

Measurement of Something

by

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Chapter 2

Structural Perturbation of Lipid Bilayers Due to Tat Peptide

2.1 Introduction

The name cell-penetrating peptide (CPP) connotes a peptide that easily penetrates cell membranes (for Reviews see [12–14]).

This thesis focuses on the transactivator of translation, Tat, from the HIV-1 virus, which plays a role in AIDS progression. Earlier work showed that the HIV-Tat protein (86 amino acids) was efficiently taken up by cells, and concentrations as low as 1 nM were sufficient to transactivate a reporter gene expressed from the HIV-1 promoter [15, 16]. It has been reported that Tat protein uptake does not require ATP [17]. Studies using inhibitors of different types of endocytosis, including clathrin and caveolae-mediated, or receptor-independent macropinocytosis reached the same conclusion that ATP mediated endocytosis is not involved in Tat protein permeation [?, 18–20]. However, this issue is controversial, as other studies found evidence for endocytosis in Tat protein import [21–29]. Still other studies have concluded that an ATP requirement for Tat protein entry depends on the size of the cargo attached to Tat protein, or on the specific cell type [30–32]. The part of the Tat protein responsible for cellular uptake was assigned to a short region Tat (48–60), G₄RKKRRQRRRPPQ₆0, which is particularly rich in basic amino acids [17]. Deletion of three out of eight positive charges in this region caused loss of its ability to translocate [17]. In this chapter, short basic regions will be called Tat, while the entire

86 amino acid protein will be called Tat protein. Tat was shown to be responsible for the Tat proteins permeation into the cell nucleus and the nucleoli [17], and this was confirmed using live cell fluorescence in SVGA cells [33]. Tat (48-60) was shown to have little toxicity on HeLa cells at 100 μM concentration [17], but the longer Tat protein (2-86) was toxic to rat brain glioma cells at 1-10 μM [34]. Interestingly, no hemolytic activity was found when human erythrocytes were incubated with a highly neurotoxic concentration (40 μM) of Tat (2-86) [34]. These results prompt the question, what is the mechanism of Tats translocation through membranes? To address this question, many biophysical studies have used simple models of biological membranes composed of a small number of lipid types. These studies are valuable because there is no possibility for ATP-dependent translocation, thus ruling out endocytosis if translocation occurs. For example, Mishra et al. reported that the rate of entry into giant unilamellar vesicles (GUVs) composed of PS/PC (1:4 mole ratio) lipids of rhodamine-tagged Tat is immeasurably slow, but it crosses a GUV composed of PS/PC/PE (1:2:1) lipids within 30 seconds [35]. This study suggests that negative curvature induced by the inclusion of PE facilitates translocation. In a subsequent study using much smaller unilamellar vesicles (LUVs), Tat did not release an encapsulated fluorescent probe in LUVs composed of lipids modeling the outer plasma membrane, PC/PE/SM/Chol (1:1:1:1.5), but did release the probe in LUVs composed of BMP/PC/PE (77:19:4) [36]; BMP (bis(monoacylglycero)-phosphate) is an anionic lipid specific to late endosomes. In that study [36], the inclusion of PE did not suffice to cause leaky fusion in LUVs in the absence of a negatively charged lipid. The contrasting results in these two experiments may also be due to the use of LUVs instead of GUVs since it was reported that Tat does not translocate across LUVS of PC/PG (3:2) but does translocate across GUVs of the same lipid composition [37]. In a similar experiment, Tat did not translocate into egg PC LUVs [38]. In another experiment confirming these results, Tat did not translocate into GUVs containing only PC with 20 mol% cholesterol, but when PS or PE was included with PC, then rapid translocation of Tat was observed [39]. These experiments demonstrate that the choice of lipids and model systems influences Tat translocation.

Is a pore formed during Tat translocation? Although direct conductance measurements of Tat and lipid membranes have not been carried out, two studies measured conductance with the somewhat similar CPP oligoarginine R₉C peptide. Using single-channel conductance of gramicidin A in planar lipid membranes consisting of anionic,

neutral or positively charged lipids, R₉C did not increase conductance, even in anionic lipid membranes [40]. By contrast, in a similar experiment using planar lipid membranes, a current was induced by R₉C in PC/PG (3:1) membranes, with increasing destabilization over time [41]. Thus questions remain about pore formation of Tat in membranes. In the GUV experiment with Tat mentioned above [39], Ciobanasu *et al.*, using size exclusion methods, suggested a pore in the nanometer range, which could only be passed by small dye tracer molecules. Thus, if a true pore forms, it is likely to be small and transitory.

The secondary structure of Tat has been characterized by many researchers. Ref. [37] carried out Circular dichroism (CD) spectroscopy on a variation of Tat where the penultimate proline on Tat (48-60) was replaced by a tryptophan [37]. Their study found a random coil secondary structure in aqueous solution as well as when Tat was mixed with PC/PG/PE (65:35:5) LUVs. Ziegler *et al.* [20] obtained the same result using CD in PC/PG (3:1) vesicles. In addition, solid state NMR has identified a random coil structure of Tat in DMPC/DMPG (8:7 mole ratio) multibilayers [42]. In the larger Tat-(1-72)-protein NMR measurements at pH 4 have determined there is no secondary structure, with a dynamical basic region [43]. Similarly, NMR was used to study the full Tat protein and found a highly flexible basic region [44]. These previous studies indicate that an alpha helix is not required for Tats translocation ability.

Regarding the mechanism of translocation of this randomly structured, short basic peptide, many models have been proposed based on the conflicting results listed above. Molecular dynamics simulations offer some insight into the molecular details of translocation. Herce and Garcia simulated the translocation of Tat (Y₄₇GRKKRRQRRR₅₇) across DOPC at various lipid:peptide molar ratios [45]. Their simulations indicated that Tat binds to the phosphate headgroups, with 1 Tat binding with 14 lipids, each positive charge on Tat associated with nearly 2 phosphate groups [45]. Translocation involved a localized thinning, and snorkeling of arginine side chains through the hydrophobic layer to interact with phosphates on the other side of the membrane. This allowed some water molecules to penetrate the membrane along with Tat, forming a pore [45]. In this simulation, performed without inclusion of counterions, pore formation was only observed at high ratios of peptide:lipid (1:18) or at elevated temperature. However, a subsequent Gromacs simulation with counterions found no thinning and no pore formation when Tat was added to DOPC

membranes [46]. Instead it found a membrane invagination associated with a cluster of Tat peptides. From their findings, the authors suggested that micropinocytosis could be the model for Tat translocation across membranes [46].

In this thesis, I combine experimental low-angle X-ray scattering (LAXS) data with MD simulations to obtain the structure of fully hydrated, oriented lipid bilayers with Tat (47-57) added at several mole ratios. The lipid systems were DOPC, DOPC/DOPE (3:1 mole ratio), DOPC/DOPS (3:1), DOPC/DOPE (1:1) and a mimic of the nuclear membrane (POPC/POPE/POPS/SoyPI/Chol, 69:15:2:4:11).

2.2 Materials and Methods

2.2.1 Stock Solutions

Synthesized lipids were purchased from Avanti Polar Lipids (Alabaster, AL). Membrane mimics for Tat experiments 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 ($\text{Y}_{47}\text{GRKKRRQRRR}_{57}$) 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.

2.2.2 Thin Film Samples

For Tat experiments, 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 μl of 4 mg mixtures in solvents were plated onto silicon wafers ($15 \times 30 \times 1$

mm) via the rock and roll method [38] to produce stacks of \sim 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 X-ray hydration chamber [39] for 0.5 to 1 hour.

2.2.3 Volume Measurements

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%.

Volumes of lipid mixtures with and without peptides in fully hydrated multilamellar vesicles (MLV) were determined at 37 ± 0.01 °C using an Anton-Paar USA DMA5000M (Ashland, VA) vibrating tube densimeter. This instrument measures the average density of a solution and compares it to the density of air using $\rho_s - \rho_0 = k(\tau_s - \tau_0)^2$ where k is an instrumental ??? that depends on the atmospheric pressure.

