

Molecular Determinants of Lipid Domain Shape

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We examined domains formed by two different lipids to investigate the influence of molecular structure on domain shape. In particular, we addressed the question as to whether domains of different lipids can exhibit a common form while maintaining their distinctive shapes. We chose dipalmitoylphosphatidylcholine and dimyristoylphosphatidylcholine because they share a common head group and differ only in the length of their hydrocarbon tails. Our results show that their domains do indeed exhibit a common form despite variations in detail imposed by the difference in chain length.

Exquisite shapes with common forms abound in nature. This commonality is often preserved despite expressions of variability. For example, leaves with a common form (e.g., maple) exhibit variations in detail according to species (e.g., red maple). Intriguing microscopic shapes are formed in lipid monolayers that exhibit phase coexistence. Each such lipid forms a unique domain shape; DPPC^{1–4} differs from DMPE,⁵ DLPE,⁶ and DMPA.⁷ While the differences between domain shapes are easily recognized, similarities between domains of different lipids are more subtle. As such, the question as to whether lipid domains can exhibit a common form while maintaining their distinctive shape has not yet been addressed.

A firm understanding of the relationship between domain shapes of different lipids would benefit the theoretical study of monolayers. Theories have been developed in attempts to predict the shapes of lipid domains. The most successful theory to date is based on the competition between electrostatic repulsion and line tension.⁸ Electrostatic repulsion between oriented head groups elongates the domain, while line tension pulls the domain into a compact shape. This theory has been successful in predicting generalized domain shapes,^{9–11} but the ability to predict domain shapes of specific lipids has not yet been achieved. Experimental data illuminating both similarities and differences between domains formed by two different lipids would add a useful piece to this theoretical puzzle.

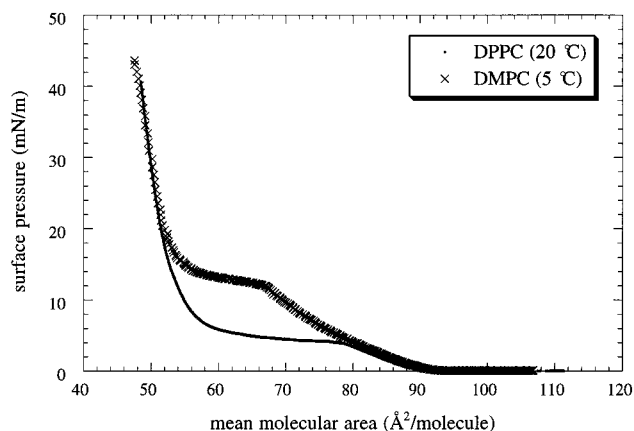


Figure 1. Isotherms of DPPC and DMPC at 20 and 5 °C, respectively. Both monolayers were compressed at a rate of $0.86 \text{ \AA}^2 \text{ molecule}^{-1} \text{ min}^{-1}$.

Lipid molecular structures can differ both in their polar head group and in the nature of their nonpolar hydrocarbon tails. Since most domain-forming lipids have saturated tails, the structure of the hydrophobic portion is generic. One would thus expect that the head group plays the largest role in determining the shapes of domains that form in a phase transition. The length of the nonpolar tails is also an important parameter, however, as different lipids with the same head group do not display the same domain shape. Our hypothesis is that the head group is the key factor in determining the form of the domain (its overall shape), while chain length impacts details within that form. Thus, we have explored the effects of chain length by studying the domain shapes formed by two different lipids with a common head group.

We have studied domain shapes in monolayers of DPPC (dipalmitoylphosphatidylcholine) and DMPC (dimyristoylphosphatidylcholine), which differ in chain length by two methylene groups. Surface pressure/mean molecular area isotherms for each lipid are shown in Figure 1. Each lipid was studied throughout its liquid expanded/liquid condensed coexistence region, originating with the kink

