The Basis of Low Surface Tensions in the Lungs

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1. Introduction

The most fundamental question concerning pulmonary surfactant is how the interfacial films that coat the alveoli achieve and sustain the very low surface tensions observed in the lungs. The low magnitude of the surface tension is now well established. Estimates based on the pressure-volume characteristics of excised lungs indicate that surface tensions reach values as low as 2-3 mN/m (1). Direct measurements, using the contact angle of fluorocarbon droplets deposited in the peripheral alveoli (2), confirm surface tensions of approximately 1 mN/m (3). These low surface tensions only occur in partially deflated lungs in which the surfactant films have been compressed by the shrinking alveolar surface area. After a single deflation, surface tensions remain essentially constant at these low values in static lungs for tens of minutes (1,4). A continuous compression of the films therefore is unnecessary to maintain the low surface tensions. These physiological observations define the unusual characteristics of the surfactant films \textit{in situ}.

The behaviors of pulmonary surfactant in the lungs and of phospholipid films \textit{in vitro} under equilibrium conditions differ considerably. For films at increasing densities, achieved either by infinitely slow decreases in surface area or by deposition of increasing amounts of material, surface tension falls only to the equilibrium spreading tension ($\sigma_e$), which for most biological phospholipids is approximately 25 mN/m (5). At $\sigma_e$, a phase transition occurs, and the two dimensional film collapses to form a coexisting three-dimensional bulk phase (6). Further decreases in surface area or increases in constituents
do not change the surface concentration of the monolayer, but instead produce the formation of more bulk phase. Collapse therefore sets a fundamental limitation on the surface tension that a monolayer can achieve at equilibrium. Under non-equilibrium conditions, obtained when the interface is compressed at finite rates, surface tensions lower than $\sigma_e$ can be reached. When compression of the films ceases, however, collapse returns surface tension towards equilibrium. The very low surface tensions observed in the lungs, and the persistence of those low values in static lungs for prolonged periods, indicate that rates of collapse far from equilibrium are slow and that the films exist in a metastable state.

This review considers two models that attempt to explain this metastability based on the behavior of simple films in vitro. The first model, referred to as the classical model, follows from the behavior of different phases within the two dimensional film, and has been widely accepted for almost three decades. The second model, based on more recent observations on the kinetics of collapse, is referred to as the supercompressed model to emphasize an analogy to three-dimensional supercooled liquids. Both models focus exclusively on the monomolecular film itself, and ignore several additional factors that might modify the behavior of the monolayer. In the lungs, for instance, the presence of additional material adjacent to the interface (7), perhaps formed during adsorption (8), might to some extent alter rates of collapse. We consider here only the fundamental behavior of the surfactant monolayer and the two known possible sources of its metastability.
2. Metastable films of pulmonary surfactant

2.1 The classical model

The different tendencies of films to collapse depend on their structure and therefore on the two-dimensional phases that they form. Interfacial films undergo phase transitions within the monolayer that are analogous to those that occur in bulk materials (6,9). For single chain surfactants, this phase behavior can be quite rich, with multiple phases distinguished by different degrees of order involving position, tilt, orientation, and chain elongation (10,11). For monolayers of the phosphatidylcholines, which constitute most of pulmonary surfactant, the phase diagram is simpler (12). Prior studies have identified only the gas, liquid-expanded (LE), and condensed phases, which have two-dimensional characteristics analogous to the gas, liquid, and solid phases of bulk materials. At physiologically relevant temperatures and surface tensions close to $\sigma_e$, single component films of constituents from pulmonary surfactant can form only the LE and condensed phases. Below $\sigma_e$, both phases collapse. Rates for the condensed phase, however, are much slower, allowing films to deviate far from equilibrium conditions. The classical model then contends that the metastability of pulmonary surfactant in situ indicates the presence of a condensed film (13-15).

1 We follow here the recommendation (11) that "liquid-condensed" and "solid" phases should instead be referred to more accurately as condensed, either tilted or untitled.
Formation of the condensed phase requires the presence of compounds that can adopt an ordered structure. Biological phospholipids commonly contain at least one unsaturated fatty acid, and the kink introduced by the cis double bond tends to disrupt ordered packing. Relative to disaturated compounds, unsaturated phospholipids melt from the gel to liquid-crystal phase in bilayer vesicles (16) at lower temperatures. Where both are readily accessible, melting temperatures in bilayers generally correlate with the condensed-to-LE transition temperature in monolayers. Early analyses, based largely on independent determination of head groups and fatty acid residues, indicated that pulmonary surfactant contains an unusually high content of disaturated phospholipids in general (17) and dipalmitoyl phosphatidylcholine (DPPC) in particular (18). More recent direct measurements of molecular species, however, indicate that DPPC is the only constituent present in significant amounts\(^2\) that has a bilayer melting transition above physiological temperatures and that can form the condensed phase in single-component films at 37°C. Recent determinations indicate that approximately 30-50% of pulmonary surfactant is DPPC (19-22).

