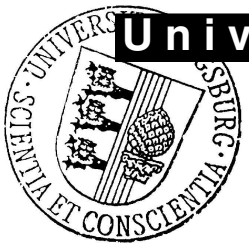

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Comparative study of the dynamics of lipid membrane phase decomposition in experiment and simulation

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Abstract

Phase decomposition in lipid membranes has been the subject of numerous investigations both experimentally and by theoretical simulation.[1] Yet quantitative comparisons of the simulated data to the experimental results are rare.[2] In this work we present a novel way of comparing the temporal development of liquid ordered domains obtained from numerically solving the Cahn–Hilliard equation and by inducing phase transition in giant unilamellar vesicles (GUVs). Quantitative comparison is done by calculating the structure factor of the domain pattern. It turns out that the decomposition takes place in three distinct regimes both in experiment and

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simulation. These regimes are characterized by different rates of growth of the mean domain diameter and there is a quantitative agreement between experiment and simulation as to the duration of each regime and the absolute rate of growth in each regime.

Introduction

The membrane of biological cells and cell organelles mainly consists of a bilayer formed by various types of lipid molecules and cholesterol. This system is highly complex due to the amount of different molecular species that are involved and interacting with each other. Giant Unilamellar Vesicles (GUVs) are used as a simpler model system. These consist only of a reduced number of lipid types and still show some of the basic properties of cell membranes. One of the main interests in research has been the coexistence of domains of separate phases with different degrees of order in the same bilayer.[3–5] Understanding the principles of this phenomenon is of great interest to biophysics and neighboring sciences, especially since it has been indicated in the past few years that for biological cells domains of different phases might play an active role in the membrane as adhesion points or transporters for proteins.[6,7] Extensive experimental effort has been put into investigating phase transitions in bilayers and e.g. for the ternary system consisting of 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) and Cholesterol a large portion of the possible mixture ratios has been investigated and charted in terms of the corresponding coexisting phases.[8] It has been found that the amount of cholesterol in the membrane sensitively influences both the dynamics of phase separation and the appearance of the domain pattern. In particular it was observed that for membranes consisting of DOPC, DPPC and varying amounts of cholesterol two different modes of phase separation appear: On the one hand nucleation and ripening of discrete domains and on the other hand spinodal decomposition.[8]

Besides experimental efforts numerous computational approaches have been applied to simulate the formation of ordered domains both in monolayer and bilayer systems. For monolayers various different models have been suggested including electrostatic line integrals,[9] Monte Carlo

methods[10] or kinetic modeling.[11] For bilayer systems Foret[12] introduced an approach based on an extended Cahn–Hilliard equation to model phase decomposition leading to domains of stable size. All of these investigations, however, lack quantitative comparison to experimental data and rather point out qualitative similarities. Krüger and Lösche[2] introduced Minkowski measures to quantify the similarity of their simulated patterns in monolayers and experimentally obtained patterns. Yet their method was limited by computational power either to small patterns of quasi 1D domains or -in the case of 2D domains- the study of insulated domains that were assumed not to interact with each other. While their results are very interesting, for real systems this assumption is only valid shortly after the onset of nucleation, however.

In this work we present a novel approach to efficiently simulate the temporal evolution of large 2D domain patterns in the regime of spinodal decomposition from the onset of phase transition until the near completion and quantitatively compare these to experimental data. Here we simulate the decomposition of two coexisting phases by applying finite element methods and solving the Cahn–Hilliard equation numerically. This proves to be a time efficient computational approach that covers the complete decomposition period. Experimentally we supercool GUVs and observe spontaneous phase decomposition using fluorescence microscopy. We compare the observed simulations and experiments by calculating the structure factor and consider its temporal development.

Methods and Materials

DPPC, 1,2-Dipalmitoyl-sn-glycero-3-phospho-rac-(1-glycerol) (DPPG) with purity >99%, DOPC as well as Cholesterol with purity of >99% were purchased from Sigma Aldrich (Munich, Germany) and used without further purification. Texas Red (TR) was applied as a fluorescent marker and obtained from Sigma Aldrich. HPLC Grade Chloroform (Avantor Performance Materials, Deventer, Netherlands) served as a solvent for all lipid mixtures.

