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

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CHAPTER 1

INTRODUCTION

In our everyday life when mixing oil and water in vinaigrette or lava lamps, we all experience liquid-liquid phase separation (LLPS), the segregation of a liquid mixture into coexisting droplets. This fascinating physical process is present in an extensive range of systems and scales (Figure 1.1). Despite its vast manifestation, it is driven by the same simple principle: the preference of molecules to interact with their same type.

At the cellular level, LLPS has been debated for a long time as a possible mechanism to drive the formation of specialised domains in the lipid membrane. The lipid membrane, a continuous barrier that surrounds cells and organelles, presents a heterogeneous structure that is required to accomplish many essential cellular functions, including signalling[1]. The alteration of membrane heterogeneity can have physiological consequences and lead to cardiovascular diseases and cancer[2].

The presence of LLPS in living cells was controversial until latterly. Recent experimental results on living yeast cells have shown the emergence of domains through LLPS at equilibrium. To prove this, Rayermann *et al.* showed that micrometre-sized domains coalesce and can mix and demix upon temperature variations[3].

Parallel to studies with living systems, much work has been conducted on artificial model systems *in vitro* to understand the biophysical principles governing LLPS in membranes. One of the fascinating features that some of these studies displayed was the sensitivity of lipid LLPS to curvature differences[4–8]. This will be the subject of investigation of this thesis.

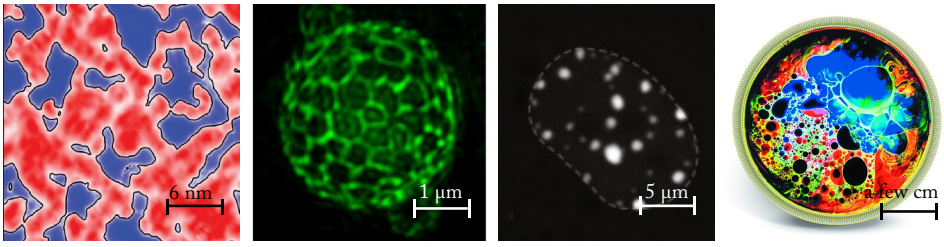

Figure 1.1: Experimental systems that show liquid-liquid phase separation. From left to right: Frozen and liquid domains coexisting in a Mott insulator[9] (reprinted with permission from Nature), lipid membrane of a vacuole of a yeast cell[3] (reprinted with permission of Elsevier), proteins in the nucleus of a HeLa cell[10] (reprinted with permission of Elsevier), and oil paint of different colours in a vase (Liquid Light Lab).

1.1 LLPS in artificial lipid membranes

The lipid membrane is a self-organised structure formed by different lipid molecules which are continuously diffusing laterally (Figure 1.2a). In this way, it can be considered a two-dimensional liquid. If a membrane is composed of a ternary mixture of phospholipids of high ($\approx 50^oC$) and low ($\approx -20^oC$) melting temperature and cholesterol, it can separate into two liquid phases[11]. One phase is rich in high melting temperature phospholipids, *e.g.* sphingolipids and cholesterol, and the other one is rich in low melting temperature phospholipids. These phases are called liquid-ordered (LO) and liquid-

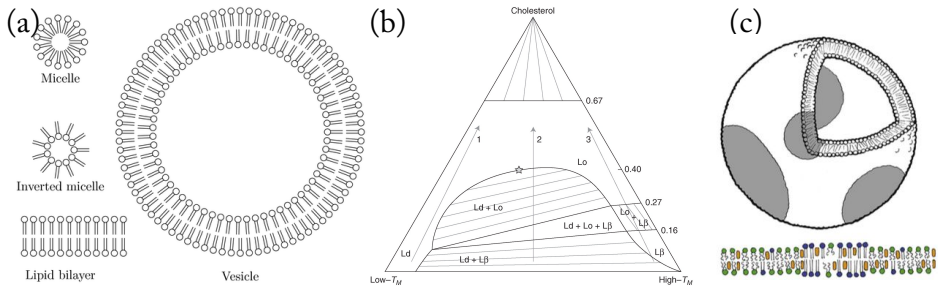

Figure 1.2: Model lipid bilayers. (a) Overview of self-assembled structures of lipids, including a vesicle and a planar lipid bilayer. **(b)** Schematic ternary phase diagram of a mixture of high melting temperature lipids, low melting temperature lipids, and cholesterol. Lβ, Ld, and Lo are the solid, liquid disordered, and ordered phases, respectively[13]. **(c)** Top: illustration of a phase-separated lipid vesicle. Bottom: phase-separated lipid bilayer composed of cholesterol (yellow), low (green) and high (blue) melting temperature lipids.[14]. Images reprinted with permission of Cold Spring Harbor laboratory press (b) and Elsevier (c).

disordered (LD), respectively, because they have a different degree of molecular order and structure. Specifically, high melting temperature phospholipids have straight acyl chains and make long-range ordered domains, whereas low melting temperature phospholipids have a kink in one acyl chain and create disordered domains[12] (Figure 1.2c, bottom). A typical phase diagram of a multi-component mixture is reported in Figure 1.2b. In this triangle-shaped diagram, every point corresponds to a specific lipid composition of cholesterol and low and high melting temperature lipids.