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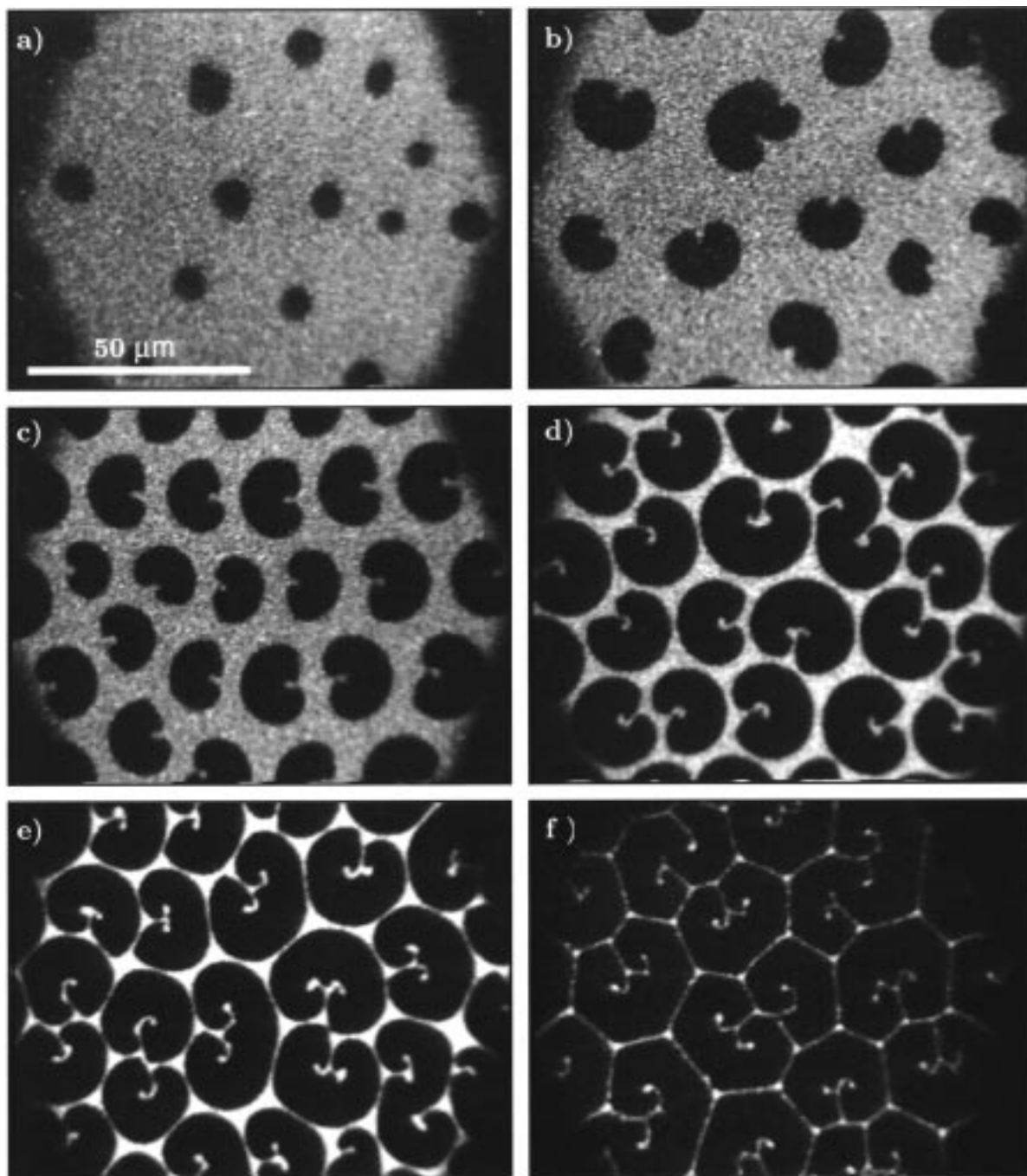


Figure 2. DPPC domains formed at 20 °C by a compression at $0.86 \text{ \AA}^2 \text{ molecule}^{-1} \text{ min}^{-1}$. Surface pressures are (a) 3.8, (b) 3.9, (c) 4.3, (d) 4.5, (e) 5.0, and (f) 7.5 mN/m.

and proceeding through the plateau in the surface pressure–mean molecular area isotherm. This kink occurs between 3.6 and 3.8 mN/m for DPPC at 20 °C and between 11.0 and 11.3 mN/m for DMPC and 5 °C. Lipids were obtained from Avanti Polar Lipids (Birmingham, Alabama), doped with 0.5% fluorescent probe (NBD-PC), and spread from chloroform (Fisher, HPLC grade). All other experimental details have been described previously.⁴

Domain shapes formed by DPPC have been well characterized in a previous study.⁴ A representative series of images acquired using fluorescence microscopy is shown in Figure 2. Domains upon nucleation appear round (Figure 2a) and only upon further compression take on their distinctive shape. The stable shape for DPPC is an asymmetric bean with a distinct cavity and a flattened

left edge (Figure 2c,d). Their asymmetry renders them chiral, reflecting the chirality of the DPPC molecule itself. (Its enantiomer forms mirror-image domains.) Multilobed domains (bilobes and trilobes) can also form, but all multilobed domains transform to beans over time. The flattened edge apparent in beans is also present in bilobed domains (Figure 2d,e). At higher surface pressures, new growth occurs within the cavity in the form of a terminal nub (Figure 2e). These nubs grow nearly simultaneously in all domains at once, do not develop into an additional lobe, and exhibit chirality (curving counterclockwise). At very high surface pressures, domains strongly repel each other and grow into interstitial spaces between domains to yield polygons (Figure 2f). Domains shown in Figure 2 are stable over time scales of days.

