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High Pressure X-ray Studies of Lipid Membranes and Lipid Phase Transitions

Abstract: Hydrostatic pressure has dramatic effects on biomembrane structure and stability and is a key thermodynamic parameter in the context of the biology of deep sea organisms. Furthermore, high-pressure and pressure-jump studies are very useful tools in biophysics and biotechnology, where they can be used to study the mechanism and kinetics of lipid phase transitions, biomolecular transformations, and protein folding/unfolding. Here, we first give an overview of the technology currently available for X-ray scattering studies of soft matter systems under pressure. We then illustrate the use of this technology to study a variety of lipid membrane systems.

Keywords: Lipid Membranes, Hydrostatic Pressure, X-ray Diffraction, Lyotropic Phases.

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1 Introduction

Hydrostatic pressure is a relatively neglected thermodynamic variable in studies of soft matter and biomolecular systems such as lipid model membranes [1–3], macromolecules and proteins [4–6]. This is surprising, given the key importance of pressure in marine biology and the origins of life [7], in biological effects such as pressure reversal of anaesthesia [8–10], and in bio-technology, for example, in high-pressure food processing [11]. Over 70% of the earth’s surface is covered with water, which, at an average depth of 3.8 km, exerts a hydrostatic pressure of 38 MPa (380 bar). Surprisingly, a wealth of marine life thrives at these high pressures.

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pressures, and life has been discovered in increasingly extreme ocean conditions. Microorganisms have been found at the bottom of the Mariana Trench which, at 11 km below sea level, exerts a pressure greater than 0.1 GPa (> 1 kbar) [12], and fish have been observed at depths of at least 7 km. Lipid membranes are key biological structures, which maintain cell integrity, and also form all of the myriad complex intracellular organelles that carry out a host of crucial biochemical functions. In addition, lipids play vital roles in cell signalling, and there is increasing evidence that the micromechanical properties of membranes can modulate the activity of proteins, peptides, channels and receptors embedded within them [13, 14]. It is known that the physical properties of lipid membranes are very sensitive to hydrostatic pressure and so it is also likely to significantly affect their function. Deep sea organisms, known as piezophiles or barophiles, must have mechanisms to adapt their lipid membranes to maintain their fluid bilayer structure and their mechanical properties under these extreme conditions [12, 15].

In addition to its relevance to extreme biological conditions, high pressure can be used to trigger structural changes in model lipid systems and has significant advantages over other triggers such as temperature or composition change: Pressure changes propagate at the speed of sound meaning that a uniform pressure is achieved over the sample extremely quickly, pressure does not usually disrupt covalent bonding below 2 GPa which is an order of magnitude higher than that required to induce lipid mesophase structure changes, and finally pressure changes can be applied extremely quickly (in milli- or even micro-seconds) both up and down in pressure meaning that any structural changes that take place on slower timescales can be decoupled from the trigger. The pressure required to cause structural changes in lipid membranes is also significantly lower than those required to alter the structure of most proteins and so high pressure experiments can allow the structural contribution of the different components in lipid-protein assemblies to be decoupled.

Fast pressure jumps unlock the potential of probing the kinetics of lipid membrane structural changes in real time, but to realise this, it is essential to couple high pressure technology to a fast structure probe technique. Synchrotron small and wide angle X-ray diffraction (SAXS/WAXS) is an ideal structural probe for these types of system and with recent developments in high flux X-ray sources and fast detectors, the time resolution of these instruments ideally complements dynamic structural studies of lipid membranes.
2 High pressure technology

Advances in the understanding of pressure effects on lipid membranes have been underpinned by development of the high pressure technology required for these experiments. Careful design of high pressure X-ray sample cells, and pressure generation and control systems have facilitated a wide range of novel high pressure and dynamic experiments. An overview of current technology and potential for future development is outlined below.

2.1 High pressure X-ray cells

2.1.1 Small angle X-ray diffraction (SAXS) cells for lipid samples

One of the challenges of carrying out high pressure X-ray experiments is designing a robust sample cell that can simultaneously withstand the required pressures while allowing X-rays into and out of the cell and maximising the diffraction angle that can be observed.

