

Measurement of the Most Important Physics Quantity Ever Discovered

by

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Chapter 2

Experiment and Simulation

2.1 X-ray optics

High resolution setup should be described.

Low resolution setup should be described.

Detector correction was applied. See references.

2.2 Hydration Chamber

The chamber is sealed very tightly. We used helium to replace air. Air scattering is strong. Mylar window, cast scattering in wide angle region. Helium. Peltier used to condense water in and out of the sample.

2.3 Sample Preparation

Synthesized lipids were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. Membrane mimics were prepared by first dissolving lyophilized lipids in chloroform and then mixing these stock solutions to create the lipid compositions DOPC, DOPC/DOPE (3:1), DOPC/DOPE (1:1), DOPC/DOPS (3:1) and nuclear membrane mimic (POPC/POPE/POPS/SoyPI/Cholesterol, 69:15:2:4:11) (based on Ref. [37]). Peptide (Y47GRKKRRQRRR57) was purchased in two separate lots from the Peptide Synthesis Facility (University of Pittsburgh, Pittsburgh, PA); mass spectroscopy revealed greater than 95% purity. This Tat peptide corresponds to residues (47-57) of the 86 residues in the Tat protein [6]. Tat was dissolved in HPLC trifluoroethanol (TFE) and then mixed with lipid stock solutions in chloroform to form mole fractions between 0.0044 and 0.108. Weight of Tat in these mole fractions was corrected for protein content (the remainder being 8 trifluoroacetate counter-ions from the peptide synthesis). Solvents were removed by evaporation in the fume hood followed by 2 hours in a vacuum chamber at room temperature.

2.4 Samples for X-ray scattering

Four mg dried lipid/peptide mixture was re-dissolved in HPLC chloroform/TFE (2:1 v:v) for most of the lipid compositions. DOPC/DOPS (3:1) mixtures required chloroform/HFP (1:1 v:v) in order to solubilize the negatively charged DOPS. 200 l of 4 mg mixtures in solvents were plated onto silicon wafers (15x30x1 mm) via the rock and roll method [38] to produce stacks of 1800 well-aligned bilayers; solvents were removed by evaporation in the fume hood, followed by two hours under vacuum. Samples were prehydrated through the vapor in polypropylene hydration chambers at 37 °C for two to six hours directly before hydrating in the thick-walled X-ray hydration chamber [39] for 0.51 h. Pre-equilibration allowed sufficient time for equilibrium binding of peptides with membrane mimics.

For ripple phase experiments, the sample was annealed at 60 °C for approximately 10 hours prior to the experiment. The sample was trimmed to 1 mm for high resolution and 5 mm for low resolution study, respectively. The temperature was set at 18 °C. The sample was dried by applying a small heating current to the Peltier.

2.5 Low Angle X-ray Scattering Experiment

The analysis of diffuse low angle X-ray scattering from oriented stacks of fluctuating fluid bilayers has been previously described [39]. Absolute form factors $-F(qz)-$ were obtained as previously described [42]. Modeling to estimate the locations of Tat and the lipid components was performed using the SDP program [44].

2.6 Transmission X-ray Scattering Experiment

Transmission experiment. The axis of rotation does not coincide with the plane of the sample, so that the sample-to-detector distance is not fixed for different motor angle. The sample-to-detector distance was estimated from the setup geometry. We used 35 μm thin Si wafer, which absorbs x-ray by only 10%.

2.7 Densimetry

2.7.1 Samples for densimetry

Multilamellar vesicles (MLVs) were prepared by mixing dried lipid mixtures with MilliQ water to a final concentration of 2-5 wt% in nalgene vials and cycling three times between -20°C and 60°C for ten minutes at each temperature with vortexing. Pure Tat was dissolved in water at 0.4 wt%.

2.7.2 Densimetry

Volumes of lipid mixtures with and without peptides in fully hydrated multilamellar vesicles (MLV) were determined at 370.01 °C using an Anton-Paar USA DMA5000M (Ashland, VA) vibrating tube densimeter [47].