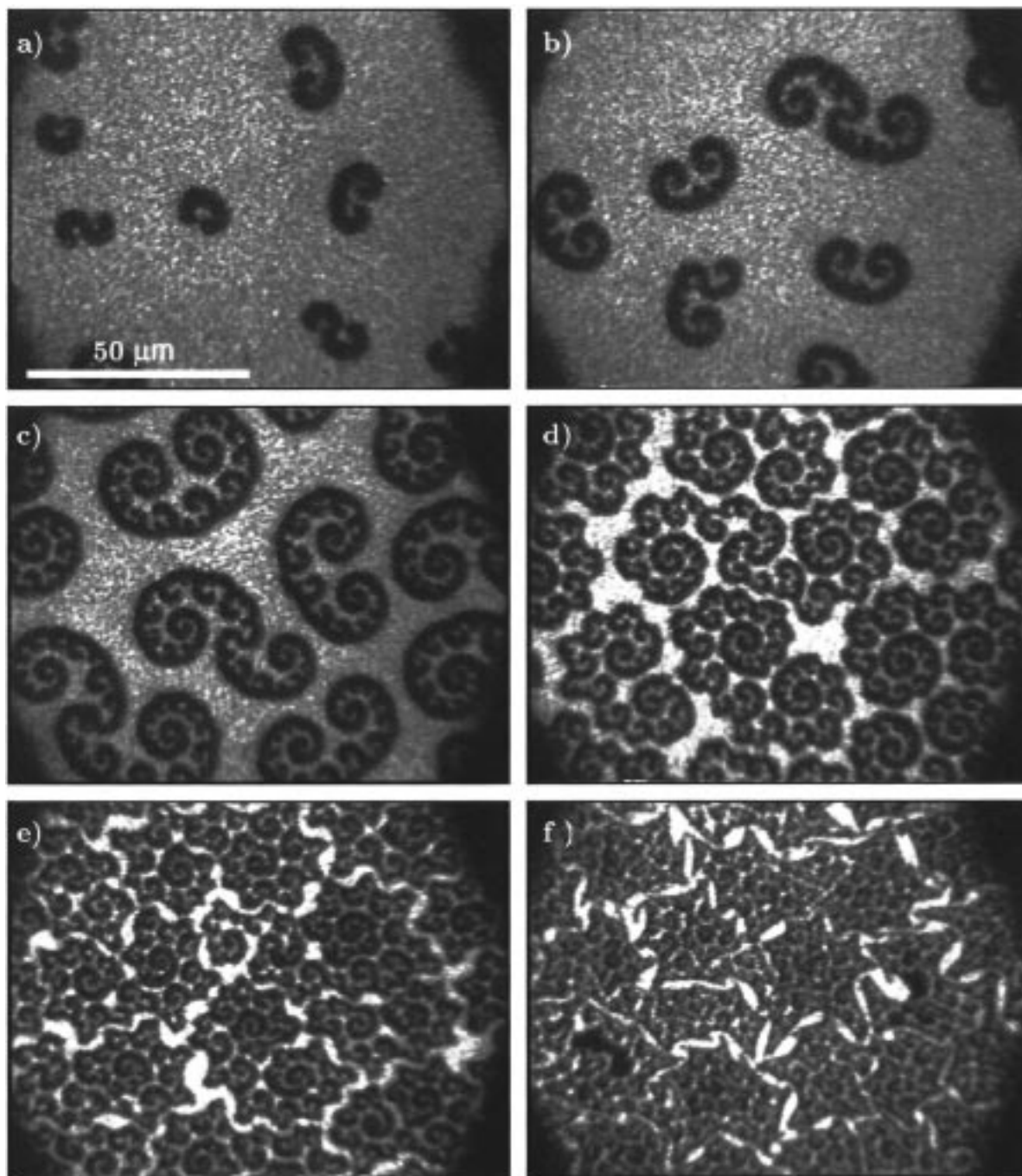


Figure 3. DMPC domains formed at 5 °C by a compression at $0.86 \text{ \AA}^2 \text{ molecule}^{-1} \text{ min}^{-1}$. Surface pressures are (a) 11.4, (b) 11.6, (c) 12.1, (d) 13.0, (e) 13.5, and (f) 13.8 mN/m.

