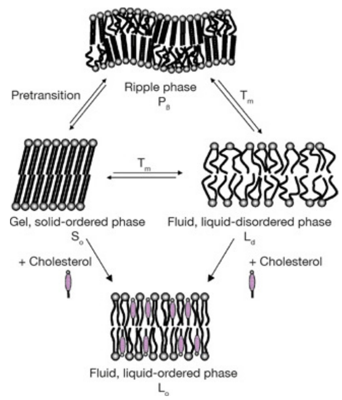
3.5: The Ripple Phase

Lipids consist of hydrophilic polar head groups attached to hydrocarbon chains and arrange themselves in bilayers to make biological membrane structures. At lower temperatures, the bilayer is in a $L_{\beta'}$ 'gel' phase and there is a transition to 'fluid' phase, L_α , at higher temperatures due to an increase in mobility of individual lipids in the bilayer. A smectic ripple phase $P_{\beta'}$ is observed in hydrated lipid bilayers between the $L_{\beta'}$ and L_α phase. This phase is characterized by corrugations of the membrane surface with well-defined periodicity with an axis parallel to the mean bilayer plane [1]. The molecular origin of ripple-phase formation is traditionally been associated with the lipid headgroup region and hence lipids can be classified into ripple-forming and non-ripple forming lipids based on their headgroups. One of the lipid families belonging to ripple-forming class is phosphatidylcholines and has been studied in extensive detail [1].

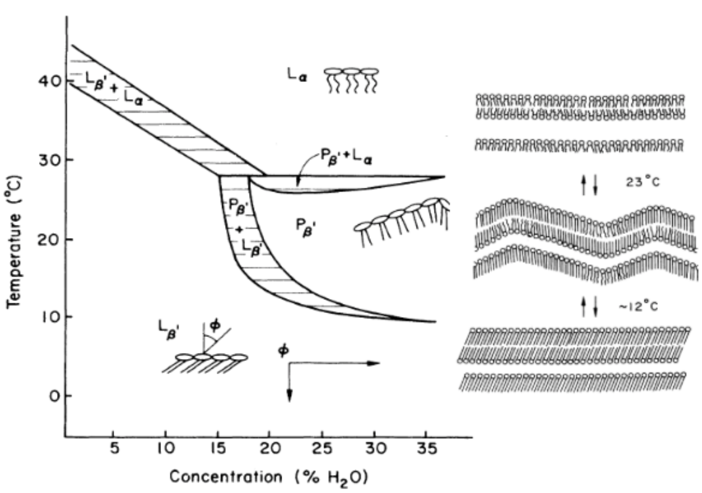


Scheme above shows different physical states adopted by a lipid bilayer in aqueous medium [2]

Thermodynamics and Existence

The existence of the ripple phase at first sight is paradoxical on thermodynamic grounds since it involves an apparent lowering of symmetry (from $L_{\beta'}$ *to* $P_{\beta'}$) on increasing the temperature. Some models suggest that ripples exist because of periodic local spontaneous curvature in the lipid bilayers formed due to electrostatic coupling between water molecules and the polar headgroups or coupling between membrane curvature and molecular tilt. It has also been speculated that ripples form to relieve packing frustrations that arise whenever the relationship between head-group cross sectional area and cross-sectional area of the apolar tails exceeds a certain threshold [1]. However, there is not one conclusive theory to explain ripple phase formation.

Phase Diagram Depicting Ripple Phase



Experimental phase diagram for (1,2-dimyristoyl-sn-glycero-3-phosphocholine) DMPC, plotted as a function of temperature and hydration. Solid lines indicate first order transitions. Arrows indicate directions of increasing tilt in the $L_{\beta'}$ phase. The rightmost schematic shows, from top to bottom, the forms of the phases L_α, $P_{\beta'}$ and $L_{\beta'}$ [3]

Types of Ripple Structures

Two different co-existing ripple phases have been reported, one is asymmetric, having a sawtooth profile with alternating thin and thick arms and a periodicity of 13-15 nms and the other one is symmetric and has a wavy sinusoidal structure with twice the periodicity of the asymmetric structure [4]. In phosphatidylcholine bilayers, asymmetric ripple phase formed is more stable which forms at the pretransition temperature upon heating from the gel phase. The metastable ripple phase is formed at the main phase transition upon cooling from the fluid phase and has approximately double the ripple repeat distance as compared to the stable phase [1].

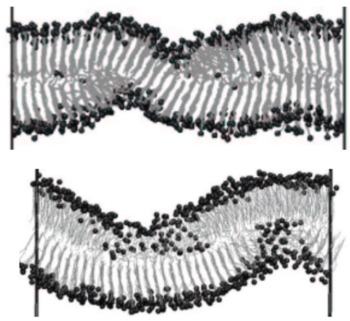


Figure above shows the model of asymmetric (upper) and symmetric (lower) ripple phase with 720 lipids molecules in a bilayer [4].