2.8 MD simulation

Systems with different DOPC/Tat mole ratios (128:0, 128:2 and 128:4, corresponding to 0, 0.015 and 0.030 mole fractions) were simulated atomistically using the Gromacs 4.6.1 package [49]. DOPC was modeled by the Slipid force field [50, 51] and HIV Tat was modeled by Amber 99SB [52]. Tip3p water was used [53]. The number of Tats was divided equally on each side of the bilayer to mimic experimental conditions. All systems were simulated at 310 K with a constant area in the x-y plane for and 1 atm constant pressure in the Z direction. Each system was simulated for 100 ns and the last 50 ns was used as the production run. At each DOPC/Tat mole ratio, we studied systems with three different area/lipid (AL). For the DOPC system, we fixed AL = 68, 70, 72 Å²; DOPC/Tat (128:2), we fixed the AL = 72, 74, 76 Å²; DOPC/Tat (128:4), we fixed the AL = 72, 74, 76 Å². For each DOPC/Tat system at fixed AL, we then conducted seven independent simulations with the center of mass (COM) of each Tat constrained at different bilayer depths from the bilayer center (18, 16, 14, 12, 10, 8 and 5 Å). In total, 45 independent simulations were conducted. The goal of constrained simulations is to find the best match between experimental and MD simulation form factors. Comparison to the X-ray form factors was performed using the SIMtoEXP software [54]. Additional details concerning the MD simulations are in Supplementary Data 6.

Chapter 4

Ripple Phase

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

4.1 Introduction

The ripple phase is the greatest thermodynamic phase of lipid bilayers. This phase was originally found by Tardiue et al. in some lipid bilayers. What did they find? Further work on the ripple?

The structure of the ripple phase bilayers can be divided into two categories: 1) the average electron density profile that characterizes how each bilayer is packed within a stack of lipid bilayers and 2) lateral lipid structure that characterizes how lipid chains are packed.

Extensive work was done by Wack and Webb (1989). The X-ray form factor for DMPC was analyzed by Sun et al. Sengupta et al studied the temperature dependence of DMPC and other lipids.

Hatchel (1991) did wide angle scattering (transmission) for DPPC. Rughnathan and Katsaras did near grazing angle WAXS on DMPC. Sengupta et al. suggested lipid chain packing in 2003 based on average bilayer electron density (ED) profile calculated from low angle X-ray scattering. de Vrie et al suggested interdigitated chain in the minor side based on molecular dynamics (MD) simulations.

Many theoretical work have been done to understand the origin of the ripple phase. Some work include Lubensky and Macintosh, ...

These theories predict chain packing in both major and minor sides. It is obviously important to determine the packing in order to validate or invalidate those theories. However, this information is not completely known. WAXS is a direct probe of the chain packing. It is difficult because scattering is rather weak compared to gel phase.

Here, we report DMPC average structure calculated from low angle X-ray scattering data collected at a synchrotron. Careful analysis allowed us to derive a high resolution ED profile. The profile suggests WHAT?

We also report wide angle X-ray scattering, and suggest chain packing based on both LAXS and WAXS. Highly brilliant synchrotron X-ray allows high resolution study on weak, possibly diffuse scattering. We determined that the peak is off the equator and tilted from the vertical q_z axis.

4.2 Materials and Methods

High resolution was achieved by the use of Germanium monochromator, 0.01% energy dispersion. Low resolution data were taken with multilayer monochromator with 1% energy dispersion.

To achieve small mosaic spread, samples were annealed at 60 °C for at least 6 hours right before the experiment. Silicon wafers were cleaned just before use. 2:1 methanol: chloroform was used to dissolve DMPC. Samples were rocked continuously during solvent evaporation and dried further inside a glove box. Samples were then dried under a hood for 24 hours and in a vacuum chamber for 2 hours. Samples were stored in a refrigerator.

A thin piece of molybdenum was used to attenuate the beam. The attenuation length μ of X-ray in Mo is 6.433 μm for 8 keV and 13.74 μm for 10.55 keV [8]. For a nominal 25 μm thick Mo attenuator, $\mu = 13.74$ gives the attenuation factor of $[\exp(-25/13.74)]^{-1} = 6.2$. The exact attenuation factor was determined by taking images with and without the attenuator and calculating a factor that needed to be multiplied for the images to match with each other.