The Tat peptide sequence used in X-ray experiments and MD simulations was Y₄₇GRKKRRQRRR₅₇. Table 2.1 lists the chemical formulas and molecular weights of these amino acids for convenience. The molecular weight of this sequence is $181.2 + 75.1 + 146.1 + 2 \times 146.2 + 6 \times 174.2 - 10 \times 18 = 1560$. The Tat peptides were synthesized in trifluoroacetic acid, which has the chemical formula CF₃CO₂H, and is made into a powder form by the freeze-dry method. Therefore, each positively charged amino acid such as an arginine and lysine was counter-balanced by a trifluoroacetate (TFA) (C₂F₃O₂). Since Tat has six arginines and two lysines, it came with eight trifluoroacetates. This complex has a molecular weight of $1560 + 113 \times 8 = 2464$. We used the molecular weight of this complex in order to calculate the molarity of Tat correctly. The same molecular weight was also used in preparing oriented samples.

The Tat volume V_{Tat} was calculated from the measured average density of a Tat-water solution in the following way. Assuming that Tat molecules in water do not change the volume of water molecules, the density of Tat-water solution is equal to the mass of Tat-water solution divided by the sum of volumes of water and Tat,

$$\rho_{\text{sol}} = \frac{m_w + m_c}{V_w + V_c N_c}, \quad (2.1)$$

Code	Amino acid	Chemical Formula	Molecular weight (g/mol)
K	Lysine	C ₆ H ₁₄ N ₂ O ₂	146.2
R	Arginine	C ₆ H ₁₄ N ₄ O ₂	174.2
G	Glycine	C ₂ H ₅ NO ₂	75.1
Y	Tyrosine	C ₉ H ₁₁ NO ₃	181.2
Q	Glutamine	C ₅ H ₁₀ N ₂ O ₃	146.1

Table 2.1: Some Amino Acids Data

where m_w and m_c are the total masses of water and Tat-TFA complex, respectively, V_w is the total volume of water, V_c is the molecular volume of a Tat-TFA complex, and N_c is the total number of this complex in the solution. Denoting $V_w = m_w/\rho_w$ and $N_c = N_A m_c/W_c$, where W_c is the molecular weight of the complex, N_A is the Avogadro's number, and ρ_w is the density of water, we have

$$V_c = \frac{W_c}{\rho_{\text{sol}} N_A} \left(1 + \frac{m_w}{m_c} \left(1 - \frac{\rho_{\text{sol}}}{\rho_w} \right) \right), \quad (2.2)$$

which allows us to calculate the molecular volume of a Tat-TFA complex from the experimentally measured quantities. Assuming that the molecular volume scales with the molecular weight gives the volume of Tat, $V_{\text{Tat}} = 1560/2464 \times V_c \text{ \AA}^3$.

2.2.4 X-ray setup

Figure 2.1 shows a schematic of the X-ray setup.

The hydration chamber is described in detail in [?]. The sample holder was mounted on a servo motor, which allowed continuous rotation of the sample between -1.6° and 7° . A Peltier cooling/heating element was attached to the sample holder and the sample was situated on this Peltier element, with which hydration level of the sample could be easily adjusted. This ability of the chamber was important especially in the ripple phase experiment, which is described in chapter 3. Hydration level of a sample was estimated by measuring the average interbilayer distance, D -spacing, which was easily calculated by indexing the diffraction peaks using the tview software developed by Dr. Yufeng Liu. The semitransparent beam stop was set to always cover the direct beam, which would otherwise saturate the charge coupled device (CCD) detector. The beam profile was measured through this semitransparent beam stop. Data reduction and correction for a CCD detector are described in detail in [47].

Figure 2.1: X-ray setup for LAXS experiments.

2.2.5 Data reduction of diffuse scattering data

2.2.6 Analysis of Diffuse Scattering

Figure 2.2 shows our typical low angle X-ray scattering (LAXS) data from oriented stacks of fluctuating bilayers in the fluid phase. Analysis of diffuse scattering intensity patterns like the one shown in Fig. 2.2 results in material parameters such as the bending modulus K_c and bulk modulus B as well as the absolute form factor $|F(q_z)|$. The form factor is the Fourier transform of the bilayer electron density profile $\rho(z)$ and directly related to the internal structure of the bilayers including Tat peptides.

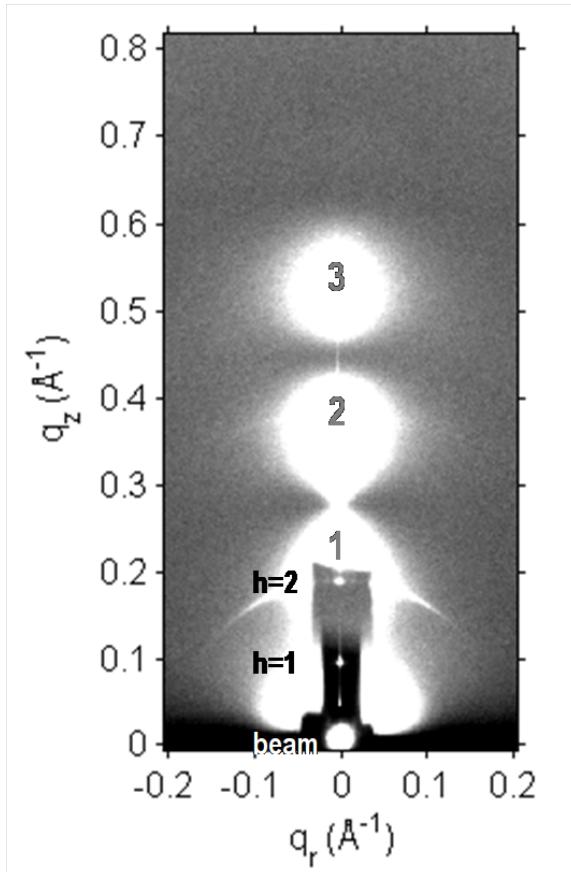


Figure 2.2: LAXS of DOPC:DOPE (1:1) with $x_{\text{Tat}} = 0.034$ at 37 °C. White lobes of diffuse scattering intensity have large grey numbers, while lamellar orders and beam are shown to the left of the molybdenum beam attenuator (short, dark rectangle). q_z and q_r are the cylindrical coordinates of the sample q -space, where q_z -axis is along the bilayer normal and q_r -axis is along the in-plane direction. The lamellar repeat spacing was $D = 66.2 \text{ \AA}$.

The form factor $F(q_z)$ is obtained by realizing that the diffuse scattering intensity pattern $I(\mathbf{q})$ is a product of the structure factor $S(\mathbf{q})$ and the form factor; $I(\mathbf{q}) = S(\mathbf{q})|F(q_z)|^2$, where $\mathbf{q} = (q_r, q_z)$, indicating that the system is in-plane isotropic. In fully hydrated multilamellar samples, $S(\mathbf{q})$ is not simple delta functions because of thermal fluctuations of bilayers. Calculating $S(\mathbf{q})$ requires a model free energy for bilayer fluctuations, from which the scattering pair correlation function is derived. A basic scattering theory, then, relates the scattering intensity $I(\mathbf{q})$ to the pair correlation function. For modeling the membrane fluctuations of a multilamellar system, the smectic liquid crystal free energy functional in the discreet form,

$$F = \frac{1}{2} \int d\mathbf{r} \sum_{n=0}^{N-1} \left\{ K_c [\nabla_r^2 u_n(\mathbf{r})]^2 + B [u_{n+1}(\mathbf{r}) - u_n(\mathbf{r})]^2 \right\}, \quad (2.3)$$

has been shown to be adequate [48]. Here, $u_n(\mathbf{r})$ is the spatial deviations of the center of the n -th bilayer from its average position in the z direction at the in-plane location $\mathbf{r} = (x, y)$ (Fig. 2.3). The first term is the bending free energy proportional to the curvature squared with the proportionality given by a bending modulus K_c and the second term is a harmonic approximation to the interactions between membranes with a modulus B . Once the two dimensional structure factor map $S(\mathbf{q})$ is calculated from Eq. (2.3), the form factor can be calculated by dividing the intensity by the structure factor. Getting the best fit of a model structure factor to the intensity results in the material parameters, K_c and B .

