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Solid-like domains in fluid membranes

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Abstract

We study model membranes in the form of giant unilamellar vesicles (GUVs) composed of two saturated lipids with different hydrophilic headgroups or different hydrophobic chain lengths. Lateral phase separation in the lipid bilayer causes solid-like 'gel' domains to nucleate and grow in the fluid membrane. We study the shape and size of these domains as well as their growth and interactions.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

In membranes containing as few as two lipid species, phase separation can lead to the coexistence of fluid and solid domains [1–4]. The properties and behaviours of solid-like domains in a fluid membrane are not only interesting from a scientific perspective, but are also of biotechnological interest [5]. For these reasons, such inclusions have been widely considered in theory and simulation [6, 7]. While some experimental studies exist [8], many phenomena remain unexplored.

Here we present a survey of observations of solid-like (or gel) domains in model membranes. We have studied mixtures of two zwitterionic lipids and mixtures containing one charged lipid and one zwitterionic lipid. We observed circular as well as stripe-like domains. Strikingly, mixtures containing lipids with no net charge display size-limited domains and inter-domain repulsion; analogous phenomena in lipid monolayers arise from electrostatic repulsion [9, 10], but this seems not to be the case in all our systems.

2. Methods

Fully saturated lipids were purchased from Avanti Polar Lipids and used without further purification: 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC), 1,2-dipalmitoyl-

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sn-glycero-3-phosphocholine (DPPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE), 1,2-dipalmitoyl-*sn*-glycero-3-[phospho-L-serine] (sodium salt) (DPPS), and 1,2-dipalmitoyl-*sn*-glycero-3-phosphate (monosodium salt) (DPPA). Lipids were mixed in chloroform in the desired ratios. For some observations, the hydrophobic salt tetrabutylammonium chloride (TBAC, from Fluka) was mixed with the lipids at 10.0 mol%.

Giant unilamellar vesicles (GUVs) with a diameter of 10–80 μm were prepared by electroformation [11]. Lipids dissolved in chloroform solution were dried on platinum wire electrodes and subsequently hydrated with deionized water. Vesicles were formed at a temperature higher than the chain-melting temperatures of both lipids in each mixture; the electroformation chamber was resistively heated and the temperature measured using a thermocouple. The electroformation voltage of 3–6 V_{pp} at 10 Hz was applied across the electrodes for 30–90 min. In the presence of salt (100 mM NaCl), this time was decreased to less than a minute to minimize photobleaching and vesicle destruction by gas bubbles liberated by hydrolysis; this short electroformation produced a few spherical GUVs and many giant unilamellar mushroom membranes, which were not closed to form vesicles. After vesicles had formed, the temperature was lowered (with cooling rates of 0.1–0.4 °C min^{-1}) so that the lipids demixed and two phases coexisted. Observations were done *in situ* with the vesicles adhering to each other and to the electrodes on which they were formed. This adhesion tensed the membranes so that thermal fluctuations were not visible with optical microscopy.

Phase separation in the vesicle membranes was visualized using trace amounts, 0.1–0.5 mol%, of preferentially partitioning amphiphilic fluorescent dyes purchased from Molecular Probes: Lissamine rhodamine B 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (Rh-DPPE), 2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-pentanoyl)-hexadecanoyl-*sn*-glycero-3-phosphocholine (BODIPY), 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate ($\mathrm{DiIC}_{18}(3)$) and 6-dodecanoyl-2-dimethylaminonaphthalene (Laurdan). For the binary PC vesicles, BODIPY and Rh-DPPE were used as complementary probes: Rh-DPPE preferentially partitions into the solid-like domains, BODIPY into the fluid phase. For all other lipid mixtures, solid-like domains exclude $\mathrm{DiIC}_{18}(3)$ and Rh-DPPE.

An inverted Nikon microscope together with a BIORAD confocal system was used for image acquisition. For some observations with Rh-DPPE only or Rh-DPPE and Laurdan, a Mira Ti–sapphire laser with wavelength 780 nm was used for multiphoton excitation. For all other observations, an Ar laser with wavelength 488 nm was used for single-photon excitation. LaserVox, LaserPix and ImageJ were used for image processing, including reconstruction of images from confocal stacks, and analysis.

3. Results and discussion

3.1. Shape and size of domains

In DPPC–DPPE membranes at temperatures where fluid and solid-like phases coexist, the solid-like domains are circular (figure 1). On cooling, they grow equally along all radial directions. However, they only grow to a certain size, before, on further cooling, new small solid-like domains nucleate and coexist with the larger domains (figure 2). As the sample is cooled further, the larger solid-like domains do not appear to increase in size, while the smaller domains grow. The solid-like domains do not appear flat, but have a curvature similar to that of the fluid-like regions (figure 3); it is therefore unlikely that elastic effects arising from membrane bending are responsible for the limited growth of the solid-like domains [6]. The solid phase is more condensed and thus the dipole density higher (by about 50%). Molecular dynamics simulations have shown that repulsive interactions dominate the interaction energy

