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Soft pinning of liquid domains on topographical hemispherical caps

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ABSTRACT

The role of lipid composition as a regulator or mediator of processes that take place in biological membranes is a very topical question, and important insights can be gained by studying in vitro model lipid mixture systems. A particular question is the coupling of local curvature to the local phases in membranes of mixed composition. Working with an experimental system of giant unilamellar vesicles of ternary composition, the curvature is imposed by approaching the membrane to a topographically (on the micron scale) patterned surface. Performing experiments, we show that domains of the more disordered phase localise preferentially to regions of higher curvature. We characterise and discuss the strength of this "caging" behaviour. In future, the setup we discuss here could prove useful as a platform to localise domains rich in membrane proteins, or to promote the onset of biochemical processes at specific locations. Finally, we note that the methods developed here could have also applications in bio-sensing, as a similar but metal coated topography can sustain plasmonic resonances.

1. Introduction

Artificial lipid bilayers are interesting systems from many different perspectives: their self-assembling nature makes them relatively easy to manipulate in ambient conditions, and they are a useful 2D model system to study new physics questions and to trial designs of hierarchical complexity. They are also useful model systems to understand biological processes, as their physical-chemical properties underpin various functional aspects of biological membranes, which in turn are a fundamental component of cells. Of particular interest to ourselves and others, multicomponent lipid membranes are the simplest model to study liquid–liquid phase separation, possibly linked to the concept of lipid "rafts" which have been proposed as an important aspect of lipid–protein interactions in the plasma membrane of cells, and linked to adhesion, endocytosis, protein complexation, apoptosis, and lipid regulation (Simons and Ikonen, 1997; Edidin, 2001; Veatch and Keller, 2002).

This paper focuses on the influence of curvature on the lateral organisation of liquid–liquid phase separated lipid bilayers. This particular aspect is very interesting for biology, as it could shed some light on the sensitivity to the curvature shown by some membrane proteins (Parthasarathy et al., 2006; Mouritsen, 2011), and more generally to the coupling of curvature to lipid composition (Sorre et al., 2009; Kamal et al., 2009; Tian and Baumgart, 2009). In turn, protein localisation is linked to complex processes, such as the growth of actin filaments (Gallop and Walrant, 2013), that seem to be influenced by curvature.

Finally, understanding how phase separated bilayers laterally organise in presence of locally induced curvature may lead us to harness this effect for practical purposes such as biosensing: for example, localising particular membrane components or membrane bound species to species a pattern on the nano- or micro-scale could make a powerful combination with plasmonic resonance probes (Christensen and Stamou, 2010).

A brief literature review and background information are provided in Section 2, then the experimental methods, which cover the crafting of the microstructured surface, the preparation of lipid bilayers, and the imaging methods are given in Section 3. The analysis of domain localisation is reported in Section 4. The main result shown here is the preferential localisation of liquid disordered domains onto topographical bumps.

2. Background

2.1. Liquid–liquid phase separation

Biological membranes are composed of thousands of lipid species, well regulated in the various subcellular organelles (van Meer et al., 2008). The protein concentration is also high (by weight and area fraction, but not as a molar fraction). Despite this

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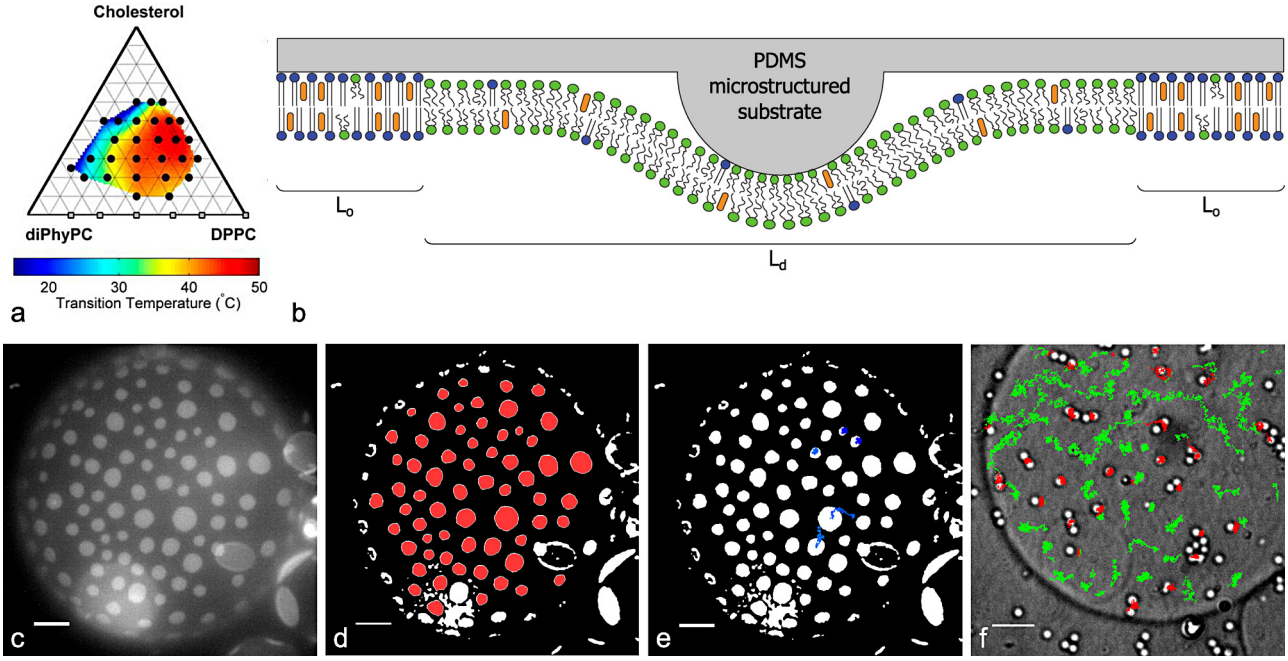