Low angle scattering was done with low resolution setup.

25 μm silicon wafer was used to deposit our sample. This wafer absorbs only 20% of the X-ray beam at 10.5 keV (Check this). This weak absorption allows transmission scattering. Transmission experiment was done with low resolution setup.

Grazing angle experiment was done with both high and low resolution setups. To minimize geometric broadening in grazing angle experiment, the sample was trimmed to either 1 or 2 mm.

4.2.1 Sample Preparation

DMPC was purchased from Avanti Polar Lipids and used without further purification. Oriented thin film was deposited following the rock and roll procedure. In previous synchrotron experiments, the samples were created and annealed more than a week in advance and stored in a refrigerator. The orientation quality of these samples were found to be worse than the quality soon after the samples were annealed. Therefore, to ensure the best sample quality, the sample was annealed for approximately 12 hours just before the X-ray experiment. Figure X compares a sample scattering in

2011 and 2013 synchrotron runs. Because of substantial degree of mosaic spread in the 2011 sample, many peaks overlapped, rendering the accurate measurement of integrated intensity difficult. Figure XX shows a picture of the annealing chamber. To achieve gentle but efficient hydration of a sample, filter papers were installed to cover the sample. For successful annealing, it must be emphasized that the annealing chamber equilibrates in the over prior to putting the samples in the chamber. When the sample was put in the chamber with its water at room temperature and then the system was placed inside the oven, warm vapor condensed on the cooler sample, causing so called flooding of oriented sample. A small drop of water on the oriented film was detrimental for the orientation because the bilayers tend to peel off, resulting in entropy-driven formation of unoriented vesicles in the water subphase.

The sample for 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 further trimmed down to 1 mm in width.

The sample for transmission study was deposited on a thin, 35 micron, silicon wafer. Because the wafer was very fragile, attaching the sample to a sticky thing was impossible. Instead, the sample was attached to a plastic cap of a small vial with a small amount of heat sink compound at a corner of the wafer. The wafer was stable enough for rocking. The sample deposited on a glass cover slip (70 microns) was prepared similarly.

4.2.2 Low Angle Diffraction Experiment

The same setup as described in Tat chapter was used for low angle diffraction experiment. In order to achieve a D-spacing comparable to that of Wack and Webb ($D \approx 57.9 \text{ \AA}$), the current to the Peltier was reversed, which heats the Peltier surface the sample was situated on.

The integrated intensity of each peak was obtained 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 side was made according to the width of each peak. Because of mosaic spread in the sameple, peaks were wider for higher orders. Accordingly, the box was made wider for higher orders. The box size was chosen so that approximately 90% of the peak intensity was counted toward the integrated intensity.

A few peaks in the ripple phase were very strong, leading to saturation of CCD pixels. A nominally 25 micron molybdenum attenuator was inserted in the upstream to reduce the intensity of the X-ray beam and one second exposure was collected. The integrated intensity of the most strong, (1,0) peak was measured from this short exposure. To measure the actual attenuation factor, a one second exposure without the attenuator was also taken. Comparison of these two images yielded an attenuation factor of 7.8. The intensity of (2,0) and (2,-1) peaks were also measured in this one

second exposure with an attenuator. All the other peaks were observed without saturation in 60 second exposure. To properly scale the strong orders, (1,0) peak intensity was multiplied by 7.8 x 60 and (2,0) and (2,-1) were multiplied by 60.

The integrated intensity, peak position in pixel, the size of box, and estimated background for all the peaks are shown in Appendix.

4.2.3 Near Grazing Angle Experiment

Plot along the peak, along the arc, horizontal, vertical, etc.

4.2.4 Transmission Experiment

No strong order on the equator. Subtraction of water scattering from the background image.

