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Application Note

Micostructured lipid bilayers

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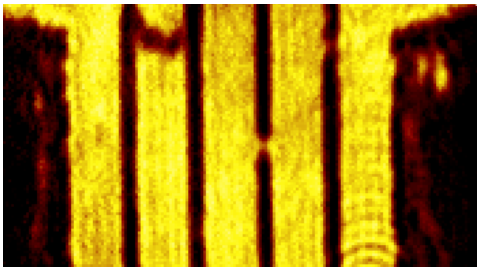

Fig.1: Thickness map of microstructured lipid bilayer stripes on silicon

Abstract

The phase transition of individually addressable microstructured lipid bilayers was investigated by means of non-contact imaging ellipsometry. 2-D membrane compartments were created on silicon substrates by micromolding in capillaries and the thermotropic behavior of various saturated diacyl phosphatidylcholines (DPPC, diC15PC, DMPC) as well as mixed DMPC/cholesterol membranes was determined measuring area expansion and thickness of the bilayer as a function of temperature. We found an increase in the phase transition temperature of 2 – 6 °C as compared with liposomes and a reduced melting cooperativity. Individually addressable microstructured lipid bilayers are clearly advantageous over conventional bilayer preparation techniques if precise measurements are needed.

Introduction

Today, solid supported membranes are among the most frequently used model systems for complex biological membranes. Although indisputable useful, the solid support itself imposes restrictions that keep researcher busy to circumvent those. The number of surface modifications to satisfy the desire to adjust a large variety of physical properties is large. Typically, high lateral mobility and facile ion transport are the key prerequisites for the establishment of useful lipid models that mimic biological membranes. However, little is known about the impact of the solid support on fundamental physical properties of lipid bilayers attached to surfaces such as the main phase temperature. It is expected that phase transitions are strongly affected if not entirely suppressed by the various different supports ranging from glass, gold,

silicon, mica to all kinds of functionalized surfaces. Since lateral expansion of the membranes might be restricted or abolished, microstructured membranes are particular useful to investigate phase changes in bilayers as a function of temperature or membrane active compounds.

Recently, we demonstrated that it is feasible to determine changes in membrane thickness and thermal expansion of SSMs during the main phase transition by means of temperature controlled AFM utilizing microstructured bilayers prepared by micromolding in capillaries. However, we also discovered that AFM might not be ideally suited to unambiguously determine thickness changes and area expansion of the bilayer due to the invasive nature of the method. In detail, we found that depending on the load force (contact mode) or the amplitude ratio (Tapping mode), the area expansion can be over-determined, since the tip spreads the lipid material across the boundaries, once the bilayer is in the liquid crystalline state. The same holds for the determination of the bilayer thickness. Depending on the bilayer stiffness, substantial indentation might lead to a systematic overestimation of the thickness change as a function of temperature. As outlined below, imaging ellipsometry, a non-contact method, appears to be much better suited to acquire changes in thickness and area expansion of microstructured lipid bilayers simultaneously. Very recently, it has also demonstrated that imaging ellipsometry of microstructured membranes allows for a quantitative determination of physical-chemical properties of supported membranes such as bilayer thicknesses, molecular areas, lateral uniformity, and ligand-receptor interactions.

Here, we present a thorough study on the thermotropic behavior of individually addressable microstructured bilayers that not only have the advantage to provide the necessary space for lateral expansion upon heating but also allow comparing the parameters with an internal standard, a lipid bilayer with known thermotropic properties. Based on this setup, it became possible to systematically detect changes of the phase transition behavior as a result of the attachment of the bilayer to the solid support. Specifically, we investigated the influence of the cholesterol content and chain length on the phase behavior of supported saturated diacyl phosphatidylcholine bilayers. Compared to results obtained with vesicles, a systematic reduction in cooperativity of the main phase transition was observed, while the main phase transition temperatures as well as the lateral diffusion coefficient depend only slightly on the solid support.

