On the geometry of demixing: A study of lipid phase separation on curved surfaces

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CHAPTER 7

CONCLUSIONS

All animal organisms are comprised of cells, whose membranes possess specialised lipid domains that differ in their chemical composition and therefore properties and function. Their appearance and behaviour have been investigated in model lipid membranes such as vesicles and supported lipid bilayers. A fascinating feature observed in these model systems is that the phase separation process is sensitive to curvature differences. Despite many experimental and theoretical discoveries have been done on the interplay between curvature and phase separation, a complete mechanistic biophysical picture is still lacking.

In this thesis, we offer a collection of experimental tools which can be used on the one hand as a framework for previous experiments, and on the other hand to discover new phenomena in membrane physics. We present experimental systems of supported lipid bilayers (SLBs) on three different scaffolds, namely colloidal particles, surfaces topographically patterned with colloidal particles, and micro-structures obtained with a combination of micro-printing and replica-molding. Crucially, in all three SLBs we preserve the lateral lipid mobility which allows the molecules to diffuse freely and self-organise into domains.

The first experimental system consists of SLBs on colloidal particles. Because of the closed surface of the colloids, we can obtain simultaneously a closed and fixed shape of the membrane. These properties were previously studied in experiments with vesicles[4] and large SLBs[5], respectively, but were not controlled at the same time. By using this experimental setup, we showed that the curvature of the substrate regulates the organisation of the lipids in two complementary ways. First, the alternation of highly and gently curved regions favours the segregation of phase-separated domains that are more or less compliant to bending (*i.e.* geometric pinning). We show that there is a strong correlation between the ratio of the two liquid phases, the shape of the membrane, and the location of the phase-separated domains. Therefore our results shed some light on the complexity of the equilibrium configurations obtained previously in experiments with vesicles[4]. Second, curvature can alter the sorting of the lipids in the domains. Curvature-induced lipid sorting has been observed previously in experiments of tube-pulling from vesicles[6–8], and here we show that is dependent on the total geometry of the membrane. We complement the experimental results with qualitative analytical and numerical findings.

In the second experimental system, consisting of SLBs on substrates topographically patterned with colloidal particles, we presented the consequences of openness *versus* closeness of the surface. In particular, we showed that when the lipid composition is not locally conserved, the geometric pinning effect is dominant. This elucidates why in a work performed previously on supported lipid bilayers on substrates topographically patterned with half spheres[5], the phase more compliant to bending was consistently observed only in the regions of higher curvature.

Finally, we overcame the limitations of shape and size of SLBs present in literature, by introducing micro-structures as substrates obtained from 3D printing. We showed that the bilayer is homogeneous and fluid, and we used it for phase separation and fluorescence recovery after photo-bleaching experiments on anisotropic surfaces. We expect that the SLBs obtained with this method could be used to analyse the effect of curvature on

localisation and diffusion of curvature-sensing proteins and colloidal particles.

We anticipate that our experimental strategies could be straightforwardly extended to any desired shape and provide a pathway to understanding how cellular geometry influences membrane composition. We, therefore, expect this work to have a significant impact on membrane science. More broadly, our methods and results could provide general insights into phase separation of complex fluids on two-dimensional curved geometries.

Outlook: Colloid supported lipid bilayers for self-assembly

In this last section, we explore the use of phase-separated CSLBs for programmable bottom-up self-assembly.

The strategy of using phase-separated lipid membranes for self-assembly has already been exploited in experiments with giant unilamellar vesicles (GUVs) to make building blocks with anisotropic distribution of adhesive functionalities[84;212]. When vesicles phase separate, textures of ordered and disordered liquid domains are produced and either of them can be further functionalised with sticky linkers to make patchy particles. DNA modified with hydrophobic anchors[84], DNA origami nanostructures[213–216], and biotin-avidin bonds[212] were used to link vesicles and assemble them into clusters.