4.2.5 Model

4.3 Lattice Structure

It has been shown from X-ray studies (ref) that ripples in in different bilayers are registered to form a two-dimensional oblique lattice as shown by the unit cell in Fig. X. The unit cell vectors in the ripple phase can be expressed as

$$\mathbf{a} = \frac{D}{\tan \gamma} \hat{\mathbf{x}} + D \hat{\mathbf{z}} \quad (4.1)$$

and

$$\mathbf{b} = \lambda_r \hat{\mathbf{x}}. \quad (4.2)$$

The corresponding reciprocal lattice unit cell vectors are

$$\mathbf{A} = \frac{2\pi}{D} \hat{\mathbf{z}} \quad (4.3)$$

and

$$\mathbf{B} = \frac{2\pi}{\lambda_r} \hat{\mathbf{x}} - \frac{2\pi}{\lambda_r \tan \gamma} \hat{\mathbf{z}}. \quad (4.4)$$

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

$$\mathbf{q}_{hk} = h\mathbf{A} + k\mathbf{B}, \quad (4.5)$$

so its Cartesian components are

$$q_k^x \equiv q_{hk}^x = \frac{2\pi k}{\lambda_r} \quad (4.6)$$

$$q_{hk}^y = 0 \quad (4.7)$$

$$q_{hk}^z = \frac{2\pi h}{D} - \frac{2\pi k}{\lambda_r \tan \gamma}. \quad (4.8)$$

4.4 Scattering Basics

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

$$\mathbf{k}_{\text{in}} = \frac{2\pi}{\lambda} \hat{\mathbf{y}}, \quad \mathbf{k}_{\text{out}} = \frac{2\pi}{\lambda} (\sin 2\theta \cos \phi \hat{\mathbf{x}} + \cos 2\theta \hat{\mathbf{y}} + \sin 2\theta \sin \phi \hat{\mathbf{z}}), \quad (4.9)$$

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

$$\begin{aligned} \mathbf{q} &= \mathbf{k}_{\text{out}} - \mathbf{k}_{\text{in}} \\ &= q (\cos \theta \cos \phi \hat{\mathbf{x}} - \sin \theta \hat{\mathbf{y}} + \cos \theta \sin \phi \hat{\mathbf{z}}), \end{aligned} \quad (4.10)$$

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

$$\hat{\mathbf{e}}_{\mathbf{x}} = \hat{\mathbf{x}}, \quad \hat{\mathbf{e}}_{\mathbf{y}} = \cos \omega \hat{\mathbf{y}} + \sin \omega \hat{\mathbf{z}}, \quad \hat{\mathbf{e}}_{\mathbf{z}} = -\sin \omega \hat{\mathbf{y}} + \cos \omega \hat{\mathbf{z}}. \quad (4.11)$$

From Eq. (4.10) and (4.11), we find the sample q -space to be

$$\begin{aligned} q^x &= \mathbf{q} \cdot \hat{\mathbf{e}}_{\mathbf{x}} = q \cos \theta \cos \phi, \\ q^y &= \mathbf{q} \cdot \hat{\mathbf{e}}_{\mathbf{y}} = q (-\sin \theta \cos \omega + \cos \theta \sin \phi \sin \omega), \\ q^z &= \mathbf{q} \cdot \hat{\mathbf{e}}_{\mathbf{z}} = q (\sin \theta \sin \omega + \cos \theta \sin \phi \cos \omega). \end{aligned} \quad (4.12)$$

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

$$X = S \tan 2\theta \cos \phi, \quad Z = S \tan 2\theta \sin \phi, \quad (4.13)$$

where S is the distance between the sample and detector. From a model for the electron density of a lipid bilayer, one calculates a X-ray scattering intensity pattern, $I(\mathbf{q})$. Then, Eq. (4.12) and (4.13) 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 ω for a fixed wavelength, which was achieved by continuously rotating the sample with a motor. In the ripple phase, because our sample has in-plane rotational symmetry, the ripple side peaks make up Bragg rings while the main peaks are still delta function like (see Fig. X) in q -space. In order for the main peak to be observed, ω must be equal to θ_B , but the side peaks are observed at any ω . 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 θ and ω . In the small angle approximation, $\sin \phi \approx Z/(2S\theta)$