Formation of a uniformly condensed film would require a significant change in composition. Analysis of compression isotherms suggests that to achieve the

\(^2\) Dipalmitoyl phosphatidylglycerol (DPPG) forms condensed films and is present in pulmonary surfactant, but in amounts that represent less than a percent of the total material (18).
characteristics of a condensed phase, mixed phospholipid monolayers must contain more than 90% DPPC (23). Microscopic studies, which determine phase behavior directly, confirm that a condensed phase formed from the complete mix of surfactant phospholipids would require a DPPC content that exceeds 95% (24). The classical model then contends that relative to freshly secreted surfactant, the condensed film that functions in the lungs must be greatly enriched in DPPC (13,14).

Selective adsorption: This enrichment could occur either by selective adsorption of DPPC (8) or by selective exclusion of other components (13-15). Selective adsorption could have either a thermodynamic or a kinetic basis. Both seem unlikely. A refinement based on thermodynamics could occur if DPPC had a lower $\sigma_e$ than other constituents. In that case, DPPC might continue to adsorb after surface tensions had fallen to a level where insertion of other components had ceased. Experimental observations, however, suggest that $\sigma_e$ is actually higher for DPPC than for other phospholipids (5). Thermodynamics therefore provides no clear basis for selective adsorption.

A mechanism for kinetic selection during adsorption is also not apparent. In contrast to classical surfactants, which adsorb as monomers, vesicles of pulmonary surfactant insert together into the interface (25,26). Therefore the different components should all adsorb at the same rate. Microscopic observations on the separated phases within monolayers forming by adsorption supports this contention, and argue against any compositional change during adsorption at surface tensions above $\sigma_e$. Rather than revealing the uniformly condensed phase required by the classical model, fluorescence microscopy
shows that at any given surface tension, films during adsorption have roughly the same ratio of LE and condensed phases as spread films that contain the complete set of surfactant constituents (27). These observations, although qualitative, argue that adsorbed films contain the full set of constituents, and that different components adsorb at the same rate with no selective advantage for any particular compound.

Selective exclusion: Enrichment of DPPC could also occur by selective exclusion of other components from the interface. Because of the insolubility of lipids in air and water (28), desorption of constituents from the films is negligible. Selective collapse, however, or squeeze-out, could change the film’s composition. The strongest evidence for this possibility has been the variation of surface tension during compression of mixed monolayers related to pulmonary surfactant. Surface tension remains relatively constant just below $\sigma_c$ during a large change in area before compressibility$^3$ falls and surface tension drops more steeply (23). These results have been interpreted as demonstrating the selective exclusion at surface tensions below $\sigma_c$ of constituents other than DPPC.

$^3$ Although the compressibility of a film is defined under equilibrium conditions, here we employ the same term in reference to the slope of a surface tension–area plot when the film is not in equilibrium during a dynamic compression. Similarly, although surface tension is defined at equilibrium (6), here we extended the concept to non-equilibrium conditions, recognizing that its value might include the effects of dynamic and static components.
which eventually results in a film that is sufficiently enriched in DPPC to form the condensed phase required to reach and sustain low surface tensions.

Microscopic observations on the collapse of surfactant films now link the composition of excluded material directly to phase behavior within the monolayer. Near $\sigma_e$, monolayers of pulmonary surfactant collapse to form a stacked smectic liquid-crystal (29), which is the same structure that hydrated phospholipids form in their bulk phase. Microscopy shows that like other liquid-crystalline materials (30,31), the surfactant monolayer likely flows from the interface as a continuous lamella into the collapsed stack (Figure 1). The collapsed phase should therefore have the same composition as the region of the monolayer from which it formed. Separation of a condensed phase containing only DPPC and a LE phase that collapses much more rapidly would provide the basis for selective exclusion and the formation of a film enriched in DPPC.