Methods and Materials

Formation of structured lipid bilayers

Individually addressable microstructured solid-supported lipid bilayers were prepared by micromolding in capillaries as described previously. In brief, all preparation steps were carried out at 40 °C, i.e. well above the main phase transition of DMPC (23°C). The silicon wafers were cleaned with diluted aqueous HF solution (1 % v/v) and activated in $NH_4OH/H_2O_2/H_2O$ (1:1:5, v/v) at 75°C for 20 minutes in order to form a thin SiO_2 layer at the same time. The oxidized wafers were stored in water and used on the same day.

Instrumentation

Imaging ellipsometer (EP^3-SW, Nanofilm Technologie, Göttingen, Germany) equipped with a a Nd:YAG laser (λ = 532 nm) was used to determine thickness and area of the structured lipid membranes.

Measurements

Phase transition of a solid supported microstructured DMPC bilayer

In the following section, we describe temperature controlled ellipsometric experiments on microstructured DMPC bilayers deposited on SiO_2-adlayers of silicon wafers. Starting at low temperature, i.e. below the main phase transition temperature of DMPC, the sample is heated up in small increments. After thermal equilibration, which took about 5-10 min after the temperature had been changed, the bilayer thickness was measured and a “Delta map” of the entire image was recorded for each temperature step. The “Delta map” was transformed into a thickness map using the corresponding optical model consisting of a thin silicon oxide layer on bulk silicon

followed by a lipid bilayer in aqueous environment (figure 2). Figure 3 shows the change in bilayer thickness t and the relative bilayer area $\Delta A/A$ as a function of temperature. The bilayer thickness is reduced from 4.6 to 3.2 nm, while the bilayer area is increased by 26 % during phase transition. Heating and cooling curves show a hysteresis of about 2 °C towards lower temperatures in the cooling cycle. The phase transition temperature is determined by fitting an empirical sigmoidal curve to the data. From the thickness change we obtain T_M = (25.0 ± 0.3) °C and from the area expansion T_M = (26.7 ± 0.4) °C (heating curves). Both curves show a rather broad transition within a regime of about 11 – 15 °C. The entire process is reversible, i.e. heating the sample once again leads to the same values for thickness and area.

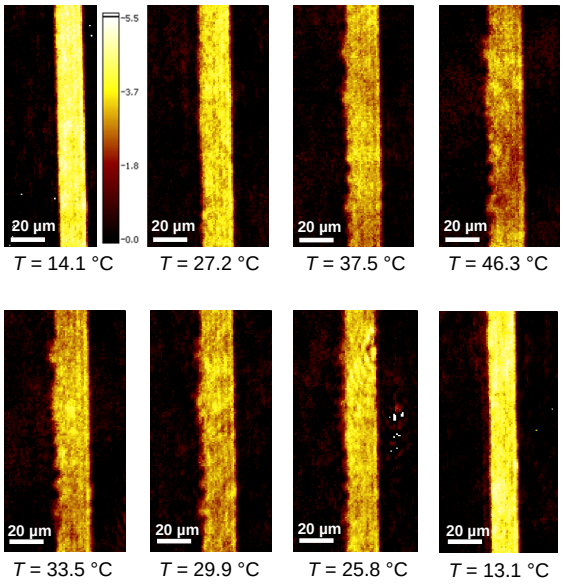

Fig.2: Ellipsometric thickness maps of structured DMPC bilayers on Si/SiO_2 at various temperatures. The height scale is valid for all images.

Phase transition temperature of phospholipids with various chain lengths

The first important benchmark of this new technique to study the thermotropic phase behavior of lipid bilayers on solid supports was to investigate the impact of fatty acid chain length on the phase transition (Fig. 4). We chose three different phosphatidylcholines with two saturated C14 (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine, DMPC), C15 (1,2-dipentadecoyl-*sn*-glycero-3-phosphocholine, diC15PC), and C16 (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, DPPC) fatty acids. From bulk measurements employing multilamellar as well as unilamellar vesicles, the phase transition temperatures and enthalpies are well known. Figure 6 shows the change in bilayer thickness and relative area expansion of DMPC, diC15PC, and DPPC as a function of temperature. All transition temperatures are shifted by 2-6 degrees to higher temperatures, while the broadness of the transition is reduced from shorter to longer chain length. Cooperativity of the phase transition, expressed as the cooperative unit size n, is at least than by a factor of 10 smaller than for MLVs and increases unexpectedly with acyl chain length from 10 to 17 molecules.

