



# **Measurement of Something**

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# Chapter 1

## Introduction

Lipids are amphiphilic molecules, consisting of hydrophilic headgroup and hydrophobic chains. There are various kinds of lipids. These can be categorized in terms of headgroup, chain length, and chain saturation.

In water lipids self-assemble into lipid bilayers to shield their hydrophobic cores. Lipid bilayers are the building blocks of cell membranes. Lipid bilayers display a wide variety of thermodynamic phases as a function of temperature and hydration. Figure 1.1 shows a generic phase diagram of phosphatidylcholines (PC). At full hydration, a lamellar phase coexists with excess water. PC lipids constitute a substantial fraction of mammalian cell membranes and have been studied for many decades. In the high temperature, fluid  $L_\alpha$  phase, the hydrocarbon chains are conformationally disordered, and intra-membrane molecular correlations are liquid-like [1]. In the low temperature, gel  $L'_\beta$  phase, hydrocarbon chains are stiff and tilted with respect to the membrane normal [2]. Between the fluid and gel phases appears a structurally modulated or rippled membrane phase. This phase has been detected in several lipids (REF: cite as many lipids as possible). The low angle diffraction pattern of this phase conforms to the symmetry of a 2D monoclinic lattice. This phase was termed  $P'_\beta$  and is commonly called the ripple phase. The topography of the membrane ripples has been directly visualized by freeze fracture electron microscopy experiments [3–7]. The wavelength of the modulation is about 140 Å for dimyristoylphosphatidylcholine (DMPC), which has 14 carbons in the hydro carbon chains [8]. There was evidence that molecular conformation in the ripple phase is not unique. NMR signals in the ripple phase [9] were consistent with a superposition of signals observed in the fluid and gel phases. Lateral diffusion measurements found two distinct populations, with

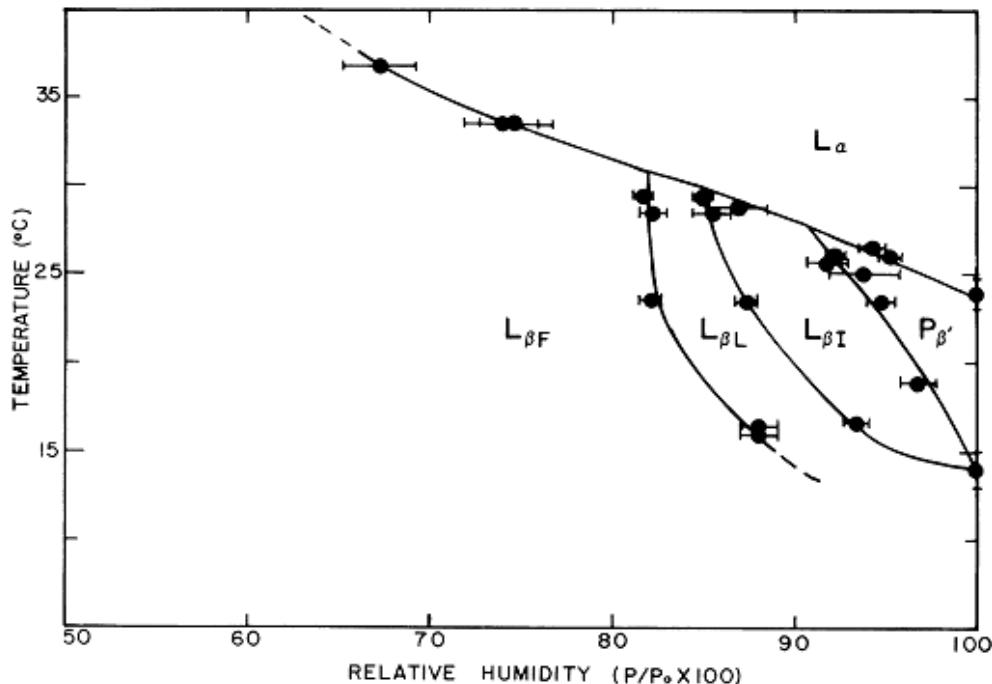


Figure 1.1: Experimental phase diagram of DMPC from Ref. [11].

diffusion coefficients characteristic of fluid and gel phases [10].

At higher temperature, lipids are in fluid phase. In this phase, chains are flexible. Lipid bilayers are also flexible and fluctuating. This flexibility of lipid bilayers make many interesting biological phenomena possible.

As the temperature is reduced, lipid bilayers go into the gel phase. In this phase, chains are straightened out and bilayers are rigid.

Between the fluid and gel phases, some lipids have the ripple phase. This phase is found in saturated lipids. In this phase, the bilayer height is modulated in a periodic manner in the in-plane direction. Each bilayer is registered along its orthogonal direction.

In this thesis, we focus on the fluid and ripple phase. In the former phase, we investigated the interaction of a peptide called Tat with lipid bilayers in the fluid phase. Tat is discussed in chapter 3. Regarding the ripple phase, we measured the electron density profile of the lipid bilayers using a stack of oriented bilayers. Using wide angle x-ray scattering technique, we also investigated the chain packing within a bilayer. The ripple phase is discussed in chapter 4. The appendices show a lot of details that will allow other people to reproduce much of the results shown in this

thesis as well as help readers understand scattering analysis employed in this work. It is my hope that these details will help future researchers, especially students, understand some of the techniques to investigate the structure of lipid bilayers in sub Angstrom resolution.

# Chapter 3

## Ripple Phase

When the temperature is reduced from the fluid phase, the ripple phase is observed in bilayers consisting of DMPC and DPPC lipids. This chapter discusses X-ray scattering experiments on the ripple phase formed by dimyristolphosphatydylcholine (DMPC) bilayers.

### 3.1 Introduction

(At some point, do some literature search and write up this section) The ripple phase has been a fascinating thermodynamic phase to many physicists and physical chemists since its discovery. It was originally observed in calorimetry study for alkanes by sturevant. Although this phase has never been reported to occur in a biologically relevant situation, it provides an interesting opportunity to study fundamental lipid interactions and their influence on the bilayer shape. (Let's find some recent papers and see if anyone says anything about biological relevance)

In the first structural study of this phase by Tardieu *et al.*, the X-ray diffraction pattern from DLPC was phased by a pattern recognition technique and the electron density map was calculated. It was shown that the structure corresponds to a 2D oblique unit cell shown in Fig. 3.1. The calculated electron density map showed that DLPC bilayers are height modulated and have a smooth, asymmetric shape. The ripple wavelength  $\lambda_r$  was reported to be 85.3 Å, the lamellar periodicity  $D$  55.3 Å, and the oblique angle  $\gamma$  110°. The electron density map reported the ripple amplitude  $A = 15$  Å in DLPC.

Various experiments have indicated the existence of two types of ripple phases: the

stable asymmetric and the metastable symmetric phase. In the asymmetric phase, a plane of reflection perpendicular to the ripple wave vector is absent. The metastable symmetric phase has been seen in DPPC bilayers, but not in DMPC.

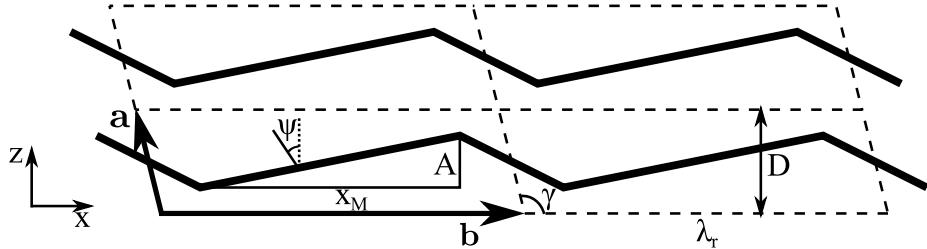


Figure 3.1: Lattice structure of the asymmetric ripple phase. Unit cells are shown in dash lines. Center of bilayers are shown by thick, solid lines. Notations in the figure are (**a** and **b**: lattice unit vectors), ( $D$ :  $D$ -spacing along  $z$ ), ( $\lambda_r = |\mathbf{b}|$ : ripple wavelength), ( $\gamma$ : oblique tilt angle), ( $A$ : ripple amplitude), ( $\psi$ : chain tilt angle with respect to the  $z$  direction), and ( $x_M$ : projected length of the major arm).

The equilibrium structure of the ripple phase has been extensively studied by X-ray diffraction [2,8,85–89], neutron diffraction [90,91], AFM [], freeze fracture electron microscopy [92], and freeze fracture scanning tunneling microscopy [] techniques. In the scanning tunneling microscopy experiment [7], the three-dimensional contours of the ripple phase  $P_{\beta'}$  of dimyristoylphosphatidylcholine (DMPC) were imaged, and a ripple wavelength of 130 Å and an amplitude of 45 Å were reported.

While many studies used multilamellar samples, the ripple phase was also found in unilamellar vesicles, where a vesicle has only one bilayer [93].

The ripple phase has been detected in a wide variety of lipids, including phosphatidylcholines, phosphatidylacetoyl (PA), and phosphatidylglycerol (PG), but no ripple phase has been observed in bilayers composed of PE headgroups. These studies suggest that the size of headgroup has something to do with the ripple formation.

From X-ray data of the DMPC ripple of unoriented samples, Wack and Webb [8] argued that the ripples have a sawtooth shape, but were unable to phase the observed pattern. Their X-ray form factor data were later phased by employing a modeling and fitting technique by Sun *et al.* [88], and the electron density map was calculated, which indicated that the ripples indeed have a sawtooth shape. The map also showed that the major arm is about twice as long as the minor arm. The bilayer thickness was found to be larger than that of the minor arm. The value of the bilayer thickness in the major arm was comparable to the thickness of DMPC bilayers in the gel phase

whereas the thickness of the minor arm was comparable to that in the fluid phase.

A structural investigation by X-ray diffraction of the ripple phase of oriented dipalmitoylphosphatidylcholine (DPPC) samples indicated that hydrocarbon chains are packed in a hexagonal lattice with chains tilted in the plane perpendicular to the ripple wave vector [94]. In that study, the oblique angle  $\gamma$  was found to be  $90^\circ$ . It is believed that the resolved structure was for the symmetric ripple, which has been shown to be thermodynamically metastable and whose occurrence depends on the sample history [95]. In [94], only symmetric ripple was observed in the low angle X-ray scattering, which seems to contradict with the metastability of this symmetric ripple.

Sengupta et al. [96] has investigated temperature dependence of the average structure of DMPC and concluded that there is no obvious change in the structure as a function of temperature. On the other hand, the ripple phase composed of POPC showed some variation in the average structure. Based on calculated electron density profiles and model parameters, they argued that chains in both major and minor arms are tilted with respect to the stacking  $z$  direction by the same amount and that chains are parallel to the local normal in the major arm. This argument was inconsistent with the findings in [88] that the thickness of major arm is almost identical to that of the gel phase where chains are tilted by  $\sim 30^\circ$ . To circumvent this discrepancy, Sengupta *et al.* speculated that chains might be titled by some amount into the direction perpendicular to the ripple direction. This type of information , however, is not well captured in low angle scattering data, and wide angle scattering is essential.

In a giant unilamellar vesicle composed of a mixture of DPPC and DOPC, co-existing domains of  $L'_\beta$  and  $P'_\beta$  have been found [97]. The  $P'_\beta$  domain had lower concentration of DPPC than the  $L'_\beta$  domain. Addition of anionic lipids (DOPG?) turned the gel phase domain into the ripple phase domains. The authors concluded that reduction of surface tension drove highly stressed gel phase to less stressed ripple phase.

**AFM** The ripple phase has also been observed in the top layer of solid supported double layers through atomic force microscopy (AFM). The effect of the bottom layer on the top layer in the ripple phase has not been thoroughly studied. It is not clear whether the structure of these ripple formation top layers is the same as that in a bulk sample such as MLVs and oriented samples.

A few MD (molecular dynamics) simulations have shed light on molecular organi-

zation in the ripple phase as well. de Vrie *et al.* [98] carried out atomistic simulations resulting in an assymetric ripple where chains are gel-like in the major arm and interdigitated in the minor side. Coarse-grain simulations performed later essentially found the same results [99].

A theory developed by Chen *et al.* [100] has been successful in describing some features in the ripple phase. In this theory, the divergence of the tilt field of lipids are coupled to the curvature of the bilayer. Increase in the divergence of the lipid tilt is compensated by increase in the curvature, leading to the observed height modulated ripple phase. This theory predicted ripple phases with different symmetry for chiral and achiral lipids. Later, Katsaras and Raghunathan [101] carried out low angle X-ray scattering experiment on regular DMPC and achiral DMPC and found that there was no structural difference between them.

Raghunathan theory (2011)

Schmidt theory (2013)

$D$ (Å)	$\lambda_r$ (Å)	$\gamma$ (deg)
55.0	159.4	99.0
57.0	140.8	97.6
57.3	151.6	97.8
57.4	148.4	97.6
57.5	144.1	97.8
57.5	141.9	98.0
58.0	140.1	98.2
<b>57.8</b>	<b>145.0</b>	<b>98.2</b>
58.0	141.7	98.4
59.8	129.6	97.3
60.6	130.1	97.0
61.5	130.8	96.5
62.4	122.0	95.9
63.9	123.1	94.9
64.9	120.3	92.3

Table 3.1: Lattice constants for DMPC at  $T = 18.0$  °C reported by Wack and Webb [8] except the one colored in blue. The data collected and analyzed in this thesis are colored blue.

## 3.2 Materials and Methods

### 3.2.1 Sample Preparation

DMPC was purchased from Avanti Polar Lipids. Four mg DMPC powder was dissolved in 140  $\mu\text{l}$  chloroform:methanol (2:1 v:v) mixture. The solution was plated onto silicon wafers following the rock and roll procedure [102]. See also Sec. 2.2.3. For all the ripple phase experiments, the temperature of the hydration chamber was set to 18 °C. In 2011 and 2012 synchrotron experiments, the samples were created and annealed more than a week in advance and stored in a refrigerator. The quality of these samples measured by their mosaic spread was found to worsen over time after the samples were annealed. Therefore, to attempt better quality, the samples were annealed for only about 12 hours just before the X-ray experiment. Figure 3.2 shows a picture of the annealing chamber. Annealing is promoted both by hydration and by elevated temperature. To achieve gentle but efficient hydration of a sample, filter papers were installed that exposed a larger surface for evaporation. The temperature was set to 60 °C. It must be emphasized that the annealing chamber should equilibrate in an annealing oven set to 60 °C, prior to putting a sample in the chamber. When a sample was put in the chamber sitting at a room temperature and then the system was placed inside the oven, warmer water vapor inside the chamber condensed on the cooler sample, causing so called flooding of oriented sample. A small drop of water on an oriented film is detrimental for the orientation quality because the entropy-driven formation of unilamellar vesicles causes oriented bilayers to peel off one by one.



Figure 3.2: Picture of an annealing chamber.

The sample for the grazing incident wide angle study was prepared in the same way as for low angle study. In order to minimize the geometric broadening, the sample

was trimmed to 1 mm in width along the beam direction.

The sample for transmission study was deposited on a thin, 35 micron, silicon wafer, and oriented following the rock and roll procedure [102]. Because the wafer was very fragile, attaching the sample to a sticky thing was impossible. Instead, the sample was attached to a plastic cap on a small vial with a small amount of heat sink compound at a corner of the wafer. The wafer was stable enough for rocking.

### 3.2.2 Instrumental Resolution

The X-ray scattering experiments were carried out at the Cornell High Energy Synchrotron Source (CHESS) G1 station in three different runs (2011, 2012, and 2013). The low angle X-ray scattering (LAXS) data analyzed in this thesis were collected in 2013. The near grazing incidence wide angle X-ray scattering (NGIWAXS) data were also collected in the 2013 run, but with smaller energy dispersion than in the LAXS experiment. The transmission wide angle X-ray scattering (TWAXS) data were collected in the 2011 run. The ripple phase experiment in the 2012 run was not successful due to low sample quality. The instrumental resolution in these X-ray experiments depended on the beam divergence, energy dispersion, and geometric broadening.

#### 3.2.2.1 Divergence

The beam divergence quantifies an angular spread of the incoming X-ray beam. We estimated the beam divergence by measuring the horizontal and vertical beam widths at two known sample-to-detector  $S$  distances with difference  $\Delta S$ . The beam widths were larger at the further distance, which indicated that the beam was divergent. We calculated the divergence as  $\text{div} = \Delta B / \Delta S$ , where  $\Delta B$  is the difference in beam widths at different  $S$  distances. Table 3.2 summarizes beam divergence.

year	type of experiment	horizontal (rad.)	vertical (rad.)
2013	LAXS	$4.2 \times 10^{-5}$	$1.6 \times 10^{-4}$
2013	NGIWAXS	$4.2 \times 10^{-5}$	$1.6 \times 10^{-4}$
2011	TWAXS	$2.5 \times 10^{-5}$	$5 \times 10^{-5}$

Table 3.2: Beam divergence

### 3.2.2.2 Energy dispersion

A W/B<sub>4</sub>C multilayer monochromator with energy bandwidth  $\Delta E/E$  of 1.3% was used in the LAXS and TWAXS experiments. The energy of the X-ray beam was 10.55 keV, corresponding to a wavelength  $\lambda$  of 1.175 Å, in the LAXS experiment. To achieve a higher instrumental resolution than that for the LAXS experiment, a (111) silicon monochromator was used for the NGIWAXS experiment, which gave  $\Delta E/E$  of 0.01%. Due to the geometry of the G1 station, the Si monochromator was placed in the G1 hutch, in series with the multilayer monochromator. Table 3.3 summarizes energy dispersion.

year	type of experiment	$\Delta E/E$ (%)	$E$ (keV)	$\lambda$ (Å)
2013	LAXS	1.5	10.55	1.175
2013	NGIWAXS	0.01	10.55	1.175
2011	TWAXS	1.5	10.54	1.176

Table 3.3: Energy dispersion

### 3.2.2.3 Geometric Broadening

The beam footprint on the sample has a finite size and this causes geometric broadening of diffraction peaks on the CCD detector.

**LAXS** In the LAXS experiment, the geometric broadening in the horizontal  $x$  direction is simply the horizontal beam width for  $k = 0$  peaks with minor additional broadening for  $k \neq 0$  peaks. Geometric broadening in the vertical  $z$  direction is due to different heights of the sample along the  $y$  direction of the beam at non zero angle of incidence  $\omega$ . It is given approximately by  $w_s \tan \theta$ , where  $w_s$  is the sample width along the  $y$  direction and  $\theta$  is the scattering angle. The beam shape, measured through a semi-transparent 200 µm thick molybdenum (Mo) beam stop, is shown in Fig. 3.3 and 3.4. The horizontal beam width was 1.7 pixels (0.12 mm). The vertical beam width was approximately 1 mm, tall enough to cover the entire sample if the sample was tilted between 0° and 11.5°. The sample was rocked during X-ray exposure between -1.6° and 7° in order to observe many diffraction peaks in one data collection and keep all the sample in the beam.

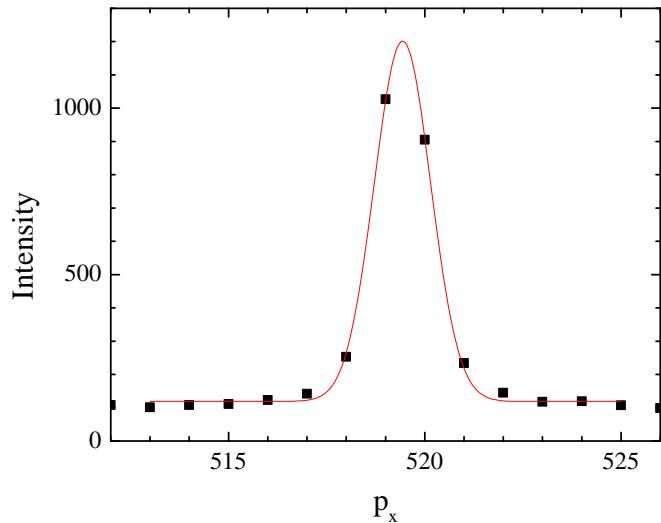


Figure 3.3: The horizontal profile of the beam used in the 2013 low resolution study. Each pixel was 0.07113 mm, which gave a CCD angular resolution  $\Delta\theta$  of 0.0057°, corresponding to  $\Delta q = 0.0011 \text{ \AA}^{-1}$  at the sample to detector distance of 359.7 mm. The beam FWHM = 1.7 pixels, giving  $\Delta\theta = 0.010^\circ$  or  $\Delta q = 0.0019 \text{ \AA}^{-1}$ .