The phase behavior within monolayers containing extracts of pulmonary surfactant and closely related model systems provides evidence that ultimately argues against selective exclusion. Films containing the complete set of surfactant phospholipids, from which the proteins and cholesterol-containing compounds have been removed, do show the expected separation of the LE and condensed phases (24). The two phases have the necessary difference in composition, with essentially pure DPPC in the tilted-condensed (TC) domains, and other constituents located in the surrounding LE film. Brewster angle microscopy also shows that the more ordered phase has the same optical anisotropy and optical thickness observed with films of pure DPPC in the TC phase. At ambient
laboratory temperatures, however, the difference between collapse rates of the two phases is less than expected (32). During relatively slow compression from $\sigma_e$ to low surface tensions, the ratio of areas occupied by the two phases remains essentially unchanged. Although the difference in rates of collapse might be substantial at 37°C, the behavior of the two phases at ambient laboratory temperatures suggests that a structural phase alone is insufficient to determine the ability of the films to reach low surface tensions.

Films with the complete set of constituents in surfactant extracts that specifically include the cholesterol-containing compounds also show phase separation. Both fluorescence microscopy (27,33) and Brewster angle microscopy (33) demonstrate coexistence, with domains of a more ordered phase that appear and grow during compression of the surfactant monolayers. The coexistence, however, is not solid-fluid as expected for the condensed and LE phases. Domains of the more ordered phase can undergo rapid changes in shape (34), indicating fluid characteristics rather than the solid behavior of the condensed phase. These domains then presumably represent a liquid-ordered phase of DPPC-cholesterol (35), comparable to the "lipid rafts" that may provide a basis for organization in cellular membranes (36,37). Under conditions at which phase separation is present at $\sigma_e$, light scattering microscopy detects collapse only from the disordered LE phase (29). The liquid-ordered and liquid-disordered phases therefore do show the difference in rates of collapse required for selective exclusion of different constituents.

The extent of phase separation at $\sigma_e$, however, is insufficient to provide the basis for producing a film that contains almost pure DPPC. Phase coexistence at $\sigma_e$ in calf lung
surfactant extract (CLSE) can be minimal or nonexistent. During compression at 20°C, initial separation of two phases terminates before reaching $\sigma_e$ (34). Remixing immediately follows dramatic changes in the shape of the ordered domains and in the extent of the interfacial boundary, consistent with behavior caused by low line tension between two immiscible fluid phases close to a critical point. This remixing requires the presence of cholesterol (34). Critical points between fluid phases are now well documented in monolayers containing mixtures of cholesterol and phospholipids (38). The termination of phase coexistence above $\sigma_e$ would of course eliminate the basis for selective exclusion.

Although the phase behavior at higher temperatures may shift to allow coexistence at $\sigma_e$, the fraction of the interface occupied by the ordered phase can be minimal. For extracts of pulmonary surfactant, the onset of phase separation at 37°C occurs only just above $\sigma_e$. Higher temperatures generally shift phase transitions to lower surface tensions, an effect that is well known for the LE-TC transition for DPPC (39). The presence of multiple additional components in surfactant films reduces transition surface tensions relative to pure DPPC by an additional 8-10 mN/m (33). The multiple components also affect the progression of the transition as well as its onset. Although the Gibbs phase rule for a single component film confines coexistence to a single surface tension, the presence of multiple components releases these constraints, and completion of the transition for films that include the surfactant phospholipids requires compression through a broad range of surface tensions (33,34). Consequently at $\sigma_e$ and 37°C, the ordered phase in complete extracts of calf surfactant first appears at approximately 26 mN/m and occupies less than
4% of the interface when collapse begins (Figure 2) (33). Exclusion of the LE phase would therefore require the collapse of 96% of the film. Whether the resulting liquid-ordered phase of DPPC-cholesterol would have the metastability of films in the lungs is unknown. A reduction in interfacial area of that extent, however, is physiologically unrealistic.

Although widely accepted, evidence supporting the classical model has been largely indirect. The monomolecular thickness of the film has complicated efforts to detect the compositional change predicted by the model. Structural evidence for the condensed phase has also been limited and inconclusive. Perhaps the strongest support for the model has simply been the absence of any film derived from pulmonary surfactant other than condensed DPPC that could replicate the behavior of films in the lungs. The supercompressed fluid model now provides one such alternative.