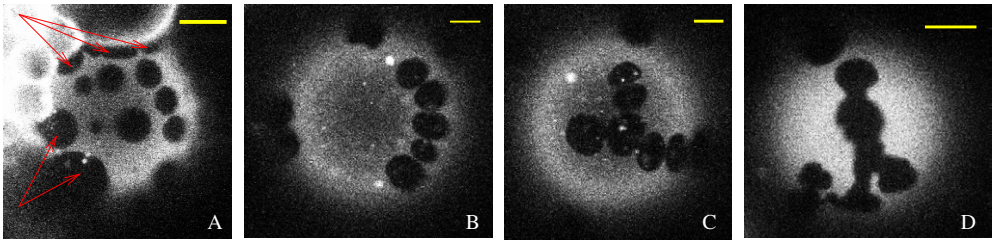

Figure 1. Circular solid-like domains in DPPC–DPPE (ratio 1:1) vesicles containing the $DiIC_{18}(3)$ dye. The images correspond to slices through the (poles of) vesicles. Static domains in part (A) are marked by arrows. The scale bar represents 10 μm.

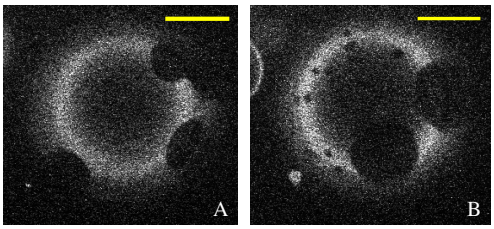

Figure 2. Circular solid-like domains in DPPC–DPPE (ratio 1:1) vesicles containing the $DiIC_{18}(3)$ dye. The images correspond to slices through the (poles of) vesicles. Image (A) showing only large domains was taken about 5 min before and about 0.5 °C higher than image (B) showing large and small domains. The scale bars represent 10 μm.

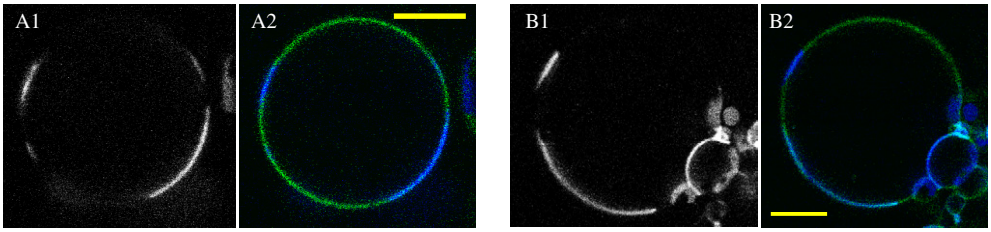

Figure 3. Images through the equators of two DPPC–DPPE vesicles (A and B). Images (A1) and (B1) show the Rh-DPPE emission (fluid phase only). Images (A2) and (B2) are false-colour images showing the Rh-DPPE emission in blue (fluid phase only) and the Laurdan emission in green (solid-like and fluid phases). The scale bars represent 10 μm.

of lipid dipoles at large distance [12]. However, we have not, to date, experimentally tested the role of electrostatic interactions in limiting the size of solid-like domains in DPPC–DPPE vesicles.

In DLPC–DPPC membranes in the coexistence region, the solid domains are stripe-like with typically a number of stripes per vesicle (figure 4). The stripes grow longitudinally, increasing in length while their width remains constant. The width of the stripes is usually 2 μm or less and, within each vesicle, stripes are usually strikingly monodisperse in width. Stripes of similar widths are also observed in flaccid vesicles, suggesting that the stripe width is not limited by membrane tension or bending. Furthermore, the widths of stripes do not significantly change according to whether vesicles are formed without added salt or with addition of 100 mM NaCl, which corresponds to a range of Debye screening lengths down to 1 nm [13]. This indicates that the widths of stripes are also not determined by electrostatic

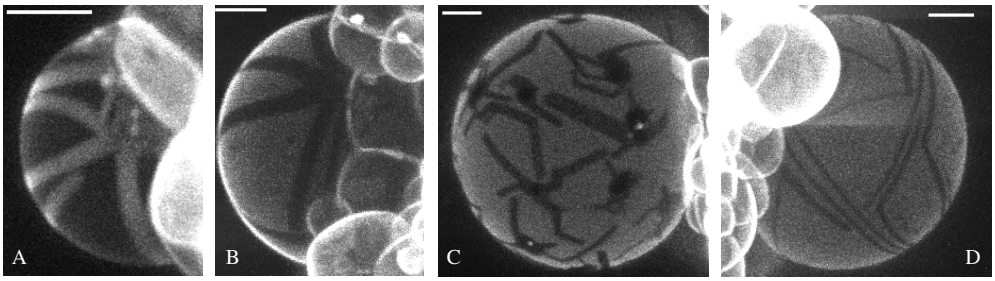

Figure 4. Stripe-like solid domains in DLPC–DPPC vesicles (ratio 1:1). The solid-like and fluid domains are bright due to the preferential partitioning of Rh-DPPE (A) and BODIPY (B),(C),(D), respectively. The domains shown in part (C) grew at a temperature close to the upper boundary of the coexistence region. The images are projections of vesicles hemispheres. The scale bars represent 5 μm.

interactions mediated through the water. Vesicles made in the presence of 10 mol% TBAC, a hydrophobic salt, similarly do not show significantly different domains. However, we cannot confidently infer from this any information about electrostatic interactions mediated through the hydrophobic portion of the bilayer because the behaviour of TBAC in such a lipid bilayer has not been characterized. In contrast, preliminary results show that varying the cooling rate can change the sizes of the domains, suggesting that the size may be influenced by nucleation and growth processes.