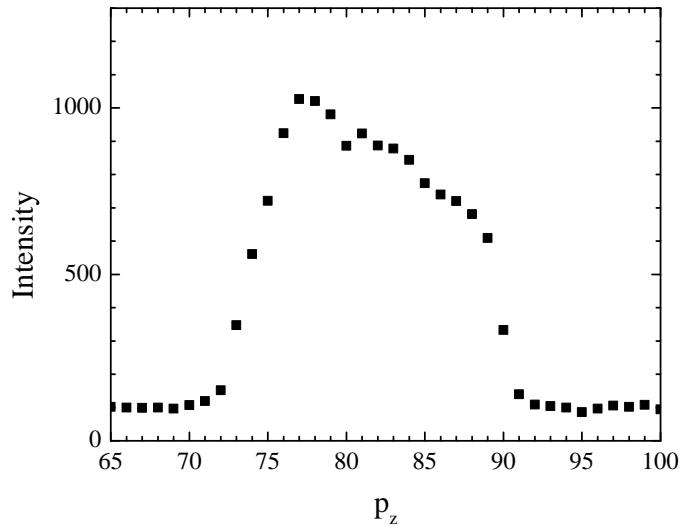


Figure 3.4: The vertical profile of the beam used in the 2013 low resolution study. The beam height = 15 pixels = 1.1 mm.

**NGIWAXS** In the NGIWAXS experiment, the horizontal geometric broadening was due to the sample width along the beam direction and the horizontal beam width. From the geometry of the experiment shown in Fig. 3.5, the geometric broadening  $\Delta x$  can be estimated, assuming simple additivity,

$$\Delta x = \Delta x_{\text{beam}} + w_s \tan(2\theta),$$

where  $\theta$  is the in-plane scattering angle. The total scattering angle  $2\theta$  for the ripple WAXS was approximately  $16^\circ$ . To minimize the contribution to  $\Delta x$  from the sample, the sample was trimmed to  $w_s = 1$  mm along the beam direction. This width was chosen because (1) I could not trim more without a more sophisticated device than a simple razor blade, (2) a very narrow sample would be a weak scattering body, and (3) disordering effect from the sample edge might become too significant to ignore. Given the above reasons and due to limited availability of synchrotron beam time, I considered a 1 mm width to be reasonable. The horizontal beam width was 4 pixels (0.28 mm) as shown in Fig. 3.6. With these experimental parameters, the resolution was estimated to be  $\Delta x = 0.57$  mm = 8 pixels, which would be the unresolved width of an intrinsically infinitely sharp wide angle peak. [Comment and refer to the gel phase data shown in the result section of NGIWAXS.](#) The sample to detector distance were 220.6 mm, measured using silver behenate. Then, the minimum peak width measured in  $q$ -space would be  $\Delta q \approx 0.014 \text{ \AA}^{-1}$ . The vertical geometric broadening was negligible because the sample width  $w_s$  was narrow and scattering of interest occurred at small  $q_z$ .

**TWAXS** In the TWAXS experiment, geometric broadening in both  $x$  and  $z$  directions was non-negligible. To calculate the broadening, let us assume that the beam has a rectangular cross section with its height  $Y_b$  and width  $X_b$  as shown in Figure 3.8. When the sample is tilted by  $\omega$ , X-rays emerging from the top edge of the sample travel extra distance compared to the distance that X-rays from the bottom edge of the sample travel. This, then, leads to distortion of the scattered beam; namely, the scattered beam will appear on the CCD screen as a parallelogram as shown in Figure 3.8. Figure 3.9 shows the top- and sideview of the projection of the beam on the sample. From simple geometry, it can be shown that  $a = Y_b / \tan \omega$ ,  $b = aX/(2S)$ ,  $c = aZ/(2S) + Y_B/2$ , and  $B = \tan^{-1}(Z/S)$ . Since  $H = 2c$  and  $W = 2b$ ,  $H$  and  $W$  in

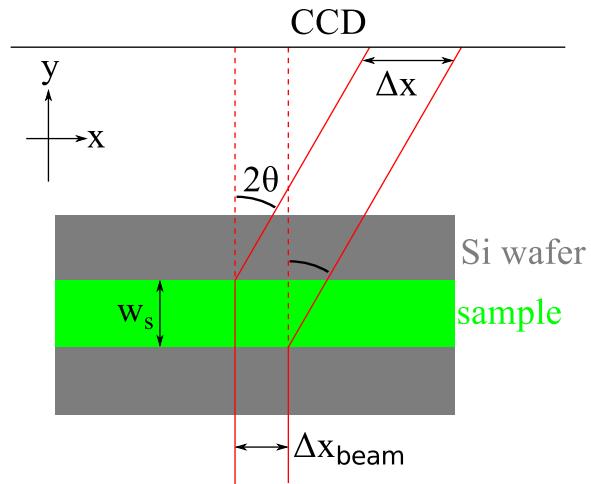


Figure 3.5: In-plane geometric broadening due to the sample width  $w_s$  and the beam width  $\Delta x_{beam}$ . A top view of the sample (green) on the Si wafer (gray) and the incoming and diffracted X-rays (bounded by red solid lines) are shown. The total in-plane scattering angle for a lipid chain-chain correlation is labeled as  $2\theta$ , and the geometric broadening as  $\Delta x$ .

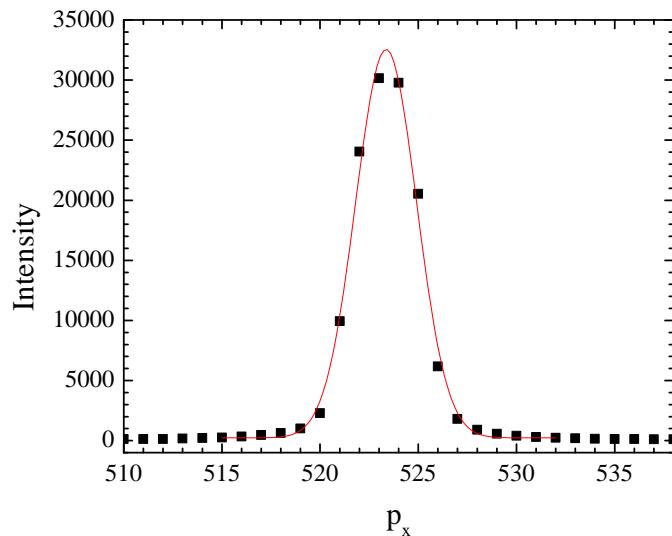


Figure 3.6: The horizontal profile of the beam used in the 2013 high resolution experiment. The CCD angular resolution  $\Delta\theta = 0.0092^\circ$  corresponding to  $\Delta q = 0.0017 \text{ \AA}^{-1}$ , at the sample to detector distance of 220.6 mm. The beam FWHM = 3.7 pixels = 0.26 mm, giving  $\Delta\theta = 0.034^\circ$  or  $\Delta q = 0.0063 \text{ \AA}^{-1}$ .

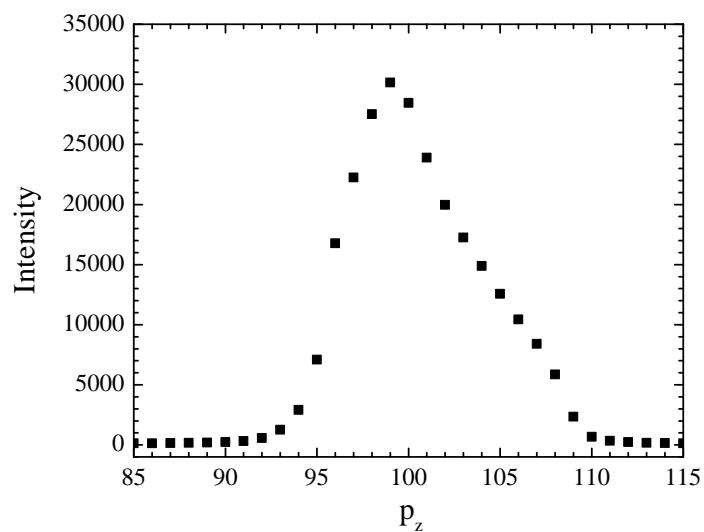


Figure 3.7: The vertical profile of the beam used in the 2013 high resolution experiment. The beam height = 9 pixels = 0.64 mm.

Figure 3.10 are given by

$$H = Y_b \left( 1 + \frac{Z}{S \tan \omega} \right) \quad (3.1)$$

$$W = Y_b \frac{X}{S \tan \omega}. \quad (3.2)$$

The sample to detector distance  $S$  was 158.6 mm, giving an angular CCD resolution of  $0.013^\circ/\text{pixel}$ , or  $0.0024 \text{ \AA}^{-1}/\text{pixel}$ . The observed wide angle peak was at  $(X, Z) = (44.0 \text{ mm}, 15.5 \text{ mm})$ . The beam width and height were both  $0.2 \text{ mm} = 2.8 \text{ pixels}$ . With this setup,  $W = 0.7 \text{ pixels}$  and  $H = 3.1 \text{ pixels}$ . Therefore, the distorted shape of the diffraction peak was negligible. Table 3.4 summarizes geometric broadening for our experiments.

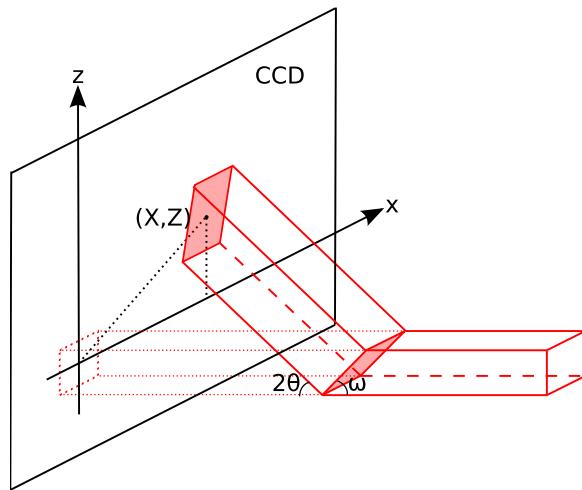


Figure 3.8: Geometric broadening in TWAXS. The cross section of the incoming X-ray with the sample and the CCD detector are both shaded in red. The sample is tilted by  $\omega$ . The red dots show the transmitted beam. The incoming beam is rectangular but upon scattering appears as a parallelogram on the CCD.

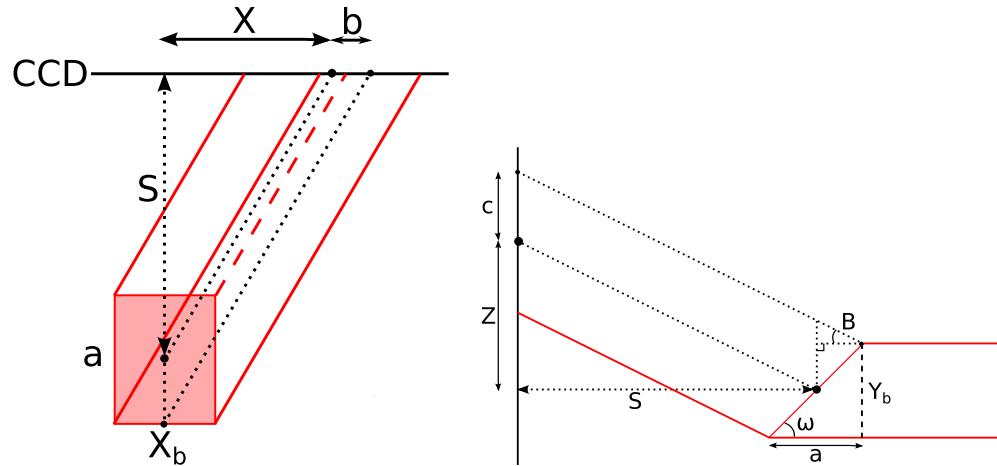


Figure 3.9: Top and side view of the beam on the sample in TWAXS. The cross section of the incoming X-ray with the sample is shaded in red.  $X_b$  and  $Y_b$  are the beam width and height, respectively.  $S$  is the sample to detector distance.  $(X, Z)$  is a position of the center of the scattered beam on the detector with respect to the center of the transmitted beam as shown in Figure 3.8.

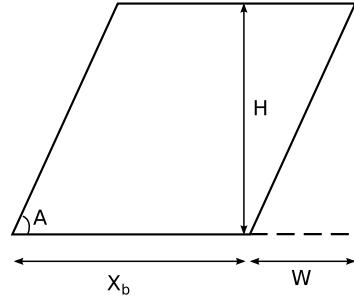


Figure 3.10: Projection of rectangular beam on the detector. Scattered beam appears as a parallelogram on the CCD.

type of experiment	horizontal (pixels)	horizontal ( $\text{\AA}^{-1}$ )	vertical (pixels)	vertical ( $\text{\AA}^{-1}$ )
LAXS	1.7	0.0019	$6.6q_z$	$0.0072q_z$
NGIWAXS	8	0.014		
TWAXS	2.8	0.0067	3.1	0.0074

Table 3.4: Geometric broadening

### 3.2.3 Low Angle X-ray Scattering Experiment

The X-ray beam for the low angle X-ray scattering (LAXS) experiment was set up by the station scientist, Dr. Arthur Woll. We chose the sample to detector distance to be 359.7 mm, measured by indexing silver behenate Bragg peaks. The D-spacing of silver behenate is known to be 58.367 Å.

Occasionally, sheets of molybdenum (Mo), each nominally 25  $\mu\text{m}$  were used to attenuate the incoming beam. These sheets were installed by Dr. Arthur Woll in the upstream of the sample chamber. The attenuation length  $\mu$  of 10.55 keV X-ray in Mo is 13.74  $\mu\text{m}$  [103]. For a 25  $\mu\text{m}$  thick Mo attenuator, the attenuation factor is calculated to be  $[\exp(-25/13.74)]^{-1} = 6.2$ . The exact attenuation factor was determined by comparing X-ray images collected with and without the attenuator, shown in Fig. 3.11. The attenuation factor of the nominally 25  $\mu\text{m}$  thick Mo was found to be 6.9 for the wavelength used (1.175 Å), indicating an actual thickness of 27  $\mu\text{m}$ .

Sheets of Mo were also used as a semi-transparent beam stop downstream of the sample, just outside the hydration chamber, to attenuate the beam and strong orders. 100 and 200  $\mu\text{m}$  were used to attenuate strong orders and either 200 or 225  $\mu\text{m}$  to attenuate the beam. To avoid saturation of CCD pixels by the very intense beam of  $10^{11}$  photons/mm<sup>2</sup>/second, the beam stop was always set to attenuate the beam.

A few Bragg peaks in the low angle X-ray scattering of the ripple phase were very strong, leading to saturation of CCD pixels for data collection with a long exposure time. In order to probe a wide range of  $q$ -space, three images were taken: 1) a short, one second exposure with a nominally 25 micron molybdenum attenuator installed in the upstream of the sample to reduce the intensity of the incoming X-ray beam, 2) one second exposure without the beam attenuator, and 3) 60 second exposure with a beam stop blocking the very intense (1,0) and (2,0) peaks. See Fig. 3.12. Then, the integrated intensity of (1,0) peak was measured from the first image. This value was multiplied by 6.9 to account for the beam attenuation and by 60 to scale with the exposure time. The intensity of (2,0) and (2,-1) were measured from the second image, also multiplied by 60 to account for the shorter exposure time. The intensities of the rest of the observed peaks were measured from the third image.

The integrated intensity of each peak was obtained using the Nagle lab tview software developed by Dr. Yufeng Liu [50] by putting a box around a peak and summing up the intensity in those pixels that fall inside the box. The background

scattering was estimated by measuring the intensity in pixels near the peak but not containing any peak tail. The choice of box size was made according to the width of each peak. Because of mosaic spread in the sample, the peaks were wider for higher orders. Consequently, the box was made wider for higher orders. The box size was chosen so that approximately 80% of the peak intensity was counted toward the integrated intensity.

### 3.2.4 Near Grazing Incidence Wide Angle X-ray Scattering Experiment

The high resolution wide angle X-ray scattering (WAXS) experiment was also carried out at the G1 station. The instrument was set up by the G1 station scientist, Arthur Woll, and the assistant scientist, Dr. Robin Baur. Wide angle X-ray scattering was collected at an incident angle of  $0.2^\circ$ . The total external reflection from an air-lipid interface occurs approximately at  $0.1^\circ$  and  $0.17^\circ$  for air-silicon interface, so  $0.2^\circ$  is not quite grazing incidence. Grazing incidence usually implies that the incident angle is less than the critical angle for a total external reflection. Therefore,  $0.2^\circ$  is called near grazing incidence (NGI) in this thesis. The background scattering was collected at  $-0.2^\circ$ . Subtraction of the negative angle data from the positive angle data resulted in a clean sample scattering image.

### 3.2.5 Transmission Wide Angle X-ray Scattering Experiment

The transmission wide angle X-ray scattering (TWAXS) experiment was also carried out at the G1 station. The incident angle  $\omega$  was set to  $-45^\circ$  for transmission data collection (see Fig. 3.13). A  $35\ \mu\text{m}$  thick silicon substrate absorbs 10.5 keV X-ray by only 20% [103], so most of the incoming X-rays penetrated the thin substrate.

Unfortunately, the axis of the rotation motor did not coincide with the sample axis, so the sample to detector distance varied as  $\omega$  was varied. To accurately measure the sample to detector distance, low angle scattering from a silver behenate (AgBe) sample was collected at a fixed  $\omega$ . Due to large mosaic spread of the AgBe sample, many orders were visible. While the relative intensity of each order was inaccurate, the positions of peaks were the same as those observed with a rotating sample. To measure the D-spacing of the sample,  $\omega$  was set to  $1^\circ$ . The sample to detector distance was measured to be 174.7 mm at  $\omega = 0^\circ$ . From the sample holder geometry shown in

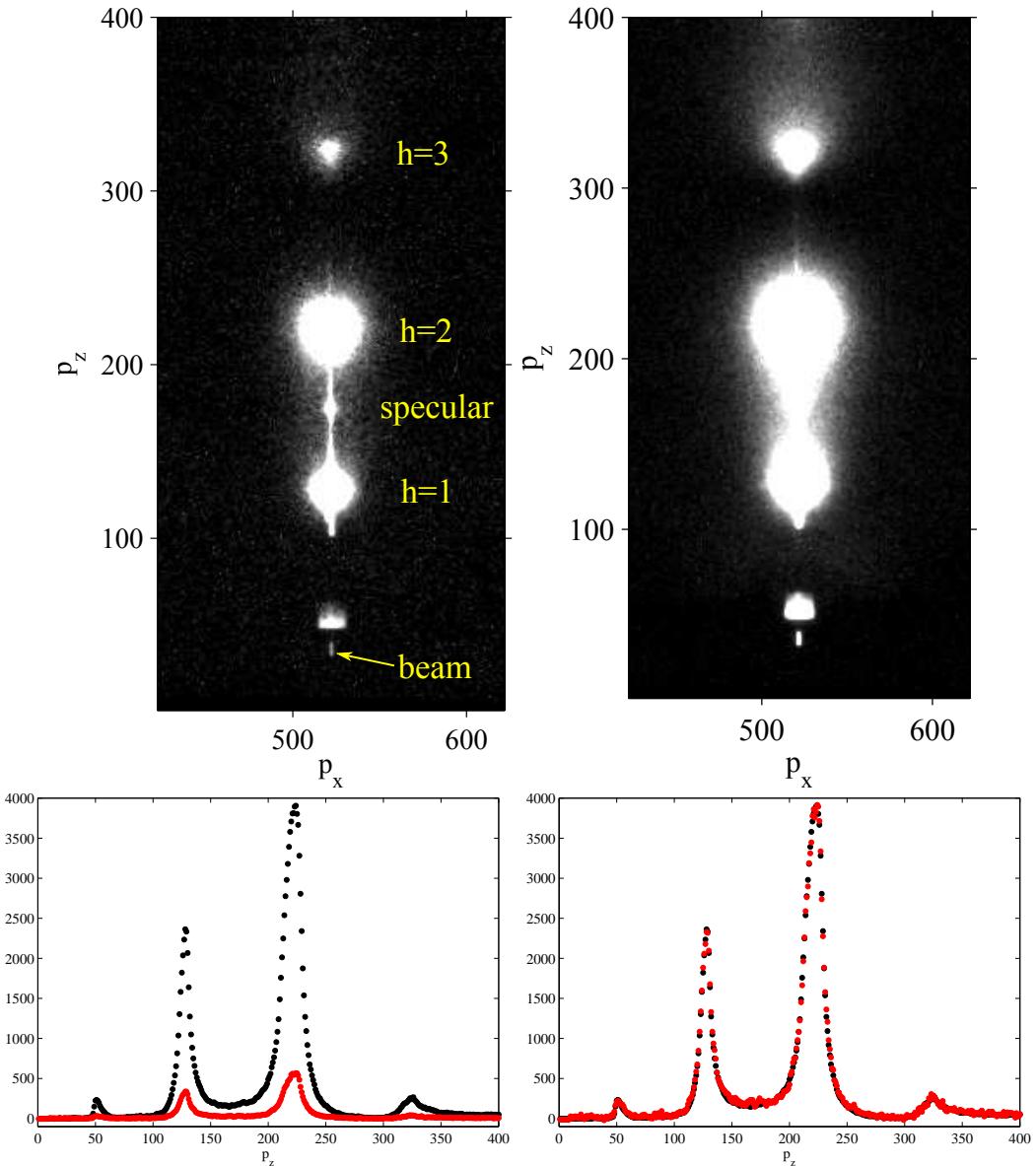


Figure 3.11: (top panels) CCD images of X-ray scattering taken with (left) and without (right) a nominally  $25 \mu\text{m}$  thick Mo attenuator. These data were taken at a fixed angle of incidence  $\omega = 0.8^\circ$ . The sample was an oriented film of DOPC:DOPE (3:1) in the fluid phase at  $37^\circ\text{C}$ . The wavelength was  $1.175 \text{ \AA}$ , the same as the one used for the ripple phase experiment. The same gray scale is used in both images.  $100 \text{ pixel} = 0.11 \text{ \AA}^{-1}$  in  $q$ . A small dot located about  $(p_x, p_z) = (520, 170)$  between the first and second orders is a specular reflection from the substrate. The exposure times were 1 second. (bottom panels) Vertical  $p_z$  slices of the X-ray images shown in the top panels (left). The scattering intensity measured with the attenuator (red solid circles) was multiplied by a factor of 6.9 and compared to the intensity measured without the attenuator (black solid circles, right).