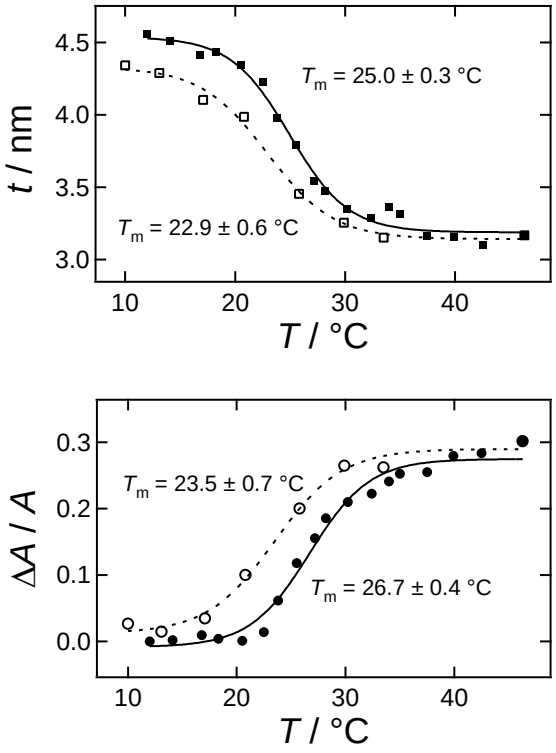

Fig.3: Changes in bilayer thickness t and relative area $\Delta A/A$ of a microstructured DMPC membrane on SiO_2/Si substrate as a function of temperature. Heating (*filled symbols*) and cooling cycles (*open*) are shown; the lines are the results of sigmoidal fits to the data of the heating (*solid*) and cooling cycle (*dashed*).

Generally, it is observed that the phase transition temperature obtained from thickness changes is lower than that extracted from the change in relative area and therefore is closer to the values that are obtained from vesicle systems. We suspect that this observation is a result of the decoupling of the two different leaflets, causing a slightly “later” response of the leaflet adjacent to the substrate, i.e. the monolayer at

the bottom melts at higher temperature than the upper leaflet. This would affect the changes in relative area to a larger extend than the shift in bilayer thickness and is a reasonable explanation for the observed reduction in cooperativity.

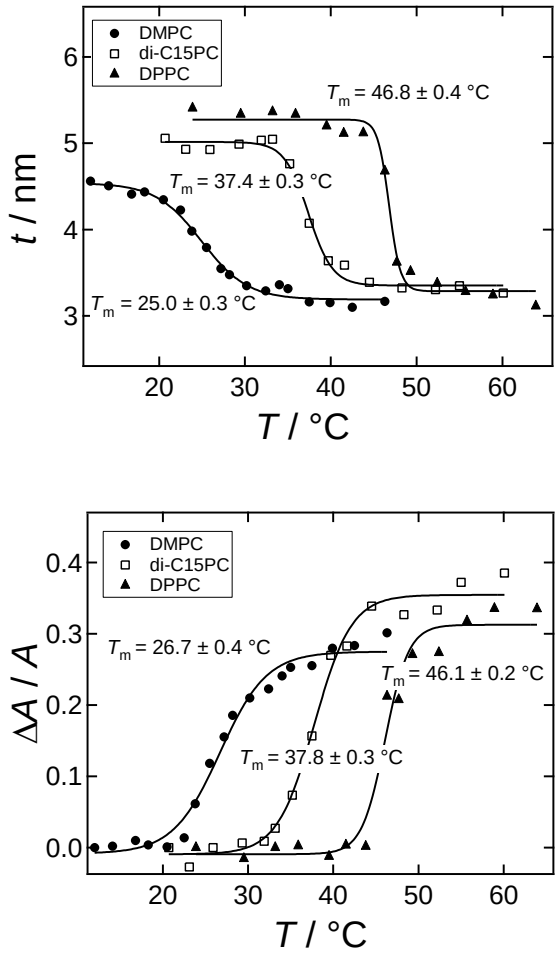

Fig.4: Changes in bilayer thickness *t* and relative area $\Delta A/A$ of various microstructured PC membrane on SiO_2/Si substrate.