and $\cos \phi \approx X/(2S\theta)$, and

$$\begin{aligned} q_x &\approx \frac{4\pi\theta \cos \phi}{\lambda} \approx kX/S \\ q_y &\approx q_z\omega - \frac{4\pi\theta^2}{\lambda} \approx q_z\omega - \frac{\lambda q_z^2}{4\pi} \\ q_z &\approx \frac{4\pi\theta \sin \phi}{\lambda} \approx kZ/S, \end{aligned} \tag{4.14}$$

with $k = 2\pi/\lambda$. For wide angle X-ray scattering, the exact relations given by Eq. (4.12) are necessary. Especially in the transmission experiment, where ω is large, an observed X-ray pattern appears nontrivial and becomes almost impossible to analyze without the use of Eq. (4.12).

4.5 Geometric (Lorentz) Correction for Ripple Phase Scattering

Our sample has in-plane rotational symmetry. This means that the sample consists of many domains with differing ripple directions, all domains being parallel to the substrate. In sample q -space, then, ripple side peaks become rings while main peaks are still points (see Fig. X). For an arbitrary incident angle, main peaks are not observed while side peaks are observed. In order to capture both main and side peaks in one X-ray exposure, the sample was continuously rotated. As a result of this rotation, main peaks become arcs that subtend an angle $2\theta_{h0}$, as shown in Fig. 4.1, with its length equal to $2\theta_{h0}q_{h0}^z$. The detector records the cross sections of these arcs

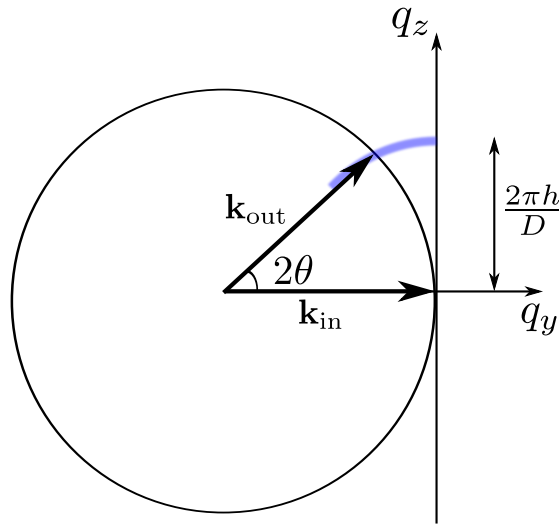


Figure 4.1: caption goes here

with the Ewald sphere, so the total scattering power is the product of the observed intensity, I_{hk}^{obs} with the arc length, that is,

$$I = 2\theta_{h0}q_{h0}^z I_{h0}^{\text{obs}}. \quad (4.15)$$

Because the sample has in-plane rotational symmetry, side peaks are represented as rings whose radius is q_{hk}^r . For a fixed incident angle, all the rings are intersected by the Ewald sphere. Because only the domains with the right ripple direction can satisfy the Bragg's condition at a given fixed angle, the scattering power of this small cross section is reduced by a factor of $2\pi q_k^r$ compared to main peaks. During an X-ray exposure, the rings cross the Ewald sphere at all incident angles. Then, the total scattering power is given by

$$I = 2\pi q_k^r I_k^{\text{obs}}. \quad (4.16)$$

Inverting Eq. (4.15) and (4.16) and realizing that the intensity is the form factor squared, we can calculate the observed intensity, I^{obs} , from a model for an electron density in the ripple phase.

Mathematically, the rotation is equivalent to an integration over ω . In low angle X-ray scattering, q_z is constant at a given pixel as ω is changed, which can be seen from Eq. (4.14). ω dependence appears only through q_y , so rotating the sample is realized by integrating over q_y . To derive the integration limits, let us consider two cases: (a) When $\omega \leq 0$, the incoming X-ray beam is blocked by the back of the substrate. This sets the lower limit to 0. (b) When $\omega \geq 2\theta$, the substrate blocks the outgoing X-ray. Within the small angle approximation, then, ω_{max} is $2 \times \lambda q_z / (4\pi)$ for scattering with q_z . Thus, the integration limits for q_y integration are $[-\lambda q_z^2 / (4\pi), \lambda q_z^2 / (4\pi)]$. We also need to integrate over X and Z to obtain integrated intensity. These lead to the observed intensity written as,