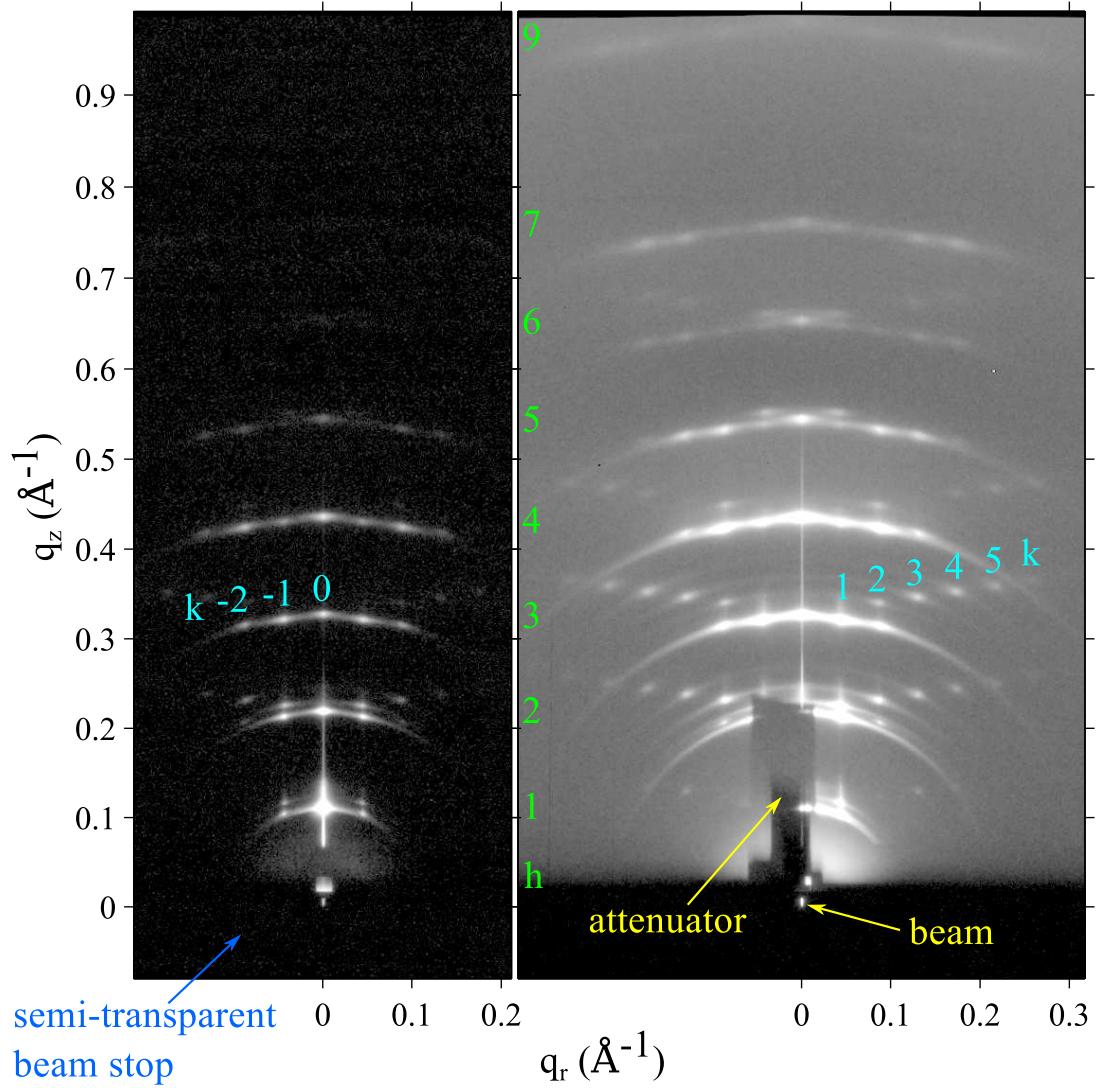


Figure 3.12: 1 second exposure (left) and 60 second exposure (right) of the low angle X-ray scattering from the DMPC ripple phase in gray log scales. The index  $h$  is labeled in green.  $(3, k)$  reflections are identified in cyan. The shadow cast by 100  $\mu\text{m}$  thick molybdenum attenuator blocking strong  $(1, 0)$  and  $(2, 0)$  orders in the right image is labeled as attenuator and extends from  $q_z = 0 \text{ \AA}^{-1}$  to  $0.2 \text{ \AA}^{-1}$ .  $D = 57.8 \text{ \AA}$ ,  $\lambda_r = 145.0 \text{ \AA}$ , and  $\gamma = 98.2^\circ$ .

Fig. 3.14, the sample to detector distance was estimated to be 158.6 mm at  $\omega = 45^\circ$ . A picture of the sample holder is shown in Fig. 3.15.

To level the sample, the sample was first leveled coarsely by watching the sample scattering. When  $\omega$  was negative, much of the incoming beam was absorbed by the flat substrate, yielding weak sample scattering. When  $\omega$  became positive, sample scattering was strong. With this procedure, we leveled the sample with an uncertainty of  $\pm 0.2^\circ$ . We then measured the beam intensity at various sample heights as a function of  $\omega$ . The sample was level when the beam intensity had the narrowest dip as the sample was moved vertically through the beam.

Background scattering was collected by replacing the sample with a bare wafer. The bare wafer was not placed exactly at the same location as the sample, which gave slightly different background scattering. This only affected the background subtraction near the beam. The wide angle scattering was not affected by this inexact placement of the bare wafer.

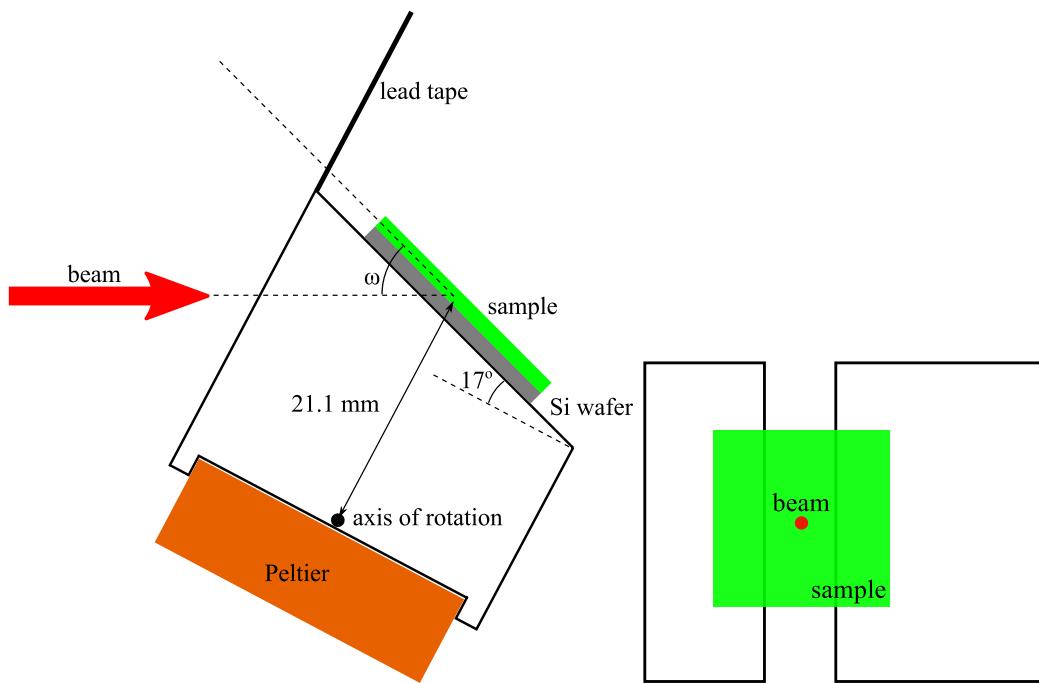


Figure 3.13: Schematics of the sample holder in the transmission mode. Side (left) and top (right) views are shown. The thickness of the Si wafer =  $35 \mu\text{m}$ . The thickness of the sample  $\approx 10 \mu\text{m}$ . The distance between the axis of rotation and sample = 21.1 mm.

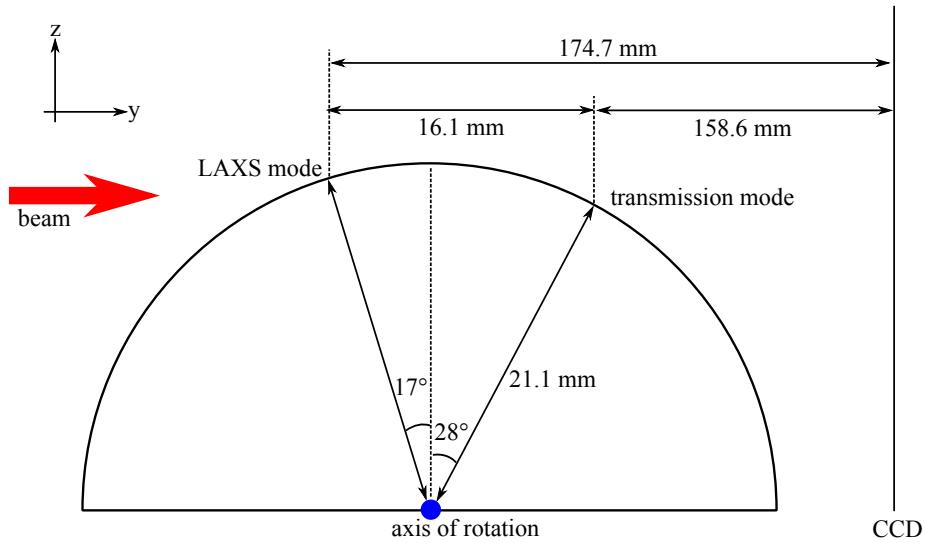


Figure 3.14: Circular path followed by the sample as the angle of incidence  $\omega$  was changed. The sample to detector distance and  $D$ -spacing of the sample were measured in the LAXS mode, where  $\omega = 1^\circ$ . WAXS images were collected at the transmission mode, where  $\omega = -45^\circ$ . The  $z$  position of the sample was slightly higher at the LAXS mode than at the transmission mode, so the sample holder was vertically shifted for different modes.

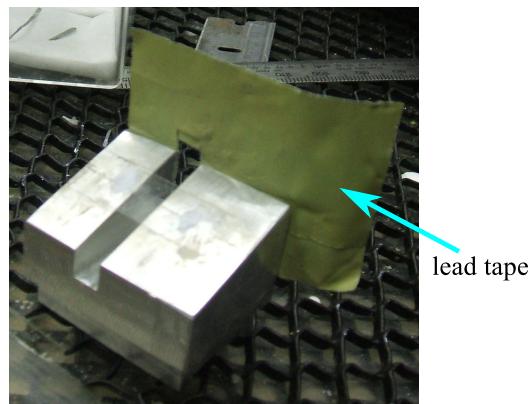


Figure 3.15: Picture of the sample holder looking from above. A lead tape was attached to the back of the sample holder to help reduce the background scattering, typically coming from the air gap between the flightpath snout and the mylar window of the chamber.

### 3.3 LAXS: analysis

#### 3.3.1 Lattice Structure

The unit cell vectors for the two-dimensional oblique lattice shown in Fig. 3.1 can be expressed as

$$\mathbf{a} = \frac{D}{\tan \gamma} \hat{\mathbf{x}} + D \hat{\mathbf{z}} \quad (3.3)$$

and

$$\mathbf{b} = \lambda_r \hat{\mathbf{x}}. \quad (3.4)$$

The corresponding reciprocal lattice unit cell vectors are

$$\mathbf{A} = \frac{2\pi}{D} \hat{\mathbf{z}} \quad (3.5)$$

and

$$\mathbf{B} = \frac{2\pi}{\lambda_r} \hat{\mathbf{x}} - \frac{2\pi}{\lambda_r \tan \gamma} \hat{\mathbf{z}}. \quad (3.6)$$

The reciprocal lattice vector,  $\mathbf{q}_{hk}$  for the Bragg peak with Miller indices  $(h, k)$  is

$$\mathbf{q}_{hk} = h\mathbf{A} + k\mathbf{B}, \quad (3.7)$$

so its Cartesian components are

$$\mathbf{q}_{hk} \cdot \hat{\mathbf{x}} = q_{hk}^x = \frac{2\pi k}{\lambda_r} \equiv q_k^x \quad (3.8)$$

$$\mathbf{q}_{hk} \cdot \hat{\mathbf{y}} = q_{hk}^y = 0 \quad (3.9)$$

$$\mathbf{q}_{hk} \cdot \hat{\mathbf{z}} = q_{hk}^z = \frac{2\pi h}{D} - \frac{2\pi k}{\lambda_r \tan \gamma}. \quad (3.10)$$

Our sample consists of many ripple domains with a uniform distribution of in-plane directions of the ripple wave vector,  $\mathbf{b}$  in Fig. 3.1. In this case,  $q_{hk}^x$  and  $q_{hk}^y$  are combined to give  $q_{hk}^r = 2\pi k / \lambda_r$ .

### 3.3.2 Sample q-space

The incoming and outgoing wavevectors of the x-ray beam in Fig. 3.16 are given by

$$\mathbf{k}_{\text{in}} = \frac{2\pi}{\lambda} \hat{\mathbf{y}}, \quad \mathbf{k}_{\text{out}} = \frac{2\pi}{\lambda} (\sin 2\theta \cos \phi \hat{\mathbf{x}} + \cos 2\theta \hat{\mathbf{y}} + \sin 2\theta \sin \phi \hat{\mathbf{z}}), \quad (3.11)$$

where  $\lambda$  is the wavelength of x-ray,  $2\theta$  is the total scattering angle, and  $\phi$  is the angle measured from the equator on the detector. The scattering vector (also called momentum transfer vector) is the difference between  $\mathbf{k}_{\text{in}}$  and  $\mathbf{k}_{\text{out}}$ ,

$$\begin{aligned} \mathbf{q} &= \mathbf{k}_{\text{out}} - \mathbf{k}_{\text{in}} \\ &= q (\cos \theta \cos \phi \hat{\mathbf{x}} - \sin \theta \hat{\mathbf{y}} + \cos \theta \sin \phi \hat{\mathbf{z}}), \end{aligned} \quad (3.12)$$

where  $q = 4\pi \sin \theta / \lambda$  is the magnitude of the scattering vector. When the sample is rotated by  $\omega$  about the lab x-axis in the clockwise direction as shown in Fig. 3.16, the sample  $q$ -space also rotates and are given by

$$\hat{\mathbf{e}}_x = \hat{\mathbf{x}}, \quad \hat{\mathbf{e}}_y = \cos \omega \hat{\mathbf{y}} + \sin \omega \hat{\mathbf{z}}, \quad \hat{\mathbf{e}}_z = -\sin \omega \hat{\mathbf{y}} + \cos \omega \hat{\mathbf{z}}. \quad (3.13)$$

From Eq. (3.12) and (3.13), we find Cartesian components of the sample  $q$ -space to be

$$\begin{aligned} q_x &= \mathbf{q} \cdot \hat{\mathbf{e}}_x = q \cos \theta \cos \phi, \\ q_y &= \mathbf{q} \cdot \hat{\mathbf{e}}_y = q (-\sin \theta \cos \omega + \cos \theta \sin \phi \sin \omega), \\ q_z &= \mathbf{q} \cdot \hat{\mathbf{e}}_z = q (\sin \theta \sin \omega + \cos \theta \sin \phi \cos \omega). \end{aligned} \quad (3.14)$$

The position,  $(X, Z)$ , of a CCD pixel is measured with respect to the beam and given by

$$X = S \tan 2\theta \cos \phi, \quad Z = S \tan 2\theta \sin \phi, \quad (3.15)$$

where  $S$  is the distance between the sample and detector.

From a model for the electron density of a lipid bilayer, one calculates the X-ray scattering intensity pattern,  $I(\mathbf{q})$ . Then, Eq. (3.14) and (3.15) relate  $I(\mathbf{q})$  to the experimentally measured intensity pattern,  $I(X, Z)$ . It is important to remember that a given pixel position,  $(X, Z)$ , corresponds to a triplet  $(q_x, q_y, q_z)$ . Fully exploring the sample  $q$ -space requires changing  $\omega$  for a fixed wavelength, which was achieved

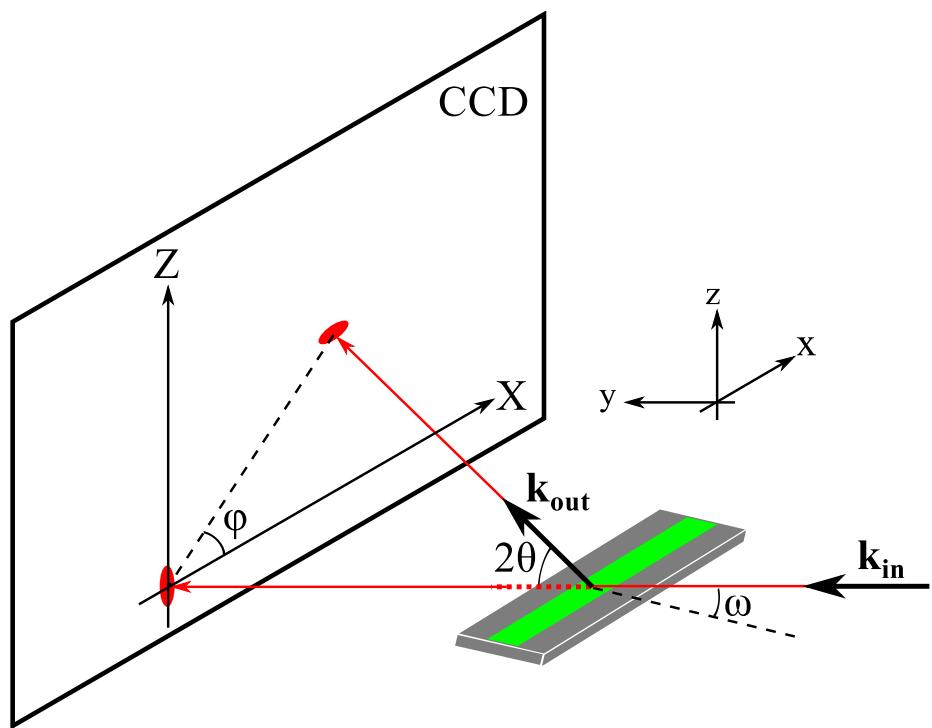


Figure 3.16: Experimental reflectivity geometry.

by continuously rotating the sample with a motor. In the ripple phase, because our sample has in-plane rotational symmetry, the ripple side peaks ( $h, k \neq 0$ ) make up Bragg rings while the main peaks ( $h, k = 0$ ) are still delta function like (see Fig. 3.17) in  $q$ -space. In order for the main peak to be observed,  $\omega$  must be equal to  $\theta_B$ , but the side peaks are observed at any  $\omega$ . Those side peaks get slightly smeared due to integration over  $q_y$ .