Influence of cholesterol on the phase transition behavior

Cholesterol is unique in its ability to change the phase transition behavior of lipid membranes, including the formation of a "liquid ordered" phase at high cholesterol concentrations. Above, the main phase transition, cholesterol causes a large increase in average orientational order of the lipid chains, accompanied by an increase in bilayer thickness. In the gel phase, however, this effect is small, because the chains are already largely in an all-trans conformation. Therefore, one major effect of the incorporation of cholesterol into lipid bilayers is to broaden and reduce the enthalpy of the main phase transition, eventually eliminating it at 50 mol % cholesterol. Additionally, the cross-sectional area of the phospholipid molecule is decreased above and increased below the main phase transition temperature, reducing the expansion of the membrane at elevated temperature. However, little is known about the quantitative impact of cholesterol on the phase transition of lipids attached to solid supports. A real quantitative measurement is necessary, if solid supported membranes are intended to be used to establish phase diagrams or the occurrence of microdomains, which becomes increasingly important in the ongoing discussion of "rafts" in model membranes. The following experiments are carefully designed to provide a standard for the thermotropic behavior of solid supported bilayers containing various amounts of cholesterol. Figure 5 *A-F* show ellipsometric thickness maps (125 × 140 µm^2) of a sample with two lipid bilayer stripes consisting of DMPC (left) and DMPC with 40 mol % cholesterol (right) at different temperatures. The thickness of the pure DMPC bilayer is strongly reduced from 5.1 nm (T = 15.6 °C) to 3.0 nm (T = 51.1 °C), whereas the DMPC/cholesterol mixture retains a height of 4.2 nm even at elevated temperatures. Also the expansion from 15 µm to 20 µm of the DMPC stripe can be clearly observed and at T = 34.6 °C even some bulges are formed at the boundary of the stripe. This effect is absent in case of the DMPC/cholesterol stripe. Figure 6 shows the change in membrane thickness and area with temperature for various DMPC/cholesterol mixtures in the range of 0 % to 40 mol % cholesterol. Up to 10 mol % cholesterol, there is no significant change in the phase transition behavior. Thickness and area changes as a function of temperature show a sigmoidal course with a phase transition temperature of 25 – 27 °C. However, a strong influence of cholesterol on the phase transition can be observed at higher cholesterol content. At 20 mol % cholesterol, the transition temperature is increased to 32 °C and the transition is broadened to more than 20 °C. With 30 mol % and 40 mol % cholesterol, a typical cooperative phase transition can no longer be observed. Change of thickness and area are only 10 % at the highest cholesterol content. We observe a steady decrease in bilayer thickness and increase in area with temperature indicating the presence of a liquid

ordered phase. All experiments are almost fully reversible upon cooling (data not shown).

It remains to be elucidated whether the substrate inhibits lateral mobility of the lipids and therefore imposes an energy barrier for diffusion, which is responsible for both, the broadening of the phase transition and the shift to higher temperatures or if the broadening is a rather intrinsic property largely independent of the bilayer substrate interaction. To investigate the lateral mobility of the lipids, we performed FRAP experiments with different DMPC/cholesterol mixtures revealing no noticeable influence of the substrate on the lateral diffusion constants.

