of the criterion is fulfilled, and also the point q itself).

Note, that this relation is not symmetric if p is at the edge of a cluster and q is within the bulk, as p might not have enough points within its neighborhood.

Definition: A point p is *density reachable* from a point q if there is a sequence of points $p_1 = q, \dots, p_N = p$ such that p_{i+1} is directly density reachable from p_i .

Again, this relation is not symmetric for the same reason that direct density reachability is not symmetric, but it is transitive.

Definition: A point p is *density connected* to a point q if there exists a point o such that p and q are both density reachable from o . As density reachability is transitive, it follows that density connectivity is symmetric.

Definition: Let D be a set of points. A *cluster* C is a non-empty subset of D with

- Maximality: $\forall p, q \in D$: If $p \in C$ and q is density reachable from p , then $q \in C$.
- Connectivity: $\forall p, q \in C$: p is density connected to q .

C. A description of the DBSCAN algorithm

Because of the definition of direct density reachability, a cluster must contain at least *MinPts* points.

Definition: The *noise* of a set of points D is defined as all points not belonging to any cluster of the set D .

Thus, we have defined clusters and noise of a given set of points. An illustration of these definitions is given in figure C.1. Ester and coworkers have shown that the clusters and the noise points can be found by the following algorithm:

- Start with an arbitrary point that has not been visited yet. (If the algorithm has just started, no points have been visited yet)
- Retrieve all points within the *Eps*-neighborhood of the point.
- If it contains at least *MinPts* points, a new cluster is started. Otherwise, the point is marked as noise. Note, that this point may later become part of a cluster, anyway, if it is within a neighborhood of another point with at least *MinPts* elements.

If a new cluster was started, it is grown:

- All points within the *Eps*-neighborhood of the starting point are also part of the cluster. These points are added to the cluster and their respective *Eps*-neighborhoods are retrieved.
 - If one of these neighborhoods contains *MinPts* or more points, the points in this neighborhood are also a part of the cluster and so forth.
 - This process continues until all points of the cluster are found.
- The whole process is repeated until no unvisited points remain.

Note that some points on the edges of clusters might swap cluster membership depending on the order in which the starting points are visited. Otherwise, the algorithm is robust against reordering of the database. Pseudocode:

```
DBSCAN(points , region_query_function , MinPoints , Eps)
{
  for all P in points:
  {
    if P was not visited:
    {
      mark P as visited

      // Get all neighbors of P from points with respect to Eps:
      P_neighbors = region_query_function(points , P, Eps)

      if (number of elements in P_neighbors) < MinPoints:
      {
        mark P as noise
      }
    }
  }
}
```

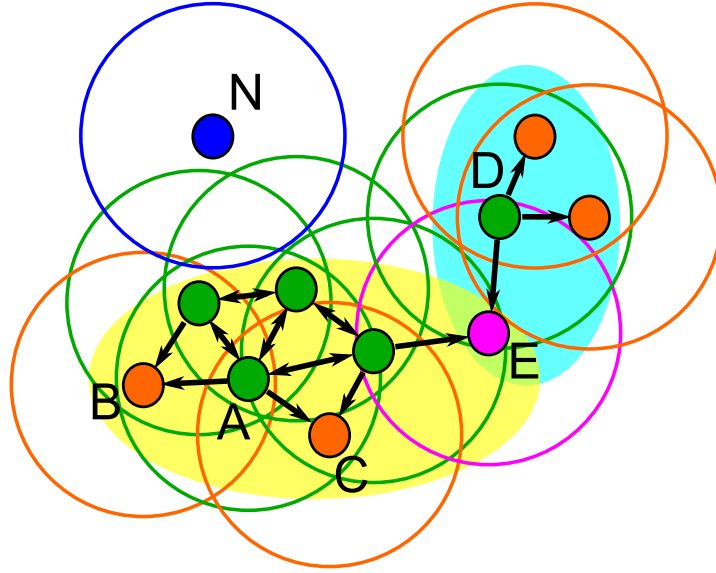


Figure C.1.: Illustration of DBSCAN concepts ($MinPts = 4$): The green points around A and the single point D are core points, having 4 or more points in their respective Eps -neighborhoods (large circles). The arrows indicate *direct density reachability*. As this relation is not symmetric, some arrows only point in one direction. The orange points are not core points, as they do not have a sufficient number of neighbors, but can be directly density reached from a core point. The points B and C are *density connected* via the green points around A and thus belong to the same *cluster* (indicated by the yellow underlay). The blue point N is neither a core point nor *density reachable* from a core point and is marked as *noise*. The purple point E is *density reachable* from both, the core points around A (forming the yellow cluster) and from the core point D (forming the center of the light blue cluster). Therefore, E would be a part of both clusters by the definition of clusters (because of maximality), but the two clusters are still distinct, because E provides no density connection between them. In practice, using the algorithm or pseudocode described in this chapter, the point E will be marked as part of either cluster, depending on the order in which the core points are visited.