### 2.2 The supercompressed fluid model:

Although collapse of monolayers limits access to surface tensions below $\sigma_e$ under equilibrium conditions, the ability of films to reach lower surface tensions during non-equilibrium compressions is well known. Like many other phase transitions, collapse of phospholipid monolayers close to $\sigma_e$ begins with an energetically unfavorable process of nucleation (9,40) that can delay its onset. Nucleation events occur at a higher frequency further below $\sigma_e$, and the surface tension at which collapse is first observed is lower during faster compression (6,41). After formation, subsequent growth of the nuclei occurs at finite rates. If compression is faster than collapse, then the density of the film
must increase, and surface tension will fall. These considerations explain the ability of rapidly compressed films containing pulmonary surfactant or related phospholipids, which collapse just below $\sigma_e$ during slow compressions, to reach the low surface tensions observed in the lungs (Figure 3) (42-45).

The surfactant films in situ not only reach very low surface tensions but also sustain them in static lungs for prolonged periods. If collapse continued, then as soon as compression ceased, surface tensions would increase and return to $\sigma_e$. At low surface tensions, however, fluid phospholipid films become metastable. Collapse becomes remarkably slow, and fluid films containing a variety of individual phosphatidylcholines (42,45) as well as CLSE (44) achieve the metastability of films in the lungs (Figure 4).

This slowing of collapse at low surface tensions, although unexpected, is analogous to the behavior of some bulk materials during cooling below a transition temperature. For those materials, rates of transformation change with decreasing temperature in the same manner that rates of collapse change with decreasing surface tension. As temperature or surface tension drops below the equilibrium value, rates of transformation initially increase to a maximum value. Further from equilibrium, however, rates decrease until they become negligible. In bulk materials, a rapid quench below a transition temperature can therefore yield metastable states for which, although the system is far from equilibrium, rates of transformation are negligible. The formation of glasses, in which a liquid is supercooled below its freezing point to form an amorphous solid, represents such a process (46). Although the equilibrium structure is a crystal, relaxation times for the glass are
extremely large, and the system is metastable. The behavior of LE monolayers, which at low surface tensions far below $\sigma_c$ develop extremely slow rates of collapse, is similar, and may reflect an analogous process.

The kinetics of phase transitions are commonly summarized in transformation diagrams. For bulk materials, time-temperature-transformation (TTT) diagrams show the time at different temperatures required to transform isothermally a given fraction of the material to the new phase (Figure 5.a) (47,48). Dividing the transformed fraction by the required time yields approximate rates of transformation. TTT plots often have a C-shape, such that the time for transformation reaches a minimum value at the “nose” of the diagram. This characteristic shape reflects conflicting effects of lower temperatures. The driving force for transformation increases as temperature drops below its transition value because the system is further from equilibrium. At the same time, however, molecular thermal motions, which allow individual molecules to transfer to the new phase by overcoming the activation energy for transformation, diminish at the lower temperatures. At some point, the slower molecular motions become the limiting factor in the transformation, producing rates that pass through a maximum and then decrease at temperatures below the nose.

Transformation diagrams can also be used to express the kinetics of collapse, but with surface tension for the two-dimensional films replacing temperature for the bulk materials. Time-surface tension-transformation ($T\sigma T$) diagrams for isothermal monolayers show the time required at different constant surface tensions for a specific
fraction of the film to collapse (Figure 5.b) (49). The extent of collapse can be measured from interfacial area, which at constant surface tension must fall because constituents are lost from the monolayer. TσT diagrams for monolayers of 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC), which melts from the gel phase in bilayers at -3°C (50) and which accounts for approximately 10% of pulmonary surfactant, show the same C-shape observed for bulk materials, with a maximum rate of collapse at the nose and slower rates at lower surface tensions (Figure 6). The similarity suggests comparable effects in the two- and three-dimensional systems. The driving force for collapse increases at surface tensions progressively further below σc. At the same time, increased surface concentration during compression may diminish molecular motions and increase the film's viscosity, reducing the rate at which the monolayer can flow into the collapsed structures. When eventually viscosity becomes dominant, rates of collapse pass through a maximum and then decrease despite greater deviations from equilibrium.

Transformation diagrams are particularly useful in predicting how systems will behave when collapse occurs under different conditions. TσT diagrams explain the variation of surface tension and the shape of surface tension-area isotherms during compression at different rates. Surface concentration increases, and surface tension falls, only if compression is faster than collapse. Compression slower than the maximum rate of collapse would therefore decrease surface tension only to the point at which the two processes occur at equal rates. The steep initial fall in times of transformation in the TσT diagrams (Figure 6), which indicate increased rates of collapse, accurately predict the minimal change in surface tension observed over a broad range of compression rates.
If compression is faster than collapse at the nose, however, surface tension would decrease continuously to low values. The TσT diagrams therefore predict a threshold rate for reaching low surface tensions that is determined by the rate of collapse at the nose. Experimental observations confirm the existence of a threshold rate required to reach low surface tensions for both CLSE and POPC (44,45), and that for POPC, the threshold rate and the maximum rate of collapse are roughly equal (45).