For low angle x-ray scattering (LAXS), it is convenient to linearize the above equations in terms of  $\theta$  and  $\omega$ . In the small angle approximation,  $\sin \phi \approx Z/(2S\theta)$  and  $\cos \phi \approx X/(2S\theta)$ , and

$$\begin{aligned} q_x &\approx \frac{4\pi\theta \cos \phi}{\lambda} \approx kX/S \\ q_y &\approx q_z \omega - \frac{4\pi\theta^2}{\lambda} \approx q_z \omega - \frac{\lambda q_z^2}{4\pi} \\ q_z &\approx \frac{4\pi\theta \sin \phi}{\lambda} \approx kZ/S, \end{aligned} \quad (3.16)$$

with  $k = 2\pi/\lambda$ . For wide angle X-ray scattering, the exact relations given by Eq. (3.14) are necessary. Especially in the transmission experiment, where  $\omega$  is large, an observed X-ray pattern appears nontrivial and becomes almost impossible to analyze without the use of Eq. (3.14). The transmission experiment is discussed in Sec.3.7.

### 3.3.3 Lorentz Correction

Our sample has in-plane rotational symmetry about the  $z$ -axis. Ignoring mosaic spread to which we will come back later, this means that the sample consists of many domains with differing ripple directions, all domains being parallel to the substrate. In sample  $q$ -space, ripple ( $h, k \neq 0$ ) side peaks are represented as rings centered at the meridian, or  $q_z$ -axis, while ( $h, k = 0$ ) main peaks are still points on the meridian (see Fig. 3.17). Then, for an arbitrary incident angle  $\omega$ , ( $h, 0$ ) peaks are not observed while side peaks are observed for a range of  $\omega$  as will now be explained.

In order to capture all ( $h, k$ ) peaks in one X-ray exposure, the sample was continuously rotated over a range of  $\omega, \Delta\omega$ , about the  $x$ -axis. As a result of this rotation, the ( $h, 0$ ) main peaks become arcs that subtend an angle  $\Delta\omega$ , as shown in Fig. 3.18, with its length equal to  $\Delta\omega q_{h0}$ . The detector records the intersections of these arcs

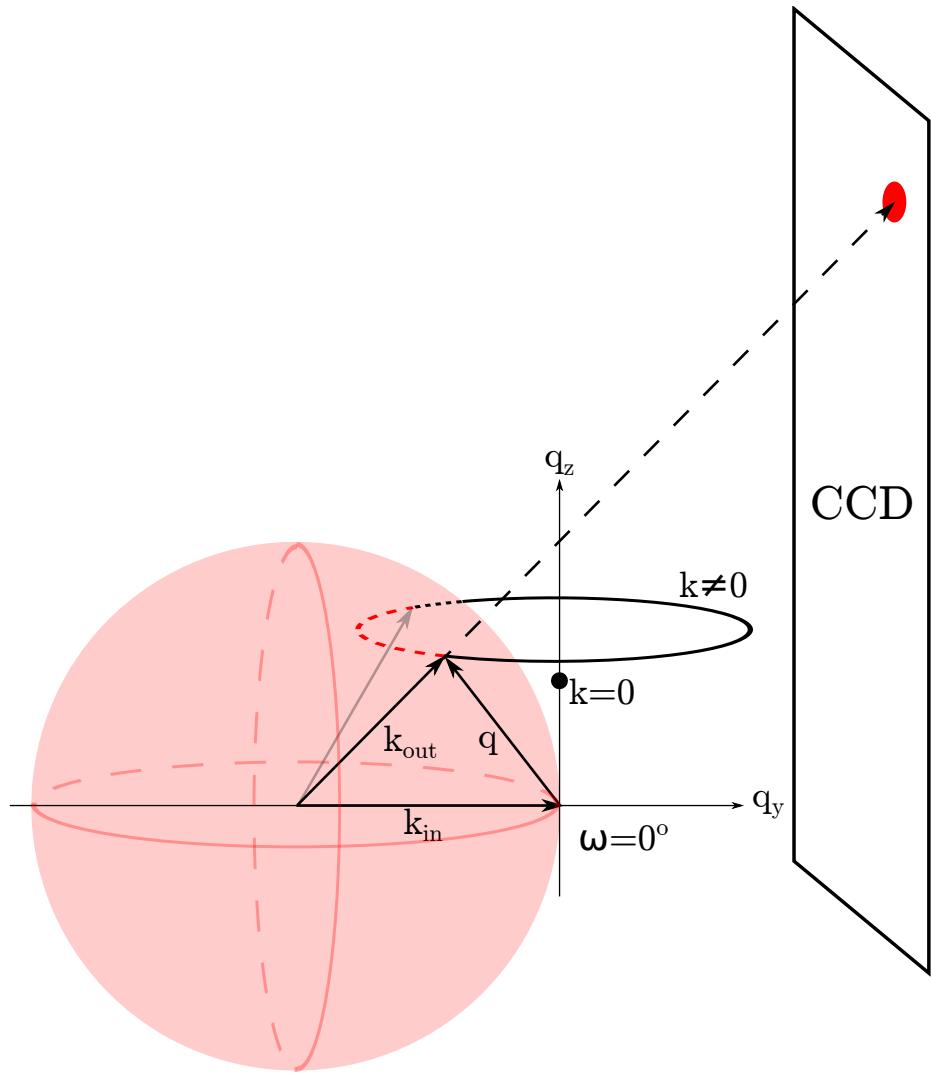


Figure 3.17: Ewald sphere construction for the ripple phase diffraction in the low angle regime. A ripple  $k = 0$  peak is the solid, black circle on the  $q_z$ -axis. A ripple  $k \neq 0$  ring is the black ring centered about the  $q_z$ -axis. The portion of the ring that is inside the Ewald sphere is shown as a red dashed line and the portion of the ring that is outside but behind the Ewald sphere is shown as a black dotted line. The magnitude of the total scattering angle is exaggerated. With a wavelength of  $1.175 \text{ \AA}$ , the magnitude  $|k_{in}| = 5.35 \text{ \AA}^{-1}$ . For a  $h = 5$  peak,  $q_{50}^z = 0.54 \text{ \AA}^{-1}$ , one tenth of  $k_{in}$ .

with the Ewald sphere, so the intrinsic scattering intensity of the  $(h, k = 0)$  reflections is the product of the observed intensity,  $I_{hk}^{\text{obs}}$  with the arc length, that is,

$$I_{h0} = \Delta\omega q_{h0}^z I_{h0}^{\text{obs}}. \quad (3.17)$$

This is the usual Lorentz correction for lamellar orders.

Now, we consider relative intensity of side peaks for a given order  $h$ . As described earlier,  $(h, k \neq 0)$  side peaks are represented as rings whose radius is  $q_{hk}^r$  in the sample  $q$ -space. Because only the domains with the right ripple direction can satisfy the Bragg's condition at a given fixed angle  $\omega$ , the intrinsic scattering intensity in this ring is reduced by a factor of  $2\pi q_k^r$  compared to the  $(h, 0)$  reflections. This reduction of intensity can be nicely visualized by the Ewald sphere construction shown in Fig. 3.17, which shows that the entire rings are not intersected by the Ewald sphere at a fixed angle. Then, the intrinsic scattering intensity in a ring is

$$I_{hk \neq 0} \propto 2\pi q_{hk}^r I_{hk}^{\text{obs}}. \quad (3.18)$$

During an X-ray exposure, the sample  $q$ -space rotates and the rings are intersected by the Ewald sphere at all our experimental incident angles  $\omega$ . However, as Fig. 3.19 shows, only small parts of the rings are actually intersected with the Ewald sphere. To obtain the full expression for  $(h, k \neq 0)$  reflections, we now turn to a more rigorous calculation.

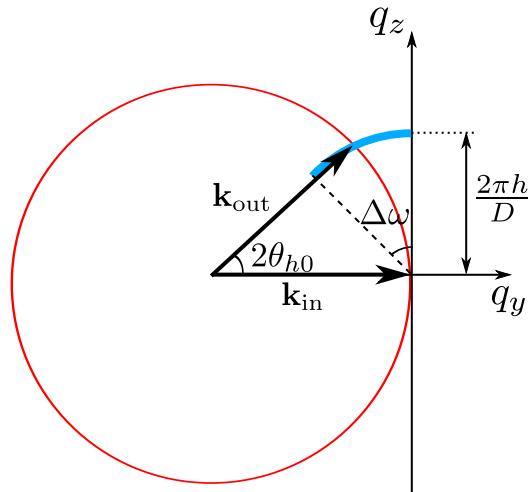


Figure 3.18: Side view of an arc of  $k = 0$  peak shown as a thick blue line.

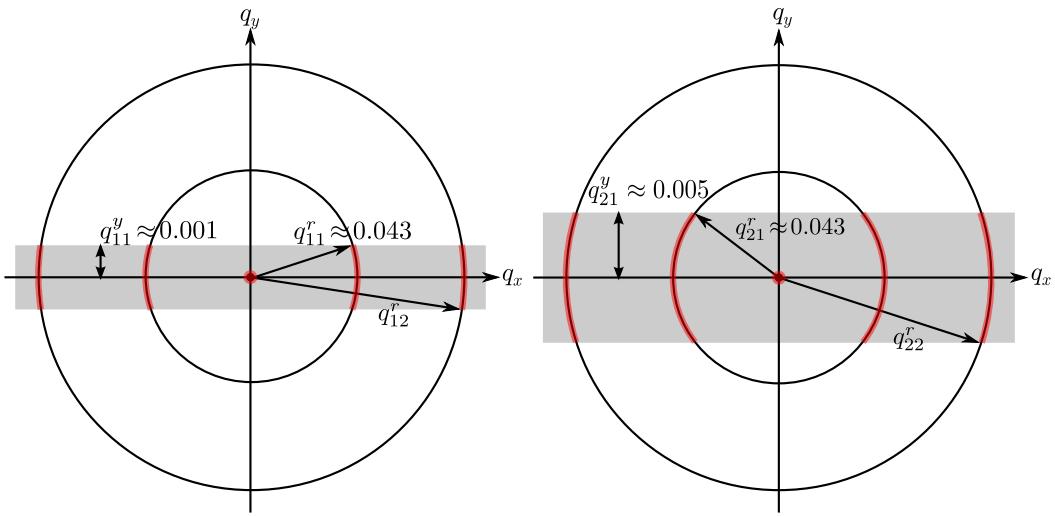


Figure 3.19:  $q$ -space representations of Bragg peaks and Bragg rings for  $h = 1$  and  $2$  and  $k = 0, 1$ , and  $2$  in  $q_{hk}^z$  planes. The intersection between the Ewald sphere and a Bragg peak/ring is indicated in red. The observed intensity for the  $k \neq 0$  orders is proportional to the fraction of the length of red arcs in the circumference. This fraction is equal to one for  $k = 0$  reflections. Because the reflections are not in the same  $q_z$  plane, the range of  $q_y$  integration indicated by the height of the gray rectangle is different for different  $h$  orders. For  $\gamma \neq 90^\circ$ , the range of  $q_y$  integration is slightly different for different  $k$  reflections with the same  $h$ . The values shown are for  $D = 58$  Å,  $\lambda_r = 145$  Å,  $\gamma = 90^\circ$ , and  $\lambda = 1.175$  Å. The magnitude of curvature of arcs is exaggerated.

Mathematically, the rotation is equivalent to an integration over  $\omega$ . In low angle X-ray scattering,  $q_z$  is nearly constant at a given pixel as  $\omega$  is changed, which can be seen from Eq. (3.16). As Eq. (3.16) shows,  $\omega$  dependence appears only through  $q_y$ , so rotating the sample is realized by integrating over  $q_y$ ; formally, we write  $d\omega = dq_y/q_z$ . To derive the integration limits on  $q_y$ , let us consider two cases: (1) When  $\omega \leq 0$ , the incoming X-ray beam is blocked by the back of the substrate. This sets the lower limit of  $\omega$  to 0. Plugging  $\omega = 0$  in Eq. 3.16), we find the lower limit of the  $q_y$  integration to be  $-\lambda q_z^2/(4\pi)$ . (2) When  $\omega \geq 2\theta$ , the substrate blocks the outgoing X-ray, so the maximum  $\omega = 2\theta$ . Within the small angle approximation,  $q_z \approx 4\pi\theta/\lambda$ . Then, the maximum  $\omega$  can be expressed as  $\lambda q_z/(2\pi)$ . Plugging this expression for  $\omega$  in Eq. (3.16), we find the upper limit of the  $q_y$  integration to be  $\lambda q_z^2/(4\pi)$ . Also integrating over the detector pixels  $X$  and  $Z$  to obtain integrated intensity, we write the observed intensity as

$$\begin{aligned} I_{hk}^{\text{obs}} &\propto \int dX \int dZ \int d\omega I_{hk} \\ &\propto \int dq_x \int dq_z \int_{-\frac{\lambda q_z^2}{4\pi}}^{\frac{\lambda q_z^2}{4\pi}} \frac{dq_y}{q_z} I_{hk}(\mathbf{q}), \end{aligned} \quad (3.19)$$

where  $1/q_z$  factor in  $q_y$  integration is the usual Lorentz polarization factor in the small angle approximation.

For a crystalline sample with in-plane rotational symmetry, the structure factor of a ripple Bragg peak is

$$S_{hk}(\mathbf{q}) = S_{hk}(q_r, q_z) = \frac{1}{2\pi q_r} \delta(q_r - q_{hk}^r) \delta(q_z - q_{hk}^z), \quad (3.20)$$

where  $q_{hk}^r = 2\pi|k|/\lambda_r$ . Thus, the scattering pattern in the ripple phase is a collection of Bragg rings for  $k \neq 0$  centered at the meridian and the Bragg peaks for  $k = 0$  located along the meridian. The scattering intensity is  $I(\mathbf{q}) = |F(\mathbf{q})|^2 S(\mathbf{q})$ , where  $F(\mathbf{q})$  is the form factor. After the  $q_z$  integration, the observed, integrated intensity of  $(h, k)$  peak is proportional to

$$I_{hk}^{\text{obs}} \propto \frac{|F_{hk}|^2}{q_{hk}^z} \int dq_x \int_{-q_{hk}^{y0}}^{q_{hk}^{y0}} dq_y \frac{\delta(q_r - q_{hk}^r)}{2\pi q_r}, \quad (3.21)$$

where  $q_{hk}^{y0} = \lambda(q_{hk}^z)^2/(4\pi)$ . For side peaks ( $k \neq 0$ ), we have

$$\begin{aligned} \int dq_x \int_{-q_{hk}^{y0}}^{q_{hk}^{y0}} dq_y \frac{\delta(q_r - q_{hk}^r)}{2\pi q_r} &\approx \int_{-q_{hk}^{y0}/q_{hk}^r}^{q_{hk}^{y0}/q_{hk}^r} d\phi \int dq_r q_r \frac{\delta(q_r - q_{hk}^r)}{2\pi q_r} \\ &= \frac{q_{hk}^{y0}}{\pi q_{hk}^r}. \end{aligned} \quad (3.22)$$

For main peaks ( $k = 0$ ), we have

$$\begin{aligned} \int dq_x \int_{-q_{hk}^{y0}}^{q_{hk}^{y0}} dq_y \frac{\delta(q_r - q_{hk}^r)}{2\pi q_r} &= \int_0^{2\pi} d\phi \int dq_r q_r \frac{\delta(q_r - q_{hk}^r)}{2\pi q_r} \\ &= 1 \end{aligned} \quad (3.23)$$

Using Eq. (3.21 – 3.23), we write the observed integrated intensity as

$$I_{h0}^{\text{obs}} \propto \frac{|F_{h0}|^2}{q_{h0}^z} \quad (3.24)$$

$$I_{hk}^{\text{obs}} \propto \frac{|F_{hk}|^2}{q_{hk}^z} \frac{q_{hk}^{y0}}{\pi q_{hk}^r} = |F_{hk}|^2 \frac{\lambda q_{hk}^z}{2\pi} \frac{1}{2\pi q_{hk}^r} = |F_{hk}|^2 \frac{2\theta_{hk}}{2\pi q_{hk}^r}, \quad (3.25)$$

where  $2\theta_{hk} = \lambda q_{hk}^z/(2\pi)$  is the incident angle at which the outgoing X-ray for the peak  $(h, k)$  is blocked by the substrate. Eq. (3.24) and (3.25) relate the form factor calculated from a model to the experimentally observed intensity, and are partially equivalent to Eq. (3.17) and (3.18).

In non-linear least squares fitting procedure, we fitted the observed integrated intensity to the calculated intensity from a bilayer model using these Lorentz corrections. This is because we can determine experimental uncertainties on observed intensity rather than the Lorentz-corrected form factors. We avoid propagating the uncertainties by fitting a model to observed intensity.

### 3.3.4 Absorption Correction for LAXS

In this section, we derive the absorption correction for an oriented sample. The calculation involves an explicit integration over the incident angle,  $\omega$ , which is necessitated by the sample rotation during an X-ray exposure. The procedure is to write down an absorption factor,  $A(\omega, \theta)$ , for a given scattering angle at a given incident angle,

and then integrate over  $\omega$ . We ignore  $q_x$  dependence because the X-ray path inside the sample is nearly within the  $y$ - $z$  plane for low angle scattering. The correction for wide angle scattering is described in a later section.

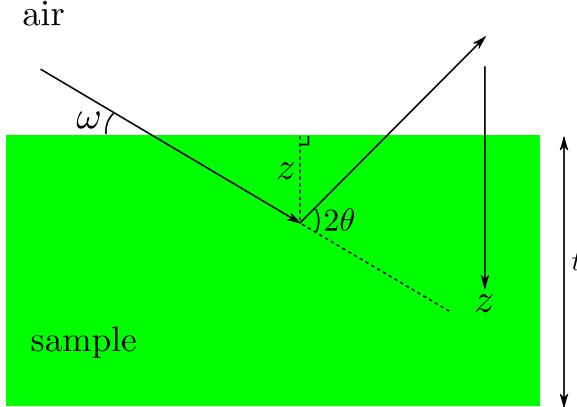


Figure 3.20: The path of X-rays within the sample. The incident angle is  $\omega$  and the total scattering angle is  $2\theta$ . An X-ray with a penetration depth of  $z$  is shown. The total thickness of the sample is  $t$ . Refraction correction is smaller than what? for  $\theta > 0.5^\circ$  ( $h = 1$ ).

Assume that all the X-rays enter the sample from the top surface. The total scattering angle is given by  $2\theta$  (see Fig. 3.20). Let the  $z$ -axis point downward. At the top surface (air-sample interface),  $z = 0$ . For X-rays that travel to  $z$  and then scatter, the total path length within the sample is

$$L(z, \omega, \theta) = \frac{z}{\sin \omega} + \frac{z}{\sin(2\theta - \omega)} = z g(\omega, \theta), \quad (3.26)$$

where  $g(\omega, \theta) = (\sin \omega)^{-1} + (\sin(2\theta - \omega))^{-1}$ . For each ray, the intensity is attenuated by the sample absorption. If non-attenuated intensity is equal to  $I_0$ , then the attenuated intensity is

$$I(z, \omega, \theta) = I_0 \exp\left(-\frac{L}{\mu}\right), \quad (3.27)$$

where  $\mu$  is the absorption length of an X-ray.  $\mu$  is about 2.6 mm for 10.5 keV for both water and lipids in all phases [103]. The observed intensity of scattering from a sample fixed at an angle  $\omega$  is equal to the integration of Eq. (3.27) over the total

thickness of the sample and given by

$$\begin{aligned} I(\omega, \theta) &= \int_0^t dz I(z, \omega, \theta) = I_0 \int_0^t dz \exp\left(-\frac{g(\omega, \theta)}{\mu} z\right) \\ &= I_0 \mu \frac{1 - \exp\left(-\frac{t}{\mu} g(\omega, \theta)\right)}{g(\omega, \theta)}. \end{aligned} \quad (3.28)$$

Defining the absorption factor at a fixed angle to be  $A(\omega, \theta)$ , the observed intensity can also be written as

$$I(\omega, \theta) = A(\omega, \theta) t I_0, \quad (3.29)$$

where  $t I_0$  is the intensity we would observe for non-absorbed X-rays. Equating Eq. (3.28) and (3.29), we get

$$A(\omega, \theta) = \frac{\mu}{t} \frac{1 - \exp\left(-\frac{t}{\mu} g(\omega, \theta)\right)}{g(\omega, \theta)}. \quad (3.30)$$

If  $\mu$  is taken to infinity (no absorption),  $A(\omega, \theta)$  goes to 1 as expected. The absorption factor  $A_{h0}$  for the  $k = 0$  peaks is given by  $A(\omega = \theta = \theta_B)$ , plotted in Fig. 3.21. As shown, this factor is about 20 % for  $h = 1$  peak relative to  $h = 4$ , so it is not negligible.