A range of high pressure X-ray sample cells have been produced that are tailored for experiments on soft matter and lipid systems. These allow fine control of pressure and temperature and in many cases allow fast pressure jumps to be performed. These allow experiments to be carried out at up to 1 GPa [16–23].

Early soft matter pressure cells were based on a beryllium tube design [16]. Due to its low atomic number, beryllium has a very low X-ray absorbance, but it is a relatively high tensile strength metal. Samples contained in a beryllium tube are connected directly to a high pressure water system (as described below) allowing diffraction patterns to be obtained from lipid samples under high pressure and dynamic pressure conditions [24, 25].

While the material properties of beryllium are extremely well suited to X-ray pressure cell construction, beryllium cells are limited in the pressure they can hold, they give relatively high background scattering and have safety problems due to beryllium oxide being highly toxic. To overcome these limitations, a number of high pressure X-ray cells have been developed with a high strength steel body and windows with low X-ray absorbance. Diamond windows offer extremely high strength while allowing reasonably high X-ray transmission, particularly at energies above 17 keV [23] and these have been used in many cases.

Pressure cells based on this design have been developed by several groups [16, 18, 26, 27], however the robust and versatile system designed by Woenckhaus [20] is particularly noteworthy as it represents a benchmark for more
recent cell development and it has facilitated a wide range of exciting high pressure and dynamic experiments on lipid systems [28–36]. This system can perform both static high pressure and pressure jump experiments between atmospheric pressure and 0.7 GPa (7 kbar) at temperatures from −40 to 100 °C. This cell employs 0.8 mm thick diamond as X-ray windows which offers a transmission of over 80% for 17 keV X-rays.

More recent cell designs have provided significant developments in sample holders which ensure a constant sample thickness [21], and dedicated sample loading ports [22]. The provision of a dedicated sample loading port (rather than removing one X-ray window to load samples as is often required in previous cells) both allows rapid exchange of samples and addresses the problem that even small rotations of the windows can cause dramatic changes in the background scattering which makes accurate background subtraction extremely difficult.

We have recently developed a high pressure cell for use at beamline I22, Diamond Light Source, UK (Figure 1) [23]. This system provides a user friendly platform for high pressure small and wide angle X-ray diffraction with fully automated pressure and temperature control and close integration with the beamline control systems. Static and millisecond pressure jump experiments can be carried out in the range 0.1 to 500 MPa and between −20 and 120 °C.

In soft matter pressure cell systems, pressure is usually generated using a fluid filled high pressure pump and pressure network [17, 18, 20, 23]. In these systems, a high pressure pump is used to compress a fluid (often water) and the pressure is then controlled via a series of valves and transferred to the sample via hydraulic...
tubing. Motor driven pressure generators [18] and electrically controlled pneumatic valves [23] allow automation of the pressure generation and control which can significantly increase the efficiency of high pressure experiments, a particularly important consideration when working at a synchrotron. Pressure jumps can be carried out by setting the sample cell to an initial pressure and a reservoir to a different pressure. These are separated using a high speed automated valve and on opening this valve, the pressure equilibrates between the cell and reservoir causing a pressure jump at the sample [16]. The speed of the jump is, to a large extent, determined by the speed at which the high pressure valve opens. Pressure jumps generated in this way are on the millisecond timescale (typically around 5 ms) and can be performed within the full pressure range of the cell. This design of pressure jump system has been refined by the use of two fast valves to initiate pressure jumps [20, 23], one for jumps up in pressure and another for jumps down. The valves are arranged so the high pressure is always pushing the valve pin out of its seat, which is an important factor in maintaining fast valve opening and hence fast pressure jumps.