During compression much faster than the maximum rate of collapse, surface tension falls rapidly, the extent of collapse is limited, and isotherms show steeply declining surface tensions and low compressibilities (45). For compression only slightly faster than the threshold, at surface tensions around the nose, rates of compression and collapse are similar. Although surface concentration increases continuously, close to the nose the films collapse extensively, resulting in isotherms with relatively flat plateaus during which surface tension changes little despite significant decreases in area. At surface tensions below the nose, rates of collapse slow, and the compressibility of the film again decreases. The resulting isotherm for a LE film of POPC shows a plateau near σe followed by a steeply falling curve. Similar isotherms obtained for mixed lipid films that model pulmonary surfactant have previously been explained by enrichment in DPPC (23). The TσT diagrams show that the variation in rates of collapse can explain the shape of isotherms even for films with a single phosphatidylcholine, and that changes in the composition of the film are unnecessary.
After reaching very low surface tension, the behavior of the films change. During subsequent expansion back to $\sigma_e$ followed by recompression, rates of collapse remain quite low. Rather than passing through the same maximum that occurs during the initial compression, collapse for films of POPC simply slows as surface tension returns toward $\sigma_e$ (51). Rates during the initial compression and subsequent expansion can differ by three orders of magnitude. Consequently, the metastability achieved by both POPC and CLSE at very low surface tensions persists during expansion at least to $\sigma_e$ (Figure 4).

During compression at the same slow rates that initially produce a collapse plateau just below $\sigma_e$, films that have previously reached low surface tensions instead follow a steeply falling isotherm (44,45). This persistent metastability is not a general characteristic of liquids supercooled to form glass. When heated, transformation rates usually increase, and crystallization frequently occurs well below the freezing temperature. For some materials, however, the onset of crystallization can be delayed at temperatures well above the glass transition for prolonged durations (52). Supercompressed films similarly do not collapse significantly even when surface tensions increase to $\sigma_e$ or remain constant at values where rates of collapse during the initial compression were maximal (Figure 4.a) (44,45,51). The analogy with supercooled liquids therefore might explain the continued resistance to collapse of the monolayers during expansion from very low surface tensions.

After the initial rapid compression to low surface tensions, supercompressed films replicate most characteristics of the surfactant films in situ. Rates of collapse in static
films are slow over the full range of surface tensions below $\sigma_c$. Cyclic changes in area that avoid expansion above $\sigma_c$ or compression to surface tensions so close to zero that the films become mechanically unstable follow the same isotherm through multiple cycles with no evidence that material is lost from the monolayer (Figure 4). Once formed, the supercompressed fluid films mimic the performance of surfactant films in the lungs.

The process of reaching low surface tensions does require speeds of compression that are apparently unnecessary in situ. Although the threshold rate required for CLSE to reach low surface tensions lies within the range that occurs during normal breathing (44), excised lungs require no comparable threshold rate of deflation to achieve normal pulmonary mechanics. Other factors could influence the behavior of surfactant films, and alter the conditions required to form the metastable films. Any process that would lower rates of collapse would diminish the threshold rate required to compress the films into the metastable state. The small thickness of the liquid film that coats the alveoli, for instance, or the presence of a surface-associated reservoir formed during adsorption (8) might significantly slow collapse. These considerations, although beyond the scope of this paper, may be crucial for understanding the behavior of films in the lungs and the achievement of metastable states without substantial changes in composition.

3. Conclusions

This review discusses two models that can partially explain the behavior of surfactant films in the lungs. In the classical model, formation of films in the condensed phase,
which have the highly ordered structure approaching that of a two-dimensional crystal, would require some process of refinement from the complete surfactant mixture to yield a composition containing essentially pure DPPC. Supercompressed fluid films, analogous to amorphous solids of three-dimensional materials, have no compositional constraints, but require an initial fast compression to reach the low surface tensions at which they become persistently metastable. Although condensed and supercompressed films each adequately replicate the behavior of pulmonary surfactant in situ, both models lack a satisfactory explanation for how the films are formed.