Lipid phase separation has been studied *in vitro* mainly using two experimental systems: vesicles, which are spherical free-standing lipid bilayers, and supported lipid bilayers (SLBs), *i.e.* planar lipid bilayers stabilised on solid supports. These systems are protein-free, easy to prepare, and can be observed and characterised with fluorescence microscopy. Because of these reasons, they have been used in soft matter physics and biophysics to investigate the many interesting properties of phase separation of lipids and two-dimensional complex fluids in general. One property which is fascinating yet elusive is the interaction between shape and phase separation patterns. We illustrate this in the next section.

1.2 Curvature and LLPS in artificial lipid membranes

When a vesicle is in the mixed state, it is always spherical because of volume minimisation. However, when it phase-separates, to minimise its free energy if the normal pressure is low it can change shape forming buds or even more exotic geometries, *e.g.* chains and star-like shapes[4]. (Figure 1.3). The origin of this behaviour is hidden in the molecular structure of the lipid domains. Since the LO and LD phases have different

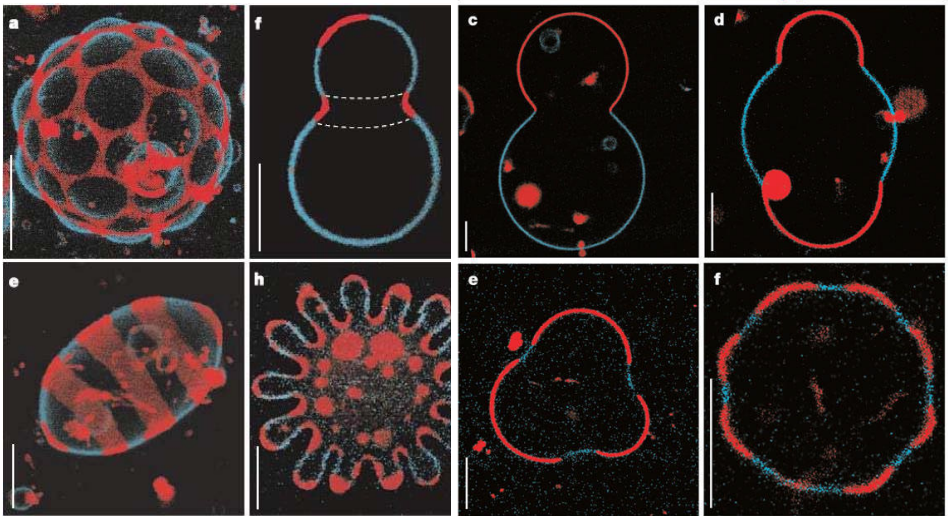

Figure 1.3: Phase-separated giant unilamellar vesicles. The liquid ordered and disordered phases are represented in blue and red, respectively. Scale bars are 5 µm. Figure reprinted from Baumgart *et al.*[4] with permission of Nature.

molecular structure and degree of order, their mesoscopic material properties are also different. This makes the disordered phase more prone to bending compared to the ordered one[15]. Simple energy minimisation arguments suggest that the disordered phase should occupy the regions of highest curvature in the membrane, while the ordered phase should avoid them. However, this simple principle does not seem sufficient to understand the heterogeneity of patterns and shapes seen in the work of Baumgart *et al.*[4]. The reason behind this is that in vesicles phase separation patterns can determine membrane shape and membrane shape can determine phase separation patterns. To get insights into this causality dilemma, different experimental systems have been developed.

In Figure 1.4, an overview of the experiments aimed at understanding the effect of curvature on phase separation in lipid membranes is shown. The experiments are grouped according to the types of model systems used: (1) vesicles in solution, (2) vesicles in contact with patterned substrates, and (3) supported lipid bilayers. Experiments on vesicles, like the ones in Figure 1.3, have performed for different lipid compositions, including lipids with non-zero spontaneous curvature[16]. In all experiments, phase separation patterns which suggest a correlation between the shape of the membrane and elastic properties were found.