GUVs with diameters of up to $200\mu m$ were prepared by electroformation following the method described by Angelova et al.[13] All GUVs consisted of 60 mol% lipid and 40 mol% cholesterol.

The lipid mixture was DOPC:DPPC:DPPG (5:4:1). The addition of DPPG as a charged lipid was necessary to enhance supercooling of the GUVs without inducing premature spontaneous phase decomposition. Although we are handling a quaternary system the membrane still separates into two distinct phases, justifying the concept of a binary mixture of immiscible fluids. The GUVs were supercooled in a custom made and temperature controlled chamber and observed directly after formation using a Zeiss Axiovert 200 inverted fluorescence microscope (Zeiss, Oberkochen, Germany) and a CCD camera (Hamamatsu, Herrsching, Germany) in combination.

Experiment

The GUVs were supercooled below the critical temperature and then illuminated with the microscope lamp to observe the spontaneous phase decomposition of the membrane. A representative sequence of images showing the temporal development of phase decomposition on a GUV is shown in the right hand column of figure 1. In the first frame a continuous and still diffuse pattern of domains can be seen which closely resembles what is expected in the regime of spinodal decomposition. In the second frame discrete domains have already formed, which are, however, still far away from their circular equilibrium shape. In the further course of the experiment (frame 3) the domains quickly relax to a circular shape and start to grow mainly by coalescing with neighboring domains.

Numerical simulation

The Cahn–Hilliard Equation[14] is a well known model to describe the properties of an interface between two coexisting bulk phases. We describe our binary phase system using the mole fraction c as the order parameter, where c = 0 and c = 1 represents pure phases. The local free energy per area is described by a function f, which depends on c and its derivatives. To model the decomposition we use a double well potential, $f(c) := f_0 c^2(c-1)^2$. In order to provide a minimum of regularity we assume $|\nabla c|$ to be small compared to the inverse of the intermolecular distance of the

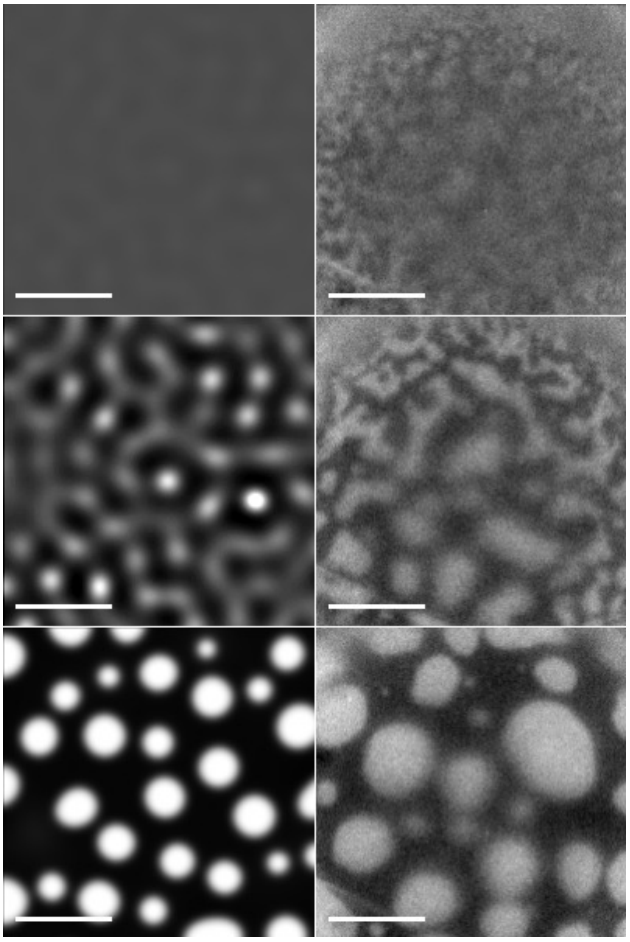

Figure 1: Comparison of domain patterns obtained from numerical simulation (left images) and in an experiment (right micrographs). Each image shows a representative pattern that is observed in the distinct phases of decomposition illustrated in figure 2. The scalebar is $10\mu m$.