3.2. Interactions of domains

Upon quenching GUVs made of mixtures of DPPC and DPPE, we see circular, solid-like domains moving in the fluid membrane. All domains on a vesicle move coherently with a centre-of-mass drift velocity of about 2–6 μm s^{-1}, which indicates that they follow convective flow in the fluid phase that is driven by small temperature differences within the sample as opposed to purely diffusive motion of the domains. During this movement, at lower temperatures the domains usually repel each other. Repulsion is observed most clearly at temperatures where we observe nucleation of new domains instead of growth of large domains. At higher temperatures, moving circular domains sometimes adhere to one another to form 'dumb-bells' or more complicated shapes, for example long chains of circular domains which might also be branched (figures 1(C), (D)). Although the domains stick together, they usually do not completely coarsen to a larger circular domain. This limited coalescence probably results from slow diffusion of lipids in the solid-like phase [14].

The stripe-like domains in DLPC–DPPC membranes also move within the membrane, following the convective flow and, due to their anisotropic shape, they can furthermore be observed to rotate. Stripe-like domains also repel each other. Due to their elongated shapes, the available 'free' surface area is significantly restricted (e.g. figure 4(C)). Upon increasing the concentration of extended stripe-like domains, we see an abrupt transition from moving to stationary domains at compositions and temperatures at which more compact stripe-like domains are still rapidly moving. This indicates that the cessation of domain movement results from repulsion between domains rather than a change in the properties of the fluid part of the membrane. Further evidence of repulsion between stripe-like domains is provided when we observe that stripe-like domains push on and distort other domains as they grow. Figure 4(D) shows non-intersecting stripes that seem to have bent while growing to avoid contact with other domains. (Stripes that appear joined as in figure 4(C) have been observed

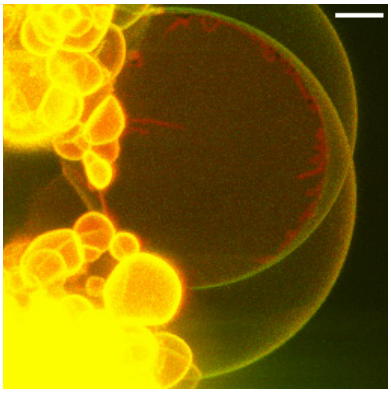

Figure 5. For DLPC–DPPC (ratio 3:1) vesicles, a false-colour image shows static solid-like stripes (red, Rh-DPPE emission) pinned at the perimeter of the area where two mostly fluid membranes (green, BODIPY emission) adhere. The scale bar represents 5 μm.

to form, if more than one stripe grows from a common nucleation site; they nevertheless repel each other.) In addition, stripes in figure 4(D) are regularly spaced, suggesting that there may be a preferred inter-domain spacing. A water-mediated electrostatic origin of the inter-domain repulsion is unlikely because the addition of salt does not significantly change the behaviour. However, repulsion may arise from elastic interactions that result from the distortion of the fluid membrane caused by the different thickness of the solid domains [15–17].

3.3. Domains at adherent areas

In vesicles with mainly mobile solid-like domains, there are nevertheless often stationary domains on the perimeter of contact areas between two vesicles (figures 1(A) and 5). This has been observed for charged DPPS-rich solid-like domains in DPPC–DPPS vesicles and DPPA-rich solid-like domains in DPPC–DPPA vesicles, and zwitterionic domains in DPPC–DPPE vesicles and DLPC–DPPC vesicles. In some cases, we have observed that domains initially diffusing in the free membrane become 'pinned' at the perimeter of a contact area. It may be that the rigid solid-like domains become stuck at this location due to the change in membrane curvature caused by adhesion. Domain relocation as a result of curvature has been shown in computer simulation [18]. It is also possible that solid-like domains are located in these regions because their thermal membrane fluctuations are suppressed and thus the additional entropy cost for the rigid domains is reduced. This does not, however, explain why domains are pinned at the edges of contact areas (rather than freely diffusing in these contact areas).

4. Outlook

Many of the phenomena that we report for solid-like domains in a fluid bilayer do not seem to depend on the structure, composition, phase or charge of the solid-like domain. We therefore infer that such solid-like domains could, in some cases, serve as optically resolvable models for hard membrane inclusions. Such models are appealing for their adaptability; for example, the shape and area fractions of these model 'inclusions' are controllable by choice of lipid components [19], relative composition and temperature [20]. Preliminary observations also suggest that the number of 'inclusions' is potentially another controllable parameter, by selection of a suitable cooling rate.

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