The first attempt to disentangle the geometry of the membrane from the phase separation process was executed in experiments with micro-manipulated vesicles[6–8;17]. By using optical tweezers, small tubes (radii $\approx 12 - 100$ nm) were pulled out of vesicles that were in the mixed state. Sorre *et al.* measured the number of lipids going into the LD phase both in the tubes and in the vesicle. They found that the more curved the tubes,

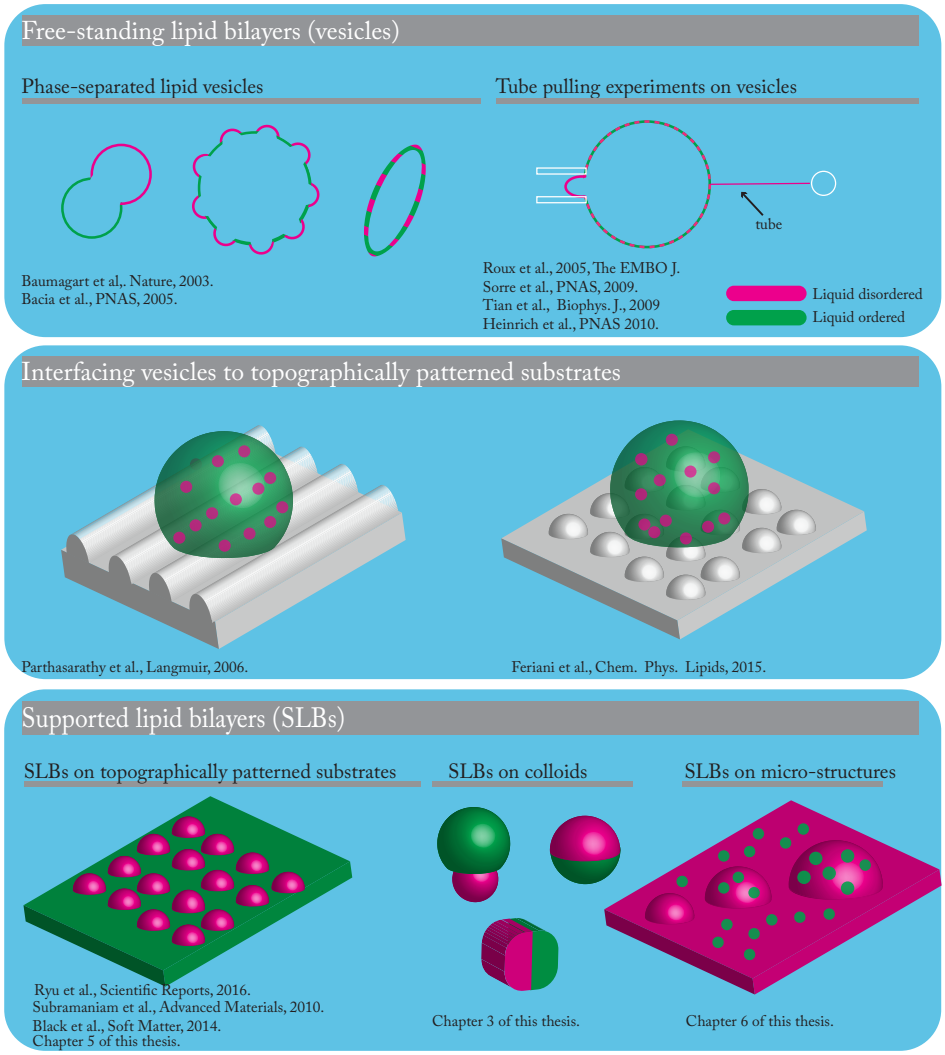

Figure 1.4: Schematic of model systems used to study the interaction between membrane curvature and local lipid composition.

the higher the number of the unsaturated lipids in the tubes[7]. Heinrich *et al.* showed that disordered domains nucleate and grow at the neck between tube and vesicle, and they ascribed this phenomenon to curvature.

This correlation between chemical composition and geometry of the membrane was further studied by approaching vesicles to topographically patterned substrates[18;19]. In these experiments, multiple circular domains were observed overall on vesicles, but the probability of finding LD domains was higher in the more curved regions, *e.g.* creases[18] and bumps[19], indicating that geometry affects the positioning of the liquid domains. However, in these vesicles, only the portion of the surface interfacing the substrate had prescribed geometry.

To achieve full control of the geometry of the membrane, supported lipid bilayers (SLBs) were used. In SLBs, the membrane shape can be fixed because the bilayer adheres to its substrate with nanoscale precision[20]. Experiments on SLBs performed on topographically patterned substrates with half-spherical[5] and step-like[21] features showed that the LD phase was pinned in regions of high curvature. This positioning was consistently regular, but the reason behind such regularity and how this regularity depends on the membrane curvature and lipid composition was not investigated. Surprisingly, Ryu *et al.* found that in SLBs on substrates with bud-like topography the ordered domains localised around the neck forming collar bands[22]. The appearance of ring-like domains in regions of high curvature seems to be in contrast to previous experiments performed in their group[21]. They ascribed the formation of these domains to minimisation of line tension and spontaneous curvature induced by fluorescent constructs.