$$\begin{aligned} I_{hk}^{\text{obs}} &\propto \int dX \int dZ \int d\omega |F_{hk}|^2 S_{hk}(\mathbf{q}) \\ &\propto |F_{hk}|^2 \int dq_x \int dq_z \int_{-\frac{\lambda q_z^2}{4\pi}}^{\frac{\lambda q_z^2}{4\pi}} \frac{dq_y}{q_z} S_{hk}(\mathbf{q}), \end{aligned} \quad (4.17)$$

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

For a crystalline sample with the in-plane rotational symmetry, the structure factor is

$$S_{hk}(\mathbf{q}) = S_{hk}(q_r, q_z) = \frac{1}{2\pi q_r} \delta(q_r - q_{r,k}) \delta(q_z - q_{z,hk}), \quad (4.18)$$

where $q_{r,k} = 2\pi|k|/\lambda_r$. Thus, the scattering pattern in the ripple phase is a collection of Bragg "rings" centered at the meridian and the Bragg peaks that are called the main peaks.

The observed, integrated intensity of hk peak is proportional to

$$I_{o,hk} \propto \frac{|F_{hk}|^2}{q_{z,hk}} \int dq_x \int_{-q_{y0}}^{q_{y0}} dq_y \frac{\delta(q_r - q_{r,k})}{2\pi q_r}, \quad (4.19)$$

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

$$\begin{aligned} \int dq_x \int_{-q_{y0}}^{q_{y0}} dq_y \frac{\delta(q_r - q_{r,k})}{2\pi q_r} &\approx \int_{-\frac{q_{y0}}{q_{r,k}}}^{\frac{q_{y0}}{q_{r,k}}} d\phi \int dq_r q_r \frac{\delta(q_r - q_{r,k})}{2\pi q_r} \\ &= \frac{q_{y0}}{\pi q_{r,k}}. \end{aligned} \quad (4.20)$$

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

$$\begin{aligned} \int dq_x \int_{-q_{y0}}^{q_{y0}} dq_y \frac{\delta(q_r - q_{r,k})}{2\pi q_r} &= \int_0^{2\pi} d\phi \int dq_r q_r \frac{\delta(q_r - q_{r,k})}{2\pi q_r} \\ &= 1 \end{aligned} \quad (4.21)$$

Using Eq. (4.20) and (4.21), we write the observed integrated intensity as

$$I_{o,h0} \propto \frac{|F_{h0}|^2}{q_{z,h0}} \quad (4.22)$$

$$I_{o,hk} \propto \frac{|F_{hk}|^2}{q_{z,hk}} \frac{q_{y0}}{\pi q_{r,k}} = |F_{hk}|^2 \frac{\lambda q_{z,hk}}{2\pi} \frac{1}{2\pi q_{r,k}} = |F_{hk}|^2 \frac{2\theta_{hk}}{2\pi q_{r,k}}, \quad (4.23)$$

where $2\theta_{hk} = \lambda q_{z,hk} / (2\pi)$ is the incident angle at which the outgoing X-ray for the peak (hk) is blocked by the substrate. Eq. (4.22) and (4.23) relate the form factor calculated from a model to the experimentally observed intensity, and are equivalent to Eq. (4.15) and (4.16), which were derived by using the Ewald sphere.

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

4.6 Contour Part of the Form Factor

As in ref, we take the ripple profile to have a sawtooth-like profile. Its amplitude is $A/2$ and the projection of the major arm on the ripple direction is x_0 as shown in Fig. X. Then, we write the ripple profile as

$$u(x) = \begin{cases} -\frac{A}{\lambda_r - x_0} \left(x + \frac{\lambda_r}{2}\right) & \text{for } -\frac{\lambda_r}{2} \leq x < -\frac{x_0}{2}, \\ \frac{A}{x_0} x & \text{for } -\frac{x_0}{2} \leq x \leq \frac{x_0}{2}, \\ -\frac{A}{\lambda_r - x_0} \left(x - \frac{\lambda_r}{2}\right) & \text{for } \frac{x_0}{2} < x \leq \frac{\lambda_r}{2}. \end{cases} \quad (4.24)$$

The ripple profile has the inversion symmetry, so that the resulting form factor is real. A and x_0 are fitting parameters that depend on the integrated intensity of each peak while D and λ_r 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 δ functions $\delta(x \pm x_0/2)$ to allow for a different electron density near the kink between the major and the minor sides.