Fig. 1. (a) The phase diagram of a system very close to the one investigated here has been published in Veatch et al. (2006), and shows an extended (and temperature dependent) region of coexistence between a liquid disordered and a liquid ordered phase; (b) schematic of the experimental condition in the experiments carried out in this work, where the top of a Giant Unilamellar Vesicle is brought close to a patterned substrate, so that the lipid membrane bilayer is pressed against the hemispherical "bump" topography. (c,d,e,f) Optical microscopy images of domains and bumps, illustrating the steps taken in image analysis: (c) is a frame, as obtained by epifluorescence; (d) is the result of filtering and thresholding, and the shaded domains are the ones that survive the rejection criteria, and are used in later analysis; (e) shows as an example five tracks, superposed to the filtered and thresholded first frame of the time lapse; (f) all the "on-bump" domain tracks are plotted in red, the "off-bump" tracks in green. The tracks are superposed to a bright field image of the vesicle in contact with the patterned substrate: the bright spots are the bumps, and the dark circle is the outline of the vesicle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

richness in composition, membranes exhibit similar thermodynamical behaviour to much simpler systems, such as ternary mixtures made of a saturated lipid, an unsaturated one and a sterol (Veatch et al., 2008; Honerkamp-Smith et al., 2008). What is observed in these mixtures is a region (bounded in composition, and temperature) of phase coexistence between two liquid regions, one enriched in the saturated lipid and cholesterol, the other enriched in the unsaturated lipid (Veatch and Keller, 2002; Veatch et al., 2008). These phases are named liquid-ordered (*Lo*) and liquid-disordered (*Ld*) respectively, see Fig. 1(a). A wide variety of ternary lipid mixtures containing a high melting temperature (T_m) lipid (usually with saturated acyl tails), a low T_m one (usually unsaturated) and a sterol have been shown to exhibit coexistence of liquid phases (Veatch and Keller, 2005). These liquid phases have been observed both in Giant Unilamellar Vesicles (GUVs) and in supported bilayers.

In model systems, phase separation can be induced for example by cooling a system prepared at close to its critical composition; then, coexisting phases are formed by spinodal decomposition, or by nucleation and growth (Stanich et al., 2013). In some experiments, this separation process proceeds to completion, i.e., domains coalesce until the system is made of only two spatially distinct regions. In other experiments, and particularly in closed systems such as vesicles, where constraining the enclosed volume imposes strong constraints to the allowed overall vesicle shape, meso-scopic domains are seen to coalesce very slowly, or even to remain stable over time Idema et al. (2010). It is not clear if these intermediate phases are metastable states, or equilibrium states of the system. In either case, domains always exist in the system for a long time (even when domain coalescence is not hindered, micron-sized domains exist for tens of minutes during coarsening).

Both phases are liquid, and characterised by fast lateral diffusion, high rotational freedom and short range order, but they present some important differences, in both composition and physical properties (Hirst et al., 2011). The L_o region of the membrane is around 1 nm thicker (characterised with AFM measurements (Burns et al., 2005; Lawrence et al., 2003)) and has higher bending modulus and viscosity; the differences between phases depend on the point of the diagram phase, vanishing as the critical point is approached (Connell et al., 2013; Yoon et al., 2010; Cicuta et al., 2007). It is important to note that the lipid domains have been shown to be in registry between the two leaflets of the bilayer (Korlach and Schwille, 1999; Marrink et al., 2007; Collins and Keller, 2008; Collins, 2008).

2.2. Physical properties

Lipid domain morphology is determined, in the plane of the membrane, by the line tension σ (the two dimensional analogue of surface tension). This tends to minimise the energy cost of the phase boundaries by maintaining circular lipid domains. Line tension has been characterised by flicker spectroscopy of fluorescently labelled domains in ternary GUVs (Honerkamp-Smith et al., 2008), and by AFM on supported lipid bilayers (Connell et al., 2013). The line tension decreases linearly as a function of the temperature difference to the critical temperature. In the work presented here the line tension is between 0.4 and 1.2 pN (Honerkamp-Smith et al., 2008).

The motility of domains is controlled by their size, and by the viscosity of the surrounding membrane. When the surrounding has high viscosity (typically, when it is in the L_o phase), then membrane viscosity can be extracted from the diffusivity of lipid domains in a Brownian regime (Cicuta et al., 2007; Petrov and Schwille, 2008). In the conditions of this work, the L_o phase has a viscosity of the order of 10^{-7}Ns/m (Cicuta et al., 2007).