We used a software called NFIT developed by Dr. Yufeng Liu [48–50] to analyze the diffuse scattering and obtain the bending modulus, bulk modulus, and form factor. Details of the analysis, including the theoretical derivation of the structure factor from Eq. (2.3) and its numerical computation, are found in Dr. Yufeng Liu’s thesis [50].

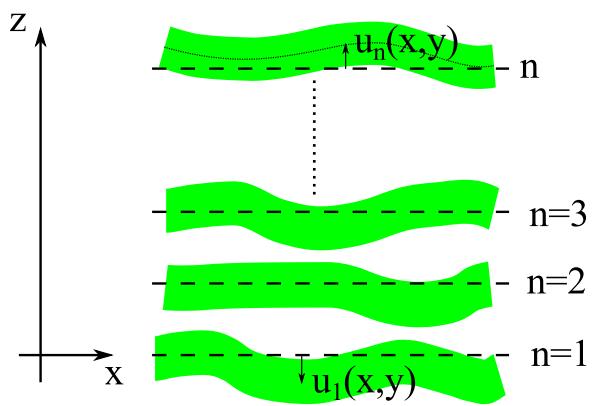


Figure 2.3: Schematic of an oriented stack of lipid bilayers. Thick green curves represent an instance of thermally fluctuating bilayers. The dashed lines show the thermally averaged positions $z = nD$ of the centers of each bilayer and $u_n(x, y)$ gives the instantaneous deviation from the average. Each bilayer extends in the $\mathbf{r} = (x, y)$ plane.

2.2.7 Modeling the Bilayer Structure

In the case of X-rays, the features with the most contrast are the electron-dense headgroups, providing the head-head spacing D_{HH} , and also the terminal methyl groups in the bilayer center with the least electron density. Modeling of the bilayer structure was done similarly to the SDP model written by Dr. Norbert Kucerka when he was a postdoc in the Nagle/Tristram-Nagle lab [52].

Parsing of DOPC into lipid components is shown in Fig. 2.4. The phosphate/choline (PC) and carbonyl/glycerol (CG) components together make up the lipid headgroup whereas the hydrocarbon chain region (HC) is divided into two components, the methylene (CH_2) and methine (CH) group combination (denoted as CH_2+CH) and terminal methyl groups (CH_3). We combine methylene (CH_2) and methine groups (CH) in order to avoid proliferation of fitting parameters.

2.2.7.1 Functional forms

Our model for the electron density profile (EDP) of Tat/lipid bilayer system consists of five structural subgroups: PC, CG, CH_2+CH , CH_3 , and Tat (see Fig. 2.5). The volume probability distributions of components PC, CG, CH_3 , and Tat are described by Gaussian functions,

$$P_i(z) = \frac{c_i}{\sqrt{2\pi}} \left(\exp\left\{-\frac{(z+z_i)^2}{2\sigma_i^2}\right\} + \exp\left\{-\frac{(z-z_i)^2}{2\sigma_i^2}\right\} \right), \quad (2.4)$$

where c_i is an integrated area underneath the curve and the two parts of the expression describe the two bilayer leaflets.

The hydrocarbon chain region (HC) is represented by error functions,

$$P_{\text{HC}}(z) = \frac{1}{2} [\text{erf}(z, -z_{\text{HC}}, \sigma_{\text{HC}}) - \text{erf}(z, z_{\text{HC}}, \sigma_{\text{HC}})], \quad (2.5)$$

where

$$\text{erf}(z, z_i, \sigma_i) = \frac{2}{\sqrt{\pi}} \int_0^{\frac{z-z_i}{\sqrt{2}\sigma}} dx e^{-x^2}. \quad (2.6)$$

The volume probability distribution for the methylene and methine group combination can then be expressed as

$$P_{\text{CH}_2+\text{CH}}(z) = P_{\text{HC}}(z) - P_{\text{CH}_3}(z). \quad (2.7)$$

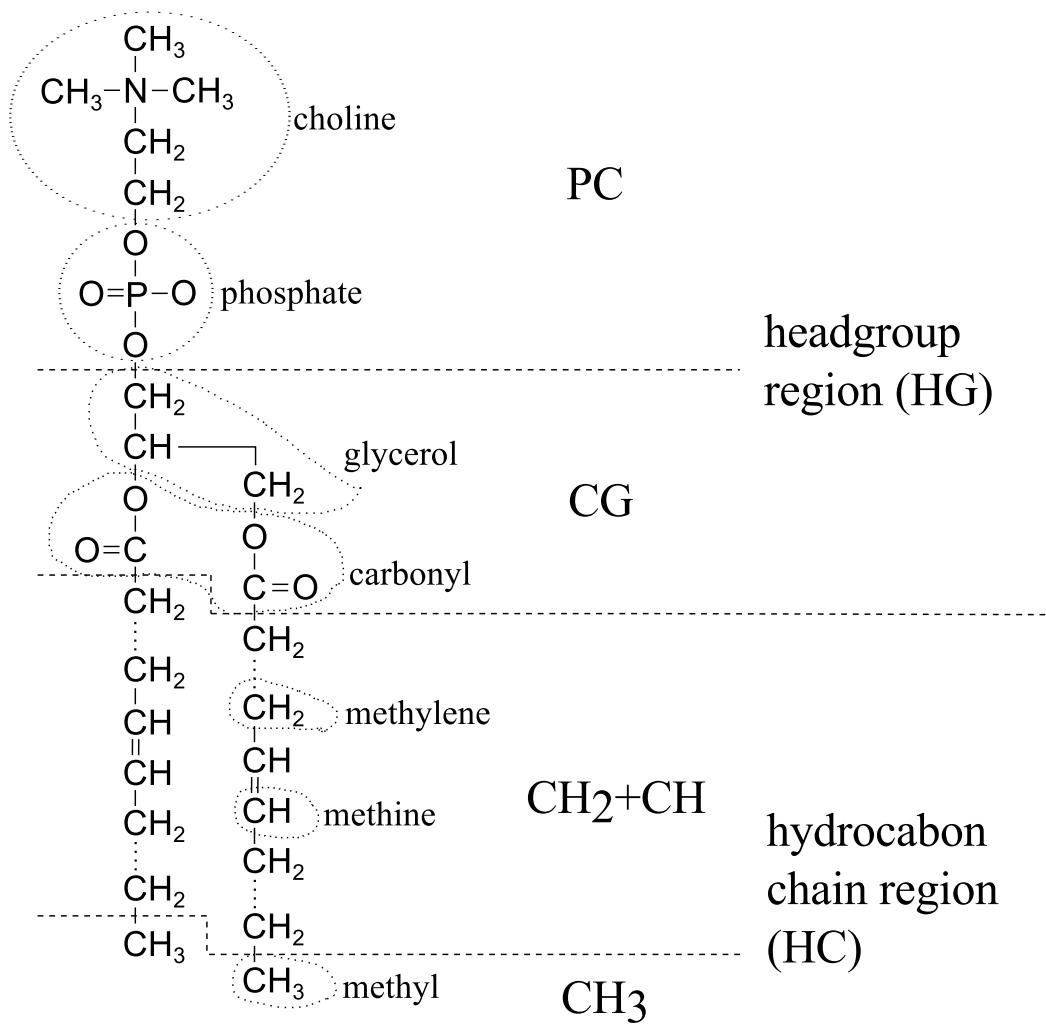


Figure 2.4: Schematic of DOPC showing each lipid component. The dash lines show where the lipid is divided into different components. The lipid headgroup is divided into two components, phosphate-choline (PC) and carbonyl-glycerol (CG). The hydrocarbon chain region is also divided into two components, methylene+methine (CH_2+CH) and terminal methyl groups (CH_3).

