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

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Summary

From the helical coiling in plants to patterns of bird flocks in the sky above us, biological systems can be elegantly described by their curvature, the measure of how much a shape deviates from flatness (Figure S.1a-b). An example of curvature is shown in Figure S.1c. When a rope is pulled from both sides it is straight and its curvature is zero, but when it is loosened to play jump, it becomes curved. In this case, the shape taken by the rope in this case is a catenary, a curve that can also be recognised in architectures of Gaudi (Figure S.1d) and in spider nets (Figure S.1e).

In cellular biology, curvature is omnipresent, for instance, in the curvature of lipid membranes. The lipid membrane is a double layer made of lipid molecules and it surrounds cells and organelles, see Figure 1.2 in the introduction for an illustration. Lipid membranes can be curved in many different ways in the cell. For example, they can be shaped to achieve specific functions by the underlying cytoskeleton or by membrane proteins. The interaction between membrane curvature and physical processes happening in the membrane is a fascinating and yet elusive subject. In this thesis, we try to shed some light on the interaction between curvature and liquid-liquid phase separation in membranes. Since natural lipid membranes are extremely complex systems and are composed by more than a thousand of different types of lipids, we use artificial membranes that are made of only three types of lipids. In these membranes, phase separation can be visualised with microscopes.

Lipids in a bilayer can segregate into coexisting domains like oil droplets in water. Oil molecules tend to neighbour with other oil molecules and minimise the contact with the surrounding water. This phenomenon is called "interface minimisation" and it is responsible for the oil and vinegar coexistence seen while preparing vinaigrette. However, differently from vinaigrette, in phase-separated membranes interface minimisation is not the only factor at play. The two liquid phases have a different stiffness because of their molecular structure. One phase is mainly composed by unsaturated lipids and the other one mostly by saturated lipids and cholesterol. The latter phase is also stiffer than the first, as saturated lipids and cholesterol molecules can pack densely. To minimise the elastic energy, the softer phase prefers to localise in high curvature regions and the stiffer phase

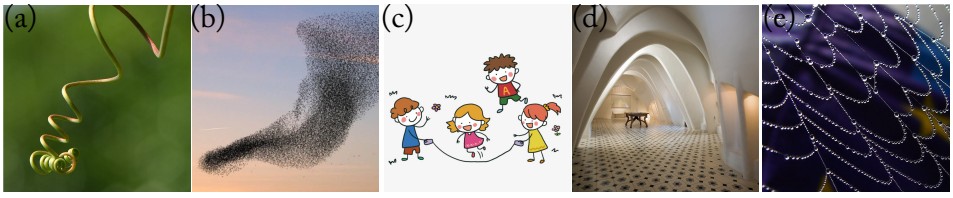

Figure S.1: Examples of curvature. (a) Helical tendrils of a cucumber plant. **(b)** Flock of birds. **(c)** Jump with a rope of catenary shape. **(d)** Catenary arches designed by Gaudi in Casa Batllo, Spain. **(e)** Spider nets of catenary shape.

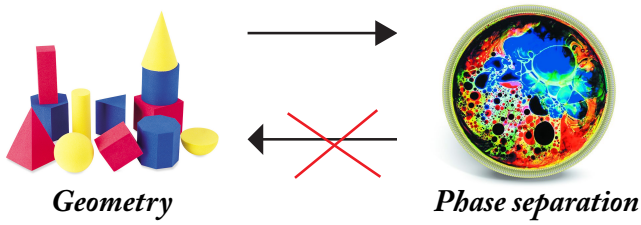

Figure S.2: In this thesis, we study how geometry affects phase separation and do not allow phase separation to affect geometry.

prefers to occupy flatter areas. We refer to this phenomenon as geometric pinning, and its competition with the minimisation of the interface governs the phase separation patterns. However, this is not the whole story because a membrane can change its shape during the phase separation process, and this makes it difficult to understand whether a membrane shape is induced by a phase separation pattern or if a phase separation pattern induces a specific membrane shape. To solve this "chicken and egg" problem, we fix the geometry of the membrane to specific shapes. In this way, we can understand how geometry influences phase separation patterns (Figure S.2).

To set the geometry of the membrane, we use particles one hundred times smaller than a human hair, called colloids. By employing different synthetic methods, we can obtain particles of various shapes. In this work, spheres, cubes, and dumbbells made of two spheres of varying radius were employed. For particle's images, see Figure 1.6 in the Introduction. Then, we fabricate small spherical lipid membranes and deposit them in contact with the colloid surface to create a lipid membrane that envelopes the particles fully.