2.1.2 Diamond anvil cells (DACs)

Diamond anvil cells [37] offer access to extremely high pressure and are routinely used for experiments at pressures up to 100 GPa (10⁵ bar). DACs consist of two opposing diamond anvils which seal against a metal gasket containing the sample. The diamonds are held in a brace which can apply a relatively small pressure which is amplified due to the anvil shape so a large pressure is applied to the sample. In simple DACs the initial pressure is applied by screws arranged around the brace; however the pressure resolution available in these systems is not ideally suited to soft matter systems. Recent advances have significantly improved pressure control in DACs, particularly with the development of gas membrane driven cells where a gas filled ‘balloon’ applies the initial pressure in place of a screw driven brace. This has a number of advantages including remote operation and higher achievable pressures due to the absence of screw friction, but particularly important for soft matter and lipid experiments is the ability to apply small, controlled pressure increments. Due to the small sample size, it is not possible to measure the pressure inside a DAC directly and instead it is usually found by placing a small ruby or α-quartz crystal in the sample and measuring the position of the R1 ruby fluorescence maximum [38] or quartz infra-red vibration [39] which shift consistently with pressure. The pressure resolution using these techniques is around 20 MPa [40]; however recent experiments using fluorescent microsphere pressure sensors have been able to improve this resolution by a factor of 30 [41].
DACs have proved extremely valuable in studying protein behaviour at high pressure [42], however they have not been extensively used to study lipids. This is primarily due to the relatively low pressure resolution and the pressure range which is often significantly higher than that required for lipid studies. However, some lipid studies have been carried out at up to 2 GPa [40, 43], and recent advances, particularly in pressure detection [41] and control [44] may open new avenues in very high pressure lipid research and structural studies of lipid-protein assemblies.

2.1.3 Future pressure-jump X-ray technology developments

As described above, the speed at which pressure jumps can take place in a hydraulic network system is limited to around 5 ms and while this is sufficiently fast to probe a wide range of lipid and biomolecular structural transformations, there are transitions, such as lipid chain ordering, that take place significantly faster than this. Effective triggering of these very fast transitions requires sub-millisecond pressure jumps, which cannot be achieved in current high pressure X-ray diffraction systems. However, two technologies that are currently used for high pressure spectroscopy show great potential for adaptation to X-ray experiments.

The first of these technologies is based on piezoelectric stack pistons [45]. These can move extremely rapidly and when incorporated into a fluid filled pressure cell, can generate extremely fast pressure jumps by direct compression of the sample. Pressure jumps can be performed in as little as 150 μs; however because of the limited movement of the piezoelectric stack, the pressure jump amplitude is currently limited to around 20 MPa. In order to take advantage of these devices’ potential to offer very fast pressure jumps, the volume of the entire pressure system must be kept as small as possible. This has previously been achieved by integrating a piezoelectric stack directly into a specially designed optical sample cell with an internal volume of around 50 mm³. This technology shows great potential for integration with X-ray diffraction systems and it may be possible to incorporate piezoelectric stacks as an addition to current soft matter pressure cells.

The second technology is based on the relatively simple burst diaphragm principle. Burst diaphragms have been used for many years to generate rapid pressure drops [46]. They act as a ‘weak spot’ in a fluid filled high pressure system which ruptures when the pressure becomes high enough, rapidly releasing fluid from the system and so creating a fast pressure decrease. The burst pressure can be approximately predicted from the thickness of the diaphragm and its material. Traditional passive burst diaphragms create millisecond timescale pressure
jumps, however recently developed electrically induced rupture diaphragms [47] allow the pressure jump to be triggered at a set pressure and pressure jumps of up to 250 MPa can be performed in less than 700 ns. This is currently the fastest pressure jump technology available and it has facilitated investigations of fast protein folding by fluorescence spectroscopy [48]. With some adaptation to minimise the water volume, it is likely that electrically induced burst diaphragm technology could be incorporated into existing soft matter X-ray cells to provide access to microsecond pressure drops.

2.2 Integration of high pressure technology with X-ray beamlines

High pressure small angle X-ray diffraction experiments have been carried out using lab based X-ray instruments [17], however the use of a synchrotron X-ray source offers significant advantages and is essential for kinetic pressure jump experiments. The tuneable X-ray energy available at many synchrotron SAXS beamlines allows the X-ray absorption of the pressure cell windows (which generally reduces with increasing X-ray energy) to be reduced to an acceptable level; for diamond windows, using an energy of 17 – 18 keV gives a transmission of over 60% through two 1 mm thick windows [23]. The high flux and fast, high sensitivity detectors that are available at a number of synchrotron SAXS beamlines allow up to 200 high resolution diffraction images to be captured per second. This timescale is ideally matched to current X-ray pressure jump technology and essential for probing fast structural transitions in real time (Figure 2). These requirements will only become more stringent as faster pressure jump techniques are implemented for X-ray diffraction.