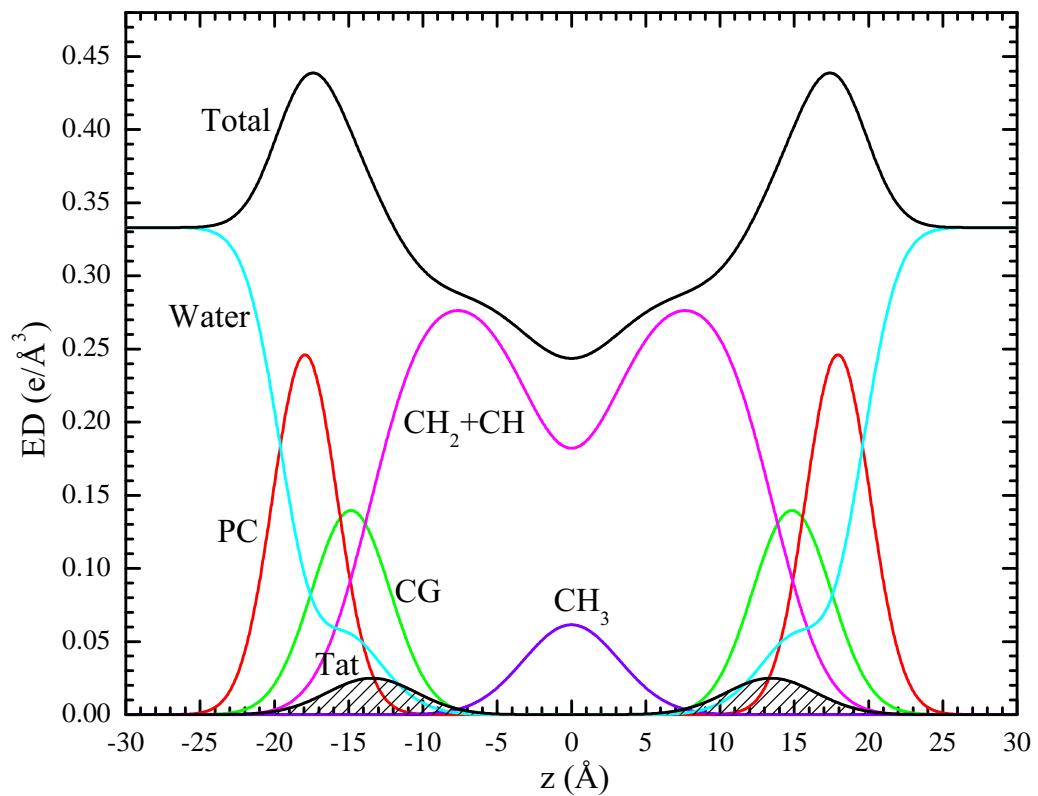


Figure 2.5: A model electron density profile for DOPC with Tat.

This definition enforces the total probability P_{HC} in the hydrocarbon chain region to equal one, which in turn means that placement of Tat in the chain region is prohibited. We call the model defined by Eq. (2.7) model A. To allow Tat to be placed inside the hydrocarbon chain region, we also consider an alternative definition,

$$P_{\text{CH}_2+\text{CH}}(z) = P_{\text{HC}}(z) - P_{\text{CH}_3}(z) - P_{\text{Tat}}(z), \quad (2.8)$$

where the volume probability of CH_2+CH combined component is reduced by the Tat volume probability distribution. We call this model B. The spatial conservation requires the water volume probability distribution to be

$$P_{\text{W}}(z) = 1 - P_{\text{PC}}(z) - P_{\text{CG}}(z) - P_{\text{Tat}}(z) - P_{\text{HC}}(z) \quad (2.9)$$

for model A and

$$P_{\text{W}}(z) = 1 - P_{\text{PC}}(z) - P_{\text{CG}}(z) - P_{\text{HC}}(z) \quad (2.10)$$

for model B.

Because X-rays measure the contrast between the bilayer and surrounding solvents, water, the experimental form factor is compared to the water subtracted model form factor,

$$F(q_z) = 2 \int_0^{\frac{D}{2}} dz \left(\sum_i (\rho_i - \rho_{\text{W}}) P_i(z) \right) \cos(q_z z), \quad (2.11)$$

where $i = \text{PC}, \text{CG}, \text{Tat}, \text{CH}+\text{CH}_2$, and CH_3 .

2.2.7.2 Constraints

The height of the hydrocarbon chain error function is fixed to one by imposing spatial conservation, whereas the mean position of the terminal methyls is fixed to $z_{\text{CH}_3} = 0$ by symmetry arguments. The total lipid volume V_L is fixed to the experimentally measured value. The headgroup volume V_{HL} was determined to be 331 \AA^3 for gel phase phosphatidylcholine (PC) bilayers [53], and we assume the same volume for the fluid phase PC bilayers. The volumes of PC and CG components satisfy

$$V_{\text{PC}} + V_{\text{CG}} = V_{\text{HL}}, \quad (2.12)$$

and the volumes of CH_3 and CH_2+CH components satisfy

$$2(16V_{\text{CH}_2+\text{CH}} + V_{\text{CH}_3}) = V_L - V_{\text{HL}}. \quad (2.13)$$

These component volumes constrain the height of the Gaussians as

$$c_{\text{PC}} = \frac{V_{\text{PC}}}{A_L \sigma_{\text{PC}}} \quad (2.14)$$

$$c_{\text{CG}} = \frac{V_{\text{CG}}}{A_L \sigma_{\text{CG}}} \quad (2.15)$$

$$c_{\text{CH}_3} = \frac{2V_{\text{CH}_3}}{A_L \sigma_{\text{CH}_3}} \quad (2.16)$$

$$c_{\text{Tat}} = \frac{V_{\text{Tat}}}{A_L \sigma_{\text{Tat}}} \quad (2.17)$$

where A_L is area per lipid.

The ratio of the carbonyl/glycerol volume to the headgroup volume V_{HL} was reported to be 0.41 [54], so we constrain the CG component volume to 135.7 \AA^3 and the PC component volume to 195.3 \AA^3 .

The most detailed structural study on DOPC to date was published by Braun *et al.* [54], and many of constraints on our model parameters can be derived from their study. However, in that work, the authors used the SDP model [52], which is specifically tailored for combined analysis of neutron and X-ray form factors. Therefore, we need to convert their structural results to the corresponding parameters in our simpler model. For example, from the reported values of the ratio of the volumes of the chain terminal methyl (CH_3) to the chain methylenes (CH_2) and the ratio of the volumes of the chain methines (CH) to the chain methylenes, we can calculate the ratio r_{CH_3} of the volumes of CH_3 to the CH_2 and CH combined component. Furthermore, the study by Braun *et al.* was at 30°C while our study was at 37°C , so our measured volume of DOPC was slightly higher.

At 30°C , the volume of DOPC was reported to be 1303 \AA^3 , so the volume of hydrocarbon chain region at the same temperature is $1303 - 331 = 972 \text{ \AA}^3$. The ratio r of the volumes of the chain terminal methyl (CH_3) to the chain methylenes (CH_2) was reported to be 1.95, and the ratio r_{12} of the volumes of the chain methines (CH) to the chain methylenes 0.91 at 30°C . Because there are 14 CH_2 groups, 2 CH groups, and 1 CH_3 group in each DOPC hydrocarbon chain, we have $2 \times (14V_{\text{CH}_2} + 2V_{\text{CH}} +$

$V_{\text{CH}_3}) = 972 \text{ \AA}^3$. Using $r = V_{\text{CH}_3}/V_{\text{CH}_2} = 1.95$ and $r_{12} = V_{\text{CH}}/V_{\text{CH}_2} = 0.91$, we get $V_{\text{CH}_2} = 27.3 \text{ \AA}^3$, $V_{\text{CH}} = 24.9 \text{ \AA}^3$, and $V_{\text{CH}_3} = 53.3 \text{ \AA}^3$. These calculated volumes lead to $V_{\text{CH}_3}/V_{\text{CH}_2+\text{CH}} = 1.97$ for 30 °C.