The temperature dependence of pulmonary mechanics should provide a basis for testing the validity of the classical model. The melting behavior of the films predicted by the two models is likely to be quite different. For the supercompressed fluid films, the restoration of rapid collapse during heating has not yet been reported. TC films of DPPC, in contrast, melt to the LE phase abruptly at discrete temperatures around 41°C and, at surface tensions below $\sigma_e$, collapse promptly (39). If low surface tensions in the lungs reflect the presence of films composed mostly of DPPC in a condensed phase, then upon melting, the films would collapse and surface tension would increase to $\sigma_e$. The larger surface tensions associated with melted films would significantly increase the pressure required to maintain the lungs at any given volume. The classical model, but probably not the supercompressed model, therefore predicts an abrupt change in pulmonary mechanics at 41°C.
Different groups that have measured the temperature dependence of pulmonary mechanics obtained results that are strikingly different. Original reports indicated the behavior predicted by the classical model, with a sharp change in pressures just above 41°C (14,53,54). Another group, however, found only a slow increase in pressures extending over a broad range of temperatures, with no particular change around 41°C (55,56). These conflicting data therefore currently provide no guidance concerning which model more accurately describes the actual films in the lungs.

In summary, the supercompressed model provides an alternative mechanism not considered previously by which metastable films might form in the lungs. Our results show that rates of collapse below $\sigma_e$ can reach a maximum and then become negligible at surface tensions far from equilibrium. The existence of a maximum rate of collapse at finite surface tensions below $\sigma_e$ results in a threshold rate of compression above which films can attain metastable states. This characteristic is analogous to many phase transitions in bulk materials, and although not exhaustively investigated before for monolayers, it is not unique to the collapse transformation. Researchers and engineers long ago found that the kinetic behavior of phase transitions is essential in achieving materials with desired properties. Although neglected in the past, the kinetics of collapse might also have a decisive role in the behavior of films in the lungs. The supercompressed model therefore provides a means for reaching persistent metastable films that opens a new perspective in the study and understanding of pulmonary function.
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References


Figure Captions

**Figure 1:** Mechanism of monolayer collapse for phospholipid and other liquid-crystalline surfactants. Collapsed structures grow by flow of the monolayer into a stacked bilayer to form trilayers. For pulmonary surfactant, the resulting structures have a circular shape (29), suggesting that surfactant molecules, N, enter the collapsed phase through a narrow region.

**Figure 2:** Phase separation of CLSE at 37°C visualized by fluorescence microscopy just above $\sigma_e$ (33). Dark regions, which show liquid-ordered domains, account for approximately 4% of the total interfacial area. Scale bar is 50 µm.

**Figure 3:** Isotherms for CLSE and POPC monolayers compressed at different rates. Similar behaviors were obtained for films of a) CLSE at 37°C (44) and b) POPC at 26°C (45). Slow compression of the films cannot achieve surface tensions much below the $\sigma_e$ of approximately 25 mN/m. By increasing the rates of compression, however, very low surface tensions are obtained. The difference in area between the points at which compression starts and ends decreases with faster rates, suggesting that the extent of collapse diminishes with faster compression. Rates of compression are expressed as initial percentage decrease in area per second.

**Figure 4:** Persistent metastability of supercompressed films. Curves show surface tension-area isotherms (top) and variations of surface tension and area with time (bottom)
for monolayers of a) CLSE at 37°C (44) and b) POPC at 26°C (45). Both films reach metastable states after a rapid compression to very low surface tensions, which can be sustained for prolonged periods without a drastic decrease in interfacial area. After a subsequent slow expansion and re-compression, the films show no evidence of further collapse.

**Figure 5:** Schematic of transformation diagrams. The panels show hypothetical experimental data (top) and the resulting transformation diagrams (bottom). a) TTT plots used for bulk materials. $\xi$ is a fixed fraction of the system transformed isothermally to the new phase at temperatures $T_1$ or $T_2$ below the transition temperature $T_c$. b) $T\sigma T$ plot for monolayer collapse. $A$ is interfacial area, and $A_0$ is the value of $A$ when surface tension first becomes constant below $\sigma_e$ after a rapid compression during which collapse is negligible. The extent of collapse is measured here as a decrease in interfacial area when the film remains at constant surface tension $\sigma_1$ or $\sigma_2$ below the transition surface tension $\sigma_c$.

**Figure 6:** $T\sigma T$ diagram for POPC. The individual points show average values obtained by rapidly compressing POPC films to the different surface tensions and then keeping surface tension constant while the films collapsed. The plot then shows the time required for the area to fall by 5, 10, 15 and 20%.
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6