As mentioned above, automation of pressure generation and control and close integration of high pressure instrumentation with beamline control systems is critical to maximising the efficiency of high pressure synchrotron X-ray diffraction experiments. A number of soft matter X-ray pressure systems have been developed or used extensively at major synchrotron SAXS beamlines including beamline ID2 at the European Synchrotron Radiation Facility [20, 28, 49], beamline A2 at the Deutsche Synchrotron [32], the Austrian SAXS beamline at Elettra [18], BL9 at DELTA [22, 50], station G1 at the Cornell High Energy Synchrotron Source [21], beamline 18ID at the Advanced Photon Source, Argonne and beamline I22 at Diamond Light Source [23].

Pressure jump experiments can very rapidly produce an enormous amount of diffraction data and there is currently a drive to develop improved tools for automated SAXS analysis in a number of areas. Lipid mesophase systems generally
used for pressure jump experiments show sharp diffraction peaks and batch analysis software [49] allows sequences of two dimensional diffraction images to be integrated to give one dimensional patterns and the diffraction peaks to be fitted providing phase identification, lattice parameters and intensity data.

3 High pressure effects on lipid membranes

3.1 Equilibrium high pressure behaviour

Lipids are amphiphilic molecules which self assemble into a variety of lamellar gel or lyotropic liquid crystalline structures when mixed with water [51, 52]. The gel phases are soft solids, with a 2-D hexagonal in-plane packing of the fully-extended hydrocarbon chains. The liquid crystal phases can form as either type I, oil in water, or type II, water in oil structures, indicated by the subscripts I or II respectively. These structures include the biologically ubiquitous flat fluid lamellar ($L_a$) phase, 2-dimensional hexagonal phases ($H_1$, $H_2$), bicontinuous cubic phases ($Q_1$, $Q_2$) and micellar cubic or 3-D hexagonal phases [53].
Figure 3: Increasing pressure causes an increase in the conformational order of lipid hydrocarbon chains. At moderate pressure, this leads to a reduction in the chain cross sectional area and an increase in the chain extension.

The structure adopted by a particular lipid system depends strongly on the lipids’ preferred curvature, as well as more subtle effects such as chain packing frustration [54], and all of these factors can be affected by pressure [49].

For any system, applying pressure will tend to promote processes or structures which reduce the overall volume [55]. In the case of lipids, increasing pressure results in a reduction in hydrocarbon chain motion and an increase in chain conformational ordering (Figure 3) [56], which will tend to reduce the cross sectional area of the lipid hydrocarbon tail region. However, the lipid head group packing is influenced far less by pressure and hence increasing pressure will tend to reduce the molecular splay, thereby increasing the spontaneous curvature of a lipid monolayer towards the chain region (or causing a decrease in the magnitude of preferred negative curvature for lipids which tend to form inverse structures) [54]. This is the opposite effect to that of increasing temperature.

For all flat or inverse fluid lipid mesophases in contact with excess water, chain ordering will tend to lead to an increase in lattice parameter, unless there is a concomitant decrease in water layer thickness. In the case of a flat lamellar phase, this swelling is simply due to an increase in chain extension (Figure 3), which causes the bilayer to thicken, generally by less than $2\text{Å kbar}^{-1}$. However in the case of inverse hexagonal and bicontinuous cubic phases, the effect may be significantly amplified, since reducing the chain volume tends to reduce the magnitude of negative curvature, thereby increasing the lattice parameter [57].

The swelling that can occur in the case of inverse hexagonal $H_{II}$ phases is limited due to chain packing frustration (Figure 4), but in this case, the lattice parameter still tends to increase slightly more than for lamellar phases. While formation of bicontinuous cubic phases induces a certain amount of chain packing frustra-
Figure 4: When cylindrical inverse micelles pack to form an inverse hexagonal phases, the lipid chains must deviate from their preferred conformation to ensure that the voids between the cylinders are filled (darker chains are compressed, lighter chains are extended). This packing frustration becomes more pronounced as the magnitude of the inverse curvature is reduced, so limits the pressure induced swelling of inverse hexagonal phases.

tion [58], this effect is far less pronounced than in the hexagonal $H_{11}$ phase, and these structures can swell by as much as 80 Å kbar$^{-1}$ at high pressure [59].