At 37 °C, the volume of DOPC was measured to be 1313.5 \AA^3 , so we have $2 \times (16V_{\text{CH}_2+\text{CH}} + V_{\text{CH}_3}) = 1313.5 - 331$. Assuming that the ratio $V_{\text{CH}_3}/V_{\text{CH}_2+\text{CH}}$ at 37 °C is the same as that at 30 °C gives $V_{\text{CH}_2+\text{CH}} = 27.3 \text{ \AA}^3$ and $V_{\text{CH}_3} = 53.9 \text{ \AA}^3$. We constrain the components for the hydrocarbon chain region in our model to these calculated values.

lipid	number of electrons	volume (\AA^3)
DOPC	434	1313.5
DOPE	410	1212.3
DOPC:DOPE (3:1)	428	1288.2

Table 2.2: Number of electrons per lipid and volume per lipid.

component	n_i^e	$V_i (\text{\AA}^3)$	$\rho_i (\text{e}/\text{\AA}^3)$
PC	97	195.3	0.497
PE	73	94.1	0.776
PC:PE (3:1)	91	170	0.535
CG	67	135.7	0.494
CH_2+CH	7.875	27.3	0.288
CH_3	9	53.9	0.167

Table 2.3: Some structural parameters for each component. n_i^e is the number of electrons and ρ_i is the average electron density.

number of electrons	838	mole fraction (x_{Tat})	n_{Tat}^e	$V_{\text{Tat}} (\text{\AA}^3)$
volume (\AA^3)	1877	0.016	13.6	30.5
$\rho_{\text{Tat}} (\text{e}/\text{\AA}^3)$	0.446	0.034	29.5	66.1
		0.059	53.0	118.8

Table 2.4: Tat basic structural parameters. The notations are the same as in Table 2.3. $x_{\text{Tat}} = \text{Tat}/(\text{Tat}+\text{Lipid})$.

2.2.7.3 Fits with Lower Bounds

Non-linear least squared fits with upper and lower bounds for the model parameters are implemented using an internal-external parameter transformation method. This

method is described in MINUIT User's Guide, section 1.3 [55]. This section briefly describes the method. The details can be found in the MINUIT website [56].

Basically, instead of a model parameter, which is also called the external variable, the minimization procedure varies a related variable called the internal variable. This internal variable can take any values between $-\infty$ to $+\infty$. At every χ^2 calculation, the internal variable is transformed to the external variable, which can take values only between the lower and upper bounds (a and b). This non-linear transformation allows an existing minimization algorithm that was developed for fits with no bounds to work for fits with bounds. This point was important because it allowed us to implement bound fits in the model fitting program called the SDP program, fully developed by Dr. Norbert Kucerka, without too many additional changes. Downsides of the transformation method include turning a linear problem into a non-linear one and some computational overhead, neither of which is particularly problematic in this study.

For variables with both lower and upper bounds (a and b , respectively), the transformation between the internal and external variables is

$$P_{\text{int}} = \arcsin\left(2\frac{P_{\text{ext}} - a}{b - a} - 1\right) \quad (2.18)$$

$$P_{\text{ext}} = a + \frac{b - a}{2}(\sin P_{\text{int}} + 1). \quad (2.19)$$

For variables with a lower bound a only, the transformation is

$$P_{\text{int}} = \sqrt{(P_{\text{ext}} - a + 1)^2 - 1} \quad (2.20)$$

$$P_{\text{ext}} = a - 1 + \sqrt{P_{\text{int}}^2 + 1}, \quad (2.21)$$

and for variables with an upper bound b only,

$$P_{\text{int}} = \sqrt{(b - P_{\text{ext}} + 1)^2 - 1} \quad (2.22)$$

$$P_{\text{ext}} = b + 1 - \sqrt{P_{\text{int}}^2 + 1}. \quad (2.23)$$

2.2.8 Molecular Dynamics Simulation

This section describes the MD simulations performed by Dr. Kun Huang, who was a graduate student of Prof. Angel Garcia at Rensselaer Polytechnic Institute.

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 [57]. DOPC was modeled by the Slipid force field [58, 59] and HIV Tat was modeled by Amber 99SB [60]. Tip3p water was used [61]. 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 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 (A_L). For the DOPC system, we fixed $A_L = 68, 70, 72 \text{ \AA}^2$; DOPC/Tat (128:2), we fixed the $A_L = 72, 74, 76 \text{ \AA}^2$; DOPC/Tat (128:4), we fixed the $A_L = 72, 74, 76 \text{ \AA}^2$. These values were based on the analysis of experimentally obtained form factors, which is discussed in Sec. 2.4.3. For each DOPC/Tat system at fixed A_L , 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 \AA). 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 written by Dr. Norbert Kucerka [62].

All simulations were conducted with a 2 fs time integration step. SETTLE [63] was used to constrain water molecules and LINCS [64] was used to constrain all other bond lengths in the system. VdW interactions were truncated at 1.4 nm with a twin-range cutoff scheme and a dispersion correction was applied to both energy and pressure. Electrostatics interactions were treated with the particle-mesh Ewald (PME) method [65]. The direct term for electrostatics was evaluated within 1.0 nm cutoff and the Fourier term was evaluated with a 0.12 nm grid spacing and a 4th order interpolation. Each system was simulated at 310 K using the V-rescale algorithm [66] with a 0.2 ps time coupling constant. The semi-isotropic parrinello-rahman barostat [67] was used to couple the system at 1 atm in the z direction with a 5 ps time coupling constant, while the projected area at the x - y plane was fixed by setting the system compressibility to 0. We inserted the Tats into the system by initially turning off all interactions between Tats and the rest of the system, with Tats constrained at different depths. Then we slowly turned on the interactions to normal strength through thermodynamics integrations. We used umbrella potentials

to constrain Tats at desired depths with a force constant of 3000 kJ/mol/nm².

The center of mass (COM) distance between each peptide and the bilayer was constrained by an umbrella potential with a force constant k of 3000 kJ/mol/nm². Essentially, this potential acts as a spring, where its potential energy depends on the deviation of the distance between the center of mass of Tat and DOPC from a preferred value, z_0 ,

$$U(z_1^{\text{Tat}}, \dots, z_1^{\text{DOPC}}, \dots) = -\frac{1}{2}k(z_{\text{cm}}^{\text{Tat}} - z_{\text{cm}}^{\text{DOPC}} - z_0)^2.$$

Then, $-\partial U / \partial z_i$ is the external force acting on atom, i .

2.3 Analysis of Molecular Dynamics Simulation Data

2.3.1 SIMtoEXP program

This section briefly describes the SIMtoEXP program developed by Dr. Norbert Kucerka [62]. Essentially, for each snapshot, positional distribution of each atom averaged over the xy plane is calculated. Then, the distribution is averaged over snapshots. The product of this distribution and the average electron density gives the electron density profile of the atom. The sum over all the atoms provide the total electron density profile. This total electron density profile minus the average electron density of water is Fourier transformed to provide the X-ray form factor.

$$F^{\text{sim}}(q_z) = \int_0^\infty dz(\rho(z) - \rho_W) \cos(q_z z). \quad (2.24)$$

Electron density profiles were symmetrized and then X-ray form factors were calculated with $\rho_W = 0.326 \text{ e}/\text{\AA}^3$, which was the average electron density of water molecules in the MD simulations. Because $\rho(z)$ is equal to ρ_W outside the bilayer, the upper integration limit takes on a finite value.