components. Then the total free energy of an isotropic binary system of nonuniform composition can be written as

$$F(c) = \int_\Omega \frac{\kappa}{2}(\nabla c)^2 + f(c)dx,$$

where the scalar κ denotes the line tension. The chemical potential $\mu(c) = \frac{\delta F}{\delta c}$ allows us to introduce a dynamical system using the Fick's first law $\mathbf{J} = -M(c)\nabla\mu$, with the mobility M depending on c. Including the temporal change of the concentration $\partial_t c = -\nabla \cdot \mathbf{J}(c)$ to ensure conservation of mass, yields the Cahn–Hilliard equation

$$\partial_t c = \nabla \cdot (M \nabla [f'(c) - \kappa \Delta c]).$$

The mobility of the Cahn–Hilliard equation is directly related to the diffusion coefficient. We can capture varying diffusion values of the mixtures components A and B by a degenerated mobility $D = D_A(1-c) + D_B(c)$. For the simulations we use the free energy factor $f_0 = 5\frac{J}{m^2}$ and the same diffusion coefficient for both phases to be $D = 2.0\frac{\mu m^2}{s}$.[15]

The simulations were performed using a C^0 interior penalty discontinuous Galerkin finite element method,[16,17] which has proved to be stable and efficient for such kind of problems. This method was particularly chosen to account for the high-order spatial derivatives of the Cahn–Hilliard equation. In this way it was not necessary to introduce a coupled system of partial differential equation and instead we could use standard fourth order Lagrangian finite elements. We solve the nonlinear equation with periodic boundary condition by a Newton method. The triangulation was carefully integrated to avoid artificial symmetry effects that could be introduced by the discretization.

Results and Discussion

For comparison we analyze the experimental and simulated images by two-dimensional fast Fourier transform. The structure factor for a given frequency q, is defined as

$$\mathscr{S}(q_j) := N^{-1} |\sum_{|q_j|=q} a(p_j) e^{-i2\pi q_j \cdot p_j}|$$

with frequency vector q_j, image position p_j, image gray value $a(p_j)$ at p_j and a scaling factor N. The mean value $1/\langle q \rangle$, with

$$\langle q \rangle := \frac{\sum_{|q_j|=q} \mathscr{S}(q_j) q_j}{\sum_{|q_j|=q} \mathscr{S}(q_j)}$$

is a reliable measure for the mean domain size and can be compared over time.[18] $1/\langle q \rangle$ was calculated for the entire course of the experiment and simulation as shown in figure 2. It is apparent from the plots that the growth of the domains occurs to develop in distinct phases both in the experiment and the simulation. We distinguish three different regimes by the different slopes of the curves as indicated in figure 2. In the first section we observe spinodal decomposition of the lipid membrane. This section ends at $\sim$1.5s and $\sim$2s for the simulation and experiment respectively. The corresponding micrograph and image of simulation are compared in the upper panels of figure 1. In this range a linear fit was performed and the slopes were $s_{exp1} = 0.06 \frac{\mu m}{s}$ in the experiment and $s_{sim1} = 0.14 \frac{\mu m}{s}$ in the simulation.

The second regime lasts for approximately 3 seconds in both experiment and simulation and is characterized by a highly accelerated rate of growth of the domains. This is again quantified by slope of the curves in this section and yields $s_{exp2} = 0.6 \frac{\mu m}{s}$ and $s_{sim2} = 0.8 \frac{\mu m}{s}$. Optically in the experiment this region is characterized by the merging of neighboring domains because after formation there are many small domains in close vicinity which are prone to coalesce. In the simulation we still observe the formation of domains, yet coalescence also occurs. Images of this phase are shown in the middle panels of figure 1.