High pressure swelling of bicontinuous cubic phases might be extremely important in biotechnical applications such as trapping of macromolecules or nanoparticles. High pressure could be used to swell the cubic water channels allowing hydrophilic cargo molecules to diffuse into the structure, the pressure could then be released allowing the cubic structure to relax back to its original dimensions and in the process trap the macromolecular cargo.

Few studies have investigated the effects of pressure on the structure and stability of type I curved lyotropic liquid crystalline phases [60]. The chain ordering in a type I system is likely to induce a complex interplay between chain cross sectional area and chain extension, making the effects of pressure difficult to predict. However, experimental results [60] have shown that pressure can induce transformations to phases of higher positive curvature in the hydrated dodecyltrimethylammonium chloride (DTAC) system, which forms type I lyotropic structures. Pressure was seen to induce a transition from a 2-D hexagonal ($H_1$) phase to a Pm3n micellar cubic phase, and from an Ia3d bicontinuous cubic phase to $H_1$. Pressure was also seen to induce a small, but significant reduction in lattice parameter in all of these structures of around 0.5–1 Å kbar$^{-1}$.

As well as influencing the structural and phase behaviour of lipids, pressure also has a large effect on the micromechanical properties of lipid bilayers.
curvature elastic energy, $g_c$, for a lipid monolayer is given by:

$$ g_c = 2\kappa(H - H_0)^2 + \kappa_G K \tag{1} $$

where $H = 1/2(c_1 + c_2)$ and $K = c_1c_2$ are the average mean and Gaussian curvatures respectively, $c_1$ and $c_2$ are the principal curvatures at a given point on the surface, $H_0$ is the spontaneous mean curvature, and $\kappa$ and $\kappa_G$ are the mean and Gaussian curvature moduli [61]. The mean curvature modulus represents the energetic cost of pure bending of the initially flat monolayer, whereas the Gaussian modulus represents the energy required to change the Gaussian curvature. This can be done without changing the mean curvature by carrying out a saddle deformation, where $K$ is negative at all points apart from certain flat points, where it is zero. A positive change in Gaussian curvature from the flat state inevitably also involves (at least locally) a change in mean curvature away from zero as well. According to the Gauss-Bonnet theorem, the average Gaussian curvature of a closed surface is constant unless there is a change in overall topology [54].

Corresponding elastic parameters can be found for bilayers. The preferred curvature $H_0$ for a symmetric composition bilayer must be zero by symmetry, and the bilayer bending modulus $\kappa^b$ is expected to be simply twice the value for a monolayer [58]. However, the expression for the bilayer Gaussian modulus, $\kappa_G^b$, is more complex [62, 63]:

$$ \kappa_G^b = 2(\kappa_G - 2\kappa H_0 l) \tag{2} $$

All of the parameters on the right of (2) refer to a monolayer, including $l$, the monolayer thickness. Previous work [59, 64] has shown that pressure increases the monolayer spontaneous curvature, $H_0$ (as described above) and it has been suggested that pressure will also increase the monolayer bending modulus [65]. It is thus to be expected [54] that pressure will also increase the bending modulus of a bilayer. It is worth noting that lipids which tend to form inverse structures have a negative monolayer spontaneous curvature, so pressure will tend to decrease the magnitude of this negative mean curvature. The observation that pressure can stabilise bicontinuous cubic lipid phases [64], which have a negative interfacial Gaussian curvature, suggests that pressure also increases the bilayer Gaussian modulus, although this is not obvious from consideration of (2), since pressure increases $\kappa$ but decreases the magnitude of $H_0$, which will tend to cancel each other out, making it difficult to predict the effect on $\kappa_G^b$.