Because the experimental form factor is on arbitrary units, it is scaled by a single constant a to produce the best fit to the simulated form factor through a linear least squared fit that minimizes the following goodness of fit

$$\chi^2 = \sum_i \left(\frac{1}{\sigma_i} (a|F_i^{\text{exp}}| - |F^{\text{sim}}(q_{z,i})|) \right)^2 \quad (2.25)$$

where σ_i is the input experimental uncertainties and F_i^{exp} is the experimental form factor measured at $q_z = q_{z,i}$. The SIMtoEXP program does not scale the input uncertainties, so the relative errors ($\sigma_i/|F_i^{\text{exp}}|$) depend on the value of the overall scaling factor a . Consequently, the χ^2 values calculated by the program had to be multiplied by $1/a^2$. These corrected χ^2 are reported in this chapter.

2.3.2 Local Thinning of Membranes

My contribution to the MD simulations was to help analyze the results.

The SIMtoEXP program only gives the average quantities for each leaflet. While our X-ray data are sensitive to the bilayer average electron density, local information

of Tat-bilayer interactions can be obtained from MD simulations. In this section, we discuss a method to extract a local membrane thickness around the Tat peptides from the MD simulation trajectories.

One of the expected effects of Tat interacting with a bilayer is compression of the lipid bilayer along the z -direction. It is reasonable to assume that this compression is greater near Tat and weaker far from Tat. If this is the case, the distance between phosphorus atoms in opposite leaflets near Tat should be different from the distance between phosphorus atoms away from Tat. For a small Tat concentration, $D_{\text{phos-phos}}$ is the same as that of pure DOPC if the distance from all Tats is large enough. For our experimental concentrations, the thinning effect may extend throughout the bilayer because the lateral effect of Tat might have a larger lateral decay length than the distance between Tats. Whether that is the case or not, we expect that the thickness near the Tats is smaller than the average thickness, so $D'_{\text{phos-phos}}$ is what we want to measure.

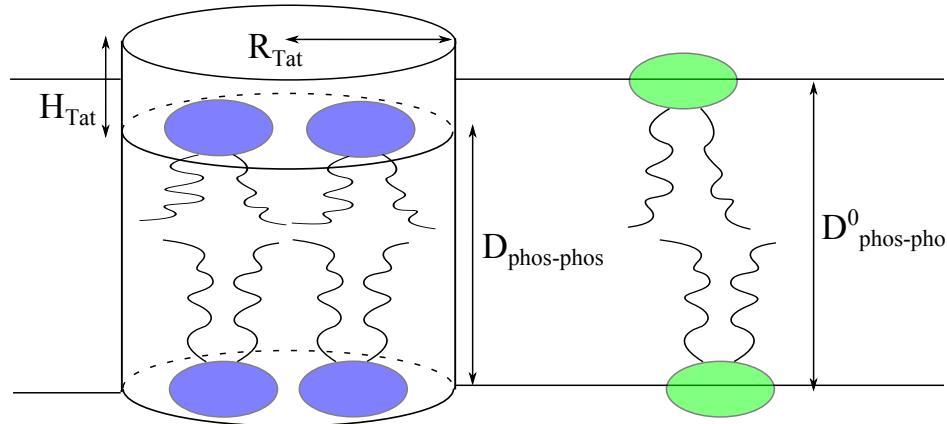


Figure 2.6: Our simple model to extract the local bilayer thickness from simulation trajectories. Tat is modeled as a cylinder with its height H_{Tat} and radius R_{Tat} . The local thickness is defined as $D'_{\text{phos-phos}}$. The thickness of the unperturbed DOPC bilayer is $D_{\text{phos-phos}}$. Blue highlighted lipids fall within the imaginary cylinder extended from the Tat. Unperturbed lipids are highlighted in green.

First, let us define what we mean by lipids close to Tat. As in Fig. 2.6, we imagine a cylinder around Tat and find all the phosphorus atoms within it. Approximating Tat as a cylinder with its height given by the FWHM of its electron density distribution, its radius $R_{\text{Tat}} = 9 \text{ \AA}$ comes from the experimentally determined volume $V_{\text{Tat}} = 1876$

\AA^3 and $H_{\text{Tat}} = 7.6 \text{ \AA}$ measured from one of the simulations (see Sec. ??). Let us define the lateral center of the cylinder as the center of mass of each Tat. Then we define $D'_{\text{phos-phos}}$ using only those lipids whose phosphorus atoms lie within these 9 \AA cylinders around the Tats. Then $D_{\text{phos-phos}} = z_{\text{phos}}^+ - z_{\text{phos}}^-$ where z_{phos}^+ and z_{phos}^- are the average z of the n_1 (n_2) lipids in the upper and lower monolayer, respectively.

The algorithm for doing the above was straightforward. For each time frame, the positions (x_i, y_i, z_i) of each Tat, i , are listed. We chose phosphorus atoms whose (x, y) lateral position lied within 9 \AA of any one of the Tat's lateral position. Then, z positions of the chosen phosphorus atoms were placed in a list. Then, z_{phos} were calculated from the list. We averaged over many snapshots to gain better statistics.

2.3.3 Lateral Decay Length of Membrane Thinning

This section describes a method to measure the lateral decay length of membrane thinning due to Tat-lipid interactions. As in the previous section, Tat is modeled here as a cylinder with its radius equal to R_1 , height H_{Tat} , and volume V_{Tat} such that $R_1 = \sqrt{V_{\text{Tat}}/(\pi H_{\text{Tat}})}$. Let $h(r)$ represent the phosphorus height profile of a leaflet as in Fig. 2.7. The two leaflets are assumed to be decoupled. In our model, lipids are separated into three regions: suppressed, boundary, and unperturbed region. The suppressed region extends from $r = 0$ to R_1 and is directly beneath (above) Tat in the top (bottom) leaflet. In this region, lipids are uniformly compressed by Tat toward the center of the bilayer, so that $h(r)$ is a constant equal to z_{phos} . From $r = R_1$ to R_2 is the boundary region, where $h(r)$ is assumed to linearly increase with the lateral distance r . The lateral decay length of membrane thinning is given by $R_2 - R_1$. In the unperturbed region ($r > R_3$), lipids do not interact with Tat, behaving identically to DOPC, so the phosphorus position is the same as that of DOPC. A continuous $h(r)$ that satisfies the above criteria is

$$h(r) = \begin{cases} z_{\text{phos}} & \text{if } 0 \leq r < R_1 \\ mr + b & \text{if } R_1 \leq r < R_2 \\ z_{\text{phos}}^0 & \text{if } R_2 \leq r < R_3 \end{cases} \quad (2.26)$$

with $m = (z_{\text{phos}} - z_{\text{phos}}^0)/(R_1 - R_2)$ and $b = (z_{\text{phos}}^0 R_1 - z_{\text{phos}} R_2)/(R_1 - R_2)$. Approximating the simulation box as a cylinder gives $R_3 = \sqrt{N A_L / \pi}$, where N is the number of lipids in a leaflet. z_{phos} can be measured directly from simulation trajectories. z_{phos}^0

is a half of the average phosphorus-phosphorus distance in a DOPC simulation, which can be easily obtained from the SIMtoEXP program. The average height profile over the monolayer, $\langle h(r) \rangle$, can be also obtained from the program in the same manner. The only unknown is R_2 .