In these works, it was reported that the spherical shape of the vesicle can be deformed in three different ways. First, the inclusion of linkers generates spontaneous curvature[213] and tube formation[126] in the membrane. Second, the phase separation process induces the formation of anisotropic shape[4]. Third, the adhesion to other vesicles mediated by the strength of the anchor moieties further deforms the membrane[84]. Since bottom-up programmable self-assembly relies on the availability of simple and highly symmetric components encoding the assembly information[217], fixing the lipid membrane to a stable and controlled shape is highly desirable. Therefore, the experimental system of phase-separated CSLBs described in this thesis is a perfect candidate for self-assembly. Since it is based on colloids it benefits of the stable and tuneable shape, low polidispersity, and high particle yield of colloidal particles. As shown in Chapter 2 and previously,[52–55] CSLBs can be functionalised with surface mobile complementary DNA linkers with cholesterol anchors to build flexibly linked self-assembled structures. Because phase separation is a temperature-dependent phenomenon, it is expected that at high temperature, DNA linkers are free to diffuse in the membrane and that at low temperature, when phase separation occurs, they preferentially localise in the cholesterol-rich phase (Figure 7.1a). In this way, the interaction between different colloids can be tuned from uniform at high temperature to site-specific at low temperature. Finally, since the patterns of the ordered and disordered domains are defined by the lipid composition and shape of CSLBs, we anticipate that depending on these quantities we can obtain specific self-assembled structures such as micelles or networks (Figure 7.1b).

To use these particles, we first must study which lipid phase can be made adhesive, or in other words in which liquid phase the DNA linkers localise. The partitioning of the linkers into either the liquid-ordered (LO) or disordered (LD) phase is indeed one of the

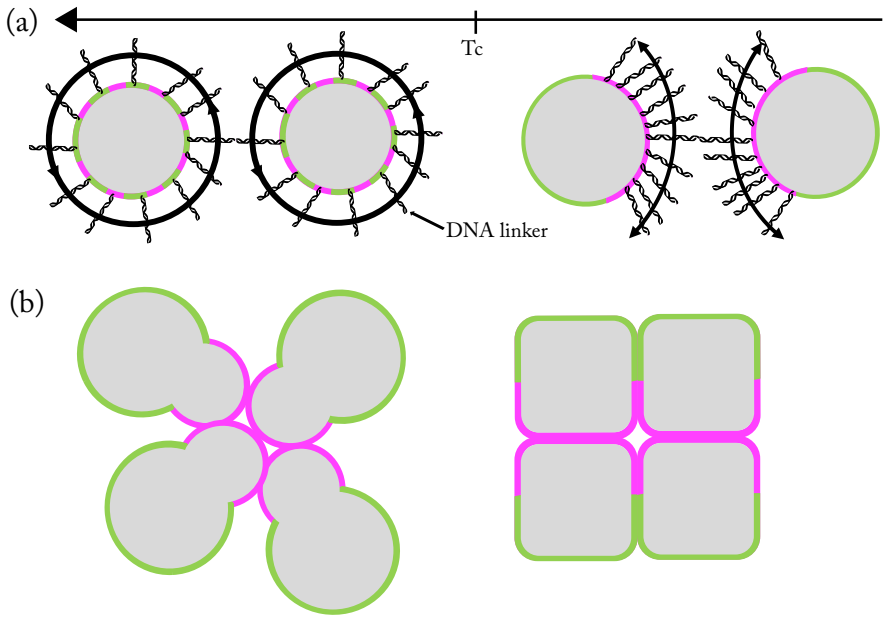

Figure 7.1: Multicomponent CSLBs for self-assembly. **(a)**Schematic representation of the experimental system. Spherical CSLBs are coated with DNA linkers. Above the transition temperature of phase separation, the linkers are free to diffuse on the surface and below, they are confined in the LD phase, shown in magenta. **(b)** Expected self-assembled structures which can be obtained using asymmetric dumbbell and cubic shape particles.

main challenge of the experiment and has been investigated in vesicles in terms of DNA structure, anchor moieties, lipid, and buffer composition[84;215]. Single and double DNA strands with cholesteryl anchors have high affinity for LO domains in membranes made of DOPC, DPPC, cholesterol, and DC[84]. Nanostructures of DNA assemble on the surface of the membrane on the LD or the LO phase depending on whether divalent ions like magnesium and calcium are present or not, respectively[214].