Summarising, while in phase-separated vesicles a large variety of complex shapes and patterns were found, in phase-separated SLBs consistent and regular pinning of the softer domains in the high curvature regions was observed. In order to understand the basic physical principles behind these behaviours, we introduce in this thesis experimental systems consisting of SLBs on (1) colloidal particles, (2) substrates topographically patterned with colloids, and (3) micro-structures. The use of colloids as scaffolds for the membrane allowed us to study the effect of the geometry and lipid composition on phase separation patterns. By connecting the membrane of the colloids to a substrate and therefore allowing lipid exchange, we could study the consequences of not conserving the lipid composition locally. This was useful to understand the effect of the connection to a substrate that was present in previous experimental studies on phase-separated SLBs[5] and for the last part of this thesis in which we used microstructures obtained with micro-printing and replica-molding as support for the lipid bilayer. These structures are always supported on flat substrates.

In the following sections, some basic experimental and theoretical concepts, which are central to understand the work in this thesis, are reviewed.

1.3 Introductory experimental concepts

1.3.1 Supported lipid bilayers. In nature, lipid membranes are connected to various supporting structures[23], in particular in cells they are coupled to the actin cortex which

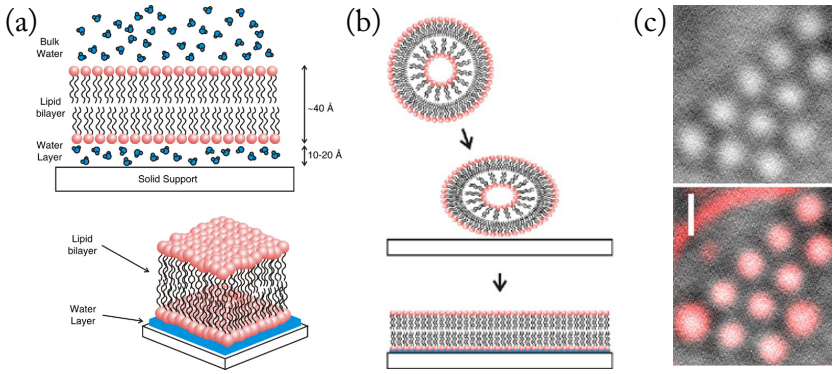

Figure 1.5: Supported lipid bilayers. (a) Schematic diagram of a solid supported phospholipid bilayer[24]. **(b)** Absorption and fusion of small unilamellar vesicles (SUVs) onto a solid substrate[24]. **(c)** Curvature modulated phase separation of a SLB obtained from GUV rupture. On a PDMS substrate with half-spherical features (bright field image on the top), the disordered phase is preferentially localised on the half-spheres, the regions of higher curvature (curvature radius $\approx$ 500 nm). Scale bar 1 µm[5]. Images reprinted with permission of Elsevier (a and b) and John Wiley and Sons (c).

provides a scaffold for the membrane. In the laboratory, such a connection can be mimicked by creating lipid membranes on top of solid substrates, so-called supported lipid bilayers (SLBs). A schematic representation of SLBs is shown in Figure 1.5a. These bilayers have stable shape and location. In this way, they can be used to model the many processes happening in the membrane and the surface interaction of the cell with the environment[24].

The main advantage of SLBs in comparison to other systems, such as black lipid membranes or monolayers, is that they are easy to prepare[24]. One of the most common methods to fabricate them, that will also be applied in this thesis, is the adsorption and fusion of vesicles from an aqueous solution to the substrate surface[24–26].
Small unilamellar vesicles (SUVs) prepared with extrusion of multilamellar vesicles through membranes with small pores or sonication are deposited onto a substrate. Under the right conditions of vesicle composition, size, surface charge, surface roughness, surface cleanliness, solution pH, ionic strength, temperature, and osmotic pressure, they rupture and then fuse forming a single bilayer[27–29] (Figure 1.5b).

Since the introduction of SLBs by Tamm and McConnell in 1985[30], their physiochemical properties have been well characterised. However SLBs have seldom been employed for studying phase separation, and understanding of the effect of substrate curvature on the phase separation process still lags behind. Inspired by previous works in which SLBs with topographical features showed localisation of disordered domains in highly curved regions[5] (Figure 1.5c), in this thesis, the power of SLBs of assuming imposed and stable shapes has been exploited. Differently from previous works, colloidal particles, flat substrates topographically patterned with colloids, and microstructures