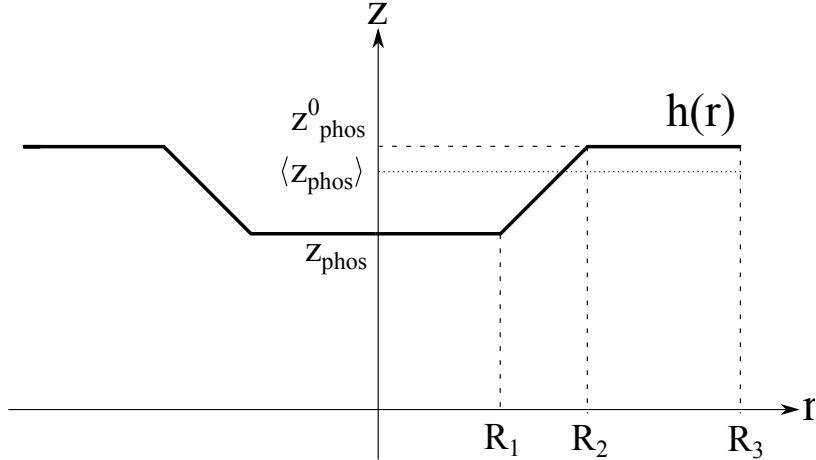


Figure 2.7: Simple model of the lateral decay of the membrane thickness perturbation due to Tat.

Let us calculate $\langle h(r) \rangle$. In cylindrical coordinates,

$$\langle h(r) \rangle = \frac{1}{\pi R_3^2} \int_0^{2\pi} d\phi \int_0^{R_3} dr r h(r) \quad (2.27)$$

The ϕ integration is trivial. The r integration is

$$\begin{aligned} & \int_0^{R_3} dr r h(r) \\ &= \int_0^{R_1} dr z_{\text{phos}} r + \int_{R_1}^{R_2} dr (mr + b)r + \int_{R_2}^{R_3} dr z_{\text{phos}}^0 r \\ &= \frac{1}{2} [z_{\text{phos}} R_1^2 + z_{\text{phos}}^0 (R_3^2 - R_2^2)] + \frac{1}{3} m (R_2^3 - R_1^3) + \frac{1}{2} b (R_2^2 - R_1^2) \\ &= \frac{1}{2} [z_{\text{phos}} R_1^2 + z_{\text{phos}}^0 (R_3^2 - R_2^2)] + \frac{1}{3} (z_{\text{phos}}^0 - z_{\text{phos}}) (R_2^2 + R_1 R_2 + R_1^2) \\ &\quad + \frac{1}{2} (z_{\text{phos}} R_2 - z_{\text{phos}}^0 R_1) (R_1 + R_2) \end{aligned} \quad (2.28)$$

Using Eq. (2.28), we get

$$\langle h(r) \rangle = \frac{(z_{\text{phos}} - z_{\text{phos}}^0)(R_1^2 + R_1 R_2 + R_2^2) + 3z_{\text{phos}}^0 R_3^2}{3R_3^2} \quad (2.29)$$

Eq. 2.29 is a quadratic equation in terms of R_2 . Solving for R_2 gives

$$R_2 = \frac{-R_1 + \sqrt{R_1^2 + 4C}}{2} \quad (2.30)$$

with

$$C = \frac{3R_3^2(z_{\text{phos}}^0 - \langle h(r) \rangle)}{z_{\text{phos}}^0 - z_{\text{phos}}} - R_1^2 \quad (2.31)$$

2.4 Results

2.4.1 Bending and Bulk Modulus

(Under construction) Show X-ray data. Show fitting boxes. Show the Kc values. Also, show the resultant form factors, which qualitatively show the membrane thinning. Also describe how I got error bars.

Fig. 2.2 shows the scattering intensity pattern from DOPC/DOPE (1:1) with mole fraction $x_{\text{Tat}} = 0.034$. The diffuse lobes are due to equilibrium fluctuations that occur in these fully hydrated, oriented lipid/peptide samples. The intensity $I(\mathbf{q})$ in the diffuse patterns provide the absolute values of the form factors $F(q_z)$, which are the Fourier transforms of the electron density profile, through the relation $I(\mathbf{q}) = S(\mathbf{q})|F(q_z)|^2/q_z$, where $\mathbf{q} = (q_r, q_z)$, $S(q)$ is the structure interference factor, and q_z^1 is the usual LAXS approximation to the Lorentz factor [68–70]. The first step in the analysis takes advantage of the q_r dependence of the scattering to obtain the bending modulus K_c with results shown in Fig. 2.8. As positively charged Tat concentration was increased, the lamellar repeat spacing D generally increased in neutral lipid bilayers and decreased in negatively charged bilayers, consistent with changes in electrostatic repulsive interactions. With few exceptions, the water space between bilayers exceeded 20 Å.

The analysis that obtains K_c also obtains the structure factor $S(\mathbf{q})$ and then the unsigned form factors $|F(q_z)|$ are obtained from the intensity $I(\mathbf{q})$ by division. Results for five different membrane mimics are shown in Fig. 2.9. Vertical lines indicate the

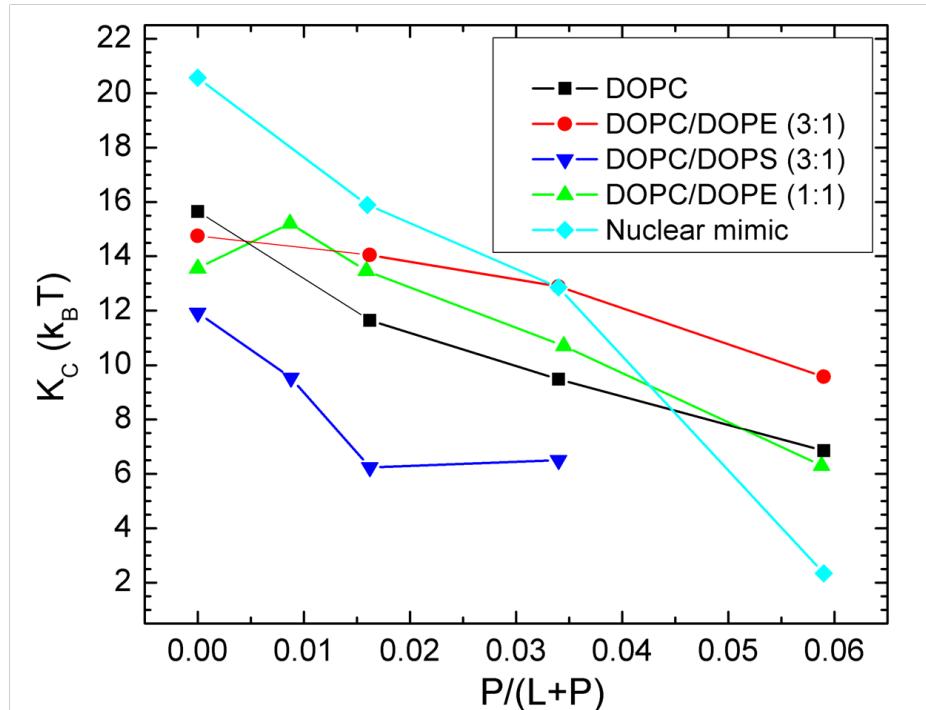


Figure 2.8: Bilayer bending modulus, K_c , vs. Tat mole fraction x_{Tat} . D -spacings for DOPC/Tat mixtures varied from 64 to 68 Å, for DOPC/DOPE/Tat mixtures from 64 to 69 Å, for DOPC/DOPS/Tat (3:1) mixtures from 57 Å to 100 Å (pure DOPS was unbound), and for nuclear mimic/Tat mixtures from unbound (nuclear mimic) to 64 Å. Estimated uncertainty in all values is about ± 2 .

zero position between the lobes of diffuse data where $F(q_z)$ change sign. In every sample, the zero positions shift to larger q_z , indicating a thinning of the membranes.