### 3.2 Lipid phase transitions

As mentioned previously, the effect of increasing pressure tends to oppose that of increasing temperature. This relationship can be quantified using the Clapeyron
equation to determine the pressure-dependence of a lipid phase transition temperature, $T_\text{i}$:

$$\frac{dT_\text{i}}{dp} = \frac{\Delta V_m}{\Delta S_m} = \frac{T_\text{i} \Delta V_m}{\Delta H_m}$$

(3)

where $\Delta S_m$, $\Delta H_m$ and $\Delta V_m$ are the molar transition entropy, enthalpy and volume changes respectively. These parameters can be determined at atmospheric pressure by using differential scanning calorimetry (DSC) to determine $T_\text{i}$, $\Delta S_m$ and $\Delta H_m$ and pressure perturbation calorimetry (PPC) to measure $T_\text{i}$ and $\Delta V_m$. If $\Delta S_m$ and $\Delta V_m$ are independent of pressure or have the same pressure dependence, the Clapeyron equation predicts a linear relationship between transition temperature and pressure and in practice, this tends to be true up to around 200 MPa.

### 3.3 Pressure jumps and structural transformation

Pressure jumps can yield valuable information about the kinetics and mechanisms of phase transitions in lipid systems. Time-resolved diffraction data can give information about possible intermediate structures or the pathway of transformations between different structures and recently, a physical kinetic model for certain lipid structure changes has also appeared [66]. If a suitable kinetic model can be fitted under different final pressure and temperature conditions, the rate at which the transition takes place can be related to the volume of activation, $\Delta^\ddagger V$, using:

$$\frac{k(p)}{k_0} = e^{-\frac{p \Delta^\ddagger V}{RT}}$$

(4)

where $k(p)$ and $k_0$ are the rate constants at pressure $p$, and at atmospheric pressure respectively. The volume of activation can be interpreted using transition state theory as the difference in volume between the transition state and the volume of the reactants at the same pressure. This can be thought of as an elastic barrier to transformation in much the same way as the activation energy for a reaction is thought of as a thermal energetic barrier to a reaction.

### 4 Examples of experimental results on lipid membranes

Self-assembled lipid structures can consist of either bulk lyotropic mesophases, or discrete vesicles. The methods for extracting structural data from each of these
types of sample are rather different. Furthermore, the effect of hydrostatic pressure on these two types of sample is also very different, and is discussed below.

### 4.1 Lyotropic mesophases

Small angle X-ray diffraction identifies the mesophase adopted by a lipid sample as well as revealing its structural parameters. Increasing hydrostatic pressure will tend to increase the conformational ordering of lipid hydrocarbon chains. This will reduce the chain splay and the cross-sectional area per lipid molecule, increase the chain length (i.e., the layer thickness), and reduce the magnitude of any preferred inverse interfacial curvature. This latter effect will tend to increase the lattice parameter of any inverse mesophase formed, such as bicontinuous cubic phases, if they can take up water from a coexisting excess aqueous region.

In addition to causing changes to the structural parameters of lipid structures, larger pressure changes can induce lipid phase transitions (in a similar way to changing temperature). By scanning hydrostatic pressure and temperature, full pressure-temperature phase diagrams can be built up and these have been determined for a variety of lipid systems [1, 64, 67, 68]

Using a combination of DSC (differential scanning calorimetry) and PPC (pressure perturbation calorimetry), $\Delta S_m$, $\Delta H_m$ and $\Delta V_m$ values have been measured for the gel to fluid transition of dipalmitoylphosphatidylcholine (DPPC) in excess water [49]. The predicted slope of the phase boundary is $0.22 \degree C \text{MPa}^{-1}$ or $22 \degree C \text{kbar}^{-1}$, in excellent agreement with the experimentally measured values [69]. Interestingly, the volume and entropy changes for lipid phase transitions tend to vary in the same way, and since they appear as a ratio in the Clapeyron equation, the pressure dependence of the transition temperature for many different phase changes is remarkably similar [1, 2, 70] with $dT_c/dp$ generally in the range $2–3 \degree C \text{MPa}^{-1}$.

Hydrostatic pressure was found to induce formation of a bicontinuous cubic phase in ditetradecyl-phosphatidylethanolamine (DTPE) in excess water which is not seen at atmospheric pressure, where a fluid lamellar – $H_{ii}$ transition is observed upon heating [64]. This effect is thought to be due to increased chain extension making the packing energy cost of forming a 2-D hexagonal phase higher as described earlier. The bicontinuous cubic phases have significantly lower packing frustration than hexagonal phases and are therefore thought to be generally favoured by pressure.