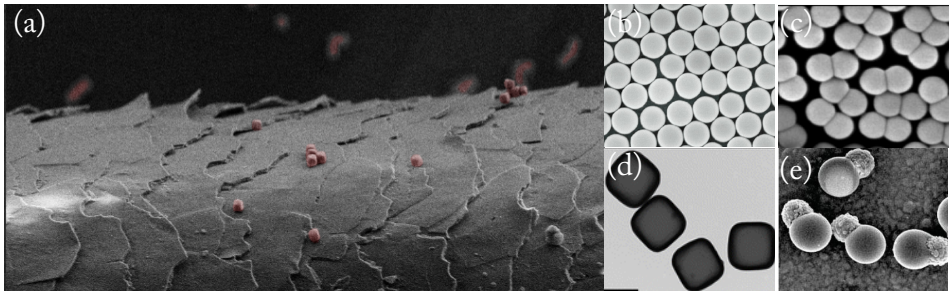

Figure 1.6: Colloidal particles. (a) Scanning electron microscopy (SEM) image of a human hair with colloidal particles (in red)[35]. **(b)** Spherical particles[36]. **(c)** Cubic shells[37]. **(d)** Symmetric dumbbell-shaped particles[38]. **(e)** Asymmetric dumbbell-shaped particles[39]. Images reprinted with permission of Iop publishing (b), Royal society of chemistry (c), MRS bulletin (d), and National Academy of Sciences (e).

obtained by combining micro-printing and replica-molding were used as a scaffold for the membrane.

1.3.2 Colloidal particles. Colloidal particles are mesoscopic sized particles suspended in a fluid. Many everyday products are made of colloids, including coffee, paint, ink, and creams. Colloids are typically one hundredth the size of a human hair or smaller (Figure 1.6a). Because of their size, they are sufficiently small that by colliding with the molecules of the surrounding fluid they undergo Brownian motion. At the same time, they are large enough to be observed with optical microscopy[31]. These particular features make them interesting model systems for atoms[32], perfect candidates for fabricating photonic crystals[33], and promising drug delivery systems[34].

Many different techniques have been developed to synthesise colloids. Each method leads to particles with a distinct shape, material, and surface properties (Figure 1.6b-e). The micrometre-size, the large availability of shapes, the tuneability of surface properties, and the possibility of being used in applications of self-assembly are the reason for our choice of colloids as substrates for SLB formation. In Chapters 2,3, and 5 silica spheres[40], hematite cubic particles[41], and polystyrene-3-(trimethoxysilyl)propyl methacrylate (PS-TPM) symmetric and asymmetric dumbbell-shaped particles[39] are used[24]. The cubic and the dumbbell-shaped particles were coated with silica using the sol-gel method in order to achieve a silica surface. As silica is highly suitable for SLB formation[29], only particles with a silica surface are used.

1.3.3 3D micro-printing. 3D printing is one of the most exciting technologies developed in our age because it allows for the top-down fabrication of designed shapes. For the first time, it enables us to create shapes described only by mathematical equations, which before could only be rendered *in silico*.

Interestingly, 3D printing has been recently extended to the microscale by using the process of two-photon polymerisation. Two-photon polymerisation is a photochemical

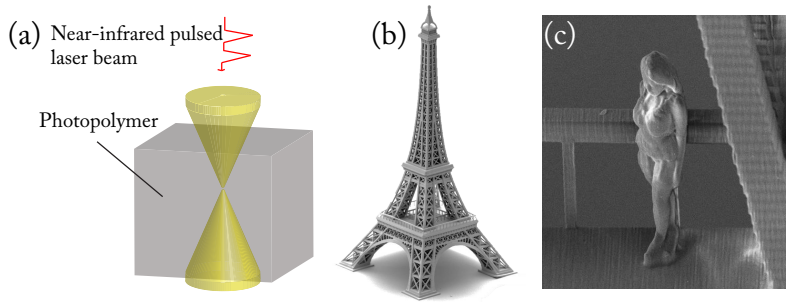

Figure 1.7: 3D micro-printing. (a) Two-photon polymerisation of resin mechanism[45]. **(b)** SEM images of 1 mm tall Eiffel tower with **(c)** a magnified view of a woman leaning from the tower. Figures reprinted with permission of John Wiley and Sons (a) and from the website of Nanoscribe GmbH (b-c).

process initiated by a femtosecond laser beam focused tightly into a photosensitive resin by a high-numerical-aperture objective[42;43] (Figure 1.7a). Three-dimensional objects are obtained by tracing the designed structure with a laser. After the printing process, the 3D printed part is surrounded by unpolymerised resin, which is removed in a developer bath. In this way, structures with sub 100 nm resolution[44] and designed geometries can be obtained, *e.g.* the Eiffel tower in Figure 1.7b-c.

In Chapter 6, we present a method in which we use 3D printing to obtain microstructures for SLB functionalisation. By using this state of the art technique, we can fabricate lipid bilayers that, unlike SLBs on colloids, can have any shape and size.

1.3.4 Self-assembly with DNA linkers. Self-assembly is the spontaneous organisation of individual disordered elements into complex ordered structures through specific and local interactions. At the microscopic scale, colloids are one of the most used elementary materials for self-assembly because they are subject to thermal fluctuations, and they can be observed by microscopy techniques. In the same manner that complex structures can be built with LEGO bricks by choosing the shape and the connections of the blocks, colloids with a specific geometry and interactions can be used to make programmable structures.