4.7 Transbilayer Part of the Form Factor

4.7.1 Delta Function Model

4.7.2 2G Hybrid Model

In the hybrid model, the terminal methyl region of the bilayer is represented as a Gaussian function [9]. 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,

$$\rho(z) = \rho_G(z) + \rho_S(z) + \rho_B(z), \quad (4.25)$$

where the Gaussian part is given by

$$\rho_G(z) = \sum_{i=1}^{1 \text{ or } 2} \rho_{Hi} e^{-(z-Z_{Hi})^2/(2\sigma_{Hi}^2)} + \rho_M e^{-z^2/(2\sigma_M^2)}, \quad (4.26)$$

the strip part is given by

$$\rho_S(z) = \begin{cases} \rho_{CH_2} & \text{for } 0 \leq z < Z_{CH_2}, \\ \rho_W & \text{for } Z_W \leq z \leq D/2, \end{cases} \quad (4.27)$$

and the bridging part is given by

$$\rho_B(z) = \frac{\rho_W - \rho_{CH_2}}{2} \cos\left[\frac{-\pi}{\Delta Z_H}(z - Z_W)\right] + \frac{\rho_W + \rho_{CH_2}}{2} \quad \text{for } Z_{CH_2} < z < Z_W. \quad (4.28)$$

with $\Delta Z_H = Z_W - Z_{CH_2}$. Here, we assume $Z_{H2} > Z_{H1}$. Table 4.1 shows some of the definitions. The transbilayer profile along $x = -z \tan \psi$ can be obtained by rotating the coordinates x and z by ψ in the clockwise direction and reexpressing $\rho(z)$ in terms of the rotated coordinates. This leads to replaing 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

$$\rho(x, z) = \delta(x + z \tan \psi) [\rho_G(z') + \rho_S(z') + \rho_B(z')]. \quad (4.29)$$

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

Table 4.1: Definitions of Z_{CH_2} and Z_{W}

Taking the two dimensional Fourier transform of Eq. (4.29) leads to the transbilayer part of the form factor,

$$F_{\text{T}} = \int_{-\frac{D}{2}}^{\frac{D}{2}} \int_{-\frac{\lambda_r}{2}}^{\frac{\lambda_r}{2}} [\rho(x, z) - \rho_{\text{W}}] e^{i(q_x x + q_z z)} dx dz \quad (4.30)$$

$$= F_{\text{G}} + F_{\text{S}} + F_{\text{B}}. \quad (4.31)$$

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 and not shown. The derivation and full expression can be found in the appendix. Here, we note that the fitting parameters in this model are Z_{Hi} , σ_{Hi} , and R_{HiM} for each of the two headgroup Gaussian functions, σ_{M} for the terminal methyl Gaussian, ΔR for the methylene region, ψ for the lipid tilt, and an overall scaling factor. The contour part of the form factor has four more parameters (A , x_0 , f_1 , and f_2). In total, the modified 2G hybrid model implements 14 structural parameters.

4.8 Results

4.9 Data

Table 4.2 summarizes data we analyzed. As shown, we measured scattering in a

	λ_r	D	γ
WW	141.7	57.94	98.4°
S1	145	57.8	98.2°
S2	?	?	?

Table 4.2: Lattice constants

almost identical conditions as the Wack and Webb's. This data allowed us to check our data obtained by using an oriented sample against an unoriented sample. As discussed earlier, these two types of samples give different Lorentz correction. We derived the Lorentz correction for our oriented sample. Applying the derived correction to our data and calculating the form factor, we were able to confirm our correction. This check is shown in Table.