Pressure has also been found to have a significant effect on lipid gel phases, causing interdigitation in phosphatidylcholine gel phases [71] and resolution of a ripple gel structure in bovine brain sphingomyelin [72].
High pressure SAXS, in combination with FTIR has also been able to give a valuable insight into liquid ordered – liquid disordered lamellar phase ($L_o - L_d$) coexistence in a dipalmitoyl-phosphatidylcholine (DPPC), ergosterol mixture [31]. It has been shown that pressure can induce fluid – fluid phase separation from a fully mixed fluid phase, and then induce formation of lipid gel structures at higher pressure.

4.2 Lipid vesicles

While pressure has exactly the same effect on lipid molecules in vesicles as in extended mesophases, pressure not only causes mesoscopic structural changes in the lipid bilayer, but may also cause large scale changes in the overall vesicle shape. The lipid bilayer which forms a vesicle is far from being a static film surrounding the water within it. In fact, both the thermal expansivity and isothermal compressibility of the lipid bilayer are significantly higher than those of water [73, 74] Consequently, it is expected that on increasing temperature or reducing pressure, the surface area to volume ratio of a spherical vesicle will increase and, as the lipid bilayer can be considered largely impermeable to water over a short timescale and away from the chain melting transition temperature, this may lead to large scale morphological changes in the vesicle. This effect has been extensively studied both theoretically and experimentally as a function of temperature [73] and a huge range of equilibrium vesicle shapes have been visualised using optical microscopy. While investigations of vesicle structure as a function of pressure have been more limited, significant morphological changes have been observed by high pressure fluorescence microscopy [75].

4.3 Dynamic structural changes

In addition to investigating the static pressure dependence of lipid mesophase structure, pressure jumps coupled to time resolved X-ray diffraction have been used to investigate the dynamic structural evolution of lipid systems as they transform from one phase to another. This is achieved by affecting a rapid pressure jump across a previously determined phase boundary. A wide range of lyotropic lipid phase transitions have been investigated in this way, including: gel – $L_{\alpha}$ [76], $L_{\alpha} - H_{II}$ [77], $L_{\alpha} -$ bicontinuous cubic [28], $H_{II} -$ bicontinuous cubic [33], and bicontinuous cubic – bicontinuous cubic [29, 32] X-ray diffraction has proved extremely useful in identifying relatively short lived structural intermediates in lipid phase transitions. For example [28], during the transition between the $L_{\alpha}$
and Pn3m bicontinuous cubic phase of monoelaidin in excess water, a series of other Im3m and Pn3m bicontinuous cubic phases have been detected over about 15 minutes before formation of the final, stable Pn3m phase. It has been proposed that these intermediate phases act as reservoirs accommodating excess water during the transition, which are then destroyed as water equilibrates within the sample. The rates at which lipid structural transitions take place can be quantified by tracking the relative intensities of diffraction peaks in a series of time resolved X-ray patterns. It should be noted that when carrying this out, if the layer spacing of a structure changes during the transition, the intensity of the peaks will be modulated as they move within the form factor envelope. However, for most transitions, this change in lattice parameter is sufficiently small to be ignored. The formation and destruction kinetics of lipid transitions have largely been fitted empirically [29, 33, 34, 78] to obtain rate constants which have been compared as a function of transition conditions. Changes in layer spacing during lamellar to bicontinuous cubic lipid structures [28] have been qualitatively attributed to a proposed stalk transition model [79]. and recently a quantitative kinetic model has been developed [66] to describe phase transitions between different bicontinuous cubic phases in lipid systems. This has been shown to fit very well to a variety of experimental data and can be used to extract useful physical parameters from pressure-jump experiments which have, until now, not been available.

5 Outlook

The development of high pressure X-ray diffraction equipment and its application to lipid systems has provided unique insights into structural transitions in model membranes. The continuing development of synchrotron SAXS beamlines with higher flux and faster time resolution, and high pressure technology that can deliver ultra-fast pressure jumps will soon unlock our ability to study lipid ordering and lipid-protein co-assembly as well as a wide range of other exciting new studies of biomembrane assemblies at the micro- or even nano-second timescale.

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