For  $k \neq 0$  side peaks, an integration over the incident angle  $\omega$  is necessary because these peaks are observable at all our experimental incident angles as described in section 3.3.3. The observed intensity for side peaks from a rotating sample is simply

$$I_{\text{obs}}(\theta) = \int_0^{2\theta} d\omega I(\omega, \theta). \quad (3.31)$$

The upper integration limit is equal to  $2\theta$  because the substrate completely blocks the scattered X-rays above this angle as discussed in section 3.3.3. Eq. (3.30), which is essentially the integrand in Eq. (3.31), is plotted in Fig. 3.22. It is maximum when  $\omega = \theta$ , meaning that the path length is shortest at the Bragg condition. The non-attenuated observed intensity is equal to  $2\theta t I_0$ . We, then, define the absorption factor  $A(\theta)$  to be the ratio of the total observed intensity to the total non-attenuated intensity,

$$A(\theta) \equiv \frac{I_{\text{obs}}(\theta)}{2\theta t I_0}. \quad (3.32)$$

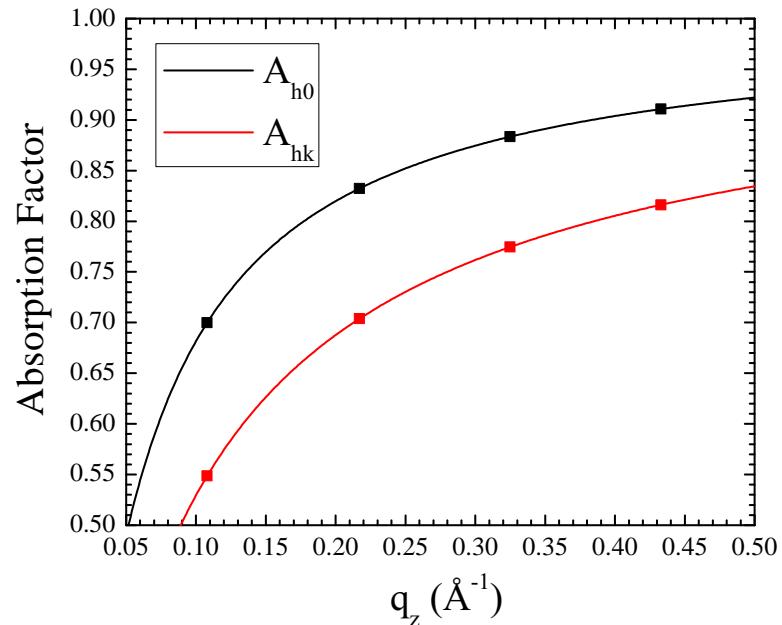


Figure 3.21: Absorption factors as a function of  $q_z \approx 4\pi\theta/\lambda$ . Values at  $q_z = 2\pi h/D$  corresponding to  $D = 57.8 \text{ \AA}$  are shown as squares.  $\mu = 2600 \mu\text{m}$ ,  $t = 10 \mu\text{m}$ , and  $\lambda = 1.175 \text{ \AA}$ .

Using Eq. (3.30) and (3.31) in (3.32), we arrive at the final absorption factor

$$A(\theta) = \frac{1}{2\theta} \int_0^{2\theta} d\omega A(\omega, \theta) = \frac{\mu}{2\theta t} \int_0^{2\theta} d\omega \frac{1 - \exp\left(-\frac{t}{\mu}g(\omega, \theta)\right)}{g(\omega, \theta)}. \quad (3.33)$$

$A_{hk} = A(\theta)$  is plotted in Fig. 3.21. The absorption correction  $A_c(\theta)$  is the inverse of Eq. (3.33).

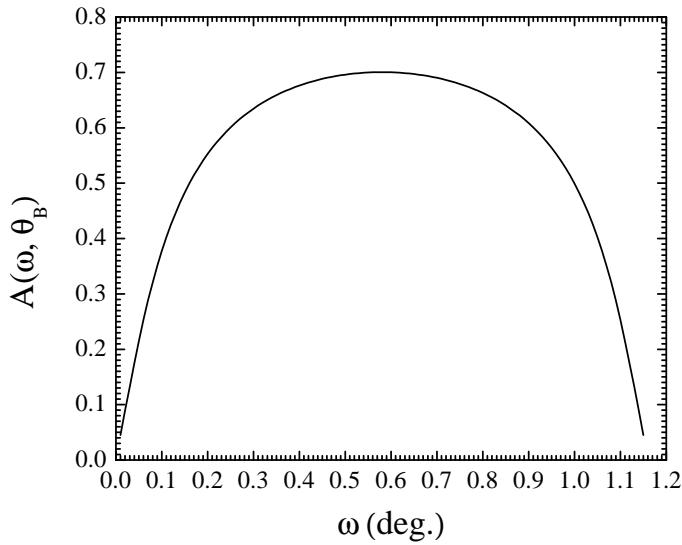


Figure 3.22: Eq. (3.30) plotted as a function of  $\omega$  for  $\theta = \theta_B = 0.58^\circ$ , corresponding to a Bragg angle for  $D = 57.8 \text{ \AA}$ .

### 3.3.5 Correction due to mosaic spread

Integrated intensity needs to be corrected for mosaic spread. During an X-ray exposure, the sample was continuously rotated. Due to this rotation, each pixel integrates intensity over a range of incident angles  $\omega$ . As described in appendix A.1.2, a mosaic spread distribution can be probed by changing  $\omega$ , so rotating the sample is essentially equivalent to integrating a mosaic spread distribution. Because the range of the distribution probed is approximately given by  $\omega = [0, 2\theta_{hk}]$  where  $\theta_{hk}$  is the Bragg angle for a  $(h, k)$  reflection, this range is larger for higher  $h$  orders. This effect is illustrated in Fig. 3.23.

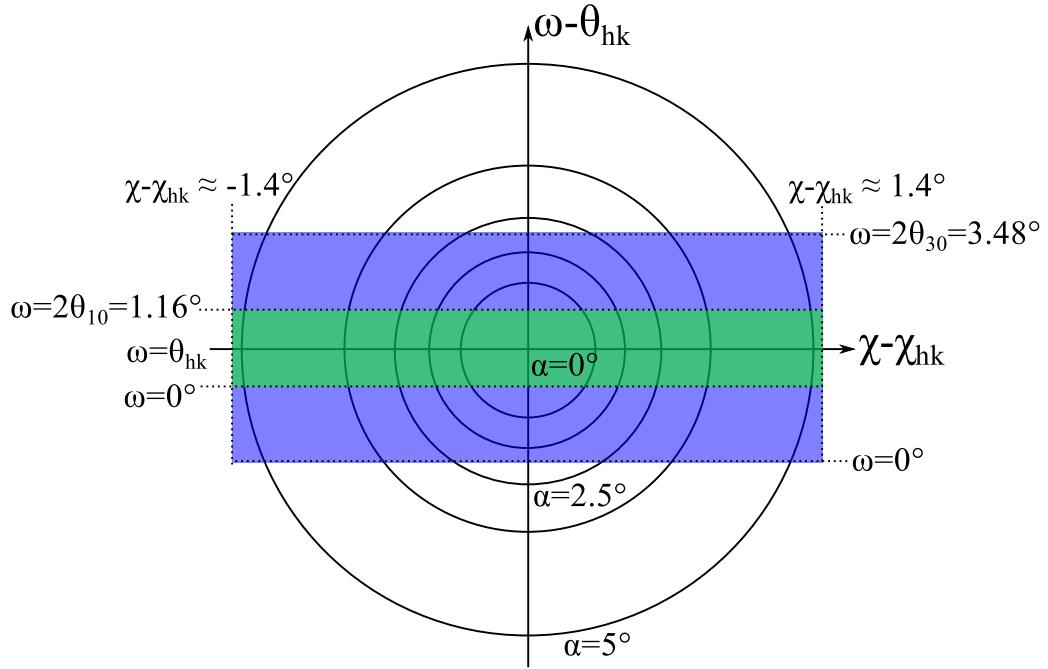


Figure 3.23: Contours of a mosaic spread distribution projected on the  $\omega\chi$ -plane, where  $\chi - \chi_{hk}$  is an angle measured from a  $(h, k)$  reflection on the detector ( $\chi = \pi/2 - \phi$  in Fig. 3.16) and  $\theta_{hk}$  is the Bragg angle for a  $(h, k)$  reflection. The distribution function takes a form of Lorentzian centered at  $\alpha = 0$ . Domains with  $\alpha = 0$  are probed at  $\omega = \theta_{hk}$  and  $\chi = \chi_{hk}$ . Integrated intensity of  $(1, k)$  reflection arises from domains in the green shaded area while that of  $(3, k)$  reflection is from the blue shaded area, which is three times larger.

We limit  $\chi - \chi_{hk}$  to go from  $-1.4^\circ$  to  $1.4^\circ$ . The effect of cutoff on  $\chi - \chi_{hk}$  is not very important because most of observed intensity was included in integration boxes. In contrast, cutoff on  $\omega$  due to substrate blocking the scattering is important, especially for lower  $h$  orders.

We take the distribution to be Lorentzian, which has been experimentally observed (REF, Dr. Nagle),

$$P(\alpha) = \frac{N}{\alpha^2 + \alpha_M^2}, \quad (3.34)$$

where  $N$  is a normalization constant and  $\alpha_M$  is the HWHM of the distribution.  $N$  satisfies

$$N \approx \frac{1}{2\pi} \left( \int_0^{\frac{\pi}{2}} d\alpha \frac{\alpha}{\alpha^2 + \alpha_M^2} \right)^{-1}. \quad (3.35)$$

We then consider a two dimensional contour map on a  $\omega\chi$  plane. Intensity for a reflection with a Bragg angle of  $\theta_B$  is given by

$$I = \int_{-\theta_B}^{\theta_B} d\omega \int_{-\chi_0}^{\chi_0} d\chi P(\alpha) = \int_{-\theta_B}^{\theta_B} d\omega \int_{-\chi_0}^{\chi_0} d\chi \frac{N}{\omega^2 + \chi^2 + \alpha_M^2} \quad (3.36)$$

After the integration over  $\chi$ , Eq. (3.36) is

$$I = 4N \int_0^{\theta_B} \frac{d\omega}{\sqrt{\omega^2 + \alpha_M^2}} \arctan\left(\frac{\chi_0}{\sqrt{\omega^2 + \alpha_M^2}}\right). \quad (3.37)$$

Eq. (3.37) is plotted in Fig. 3.24.

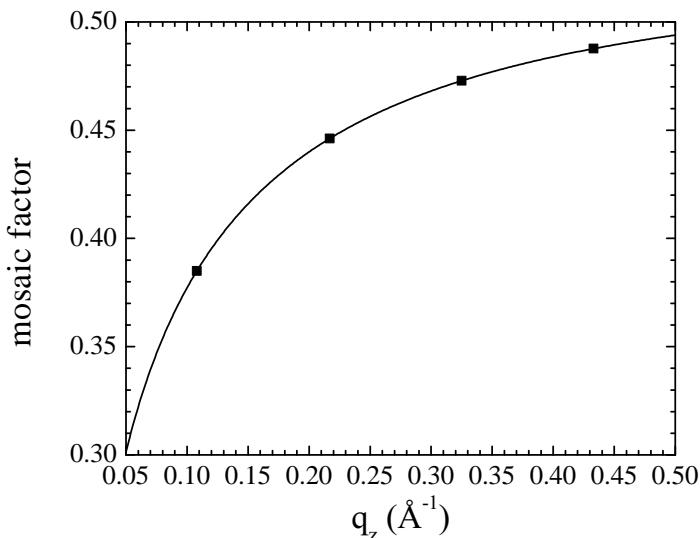


Figure 3.24: Mosaic factor given by Eq. (3.37) as a function of  $q_z \approx 4\pi\theta/\lambda$ . Values at  $q_z = 2\pi h/D$  corresponding to  $D = 57.8 \text{ \AA}$  are shown as squares.  $\alpha_M = 0.05^\circ$  and  $\chi_0 = 1.4^\circ$ . Eq. (3.37) reaches  $\sim 0.54$  at  $\theta_B = \pi/2$  and  $\chi_0 = 1.4^\circ$  and reaches  $\sim 1$  at  $\theta_B = \pi/2$  and  $\chi_0 = 1.4^\circ$  as expected.

## 3.4 LAXS: model

### 3.4.1 Contour Part of the Form Factor

As in Ref. [88], we take the ripple profile to have a sawtooth profile. Its amplitude is  $A$  and the projection of the major arm on the ripple direction is  $x_M$  as shown in Fig. 3.1. Then, we write the ripple profile as

$$u(x) = \begin{cases} -\frac{A}{\lambda_r - x_0} \left( x + \frac{\lambda_r}{2} \right) & \text{for } -\frac{\lambda_r}{2} \leq x < -\frac{x_0}{2}, \\ \frac{A}{x_0} x & \text{for } -\frac{x_0}{2} \leq x \leq \frac{x_0}{2}, \\ -\frac{A}{\lambda_r - x_0} \left( x - \frac{\lambda_r}{2} \right) & \text{for } \frac{x_0}{2} < x \leq \frac{\lambda_r}{2}. \end{cases} \quad (3.38)$$

The ripple profile has inversion symmetry, so that the resulting form factor is real.  $A$  and  $x_M$  are fitting parameters that depend on the integrated intensity of each peak while  $D$ ,  $\lambda_r$ , and  $\gamma$  are determined from measuring the positions of the Bragg peaks.

In order to allow the electron density along the ripple direction to modulate, we include two additional parameters, one to allow for the electron density across the minor side to be different by a ratio  $f_1$  from the electron density across the major side and a second parameter  $f_2$ , which is multiplied by  $\delta$  functions  $\delta(x \pm x_M/2)$  to allow for a different electron density near the kink between the major and the minor sides.

The contour part of the form factor  $F_C$  calculated from Eq. (3.38) is plotted in Fig. (3.25).

### 3.4.2 Transbilayer Part of the Form Factor

#### 3.4.2.1 1G and 2G hybrid model

In the hybrid model, the terminal methyl region of the bilayer is represented as a Gaussian function [104]. The headgroups are represented by one and two Gaussian functions in 1G and 2G hybrid model, respectively. The methylene and water regions are each treated as a constant. The gap between the two constants is represented by a sine function. Then, for half of the bilayer,  $0 \leq z \leq D/2$ , the electron density has the form,

$$\rho(z) = \rho_G(z) + \rho_S(z) + \rho_B(z), \quad (3.39)$$

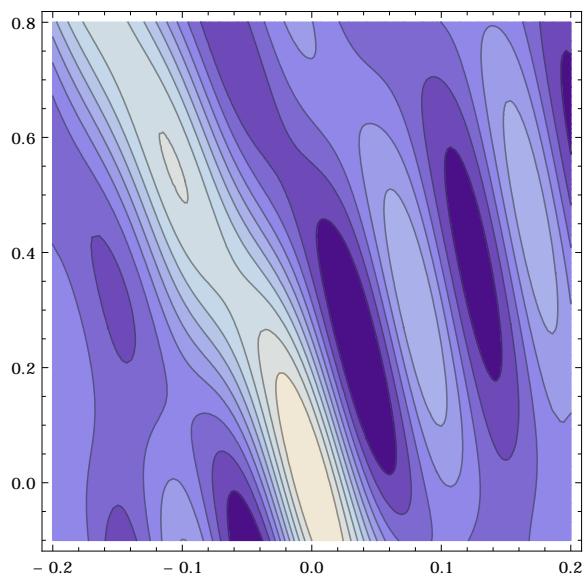


Figure 3.25: Not sure if this plot is useful. The horizontal axis is  $q_x$  and the vertical axis is  $q_z$ .

where the Gaussian part is given by

$$\rho_G(z) = \sum_{i=1}^{1 \text{ or } 2} \rho_{Hi} e^{-(z-Z_{Hi})^2/(2\sigma_{Hi}^2)} + \rho_M e^{-z^2/(2\sigma_M^2)}, \quad (3.40)$$

the strip part is given by

$$\rho_S(z) = \begin{cases} \rho_{CH_2} & \text{for } 0 \leq z < Z_{CH_2}, \\ \rho_W & \text{for } Z_W \leq z \leq D/2, \end{cases} \quad (3.41)$$

and the bridging part is given by

$$\rho_B(z) = \frac{\rho_W - \rho_{CH_2}}{2} \cos \left[ \frac{-\pi}{\Delta Z_H} (z - Z_W) \right] + \frac{\rho_W + \rho_{CH_2}}{2} \quad \text{for } Z_{CH_2} < z < Z_W. \quad (3.42)$$

with  $\Delta Z_H = Z_W - Z_{CH_2}$ . Here, we assume  $Z_{H2} > Z_{H1}$ . Table 3.5 shows some of the definitions.

	1G	2G
$Z_{CH_2}$	$Z_{H1} - \sigma_{H1}$	$Z_{H1} - \sigma_{H1}$
$Z_W$	$Z_{H1} + \sigma_{H1}$	$Z_{H2} + \sigma_{H2}$

Table 3.5: Definitions of  $Z_{CH_2}$  and  $Z_W$

The transbilayer profile along  $x = -z \tan \psi$  can be obtained by rotating the coordinates  $x$  and  $z$  by  $\psi$  in the clockwise direction and reexpressing  $\rho(z)$  in terms of the rotated coordinates. This leads to replacing  $x$  with  $x' = x \cos \psi + z \sin \psi$  and  $z$  with  $z' = -x \sin \psi + z \cos \psi$ . Then, the rotated transbilayer profile is

$$\rho(x, z) = \delta(x + z \tan \psi) [\rho_G(z') + \rho_S(z') + \rho_B(z')]. \quad (3.43)$$

Taking the two dimensional Fourier transform of Eq. (3.43) leads to the transbilayer part of the form factor,

$$F_T = \int_{-\frac{D}{2}}^{\frac{D}{2}} \int_{-\frac{\lambda_r}{2}}^{\frac{\lambda_r}{2}} [\rho(x, z) - \rho_W] e^{i(q_x x + q_z z)} dx dz \quad (3.44)$$

$$= F_G + F_S + F_B. \quad (3.45)$$

The form factor is calculated in the minus fluid convention, where the bilayer electron

density is measured with respect to the electron density of the surrounding solvent. The expression for  $F_T$  is rather messy, so the derivation and full expression are in the appendix. Here, we note that the fitting parameters in this model are  $Z_{Hi}$ ,  $\sigma_{Hi}$ , and  $R_{HiM}$  for each of the two headgroup Gaussian functions,  $\sigma_M$  for the terminal methyl Gaussian,  $\Delta R$  for the methylene region,  $\psi$  for the lipid tilt, and an overall scaling factor. The contour part of the form factor has four more parameters ( $A$ ,  $x_M$ ,  $f_1$ , and  $f_2$ ). In total, the modified 2G hybrid model implements 14 structural parameters.

## 3.5 LAXS: results

### 3.5.1 Data and Electron Density Profile

We measured scattering on oriented samples in almost identical conditions as the best unoriented sample of Wack and Webb. As discussed earlier, these two types of samples have different Lorentz corrections, so this allowed us to check our data obtained on oriented samples against an unoriented sample. As Table 3.6 shows, agreement between our oriented data and the unoriented data was good, but form factor from our oriented sample was in many cases slightly larger than that from the unoriented sample. We attribute this discrepancy to the way intensity was extracted. In X-ray data from an oriented sample, each peak was nicely separated, so integrating a peak intensity was trivial. In contrast, some reflections in unoriented data were overlapping with each other (three pairs of overlapping peaks are highlighted in Table 3.6), making separation of intensity difficult. If the  $(1, 0)$  peak in the unoriented data had been overestimated, that would account for the observed discrepancy. Indeed, the microdensitometer trace in [8] suggests that the  $(1, 0)$  and  $(1, -1)$  reflections could have similar intensity as we observed in our oriented sample.