The last regime of decomposition that we observed sets in at $\sim$4.5s in the simulation and at

$\sim$5.5s in the experiment. Here the rate of growth decreases significantly yielding $s_{exp3} = 0.2\frac{\mu m}{s}$ and $s_{sim3} = 0.2\frac{\mu m}{s}$. At this stage the domains have matured and adopted a circular equilibrium shape (compare lower panels in figure 1). As the average inter-domain distance is larger coalescence is less probable to occur in this stage than in the second stage and is likely to be one of the factors that slows down growth in this regime. The mean domain diameter at the end of the considered time interval is $\sim 5\mu m$ in the experiment and $\sim 4.5\mu m$ in the simulation.

Considering the above results we see that there are both qualitative and quantitative similarities of the simulations and experiments. In both cases decomposition occurs in three distinct stages each of which is characterized by different rates of growth. The absolute rate of growth as well as the temporal length of these regimes in both systems shows a strong correlation. This suggests that the mathematical approach to apply the Cahn–Hilliard equation to model phase decomposition in lipid membranes captures the basic physical phenomena. When comparing both curves in figure 2, the relative size differences of domains for theory and experiment are in good agreement. However the absolute values for the domain size obtained for our simulations shifts to slightly smaller values. We were not able to precisely attribute this to a specific parameter used in our simulation. We would like to point out that the experimental values of D and f_0 enter the simulation and have been taken from literature. A direct measure of these parameters for our system therefore may reduce the gap between theory and experiment.

The most notable qualitative difference between experiment and simulation concerns the second regime. While in the experiment domain growth is mainly caused by coalescence in the simulations coalescence occurs but is suppressed. In fact it seems that in the simulation the maturation of the domain pattern is somewhat lagging behind. However, the slopes in this regime have comparable values ($s_{exp2} = 0.6\frac{\mu m}{s}$, $s_{sim2} = 0.8\frac{\mu m}{s}$). This suggests that we have to refine our model for example by effectively taking into account the role of the charged lipid.

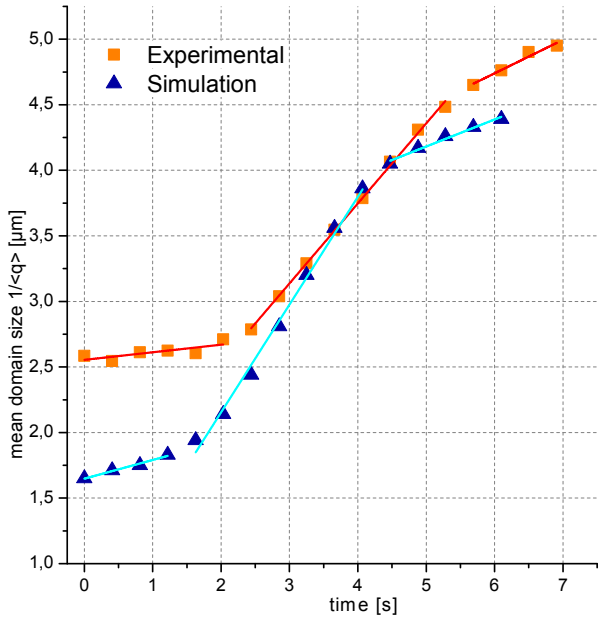

Figure 2: Evolution of the mean domain diameter during phase transitions. Orange squares mark experimental data while blue triangles represent results of numerical simulations.

Conclusion

In this work we have presented a Cahn–Hilliard approach to simulate the growth of ordered domains in lipid bilayer membranes and compare the structure of the simulated data quantitatively to microscopic observation from experiments. We demonstrated that both in experiment and simulation there are distinct regimes in phase separation where the average domain size grows with different speeds. Both the temporal duration of these regimes and the absolute growth rates are in good quantitative agreement between simulation and experiment. Furthermore the value of the experimentally obtained domain size is nicely reproduced by the simulations. We demonstrate that the mathematical approach of applying the Cahn–Hilliard equation to model interfaces is particularly useful to simulate the phase decomposition in lipid bilayer systems and provides a time efficient tool for modeling.

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