One way to program interactions between colloids is to use deoxyribonucleic acid (DNA), which can be grafted on the surface of colloidal particles[46–48] or diffuse on the surface of liquid droplets[49–51] and in the outer leaflet of lipid bilayers supported on colloids[52–55]. In the latter two systems (Figure 1.8), the linkers are free to laterally diffuse on the surface. Therefore, the resulting self-assembled structures are flexible.

In Chapter 2, a detailed analysis of the method to fabricate lipid bilayers on colloidal particles with DNA linkers is reported. In Chapter 7, the possibility of using phase-separated lipid bilayers on colloidal particles for self-assembly is explored.

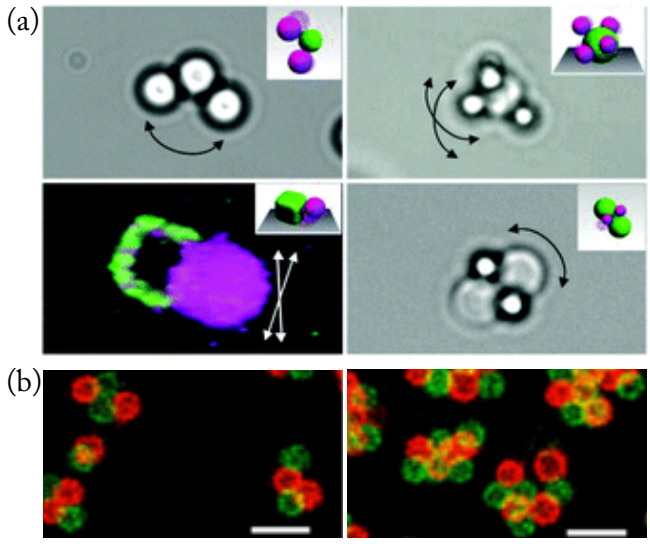

Figure 1.8: Self-assembly with flexibly linked colloids. (a) Flexible structures, including a slider (bottom left) and a hinge (bottom right) realised with self-assembled colloids coated with a lipid bilayer and DNA[55]. **(b)** Chains (left) and networks (right) created by assembly of DNA coated emulsions[51]. Image reprinted with permission of the American Physical Society. Scale bars are 5 μm.

1.4 Introductory theoretical concepts

In the last fifty years, much theoretical effort has been put into describing the process of phase separation in lipid membranes and two-dimensional physical systems in general. The approaches can be grouped into two classes. The first one focuses on the statistical nature of phase separation, treating the membrane as a set of concentration fields interacting with the environment[57;58]. The second one is a continuum elastic description[59;60] which treats lipid domains as regions on a two-dimensional surface bounded by one-dimensional interfaces. In this second approach, which is also used in this thesis, lipid membranes are described as two-dimensional fluids compliant to bending. Therefore, they can be naturally studied with the formalism of parametrised surfaces which allows for a geometrical and topological characterisation.

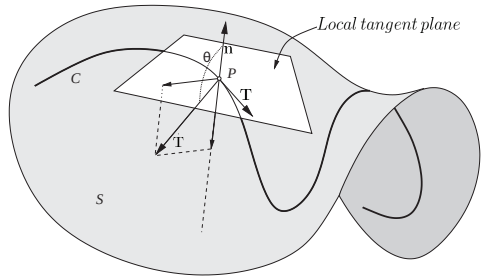

Figure 1.9: Representation of a surface S with its normal unit vector $\mathbf{n}$. Figure reproduced from Deserno[56].

1.4.1 Differential geometry of surfaces*. In this section, we introduce the elements of differential geometry necessary for the description of lipid membranes on curved surfaces and define the notation used in this thesis.

A parametrised continuous surface S in $\mathbb{R}^3$ is a continuous map $\mathbf{r} : U \to \mathbb{R}^3$, where $U \subset \mathbb{R}^2$ is an open and non-empty set. We will use only maps that are smooth, or in other words, maps in which the components of $\mathbf{r}$:

$$\mathbf{r}(u^1, u^2) = (\mathbf{r}_1(u^1, u^2), \mathbf{r}_2(u^1, u^2), \mathbf{r}_3(u^1, u^2)) \tag{1.1}$$

have continuous partial derivatives with respect to u^1 and u^2, up to all orders. For example, in the case of a sphere we can consider the parametrisation:

$$\mathbf{r}(u^1, u^2) = (\cos u^1 \cos u^2, \cos u^1 \sin u^2, \sin u^1), \tag{1.2}$$

where $(u^1, u^2) \in \mathbb{R}^2$ are usual azimuthal and polar angles on the sphere, respectively. Since the components of $\mathbf{r}$ are differentiable, we can define some useful vectors:

$$\mathbf{t}_i = \frac{\partial \mathbf{r}}{\partial u^i} \quad i = 1, 2 \tag{1.3}$$

and:

$$\mathbf{n} = \frac{\mathbf{t}_1 \times \mathbf{t}_2}{|\mathbf{t}_1 \times \mathbf{t}_2|}. \tag{1.4}$$

* This section is based on "Notes on Differential Geometry with special emphasis on surfaces in $\mathbb{R}^3$" written by Markus Deserno (2014).