Table 3.7 and 3.8 summarize observed intensity from our data shown in Fig. 3.12.  $q_z$  values for observed peaks were corrected for index of refraction (Appendix A.5).

Figure 3.26 plots a two dimensional electron density profile calculated using the phases obtained from the M1G model and our experimental form factors. It is clear that the terminal methyl exists along the bilayer center in the major arm. The thickness along the local bilayer normal in the major arm was about 40 Å. This value is slightly larger than the previously published value of 37.9 Å obtained from the unoriented data [88]. The DMPC gel phase profile published in [53] also has shown that the distance between the headgroup centers in opposing monolayers is approximately 38 Å. Therefore, our experimental electron density profile suggests that chains in the ripple major arm are less tilted than in the gel phase, where chains are tilted by 30°. Incidentally, the maximum peak in the gel phase headgroup corresponding to the phosphate and choline components has been shown to be located at 20 Å from the bilayer center [53].

Figure 3.26 also shows absence of the terminal methyl trough in the minor arm. This absence is consistent with chains being interdigitated like in ref. [98]. Indeed, our electron density profile shows a small peak at the bilayer center of the minor

$h$	$k$	$q^*$ (Å $^{-1}$ )	unoriented $ F_{hk} ^*$	oriented $ F_{hk} $	error
1	-1	0.111	60.8	86.3	3.7
1	0	0.108	100.0	100.0	0.5
1	1	0.123	26.9	43.1	2.6
1	2			0.0	3.9
1	3	0.185	7.6	8.8	0.2
2	-2	0.224	15.1	18.0	0.6
2	-1	0.215	71.2	76.0	0.4
2	0	0.217	39.7	28.7	0.2
2	1	0.228	33.9	39.5	0.4
2	2	0.246	22.7	24.6	0.3
2	3	0.271	14.2	14.6	0.1
2	4	0.301	7.8	9.2	0.2
2	5	0.329		5.6	0.7
2	6			4.1	0.3
3	-2	0.325	29.3	33.2	0.8
3	-1	0.322	44.2	45.9	0.4
3	0	0.325	12.0	13.2	0.5
3	1			0.0	7.1
3	2	0.350	10.5	10.2	0.2
3	3	0.370	14.9	13.6	0.2
3	4	0.394	10.0	13.0	0.2
3	5			9.6	0.1
3	6			5.6	0.4

Table 3.6: Comparison of form factor obtained in two different methods.  
 \*Unoriented data are from Wack and Webb [8].

$h$	$k$	$q_z$ (Å $^{-1}$ )	$q_r$ (Å $^{-1}$ )	$I_{hk}^{\text{obs}}$	$\sigma_I$	$ F $	$\sigma_F$	box size (pixels)
1	-1	0.102	-0.043	726	63	86.3	3.7	10 × 7
1	0	0.109	0	180818	1759	100.0	0.5	10 × 7
1	1	0.114	0.043	228	28	43.1	2.6	10 × 7
1	2			0	1	0.0	3.9	
1	3	0.128	0.13	3.8	0.2	8.8	0.2	10 × 7
2	-2	0.206	-0.087	49.2	3.5	18.0	0.6	10 × 7
2	-1	0.212	-0.044	1818	20	76.0	0.4	10 × 7
2	0	0.218	0	10200	174	28.7	0.2	10 × 7
2	1	0.224	0.043	550	10	39.5	0.4	10 × 7
2	2	0.231	0.086	112	3	24.6	0.3	10 × 7
2	3	0.237	0.129	27	0.2	14.6	0.1	10 × 7
2	4	0.243	0.173	8.2	0.4	9.2	0.2	10 × 7
2	5	0.25	0.214	2.6	0.7	5.6	0.7	10 × 7
2	6	0.256	0.257	1.2	0.2	4.1	0.3	10 × 7
3	-2	0.314	-0.087	305	15	33.2	0.8	15 × 7
3	-1	0.321	-0.043	1205	22	45.9	0.4	15 × 7
3	0	0.326	0	1566	110	13.2	0.5	15 × 7
3	1			0	31	0.0	7.1	
3	2	0.339	0.086	32.4	1.6	10.2	0.2	15 × 7
3	3	0.345	0.129	39.1	0.9	13.6	0.2	15 × 7
3	4	0.352	0.172	27.7	0.7	13.0	0.2	15 × 7
3	5	0.358	0.215	12.2	0.3	9.6	0.1	15 × 7
3	6	0.364	0.258	3.5	0.5	5.6	0.4	15 × 7
4	-3	0.417	-0.131	142	8	23.0	0.6	20 × 8
4	-2	0.423	-0.087	755.4	19	42.8	0.5	20 × 8
4	-1	0.429	-0.043	429.6	34	22.6	0.9	20 × 8
4	0	0.435	0.000	1917	23	16.2	0.1	20 × 8
4	1	0.441	0.043	45.3	7.2	7.2	0.6	20 × 8
4	2	0.448	0.085	43.6	2.4	9.9	0.3	20 × 8
4	3			0	1.3	0.0	2.1	
4	4	0.461	0.173	2.1	0.4	3.0	0.3	20 × 8
4	5	0.467	0.215	3.2	0.3	4.1	0.2	20 × 8
4	6	0.473	0.259	1	1.1	2.5	1.1	20 × 8

Table 3.7: Observed intensity for  $h = 1$  to 4 at  $D = 57.8$ ,  $\lambda_r = 145$ , and  $\gamma = 98.2^\circ$ .

$h$	$k$	$q_z$ (Å $^{-1}$ )	$q_r$ (Å $^{-1}$ )	$I_{hk}^{\text{obs}}$	$\sigma_I$	$ F $	$\sigma_F$	box size (pixels)
5	-3	0.525	-0.132	86.2	6.8	15.6	0.6	25 × 9
5	-2	0.532	-0.087	145	4	16.3	0.2	25 × 9
5	-1	0.538	-0.042	63.4	3.4	7.5	0.2	25 × 9
5	0	0.544	0.000	260	4	6.5	0.1	25 × 9
5	1	0.550	0.040	50	2.8	6.4	0.2	25 × 9
6	-4	0.628	-0.175	11.4	0.8	5.9	0.2	30 × 10
6	-3	0.635	-0.131	15.6	0.9	5.9	0.2	30 × 10
6	-2	0.641	-0.085	10.1	1.8	3.8	0.3	30 × 10
6	-1	0.647	0.043	16.3	3	3.4	0.3	30 × 10
6	0	0.653	0.000	60.2	4.7	3.4	0.1	30 × 10
6	1	0.659	0.044	20.4	1.5	3.9	0.1	30 × 10
6	2			0	0.6	0.0	0.9	
6	3	0.672	0.128	5.9	0.3	3.5	0.1	30 × 10
6	4	0.679	0.170	4.2	0.3	3.4	0.1	30 × 10
7	-4	0.737	-0.174	40	1.1	10.0	0.1	35 × 10
7	-3	0.743	-0.130	36	1.8	8.1	0.2	35 × 10
7	-2	0.749	-0.085	15	7.3	4.2	0.9	35 × 10
7	-1	0.755	-0.042	22	2.3	3.6	0.2	35 × 10
7	0	0.760	0.000	36	1.8	2.8	0.1	35 × 10
8	0			0	3	0.0	0.9	
9	-5	0.951	-0.215	16	3	6.1	0.5	35 × 10
9	-4	0.957	-0.173	16.9	3	5.6	0.5	35 × 10
9	-3			0	8	0.0	3.3	
9	-2	0.969	-0.086	10	2.9	3.0	0.4	35 × 10
9	-1			0	6	0.0	1.7	
9	0	0.981	0.000	17	10	2.2	0.6	35 × 10

Table 3.8: Observed intensity for  $h = 5$  to 9 at  $D = 57.8$ ,  $\lambda_r = 145$ , and  $\gamma = 98.2^\circ$  (continued from Table 3.7).

arm, rather than depletion of electron density as in the gel and fluid phases. We also fitted the data to M1G models with more fitting parameters that allow the model parameters in the minor arm to be different from those in the major arm. In those cases also, we observed the same absence of the terminal methyl trough. However, when  $f_2$  was fixed to zero in the M1G model, we obtained a quite different profile, plotted in Fig. 3.27. There, the electron density shows a dip at the center of the bilayer in the minor arm, a usual feature seen in the fluid phase. Also, the headgroup in the minor arm appears to be more smooth compared to the profile shown in Fig. 3.26. Although the profile in Fig. 3.27 was obtained using a model yielding a worse chi-squared value, some features are seen to be more physical. Therefore, we argue that the ripple profile is closer to the one plotted in Fig 3.27 than that in Fig 3.26. It is quite mysterious as to why fixing or freeing the parameter  $f_2$  led to quite different profiles because this parameter was implemented to affect only the kink region. It may be that even higher order reflections than ones we observed might be necessary to obtain a robust minor arm profile. Since  $h = 9$  was quite strong, we expect to see more orders with the same instrument by simply probing a larger  $q$ -space.

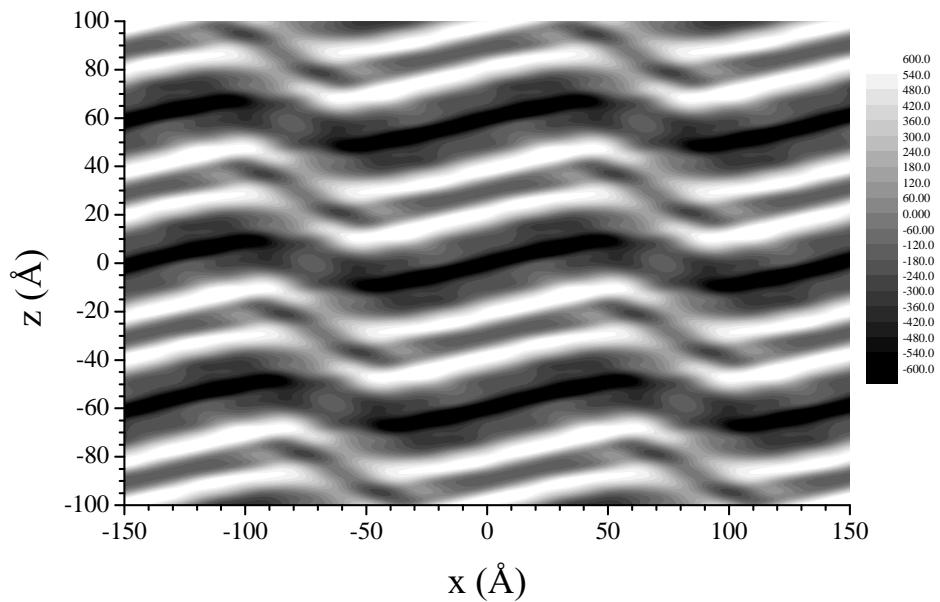


Figure 3.26: Electron density profile calculated using the phases obtained from the M2G model and experimental form factors. White is most electron dense and black is least electron dense. The thickness along the local bilayer normal in the major arm was about 40 Å. Depletion of electron density due to the terminal methyl is visible in the major arm but not in the minor arm.

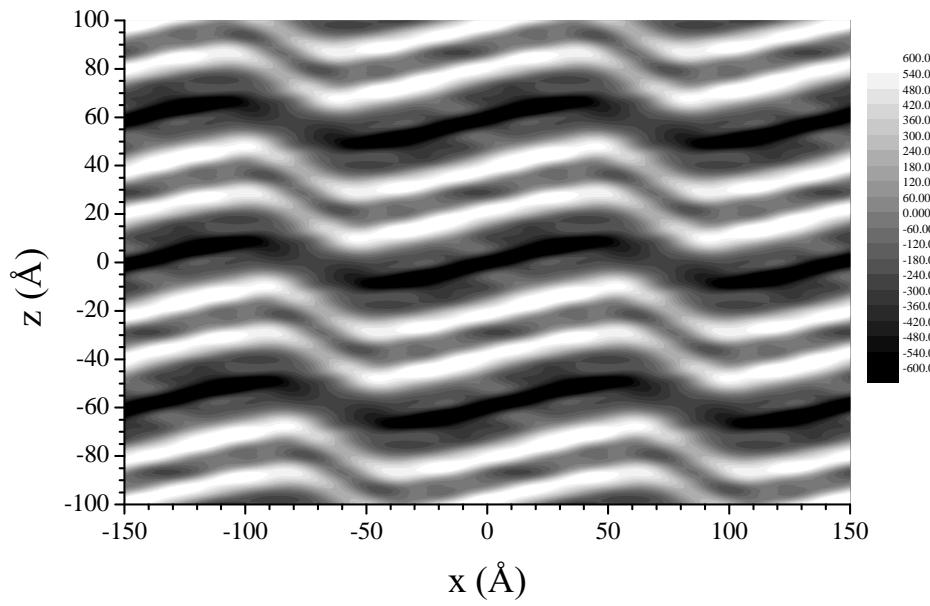


Figure 3.27: Electron density profile calculated using the phases obtained from the M2G model with  $f_2 = 0$  and experimental form factors. White is most electron dense and black is least electron dense. The thickness along the local bilayer normal in the major arm was about 40 Å. Depletion of electron density due to the terminal methyl is visible in the major arm but not in the minor arm.

### 3.6 NGIWAXS: results

As a first step in data reduction of near grazing incidence wide angle X-ray scattering (NGIWAXS) data, we subtracted background scattering data taken at incident angle of  $-\omega$  from the data taken at  $\omega$ . This background subtraction resulted in a clean image of sample scattering. A background subtracted CCD image was then transformed to the sample  $q$ -space using the relationship between the CCD pixel positions and the sample  $q$ -space given by Eq. 3.14 and Eq. 3.15. The nonlinearity of this relationship is not negligible and must be taken into account for wide angle scattering data. Figure 3.28 shows an example of the data reduction. The original scattering image taken at  $\omega = 0.2^\circ$  had unwanted scattering due to mylar windows in the hydration chamber which overlapped with the ripple WAXS. The background subtraction removed these unwanted features in the scattering data, resulting in a sample scattering image. This sample scattering image was then transformed to the sample  $q$ -space. The black regions in the sample  $q$ -space image are the regions of  $q$ -space that were not probed by the detector. Because of the nonlinearity in the transformation, straight detector edges were turned into curves, the effect of which was most visible near the meridian  $q_r = 0$ .

Figure 3.29 shows NGIWAXS from an oriented DMPC film in the ripple phase for  $D = 60.8 \text{ \AA}$ , collected with the high resolution setup. We observed a stronger peak off the equator and a weaker one, the center of which was not unambiguously determined. The maximum intensity of the stronger peak was at  $(q_r, q_z) \approx (1.478 \text{ \AA}^{-1}, 0.20 \text{ \AA}^{-1})$  as shown in Fig. 3.30. The weaker peak was observed near the equator, and separation of this peak from the stronger one was most visible at  $q_z = 0.12 \text{ \AA}^{-1}$ , indicating that the center of this peak was near  $(q_r, q_z) \approx (1.452 \text{ \AA}^{-1}, 0.12 \text{ \AA}^{-1})$ . However, because of absorption of X-rays due to the sample, intensity near the equator was attenuated. Therefore, we could not eliminate a possibility that the weaker peak is centered at  $q_z = 0 \text{ \AA}^{-1}$ . This is further investigated in the next section for transmission wide angle X-ray scattering.

We also investigated dependence of the ripple WAXS on the interbilayer  $D$ -spacing. Figure 3.31 compares NGIWAXS at two different  $D$ -spacing, showing that chain scattering did not depend on the  $D$ -spacing in this range. A weak feature that looks like an arc coming from the chain peak was observed. This feature extended from  $\phi = 0^\circ$  to at least  $70^\circ$ . This feature is perhaps mosaic spread scattering.

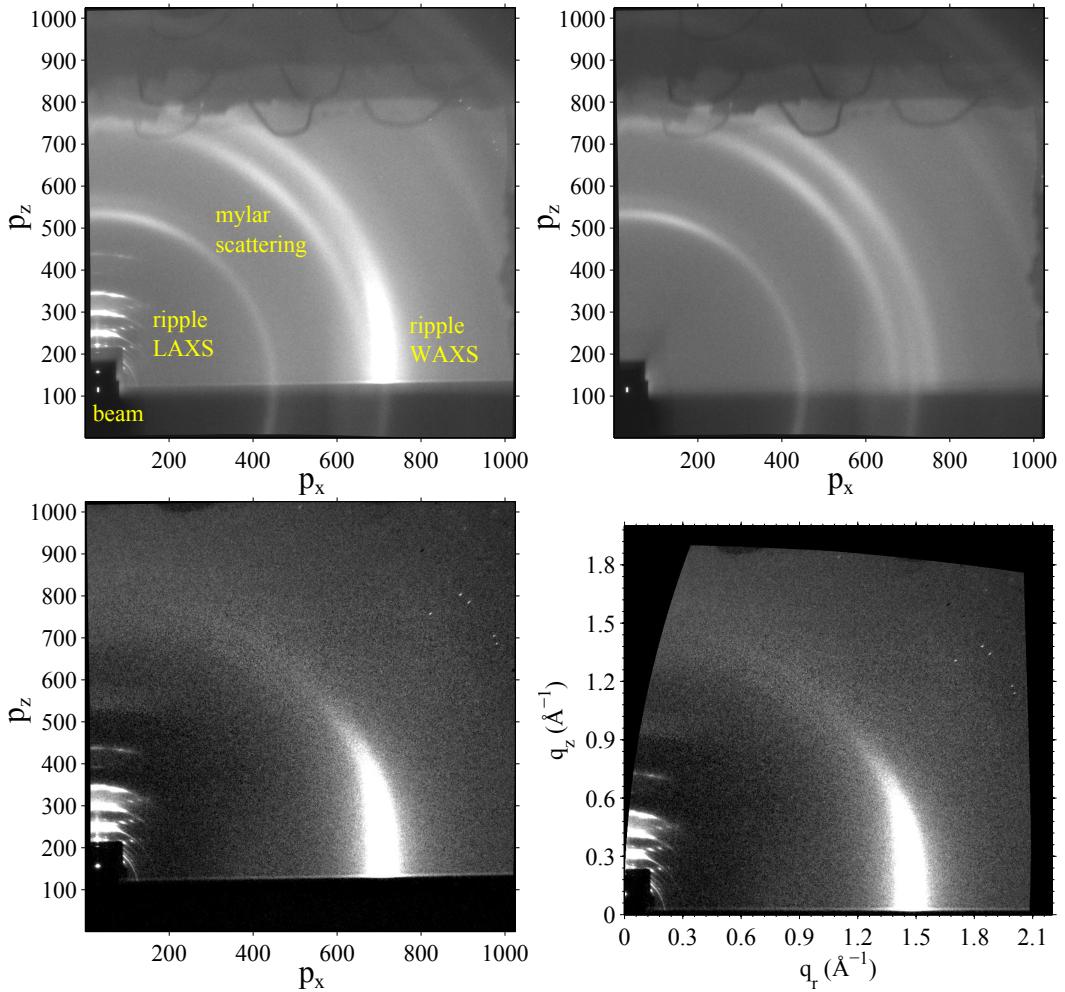


Figure 3.28: Data reduction of NGIWAXS data. (top) Data taken at  $\omega = 0.2^\circ$  (left) and at  $-0.2^\circ$  (right) with the low resolution setup at the 2011 run. Because of mosaic spread present in the sample,  $(h, 0)$  main peaks as well as  $(h, k \neq 0)$  side peaks in the ripple LAXS were observed at fixed  $\omega$ . The darker region below the equator defined by the beam vertical position  $p_z$  was due to the substrate. The beam and  $(h = 1, k = 0)$  reflection were visible through the semitransparent beam stop. Scattering at  $p_z > 700$  was the shadow cast by the electrical wires and thermal shielding in the hydration chamber. (bottom) The background subtracted image (left) and corresponding image in the sample  $q$ -space (right). Except some minor left over scattering, all the background scattering was removed very nicely. Because the meridian was not exactly along the vertical pixels, the background subtracted image was rotated by  $\sim 1^\circ$  in the clockwise direction before the  $q$ -space transformation. The data reduction was done using MATLAB.