Figure shows a LAXS pattern from DMPC at 18 °C. $D = 57.9 \text{ \AA}$. Low resolution experiment. Up to $h = 9$ orders were observed in this data set. Because of a non-negligible degree of mosaicity in the sample, strong orders cast their arcs over weaker orders. A care must be taken to decompose the intensity at a given pixel to intensity due to a strong order's arc and to that due to a weak peak. This was achieved by taking a q_z swath and fitting the intensity to two Gaussian functions whose widths were determined from the known instrumental resolution. Figure shows an example of this operation. Table and Table show with and without the decomposition operation, respectively. For many of the orders observed, errors one would expect from neglecting the mosaicity effect were small. For higher orders, however, this was crucial to obtain the correct integrated intensity.

In order to test the decomposition effect, fits were also performed for the sum of intensity for orders that overlap.

First, we fitted the data using only up to $h = 3$ orders. What did we get? How did each model do? Any inconsistency with Sun PNAS?

Next, we fitted every peak we observed. Which model failed?

4.10 Electron Density Profile

Table X shows the best fit for each model. It shows that the delta function model fails. Its failure is obviously due to its lack of fine structural details. In ref. (SUN), the model marginally worked because only up to the third orders were available. With the high flux synchrotron X-ray beam, many more higher orders were observed, whose intensity is dominated by finer details in the bilayer electron density. The table shows that 1G model also fails. 2G model works, but simple 2G model failed. $k = 6$ orders clearly require the modulation in the electron density along the ripple direction. The phase of lower orders tends to be the same throughout the different models while higher orders vary widely. These are just ideas. I need to do actual fitting.

4.11 Discussion

Comparison with previous unoriented/oriented stuff?

Which theories are consistent/inconsistent with the results of this study?

4.12 Conclusion

Well, the ripple phase is the greatest phase in the lipid bilayers. Our detailed work lead to deeper insight into the formation of this phase. Future experiments include the 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 to get a full 2D map of wide angle scattering is time consuming.

Appendices

Bibliography

- [1] Norbert Kuerka, John F. Nagle, Jonathan N. Sachs, Scott E. Feller, Jeremy Pencer, Andrew Jackson, and John Katsaras. Lipid bilayer structure determined by the simultaneous analysis of neutron and x-ray scattering data. *Biophysical Journal*, 95(5):2356 – 2367, 2008.
- [2] Stephanie Tristram-Nagle, Yufeng Liu, Justin Legleiter, and John F. Nagle. Structure of gel phase DMPC determined by x-ray diffraction. *Biophysical Journal*, 83(6):3324 – 3335, 2002.
- [3] Anthony R. Braun, Jonathan N. Sachs, and John F. Nagle. Comparing simulations of lipid bilayers to scattering data: The gromos 43a1-s3 force field. *The Journal of Physical Chemistry B*, 117(17):5065–5072, 2013.
- [4] Berk Hess, Carsten Kutzner, David van der Spoel, and Erik Lindahl. Gromacs 4: Algorithms for highly efficient, load-balanced, and scalable molecular simulation. *Journal of Chemical Theory and Computation*, 4(3):435–447, 2008.
- [5] Joakim P. M. Jmbeck and Alexander P. Lyubartsev. Derivation and systematic validation of a refined all-atom force field for phosphatidylcholine lipids. *The Journal of Physical Chemistry B*, 116(10):3164–3179, 2012.
- [6] Joakim P. M. Jmbeck and Alexander P. Lyubartsev. An extension and further validation of an all-atomistic force field for biological membranes. *Journal of Chemical Theory and Computation*, 8(8):2938–2948, 2012.
- [7] Viktor Hornak, Robert Abel, Asim Okur, Bentley Strockbine, Adrian Roitberg, and Carlos Simmerling. Comparison of multiple amber force fields and development of improved protein backbone parameters. *Proteins: Structure, Function, and Bioinformatics*, 65(3):712–725, 2006.
- [8] http://henke.lbl.gov/optical_constants.
- [9] M.C. Wiener, R.M. Suter, and J.F. Nagle. Structure of the fully hydrated gel phase of dipalmitoylphosphatidylcholine. *Biophysical Journal*, 55(2):315 – 325, 1989.