Experiments to Understand Ripple Phases

Freeze-fracture electron microscopy (FFEM) has been utilized to understand the structure of ripple phases. Freeze-fracture preparation rapidly freezes the lipid bilayer suspension at certain temperature (cryofixation) which is then fractured. The cold fractured surface is then shadowed with evaporated platinum or gold at an average angle of 45° in a high vacuum evaporator. A second coat of carbon, evaporated perpendicular to the average surface plane is often performed to improve stability of the replica coating. The specimen is returned to room temperature and pressure, then the extremely fragile "pre-shadowed" metal replica of the fracture surface is released from the underlying biological material by careful chemical digestion. The still-floating replica is thoroughly cleaned from all the chemical residues, dried and then viewed in the TEM (Transmission electron microscopy) [6]. T he results obtained through FFES show periodic linear arrays of ripples which change direction by characteristic angles of 60 or 120 degrees reflecting the hexagonal packing in the lipids [1].

Atomic Force Microscopy (AFM) allows for direct visualization of the ripple phase in supported hydrated bilayers and dynamics of formation and disappearance of ripple phases can be studied at pretransition temperatures [1]. Some examples of AFM images depicting Ripple phase are shown below from Kaasgaard *et al* [1]. In the image descriptions, Λ/2 represents the asymmetric phase and Λ shows the symmetric phase because of twice the periodicity of metastable phase than the stable phase. A phase with periodicity 2Λ has also been observed.

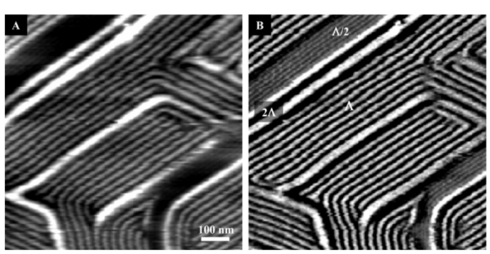


FIGURE 1 (*A*) Height mode AFM image showing different ripple phases in a DPPC-supported double bilayer at 37°C. (*B*) Deflection mode image of the same bilayer region. Three different ripple types are observed. The ripple types are denoted Λ/2-, Λ-, and 2Λ-ripples in the deflection mode image.

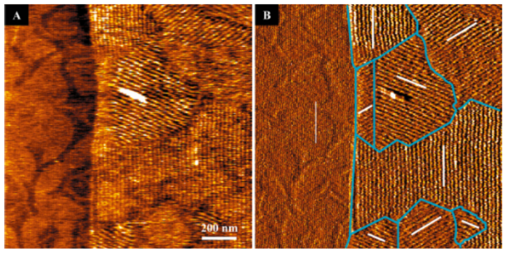
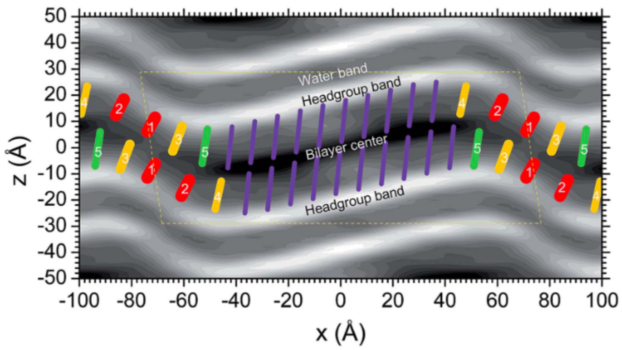


FIGURE 5 Height mode (*A*) and deflection mode (*B*) images of the interfacial region between a Λ/2-ripple domain and several Λ-ripple domains in a 7:3 DMPC-DSPC lipid bilayer at 26.0°C. The Λ/2-ripple domain emerged from within a region of Λ-ripples by an interconversion of Λ-ripples to Λ/2-ripples. The Λ/2-ripple domain is visible as the darker region on the left in the height mode image (*A*) and as the short-wavelength ripples on the left in the deflection mode image (*B*). Whereas the Λ/2-ripples were all oriented in the same direction, the Λ-ripples were present as a number of different regions that each had their own ripple direction. The different ripple regions and directions have been outlined in *B*. The rounded structures that are also seen, and are particularly visible in the Λ/2-ripple domain, correspond to domains in the first bilayer in close contact with the mica support.



Low Angle and Wide Angle X-ray scattering have also been utilized to understand the ripple phase behavior by mapping electron density as shown above [5].

Future Research

Although many theories, simulations and experiments have been conducted on Ripple phase, the exact parameters affecting its formation are still unknown for different systems. It is still not known how the hydrocarbon chains are oriented in the bilayer in $\boldsymbol{P_{\beta'}}$ phase [8]. The existence of ripple phase still remains enigmatic and determining the detailed molecular structure would seem to be prerequisite to understanding the interactions that are responsible for its formation [7].

References

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