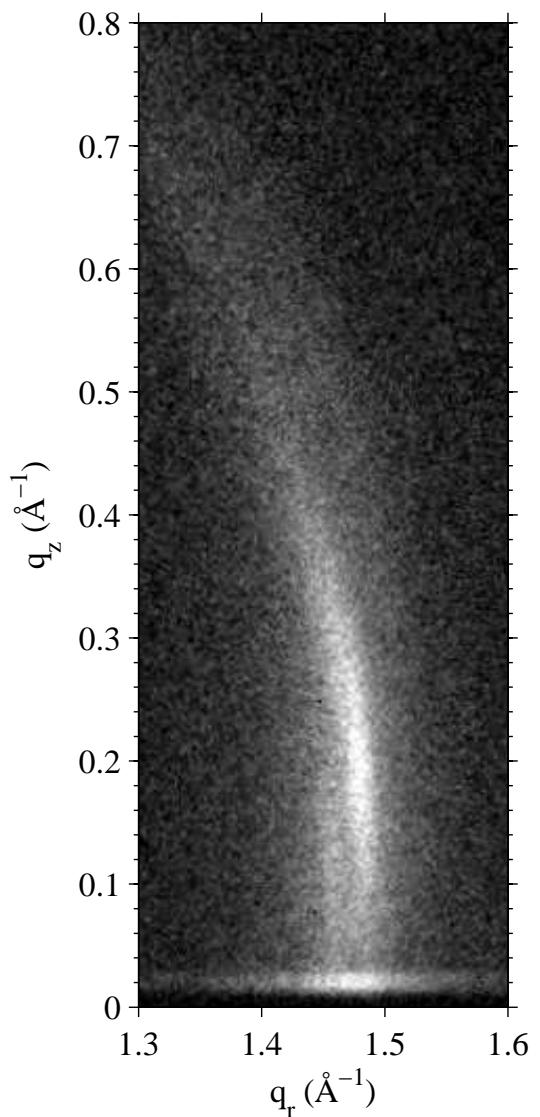


Figure 3.29: High resolution NGIWAXS of the DMPC ripple phase for  $D = 60.8 \text{ \AA}$ . The angle of incidence  $\omega$  was  $0.2^\circ$ . The stronger peak was at  $(q_r, q_z) \approx (1.478 \text{ \AA}^{-1}, 0.20 \text{ \AA}^{-1})$ . The weaker peak was at  $(q_r, q_z) \approx (1.452 \text{ \AA}^{-1}, 0.12 \text{ \AA}^{-1})$ . The scattered intensity along the line slightly above  $q_z = 0 \text{ \AA}^{-1}$  is the Vineyard-Yoneda peak [105, 106].

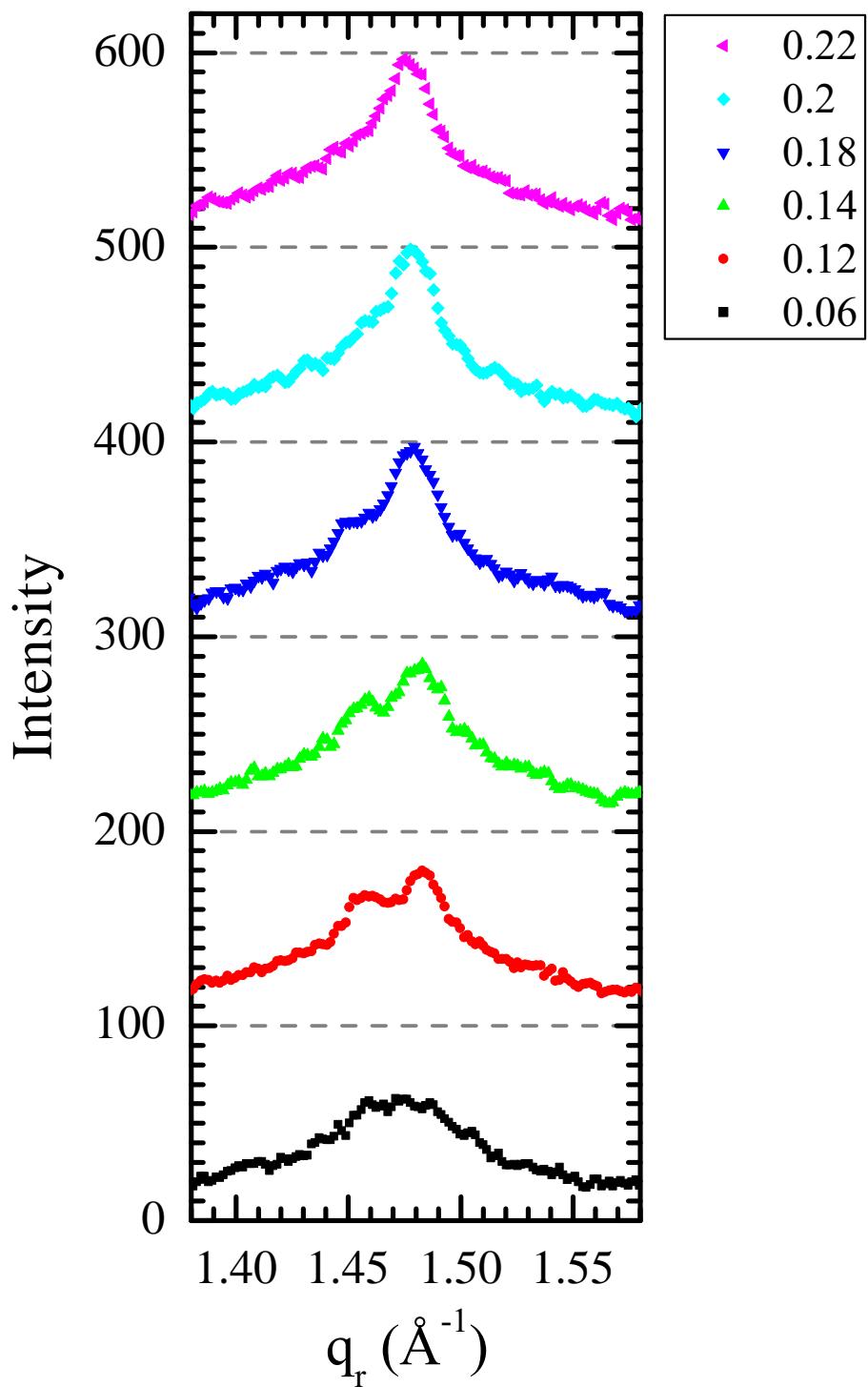


Figure 3.30:  $q_r$  swaths of the ripple WAXS, each averaged over  $0.02 \text{ \AA}^{-1}$ . Each curve is shifted by 100 vertically. The center  $q_z$  values of swaths are shown in the figure legend.

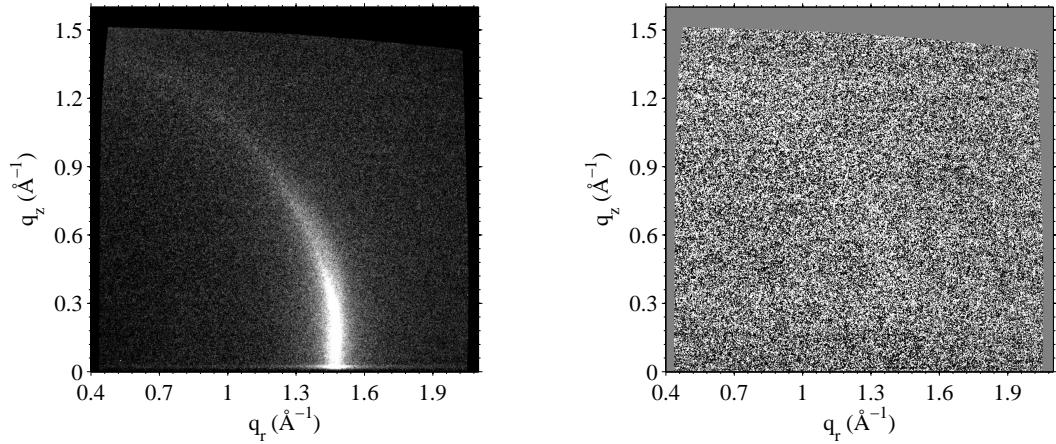


Figure 3.31: NGIWAXS of the DMPC ripple phase for  $D = 59.2 \text{\AA}$  (left) and difference between  $D = 59.2 \text{\AA}$  and  $60.8 \text{\AA}$  (right). The angle of incidence  $\omega$  was  $0.2^\circ$ . The data were taken with the high resolution setup.

The chain-chain correlation length in the ripple phase can be studied by measuring the widths of the ripple peaks, which are determined by the convolution of the intrinsic width and the instrumental resolution. To characterize the instrumental resolution, we also collected high resolution NGIWAXS for the DMPC  $L_{\beta I}$  gel phase that occurs at the highest hydration [11,53] shown in Fig 3.32. When the peak profile of the (2,0) peak in  $q_r$  was fitted to a Lorentzian, we obtained an excellent fit with its full width half maximum (FWHM) being the same as the instrumental resolution,  $\Delta q = 0.014 \text{ \AA}^{-1}$ . The center of the peak was estimated to be at  $q_r = 1.479 \text{ \AA}^{-1}$ . The chain-chain correlation length can be estimated by using the Scherrer equation [107],

$$B = \frac{0.94\lambda}{L \cos \theta},$$

where  $B$  is the observed FWHM of a Bragg peak,  $\lambda$  is the wavelength,  $L$  is the length over which chains are positionally correlated, and  $\theta$  is the Bragg angle. For  $\Delta q = 0.014 \text{ \AA}^{-1}$ ,  $q = 1.479 \text{ \AA}^{-1}$ , and  $\lambda = 1.175 \text{ \AA}$ , the Scherrer equation yields  $L = 426 \text{ \AA}$ . Because the width of the (2,0) gel phase peak was not instrumentally resolved, the correlation length of chains was greater than 426  $\text{\AA}$ . The width of similar lipid, DPPC, was resolved and had a correlation length of 2900  $\text{\AA}$  [108].

In contrast, the observed peaks in the ripple phase were instrumentally resolved (Fig. 3.33). The FWHM of the stronger peak was estimated to be  $0.025 \text{ \AA}^{-1}$ , corresponding to the correlation length of  $\sim 240 \text{ \AA}$ , indicating that the correlation length in the ripple phase is shorter than that in the gel phase. This observation can be understood by supposing that chains in the major and minor arms are not correlated, so that gel phase like chains in the major are only correlated within the major arm, limiting the correlation length along the ripple direction to be less than the length of the major arm,  $\sim 100 \text{ \AA}$ . Although it is possible that chains are correlated over a much longer distance along the direction perpendicular to the ripple direction leading to a sharp reflection along  $q_y$ , what is observed in our in-plane powder sample is a convolution of a broad width along  $q_x$  and sharp one along  $q_y$ . Such convolution would result in a broad Bragg rod as observed in our NGIWAXS data.

Figure 3.33 also shows that there was a broader component in the observed peak profile. A fit with a single Lorentzian was not very good, suggesting that the broader component is not artifact. We also fitted the peak profile in  $q_r$  along  $q_z = 0.12 \text{ \AA}^{-1}$ , where two distinct peaks were observed (Fig.3.34). The two sharp peaks fitted

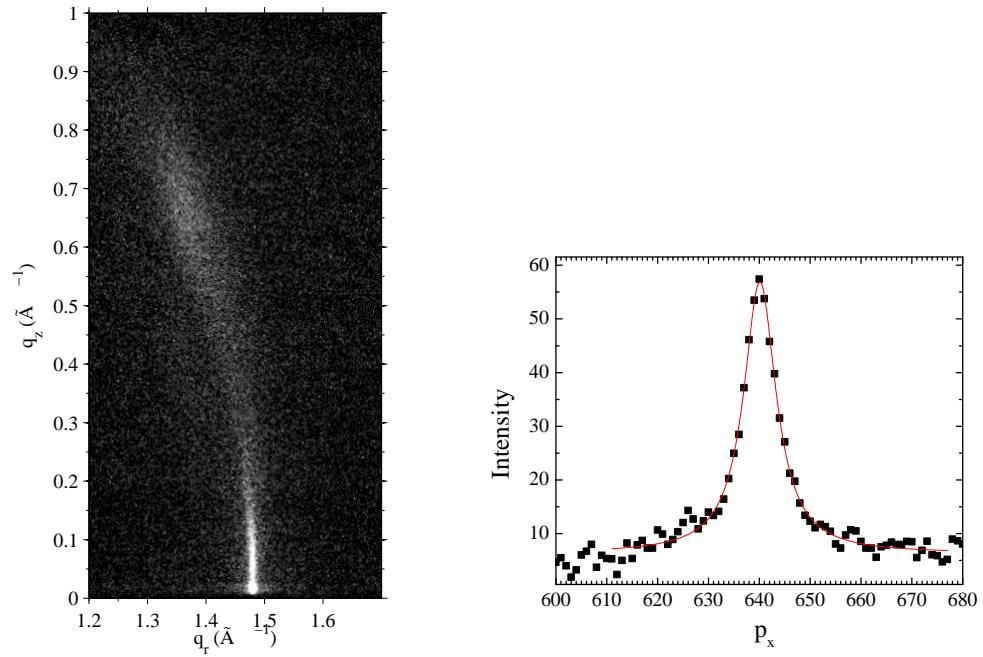


Figure 3.32: (left) NGIWAXS image of the DMPC gel phase at 10 °C for  $D = 57.7 \text{ \AA}$  where the sample was in the  $L_{\beta I}$  phase. The (2,0) peak was at  $q_r = 1.479 \text{ \AA}^{-1}$ , corresponding to  $d_{20} = 4.25 \text{ \AA}$ . (right) The (2,0) peak plotted along horizontal pixels  $p_x$  averaging over 8 pixels in  $p_z$ . The solid red line is a Lorentzian fit to the data, resulting in the FWHM of  $\sim 8$  pixels, corresponding to  $\Delta q = 0.014 \text{ \AA}^{-1}$ , which is an unresolved width of intrinsically infinitely sharp peak estimated in Sec. 3.2.2.

with Lorentzian yielded the FWHM of about  $0.025 \text{ \AA}^{-1}$ , consistent with the FWHM obtained for the stronger peak. This result suggests that both stronger peak and weaker peak are due to the gel-phase like major arm. However, as Fig. 3.34 shows, the double Lorentzian fit was only successful within a limited range in  $q_r$ . This could be due to an underlining broad peak like the one shown in Fig. 3.33. To investigate this possibility, we fitted the same peak profile to triple Lorentzian with fixed widths. Two of the Lorentzian had fixed widths of  $0.025 \text{ \AA}^{-1}$  representing the sharp peaks and the last one had a fixed width of  $0.14 \text{ \AA}^{-1}$  representing the broad peak. Figure 3.34 shows an excellent fit obtained over a large range in  $q_r$ . Curiously, the center of the stronger Bragg rod was different at the two different  $q_z$  ( $1.485 \text{ \AA}^{-1}$  at  $q_z = 0.012 \text{ \AA}^{-1}$  and  $1.478 \text{ \AA}^{-1}$  at  $q_z = 0.2 \text{ \AA}^{-1}$ ). **I feel like I need a good table**

Particle broadening is sometimes represented by pseudo Voigt. The peak profile including the weaker peak was also fitted to double pseudo Voigt. Figure 3.35 shows that a fit with double pseudo Voigt was also excellent. Given that one of the sharp Lorentzian and the broad one in the triple Lorentzian fit had the same center value, it is possible that these two Lorentzian are parts of the same pseudo Voigt representing the weaker peak.

We also measured NGIWAXS of the DMPC fluid phase at  $T = 30 \text{ }^\circ\text{C}$  (Fig. 3.36). Fig. 3.37 compares the ripple and fluid phase WAXS. The maximum intensity of the fluid phase WAXS was observed at  $q_r \approx 1.4 \text{ \AA}^{-1}$ , a smaller  $q$  than the ripple peaks. The fluid phase WAXS is smoother because the data were taken with the low resolution setup with much more intensity. Because the width of the fluid phase WAXS was very broad, it was instrumentally resolved with the low resolution setup. Figure 3.37 shows that the WAXS pattern in the fluid and ripple phases are quite distinct, suggesting that the ripple WAXS cannot be understood by assuming that chains in the minor arm are disordered like in the fluid phase.

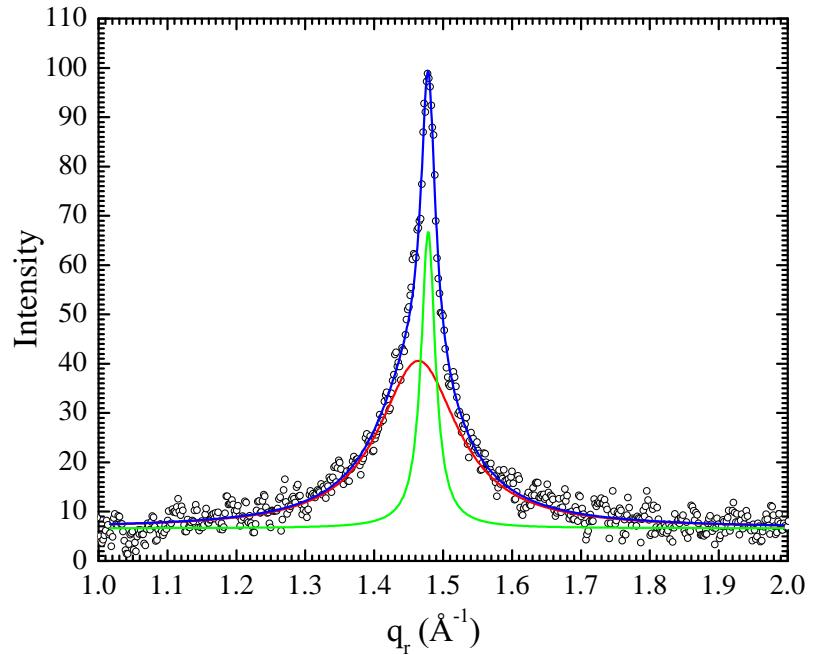


Figure 3.33: Peak profile in  $q_r$  at  $q_z = 0.2 \text{ \AA}^{-1}$  fitted to double Lorentzian functions. The FWHM and center obtained were  $0.025 \text{ \AA}^{-1}$  and  $1.478 \text{ \AA}^{-1}$  (green) and  $0.140 \text{ \AA}^{-1}$  and  $1.464 \text{ \AA}^{-1}$  (red), respectively.

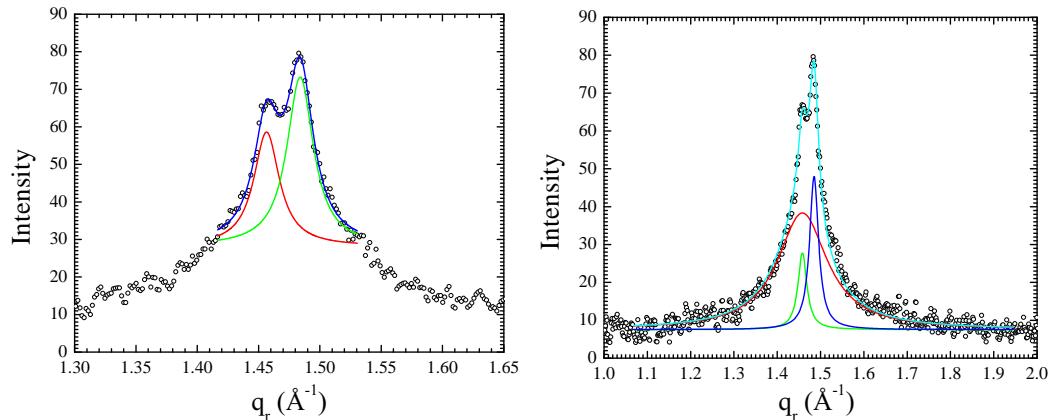


Figure 3.34: (left) Peak profile in  $q_r$  at  $q_z = 0.12 \text{ \AA}^{-1}$  fitted to double Lorentzian functions. The FWHM and center obtained were  $0.025 \text{ \AA}^{-1}$  and  $1.457 \text{ \AA}^{-1}$  (red) and  $0.026 \text{ \AA}^{-1}$  and  $1.484 \text{ \AA}^{-1}$  (green), respectively. The fit was limited within the range in which the fit was reasonable. (right) The same peak profile fitted to triple Lorentzian. The FWHM was constrained to  $0.025 \text{ \AA}^{-1}$  (blue),  $0.025 \text{ \AA}^{-1}$  (green), and  $0.14 \text{ \AA}^{-1}$  (red). The center was found to be  $1.485 \text{ \AA}^{-1}$  (blue),  $1.458 \text{ \AA}^{-1}$  (green), and  $1.458 \text{ \AA}^{-1}$  (red).