C. A description of the DBSCAN algorithm

```
    }
  else
  {
    add P to current cluster
    for all Q in P_neighbors:
    {
      if Q was not visited:
      {
        mark Q as visited
        Q_neighbors = region_query_function(points , Q, Eps);
        if (number of elements in Q_neighbors) >= MinPoints:
        {
          add all elements in Q_neighbors to P_neighbors
        }
      }
      if Q is not in a cluster:
      {
        add N to current cluster
      }
    }
  }
}
}
```

D. Equilibration and autocorrelation times

The equilibration and autocorrelation times of simulations have to be established in order to be sure, that configurations – and thus the results obtained from them – taken from these simulations are both, valid equilibrium configurations and independent from each other. For equilibration, the observables with the longest relaxation times have to be watched. As was already found by Lenz [70], the total energy of the system has one of the slowest dynamics of all observables and we could reestablish this result for our extended model.

The equilibration times depend on the exact structure and configuration of the systems and are too numerous to list them all. Some rough values are:

- $9 \times 9 \times 2$ systems: $< 100k$ MC-steps (i.e. already equilibrated after prerun)
- $12 \times 12 \times 2$ systems: $< 100k$ MC-steps
- $15 \times 15 \times 2$ systems: $50k - 1M$ MC-steps
- $18 \times 18 \times 2$ systems: $100k - 2M$ MC-steps

Most of the systems at 18×18 still equilibrate within a few $100k$ steps, millions of steps are only needed in rare cases, such as membranes with high temperature liquid structures, which need significant configurational changes from the usual starting point (a perfectly ordered structure).

The data for the small systems was automatically extracted by a computer program, checking for changes in the total energy over long periods of simulation steps. With the large, $100 \times 100 \times 2$ membranes, greater care was taken and (almost) all simulations were hand checked for equilibration. Only when a specific number of steps was deemed a safe equilibration time, were configurations extracted automatically starting at this time. Usual numbers are given in table D.1.

As for autocorrelation of the membranes: While the microscopic structure changes quickly, the large scale structures, as they are observed in the large membranes, are practically frozen and do not change a lot, even over millions of simulations steps. Therefore, most independent configurations were generated by massive parallel equilibration from scratch, using different starting configurations and different random numbers for each simulation. However, at high temperatures and low cholesterol content it was still feasible to just go on with the simulation, as the large scale structures appear and disappear on a scale of $500k - 1000k$ MC-steps.

D. Equilibration and autocorrelation times

T	$\mu[k_B T]$	#sweeps
1.3	4.6	300k
1.3	6.2	700k
1.3	6.9	700k
1.3	8.5	700k
1.3	9.2	2M
1.3	11	2M
1.3	12	>5M ¹
1.3	13	700k
1.4	4.6	1M
1.4	6.2	1.2M
1.4	6.9	1.5M
1.4	7.8	>2.5M ¹
1.4	8.5	500k
1.4	9.2	1M
1.4	9.9	>3M ¹
1.4	10.3	2.5M ¹
1.4	11	700k
1.4	12	500k
1.4	13	500k
1.4	14	300k

¹ very few simulations
could be equilibrated
at these parameters.

Table D.1.: Typical equilibration times in number of MC sweeps for $100 \times 100 \times 2$ systems.
One identity switch move is done for every sweep in these simulations.

E. Trench detection algorithms

While the algorithm for detecting cholesterol enriched clusters worked well over all parameter sets, the reverse procedure, detecting the small trenches within large gel structures, could not be achieved with an equally general method. This is part due to the size of the trenches, sometimes being smaller than the usual grid length (3σ), and part due to the unclear definition, what constitutes a trench and what does not. The criterion used in section 5.1 to find lipid areas (defined to be areas without any cholesterol) is too strict to be applied to the trench structures which often contain stray cholesterol chains or are located closely to cholesterol enriched structures which fall into the same grid sector.

Therefore, a rather generic algorithm was developed, with the drawback, that the thresholds used in the method had to be fine-tuned for every set of simulation parameters, by comparing the programs output with a visual impression of the membrane. The basic idea of the algorithm is:

- Determine whether a chain molecule belongs to the top or bottom side of the membrane.
- Divide each side into squares of side length l .
- For each side look at each square and count the number of lipid beads and cholesterol beads within the square (beads, not just heads!).
 - For each lipid bead add a score of N_L to the square.
 - For each cholesterol bead subtract a score of N_C from the squares total score.
- If the total score of a grid square is greater than N , the square is considered to contain a trench.

The values used for l , N_L , N_C and N are listed in table E.1.

$\mu/k_B T$	l/σ	N_L	N_C	N
2.3	3	2	0	10
4.6	3	2	0	20
6.2	2	4	2	10
6.9	2	4	3	10

Table E.1.: A list of parameters used for the trench detection algorithm. The temperature is $T = 1.4$.

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