Domain shapes formed by DMPC are shown in Figure 3. In this case, domains upon nucleation take on a distinctive shape: beans and bilobes are clearly visible (Figure 3a). Again, the chirality of the molecule is mirrored by the chirality of the domain shapes. As the compression proceeds, growth of these domains yields more intricate shapes. Similar to the behavior seen in DPPC, nubs form and grow into the cavity (Figure 3b). In the case of DMPC, however, multiple nub growth is the norm. DMPC nubs grow in a counterclockwise direction like their DPPC counterparts. At higher surface pressures, nub growth supersedes growth of the main backbone, which gets thinner as compression proceeds (Figure 3c,d). This continues until the backbone connecting the nubs is very thin indeed, and the nubs resemble a series of beans

connected along a line (Figure 3d). Further compression reveals the repulsive nature of these domains as they rearrange to fill available space and resemble interlocking cogs (Figure 3e). At very high surface pressures, the delicate interior structure of the domain is crushed (Figure 3f). DMPC domains presented are stable for at least 20 h; after this time, air currents serve to unravel the spiraled domains.

Differences between domains formed by DPPC and DMPC are clear. Decreased van der Waals forces due to the shorter chain length of DMPC serve to shift the electrostatics/line tension balance to favor less compact domains. Line tension in a DMPC domain is clearly lower (with respect to DPPC) as compression greatly increases the perimeter of the domain by thinning its features. The

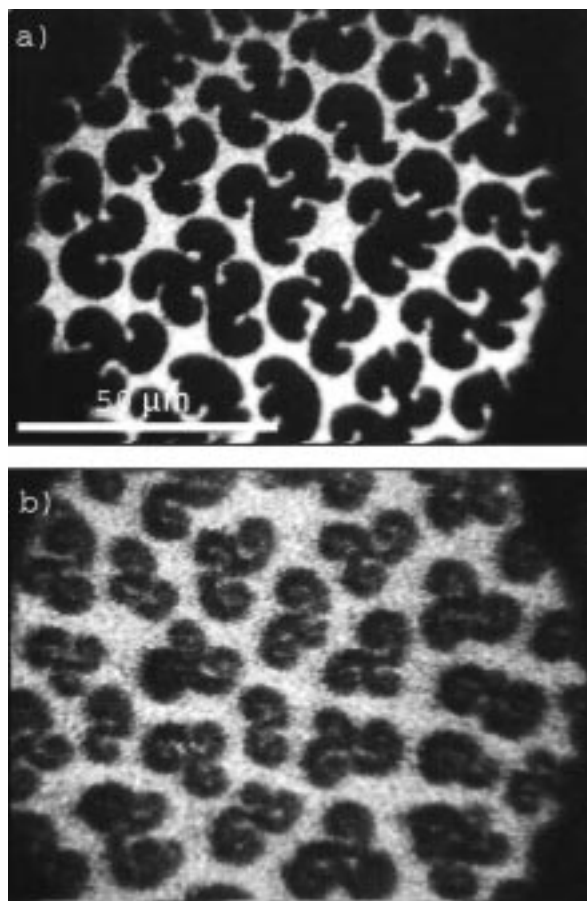


Figure 4. Domains formed by a compression at $8.6 \text{ \AA}^2 \text{ molecule}^{-1} \text{ min}^{-1}$: (a) DPPC ($20 \text{ }^\circ\text{C}$) at 5.0 mN/m ; (b) DMPC ($5 \text{ }^\circ\text{C}$) at 13.0 mN/m .

resulting complex shapes do not pack easily at high surface pressures, leading to the eventual collapse of their internal structure.

Despite these differences in detail, domains formed by DPPC and DMPC share a common form. The chirality of each molecule is reflected in the shape of the domains it forms: all lobes grow in a counterclockwise direction. Moreover, small DMPC domains strikingly resemble larger, well-developed DPPC domains. In mature DMPC domains, the backbone of the domain (excluding nubs) exhibits a form common to DPPC—that of beans and bilobes. DMPC bilobes even display the flattened edge common to their DPPC counterparts. This commonality of form between DPPC and DMPC is self-evident when the monolayers are compressed at a faster rate. Figure 4 shows domains formed for each lipid compressed at ten times the normal rate. The resulting shapes are surprisingly similar and do not display the differences in detail present in more stable domains formed at the lower compression rate. Because these shared features are not present in domains of other lipids, they are intrinsic to the phosphocholine head group.

Connections between the domain shapes formed by different lipids are thus possible. The lipid head group is the dominating influence in defining the form of a lipid domain. Chain length, on the other hand, manipulates details within this form, ensuring that each lipid exhibits a unique behavior. With better understanding how common forms arise in domain shapes and what conditions change the particular details of lipid domains, prediction of domain shape from molecular structure will become an attainable goal.

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