The vectors $\mathbf{t}_i$ are tangent to the surface and $\mathbf{n}$ is the normal unit vector. In order to calculate the curvatures of the surface, we need to calculate the first and the second fundamental forms. These forms are uniquely set by the vectors $\{\mathbf{t}_i, \mathbf{n}\}$. The first fundamental form of a surface S is defined as:

$$h_{ij} = \mathbf{t}_i \cdot \mathbf{t}_j, \tag{1.5}$$

and has the following properties:

1. it is symmetric,
2. it can be shown that the square root of the determinant $\sqrt{h}$ is equal to $|\mathbf{t}_1 \times \mathbf{t}_2|$.

In order to define the second fundamental form, we need to first specify a curve C defined on the surface S, which crosses the point P, where the tangent vector is $\mathbf{T}$, and the principal normal vector is $\mathbf{Q}$. At this point, the surface has normal vector $\mathbf{n}$, as shown in Figure 1.9. From here, we can write the following equations:

$$\mathbf{Q} \cdot \mathbf{n} = \cos\theta \qquad \text{and} \qquad \dot{\mathbf{T}} = \kappa \mathbf{Q}. \tag{1.6}$$

We use an overdot to indicate differentiation with respect to the arc length s, namely, $\dot{(...)} = \partial(...)/\partial s$, with s the arc-length along the curve. The equation on the left shows that the angle θ is the angle between the two unit vectors $\mathbf{Q}$ and $\mathbf{n}$. The equation on the right defines the curvature of the curve C, κ.

If the curve is parametrised as $u^i(s)$, we obtain:

$$\dot{\mathbf{T}}(s) = \mathbf{t}_{i,j}\dot{u^i}\dot{u^j} + \mathbf{t}_i\ddot{u^i}. \tag{1.7}$$

Since $\mathbf{t}_i \cdot \mathbf{n} = 0$ and $\kappa\cos\theta = \dot{\mathbf{T}} \cdot \mathbf{n}$, we obtain:

$$\kappa\cos(\theta) = b_{ij}\dot{u^i}\dot{u^j}, \tag{1.8}$$

where $b_{ij} = \mathbf{t}_{i,j} \cdot \mathbf{n}$, and is called the second fundamental form.

With this description, we can observe that the curvature κ of a curve at the point P is due to the curvature of the curve and the curvature of the surface to which it belongs. In order to disentangle these two contributions, two additional quantities starting from the second form can be defined, namely the normal curvature and the geodesic curvature. The normal curvature measures how much a surface bends in space and the geodesic curvature describes how much a curve curves on a surface. The normal curvature κ_n of the surface is defined as:

$$\kappa_n = \kappa\cos(\theta) = \frac{b_{ij}u'^i u'^j}{h_{ij}u'^i u'^j} = \frac{b_{ij}du'^i du'^j}{h_{ij}du'^i du'^j}, \tag{1.9}$$

where in the last step we did a re-parametrisation of the surface:

$$\dot{u^i} = (du^i/dt)(dt/ds) = u'^i/s'. \tag{1.10}$$

From here, we can calculate in which directions the normal curvature is extremal. First, we rewrite the last equation as:

$$(b_{ij} - \kappa_n h_{ij})v^i v^j = 0. \tag{1.11}$$

Second, to differentiate with respect to v^k, we treat κ_n as a constant because $d\kappa_n$ is zero for the extremal values of curvature and we obtain:

$$(b_{ik} - \kappa_n h_{ik})v^i = (b_i{}^k - \kappa_n \delta_i{}^k)v^i = 0, \tag{1.12}$$

where in the last step we rose the k index. The directions along which the normal curvature is extremal are given by the eigenvectors of the matrix $b_i{}^k$. Their corresponding eigenvalues are the principal curvatures which we define as κ_1 and κ_2. The mean and the product of the two principal curvatures are the mean curvature H and Gaussian curvature K, respectively:

$$H = \frac{1}{2}(\kappa_1 + \kappa_2) = b_i{}^i \qquad K = \kappa_1 \kappa_2 = \frac{b}{h}, \tag{1.13}$$

where b is the determinant of the second fundamental form. In membranes, the mean curvature is associated to the cost of bending of a surface and the Gaussian curvature is associated to the cost of applying splay to a surface.