We performed a preliminary experiment to test in which liquid phase linkers can be incorporated. Spherical CSLBs were prepared according to the methods described in Chapter 3 with two modifications. To image the DNA with our current microscope, only one dye was used for the lipid bilayer, namely the TOP Fluor SM which labels the LO phase. A 100 mM HEPES buffer without divalent ions was used to improve the structural integrity of the DNA. Subsequently, following the methods in Chapter 2, CSLBs were functionalised with single DNA linkers modified with a cholesteryl anchor at 5' and a Cy3 fluorophore at 3', namely Cholesteryl-TEG-TTTAT CGCTA CCCTT CGCAC AGTCA ATCTA GAGAG CCCTG CCTTA CGAGT AGAAG TAGG-Cy3. High DNA concentration, 15 µL of 4.5 µM DNA for 2.5 µg of 2 µm diameter size silica particles,

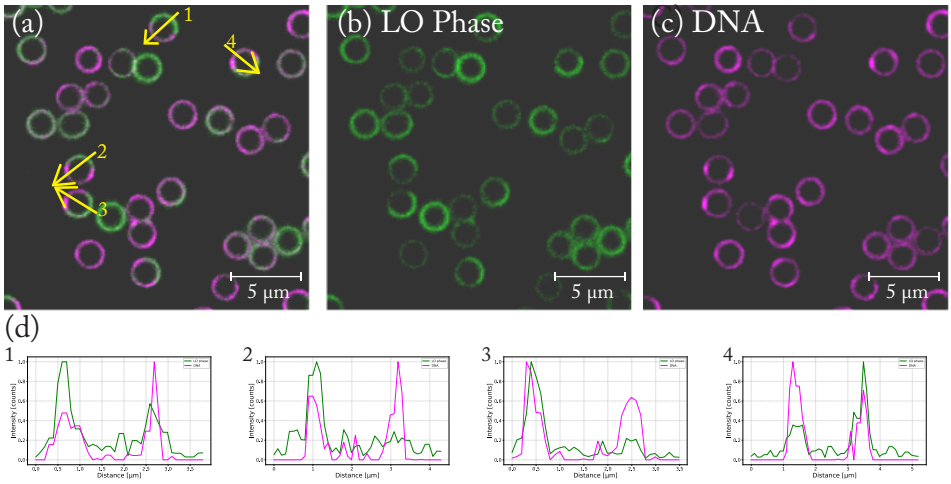

Figure 7.2: CSLBs functionalised with single-stranded DNA linkers. (a) Fluorescence image obtained by overlapping the signal from **(b)** the LO labelled with the phospholipid TopFluor SM and **(c)** the single-stranded DNA labelled with the dye Cy3. It can be observed from the figures that the two channels overlap. **(d)** Normalised fluorescence intensity profiles along the yellow lines of the four particles in Figure A. The intensity peaks of the two dyes have different heights, indicating partitioning of the DNA into the (unlabelled) liquid-disordered phase.

was used to obtain strong fluorescent signal to identifying the partitioning of the DNA.

Figure 7.2 shows that the DNA strands preferentially partition into the LD phase. This result was unexpected since the LD phase is less abundant in cholesterol than the LO phase. However, interestingly, a similar result has also been reported in experiments with GUVs made of the same lipids, namely POPC, SM, and cholesterol mixed in 1:1:1 ratio and functionalised with DNA strands modified by two tocopherol membrane anchors [218;219].

Although this preliminary result is promising, further experiments are needed. In particular, experiments with four fluorescence labels, two for the LO and LD phases and two for the complementary DNA linkers for self-assembly are required to test at the same time DNA partitioning and specificity of self-assembly. We expect that the membrane lipid composition, the DNA length and anchor moiety, and the buffer conditions can be tuned to increase the partitioning of the DNA strands into one phase.

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