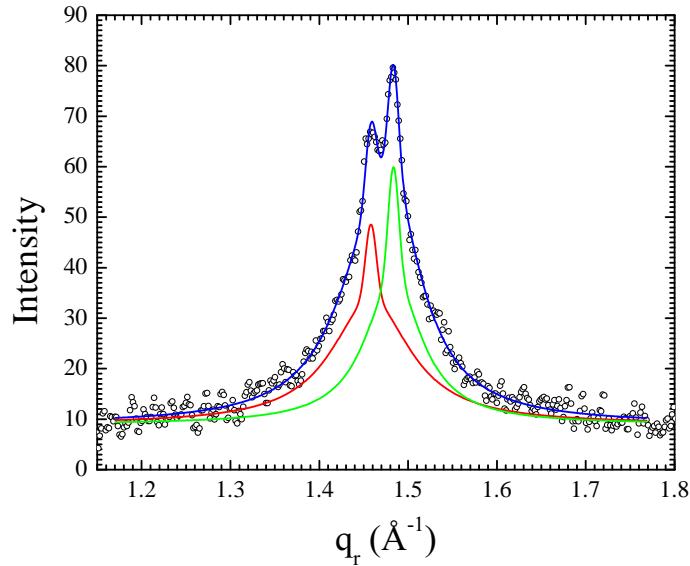


Figure 3.35: The peak profile fitted to double pseudo Voigtian functions. The FWHM and center obtained were  $0.107 \text{ \AA}^{-1}$  and  $1.458 \text{ \AA}^{-1}$  (red) and  $0.077 \text{ \AA}^{-1}$  and  $1.484 \text{ \AA}^{-1}$  (green), respectively. The Gaussian FWHM in the pseudo Voigt was fixed to  $0.014 \text{ \AA}^{-1}$ .

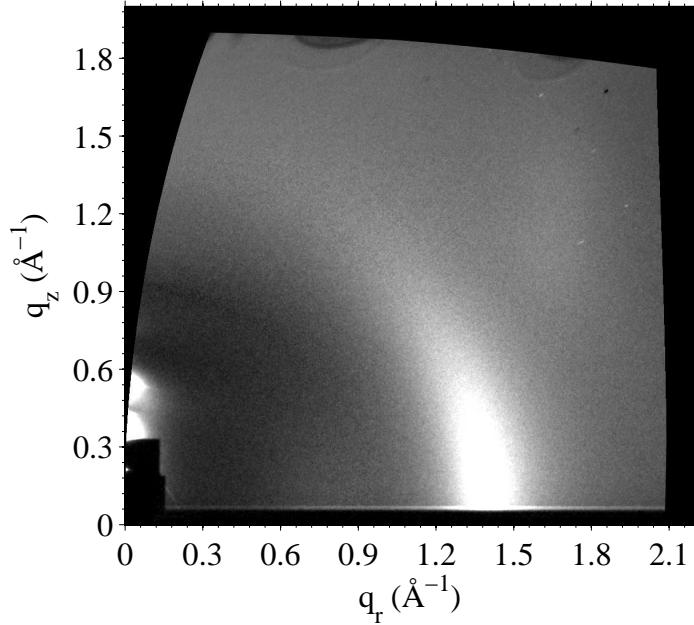


Figure 3.36: NGIWAXS of the DMPC fluid phase at  $30^\circ\text{C}$ .  $\omega = 0.5^\circ$ . The data were taken in the 2011 run with a low resolution setup. The sample width,  $w_s = 2 \text{ mm}$ .

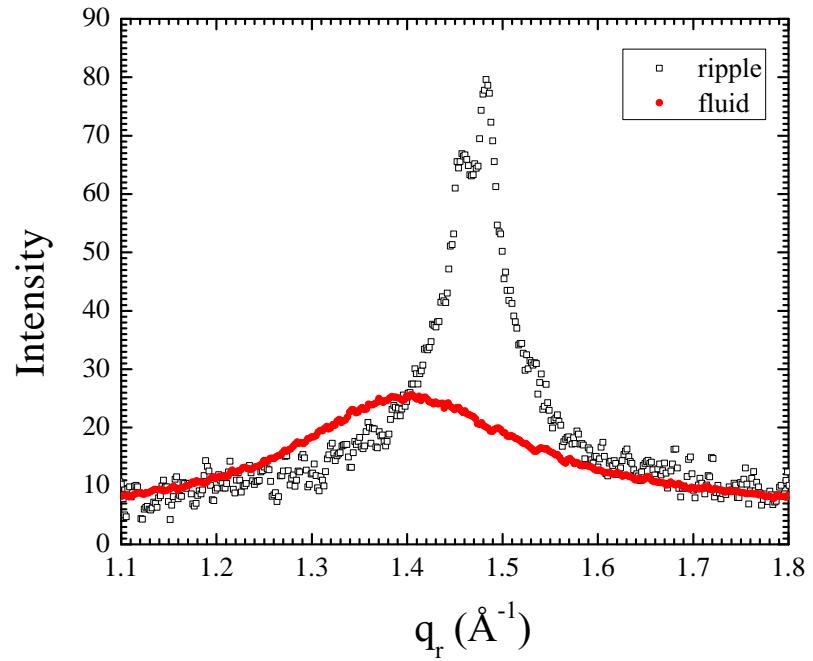


Figure 3.37: Comparison of the ripple (black) and fluid (red) phase WAXS at  $q_z = 0.012 \text{\AA}^{-1}$ . The fluid phase data were taken with the low resolution setup and scaled vertically to enable visual comparison.

### 3.7 TWAXS: results

Just like in near grazing incidence wide angle scattering, the background subtraction is the first step in analysis of transmission wide angle X-ray scattering (TWAXS) data. The background scattering data were collected by replacing the sample with a bare Si wafer. Although the background subtraction was not as good as for the NGIWAXS, this did not affect scattering in the wide angle region. Because the data were taken at  $\omega = 45^\circ$ , the ripple WAXS pattern in the CCD space is very different from the respective pattern in the sample  $q$ -space. Therefore, the CCD to  $q$ -space transformation is crucial in analyzing the TWAXS data.

Figure 3.38 shows the transmission wide angle X-ray scattering pattern after the CCD to  $q$  transformation. The stronger peak observed in NGIWAXS was also observed at approximately the same location. Because of a larger instrumental resolution than in the NGIWAXS experiment, the weaker peak was not as well separated. Figure 3.39 shows a slight hint of the weak peak at  $q_z = 0.12 \text{ \AA}^{-1}$  and Fig. 3.40 shows many more  $q_r$  swaths. This data set taken in the 2011 run motivated me to try an experiment with a higher instrumental resolution, which led to the NGIWAXS experiment in the 2013 run. Figure 3.41 shows intensity along  $q_z$ , averaged for  $q_r$  between  $1.46 \text{ \AA}^{-1}$  and  $1.52 \text{ \AA}^{-1}$ . It shows absence of a sharp peak at  $q_z = 0 \text{ \AA}^{-1}$ , complementing the NGIWAXS data. While the instrumental resolution here was not great, Fig. 3.40 and 3.41 indicate that the second, weaker peak is located at or above  $q_z = 0.12 \text{ \AA}^{-1}$ .

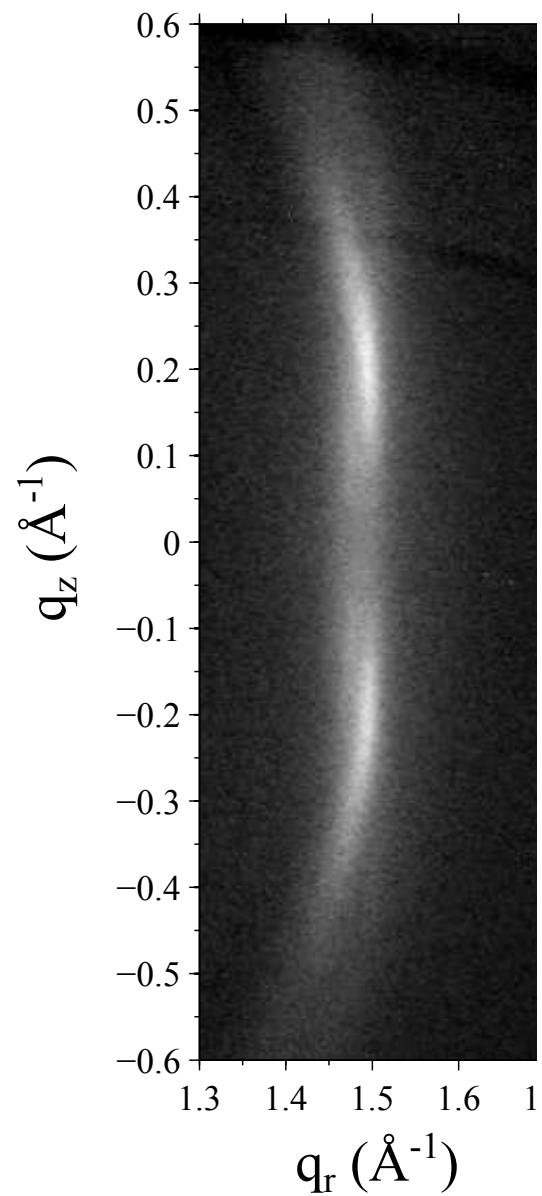


Figure 3.38: TWAXS image of the DMPC ripple phase at 18 °C and  $D = 60.3 \text{ \AA}$ .

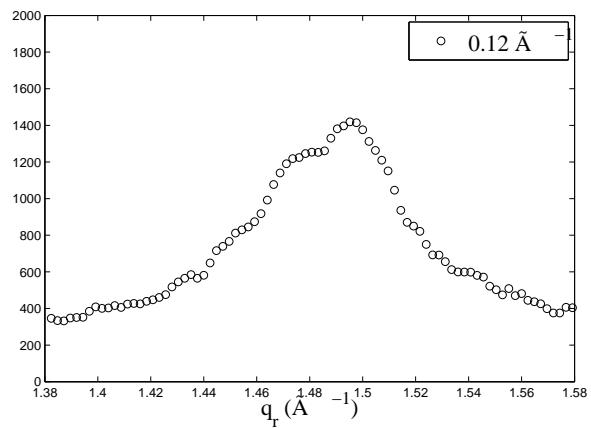


Figure 3.39:  $q_r$  swath of the TWAXS image averaged over  $0.02 \text{ \AA}^{-1}$  centered at  $q_z = 0.12 \text{ \AA}^{-1}$ .

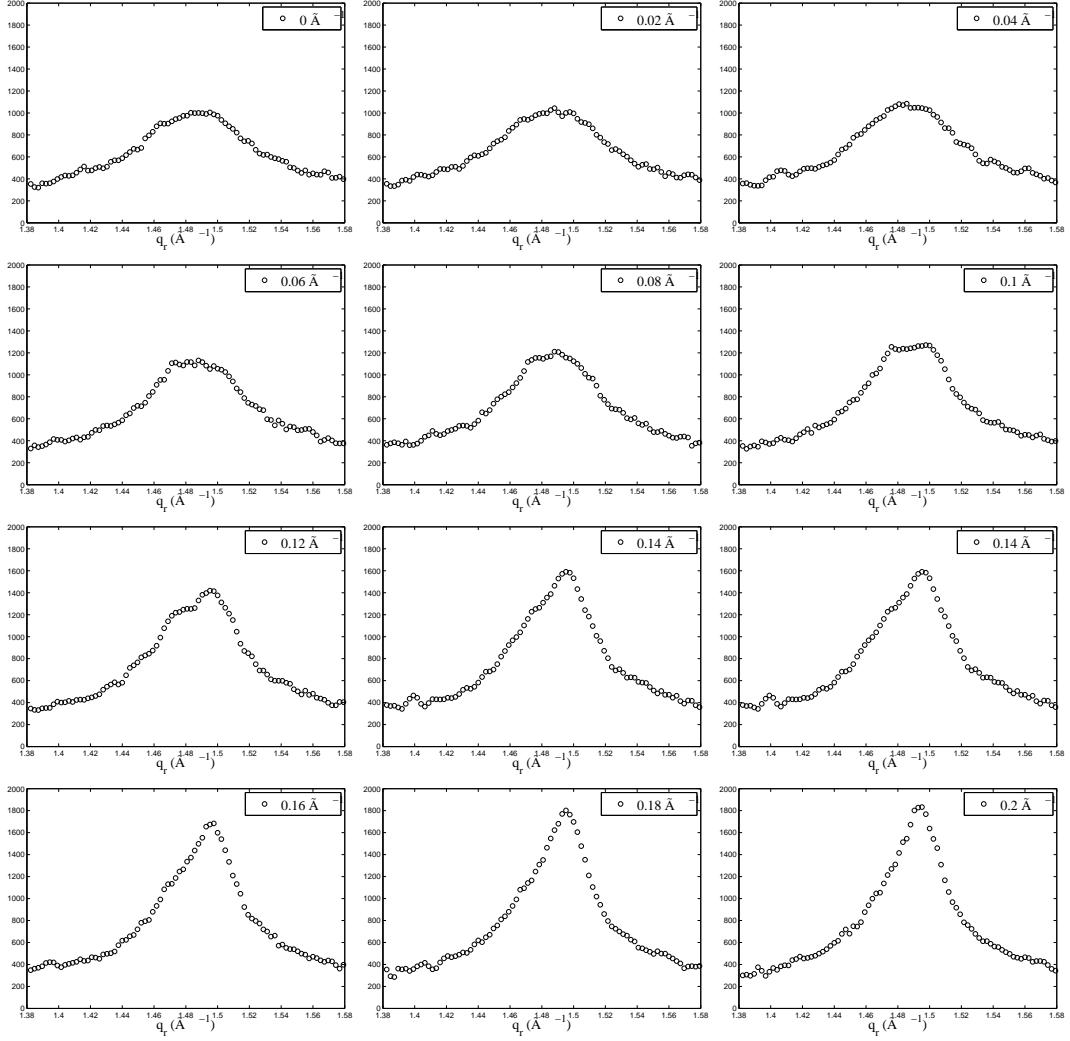


Figure 3.40:  $q_r$  swaths of the TWAXS image, each averaged over  $0.02 \text{ \AA}^{-1}$ . The center  $q_z$  value of a swatch is shown in the figure legends.

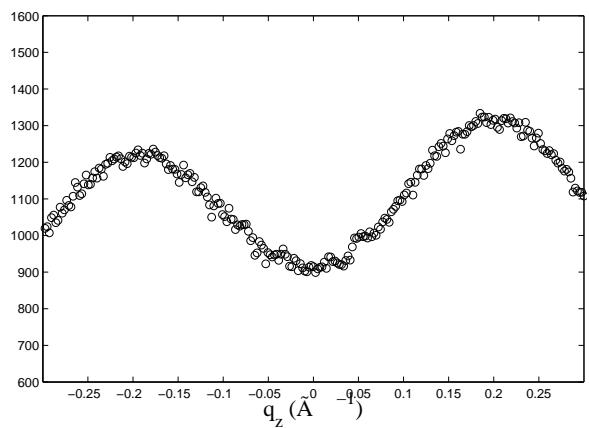


Figure 3.41:  $q_z$  swath averaged between  $1.46 \text{ \AA}^{-1}$  and  $1.52 \text{ \AA}^{-1}$ .

## 3.8 Discussion

The ripple phase has attracted many researchers since its discovery in 1967, and extensive structural work on average structural properties such as the ripple wavelength, oblique angle,  $D$ -spacing, and electron density profiles have been published. From those experimental measurements, many theoretical models to explain the origin of the ripple phase have been published with some models resulting in predictions inconsistent with the experimental data. Yet, from equilibrium structural study such as X-ray scattering, chain packing for the asymmetric ripple has only been suggested based on the measured thicknesses of the major and minor arms. For example, based on the calculated electron density profile, Sun *et al.* [88] has suggested that the chains in the minor arm are disordered like in the fluid phase. Sengupta *et al.* [96] has argued, also based on the electron density profile calculated using the data by Wack and Webb, that chains are packed like in the gel phase throughout both arms with the same tilt angle with respect to the stacking  $z$  direction. Since the thickness of the major arm has shown to be almost the same as that of the gel phase, they argued that chains in the major arm should be tilted about  $30^\circ$  with respect to the bilayer local normal.

Since the millennium, a few simulations have predicted different type of chain packing. For example, all atomistic simulations done by de Vrie *et al.* [98] have predicted existence of interdigitated state in the minor arm of the DMPC asymmetric ripple while lipids in the major arm are packed like in the gel phase with the monolayers decoupled from each other.

Previous predictions and suggestions so far have not been able to directly be verified because of the lack of quantitative wide angle scattering data. Although the DMPC WAXS was measured by Katsaras and Raghunathan [101] using a rotating anode, the wide angle peak measured was broad and ambiguous to answer the question of lipid packing. Therefore, we sought to fill the gap with synchrotron X-ray, which has been successful in describing the fluid phase chain-chain correlation. Our strength were three fold: 1) brilliant synchrotron beam that allowed use of Si monochromator with a very small energy dispersion, 2) stacks of  $\sim 2000$  bilayers oriented on the substrate that scattered strongly and anisotropically, and 3) hydration chamber that allowed us to control the hydration of the sample.

By careful analysis of low angle X-ray scattering data, we have shown that chains

in the major arm are straightened like in the gel phase, the finding of which is consistent with previous publications. The thickness of the major arm was measured to be 40 Å, slightly larger than the previously published value of 37.9 Å [88]. The electron density profile in the minor arm we obtained was similar to that of interdigitated chains. However, the wide angle X-ray scattering data is inconsistent with this. As de Vries *et. al.* calculated, interdigitated chains in the minor arm would result in a wide angle peak at  $q_z \approx 0.5 \text{ \AA}^{-1}$ , much larger  $q_z$  than 0.2 Å<sup>-1</sup> that we observed.

By comparing the wide angle pattern from the ripple and fluid phases, we showed that scattering intensity around 1.4 Å<sup>-1</sup> in the ripple phase is probably the tail of the stronger peak coming from the major arm and not due to the fluid-like chain packing in the minor arm.

Given existence of gel-like packing in the major arm, we argue that the stronger peak observed in the NGIWAXS data is the Bragg rod from the major arm. It is not clear, however, whether or not the weaker peak is also due to packing in the major arm. Resolving this issue seems to require modeling of various types of chain packing for both major and minor arms.

## 3.9 Conclusion

We have attempted to solve the lateral structure of the lipids within the bilayer using synchrotron X-rays. While we could not calculate the electron density profile unambiguously or solve the chain packing based on the measured wide angle scattering data, previously suggested structure of the ripple phase can be tested against our data. For example, Monte Carlo simulations based on a model free energy can be tuned to obtain a good fit to our measured wide angle data. One could also consider some exotic packing such as swirling pattern observed by Watkin et al. (PNAS) or tilt modulation field. Predicting the scattering intensity pattern from these structures might lead to a different way to analyze our LAXS and NGIWAXS data and possibly more improved study of the ripple phase.

Future possible experiments include a high resolution transmission experiment, where both geometric broadening and energy dispersion are minimized. The expected resolution is the width of the X-ray beam, which is about 3 pixels. This experiment doubles the best resolution achieved in this work. Another slightly different high resolution experiment is to use silicon crystal analyzer downstream of the

sample, which completely remove geometric broadening. The downside of this type of high resolution experiment is that only one point in q-space is probed at any given exposure, so getting a full 2D map of wide angle scattering is time consuming.

Also highly speculative, but the ripple phase might be an interesting phase to study curvature sensing peptides. The description of curvature in the ripple phase has been around for a while. Those curvature sensing peptides may accumulate at the kink regions. Then, the electron density profile can be calculated with the analysis detailed in this work. It would be very interesting if peptide-lipid interactions also significantly modify the wide angle pattern. With a known perturbation property of a peptide on lipids, it could shed light on the structure of the minor arm. For example, if indeed chains are fluid like in the minor, some peptides might have tendency to accumulate in the minor arm because of ease of insertion compared to the gel-like major arm. Then, the ripple phase might be used to study biologically relevant problems.

# **Appendices**

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