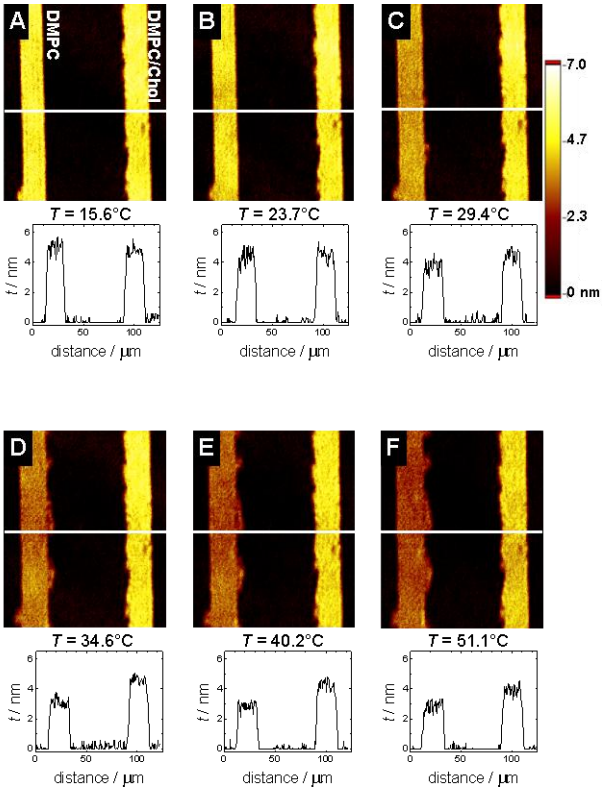

Fig. 5: Ellipsometric thickness maps (125 × 140 µm^2) of microstructured DMPC (left) and DMPC/cholesterol (40 mol % cholesterol, right) bilayers at various temperatures. A) T = 15.6 °C; B) T = 23.7 °C; C) T = 29.4 °C; D) T = 34.6 °C; E) T = 40.2 °C; F) T = 51.1 °C. The white line shows the position of the height profile given below the images. The height scale is valid for all images.

In summary, solid supported membranes display the same qualitative thermotropic phase behavior as vesicles with respect to the acyl chain dependence and cholesterol content. However, cooperativity of the phase transition is substantially reduced, which might be either an intrinsic property or a result of the presence of the solid interface. Energetically, the solid support reduces the boundary tension between the two coexisting phases. Depending on the preparation of the solid support, the main phase transition of the attached membrane may be shifted to elevated temperature as much as 2 - 6 °C. We found a slight increase in melting-cooperativity with the length of the acyl chains as opposed to studies in which vesicles were used.

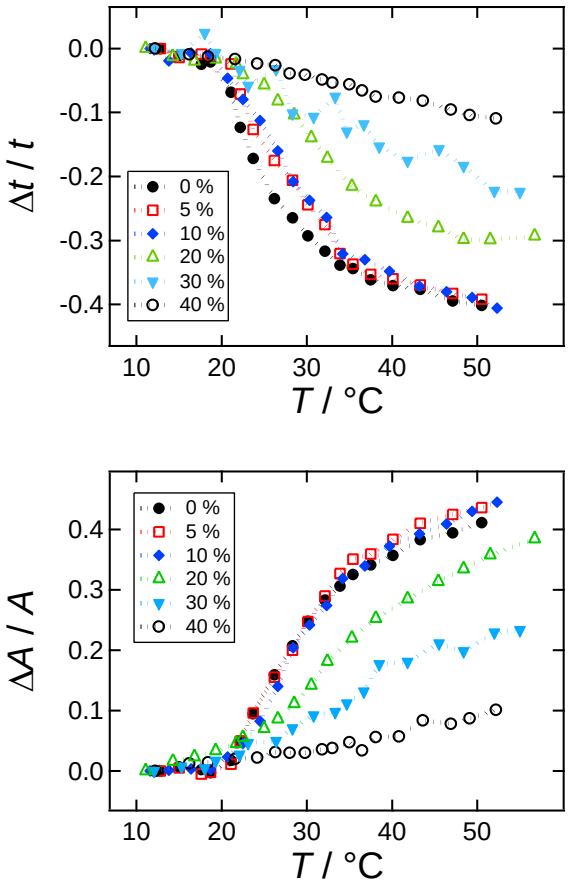

Fig. 6: Course of normalized bilayer thickness $\Delta t/t$ and area $\Delta A/A$ of solid supported microstructured membrane stripes composed of different DMPC/cholesterol mixtures as a function of temperature. The molar fractions of cholesterol (0 – 40 %) are given in the insets.

References

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[2] Gedig, M.; Faiss, S., Janshoff, A. (2008) Melting and interdigitation of microstructured solid supported membranes quantified by imaging ellipsometry. *Biointerphases* **3**, 51-58.