The geodesic curvature can be obtained in a similar way by projecting the curvature deformations to the surface and it is defined as:

$$\kappa_g = \kappa \sin(\theta). \tag{1.14}$$

1.4.2 The Canham-Helfrich model. The theoretical study of lipid membranes at the cell scale started with Cahnham[59] and Helfrich[60]. Given that the thickness of a lipid membrane is smaller than the length, they modelled the membrane as a two-dimensional sheet. Helfrich proposed a curvature energy in which only bending deformations play a relevant role in membrane elasticity. This model was successful in the explanation of many experimental results. For instance, it showed good agreement with experiments on the stomatocyte-echinocyte transition, the tubulation of vesicles driven by polymers, and the stretching of red blood cell with optical tweezers[61].

The Canham-Helfrich model was later generalised for a phase-separated lipid membrane by Seifert[62], Jülicher, and Lipowsky[63;64].

We follow this description and model the lipid membrane as a closed surface S, whose free energy is:

$$F = \sum_{\alpha=\pm} \int_{S_\alpha} \mathrm{d}A \, \left(\lambda_\alpha + k_\alpha H^2 + \bar{k}_\alpha K\right) + \sigma \int_L \mathrm{d}s \,, \tag{1.15}$$

where we label with $\{+, -\}$ the two lipid phases, and with $\{S_+, S_-\}$ the portions of the surface that they occupy respectively. These regions are not necessarily simply connected, and the interface between them is defined by L. The material parameters are k_α and $\bar{k}_\alpha$, that are the bending and the saddle-splay modulus, respectively. The interface line tension

between "+" and "-" domains is denoted with σ. We note that the bending modulus defined here is twice as much as the one often used in physics literature[65;66]. While the interfacial line tension and the bending modulus are concepts simple to picture, the saddle-splay modulus expresses intuitively how easy it is to make a saddle or a neck, *i.e* a region of negative curvature, on the surface. The geometry of the surface in the equation is captured by the mean and Gaussian curvatures. Finally, λ_α are Lagrange multipliers, and enforce (1) the ratio between S_+ and S_- and (2) the total surface area to be constant. They are chosen such that:

$$\int_{S_-} \mathrm{d}A + \int_{S_+} \mathrm{d}A = \varphi A_S + (1-\varphi)A_S, \tag{1.16}$$

where φ represents the area fraction occupied by the " $-$ " phase, $1-\varphi$ is the area fraction occupied by the " $+$ " phase, and $A_S = \int_S \mathrm{d}A$ is the total surface area. Equation 1.15 can be generalised by adding a spontaneous curvature term, but this is neglected here under the assumption that the two leaflets forming the lipid bilayer have identical geometry and chemical composition.

The expression of the Canham-Helfrich energy modified for phase separation has been successfully used to determine the material parameters of phase-separated vesicles[65;66]. In Chapter 3 and 4, we use Equation 1.15 to predict the equilibrium configuration of phase-separated lipid membranes.

1.5 Scope and outline of this thesis

This thesis presents a study of liquid-liquid phase separation in curved lipid membranes at equilibrium. Two new approaches to confine the lipid membrane onto curved scaffolds were developed, closed anisotropic colloidal particles and open 3D micro-printed surfaces. A detailed experimental and theoretical analysis of phase separation on curved surfaces is presented.

The thesis starts with **Chapter 2** in which the methods of the fabrication process of colloid supported lipid bilayers are described. The influence of the physical and chemical properties of the colloidal particles on the quality and mobility of the supported bilayer is characterised. We show that the insertion of lipopolymers in the bilayer in varying molar ratios and lengths affects its homogeneity and fluidity. We characterise every step in the use of CSLBs for self-assembly with DNA linkers. In **Chapter 3**, we use this experimental system to study the influence of geometry and conserved total lipid composition on phase separation. We make multicomponent CSLBs and we show that liquid domains can be geometrically pinned depending on the relative area fraction of the liquid phases and the shape of the colloidal support. Furthermore, we show that fixing the geometry of the membrane induces lipid sorting. The theoretical descriptions of phase-separation on curved geometries of a fixed shape is reported in detail in **Chapter 4**, where we consider the membrane as a two-dimensional fluid. First, from the Canham-Helfrich free energy on curved surfaces we derive a free energy functional which depends only on the position of the interface on the membrane and describes the lateral displacement of the liquid domains. Second, we introduce a curvature modified lattice gas model which allows us to account for curvature-induced lipid sorting.

In **Chapter 5**, we use colloidal particles to make topographically patterned substrates, *i.e.* flat substrates with attached colloidal particles. In this system, the membrane on the colloids is open because it can exchange lipids with a the bilayer on the flat substrate that acts as a reservoir. We show how lipid exchange affects the phase separation patterns. In **Chapter 6**, we take a step further developing a novel experimental system of lipid bilayers supported on microstructures of designed shape obtained by combining micro-printing and replica-molding lithography. By using these substrates, we show how curvature affects liquid-liquid phase separation and fluorescence recovery after photobleaching measurements.

This thesis ends with **Chapter 7** where we show preliminary experiments on phase-separated CSLBs functionalised with DNA linkers to study anisotropic distributions of adhesive linkers in flexible self-assembly.