In **Chapter 2**, we explain how we fabricate colloids functionalised with lipid membranes and characterise the homogeneity and fluidity of the bilayer. These properties are essential to obtain phase separation and depend on the colloids and lipids that are used. We show that the functionalised particles can also be used for self-assembly. Self-assembly is the ability of a material to build complex structures by itself. It is a promising novel way to obtain functional materials, *e.g.* it is studied to make photonic crystals. To assemble colloids coated with a lipid bilayer, short DNA strands are used as "molecular glue" to bind the particles together. This method has two advantages: (1) DNA sequences can be specifically designed to induce binding between particles and (2) the strands freely diffuse in the lipid bilayer allowing the particles to rearrange. In the chapter, the details of DNA functionalisation and particle binding are characterised. Then, in **Chapter 3**, we use these particles functionalised with a lipid membrane to study phase separation. We find that the softer phase is attracted to regions of high curvature, as expected. However, surprisingly, this only happens for specific lipid compositions and membrane curvatures. In general, we see that not only geometry but the interplay between the geometry and lipid composition determines the phase separation patterns.

In **Chapter 4**, we describe phase separation on curved surfaces theoretically. We show

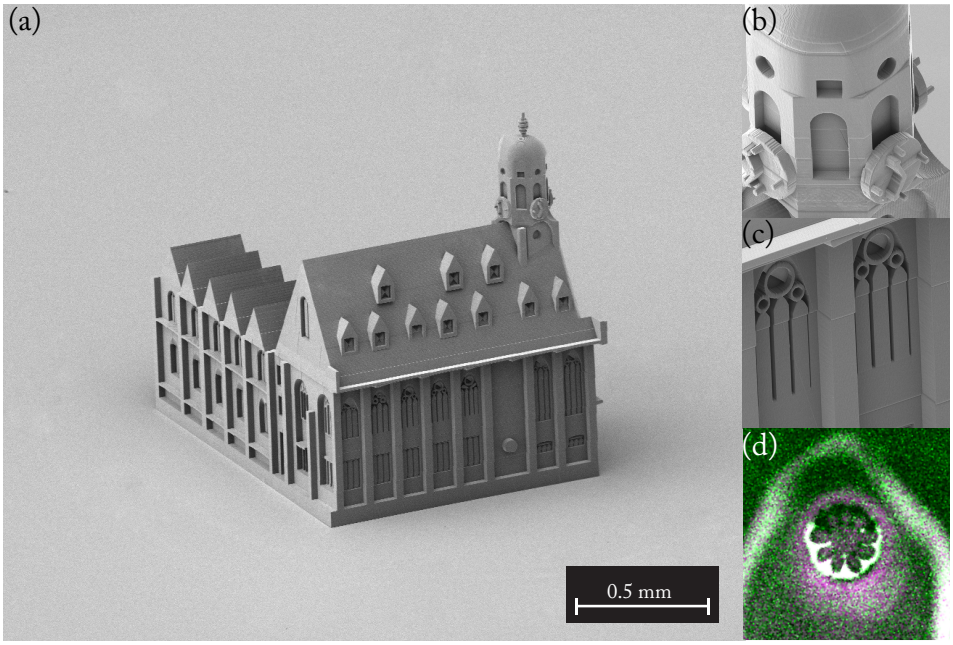

Figure S.3: 3D printed Academy building of Leiden University. (a) Scanning electron microscopy (SEM) image of the building. **(b)** SEM image of the clock tower. **(c)** SEM image of the windows. **(d)** Fluorescence microscopy image of a phase-separated lipid bilayer on one window at the entrance.

that the distribution of the liquid domains can be described by studying the interface position and that the sorting of the lipids is mediated by the interaction with curvature.

In **Chapter 5**, we fabricate supported lipid bilayers on substrates patterned with colloids, *i.e.* substrates onto which colloidal particles are attached. Since the membrane on the colloids is in contact with the membrane of the substrate, lipids can be exchanged between the colloids and the substrate. As a result, the lipid composition of the membrane on the colloids is not conserved. This is different from Chapter 3, where the lipid composition of the membrane on the colloids was conserved. We observe that because of the lipid composition is not conserved, the high curvature regions attract very strongly the softer phase. We further use these insights in **Chapter 6**, where we employ as support for the bilayer structures obtained from micro-printing and replica-molding. We show that we can fabricate lipid bilayers of arbitrary shape and we demonstrate that they are homogeneous and fluid. We can design any structure for the lipid bilayer, even the Academy Building in Leiden (Figure S.3), and perform phase separation experiments.

In **Chapter 7**, we present preliminary experiments on colloids functionalised with a phase-separated lipid membrane and DNA linkers. We show that by using phase separation we can confine single-stranded DNA into one phase and we propose further experiments to exploit phase separation to fabricate particles with site-specific binding properties.

Our findings may have a profound impact on our understanding of cellular membranes and can lead to new biomedical applications, such as drug delivery vehicles, imaging, immunochemistry, and smart materials.