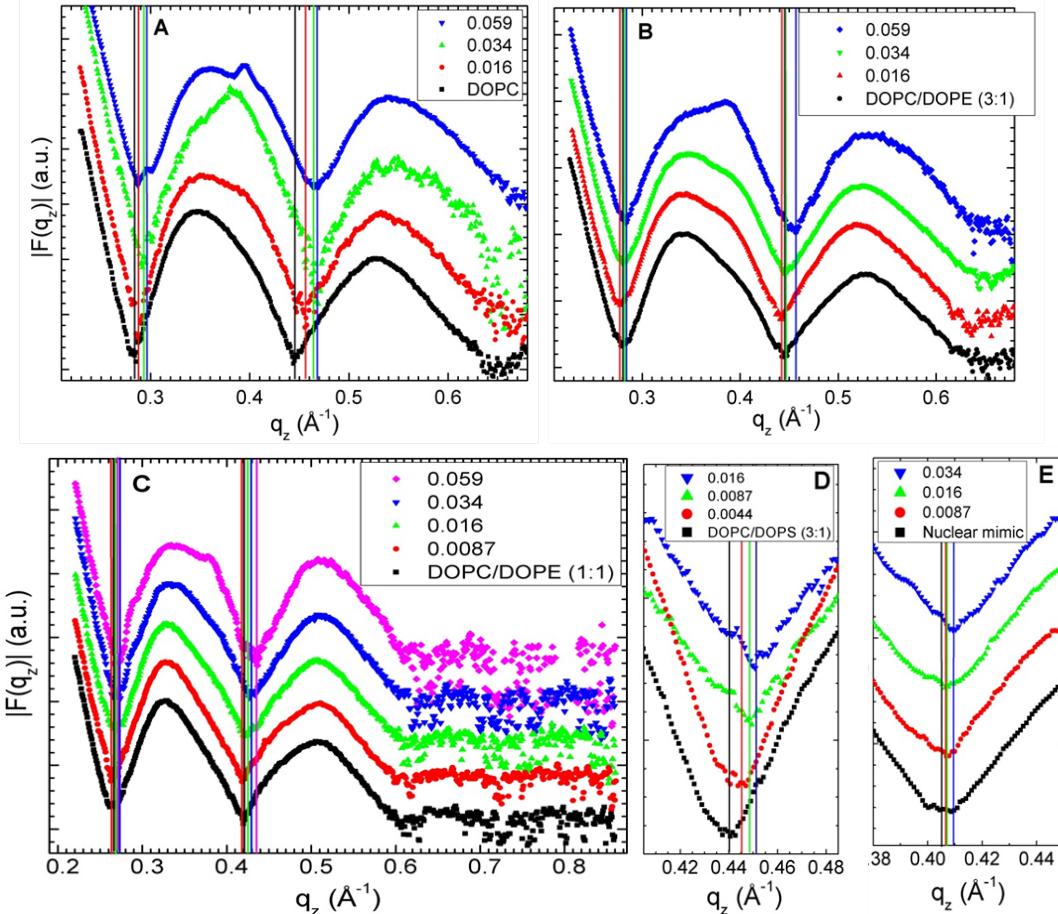


Figure 2.9: Form factors of lipid mixtures (arbitrarily scaled and vertically displaced) with increasing Tat mole fractions x_{Tat} indicated on figure legends. Lipid mixtures: A. DOPC B. DOPC/DOPE (3:1) C. DOPC/DOPE (1:1) D. DOPC/DOPS (3:1) E. Nuclear mimic. The entire q_z range is shown in C, while others show partial ranges. Solid vertical lines indicate the q_z values where the form factors equal zero between the lobes of diffuse data.

2.4.2 Volume results

Experimental and simulated volumes are given in Table 2.5. The simulated volume was obtained using the volume app in the SIMtoEXP program. The experimental Tat volume was calculated from the measured density assuming that the lipid volume was

the same as with no Tat. In general, there may be an interaction volume between the peptide and the lipid membrane as previously reported for bacteriorhodopsin [71]. As lipid was present in excess to Tat, the partial molecular volume of the lipid should be the same as with no Tat, so this way of calculating includes all the interaction volume in V_{Tat} . Comparison of V_{Tat} in water with the result for 5:1 Lipid:Tat suggests that the interaction volume may be negative, consistent with a net attractive interaction with lipid. Understandably, values of V_{Tat} were unreliable for small mole ratios of Tat:Lipid. Therefore we used simple additivity for those mimics not shown in Table 2.5 for the volumes used in the electron density profile modeling. All volumes obtained from the Gromacs MD simulations were somewhat smaller than the measured volumes, but it supports the Tat volume being closer to 1877 \AA^3 than the outlying values obtained experimentally at small Tat concentrations. The measured volume was in a good agreement with the value calculated from a peptide calculator website [72], which gave 1888 \AA^3 .

Experiments			
Tat in:	$V_{\text{lipid}} (\text{\AA}^3)$	Lipid:Tat	$V_{\text{Tat}} (\text{\AA}^3)$
water		1877	
DOPC:DOPE (3:1)	1288	5:1	1822
DOPC	1314	39.6:1	676
DOPC:DOPS (3:1)	1298	39.6:1	2613

Simulations			
Tat in:	$V_{\text{lipid}} (\text{\AA}^3)$	Lipid:Tat	$V_{\text{Tat}} (\text{\AA}^3)$
DOPC	1283	128:2	1694
DOPC	1294	128.4	1699

Table 2.5: Volume results at $37 \text{ }^\circ\text{C}$

2.4.3 Electron Density Profile Modeling

We fitted our measured X-ray form factors to the Tat-in-headgroup (THG) model described in Sec. 2.2.7. In all fits, the positions of component groups were free parameters, but we assumed that the lipid headgroup is somewhat rigid so that it cannot compress or expand. This assumption led to fixing the distance $z_{\text{PC}} - z_{\text{CG}}$ between the PC and CG components as well as the distance $z_{\text{CG}} - z_{\text{HC}}$ between the CG component and the Gibbs dividing surface for the hydrocarbon chains. We also constrained the

width of Tat Gaussian σ_{Tat} . We fitted with three different values of widths, 2.5, 3.0, and 3.5, to study the range of variation due to the Tat width. We constrained the Tat width because this parameter tended to become too small to be physical when it was set free. Without higher q_z data points, a very narrow feature in an electron density profile, which resulted in large form factors, did not get penalized.

Figure 2.10 shows the best fits and corresponding electron density profiles for DOPC with Tat, and Table 2.6 shows the best fit parameters for these fits. In most cases, a better χ^2 was obtained for smaller σ_{Tat} , consistent with its tendency to become too small to be physical as noted in the previous paragraph. The widths of the headgroups σ_{PC} and σ_{CG} decreased from those of pure DOPC when Tat was added. It is also seen from Table 2.6 that the area per lipid A_L increased as the Tat concentration was increased. An increase in A_L implies thinning of a bilayer because a lipid bilayer is an incompressible fluid membrane. Another observed trend was that z_{Tat} increased as x_{Tat} was increased.

x_{Tat}	0	0.016	0.016	0.016	0.034	0.034	0.034	0.059	0.059	0.059
χ^2	2961	1554	1570	1581	1563	1587	1607	2342	2338	2363
z_{PC}	18.1	18.0	17.9	17.9	17.8	17.7	17.6	17.8	17.8	17.7
σ_{PC}	2.52	2.14	2.17	2.18	1.86	1.92	1.93	2.02	1.97	1.93
z_{CG}	15.0	14.9	14.8	14.8	14.7	14.6	14.5	14.7	14.7	14.6
σ_{CG}	3.00	2.62	2.64	2.66	2.22	2.30	2.31	2.58	2.27	2.14
z_{HC}	13.7	13.6	13.5	13.5	13.4	13.3	13.2	13.4	13.4	13.3
σ_{HC}	3.00	2.69	2.84	2.95	2.65	2.82	3.01	2.47	2.58	2.83
σ_{CH_3}	3.20	3.19	3.22	3.24	3.37	3.43	3.47	2.70	2.70	2.74
z_{Tat}	NA	12.9	13.4	14.2	13.1	13.8	14.4	15.2	15.2	15.7
σ_{Tat}	NA	2.5	3.0	3.5	2.5	3.0	3.5	2.5	3.0	3.5
A_L	71.5	72.4	72.5	72.7	73.6	74.0	74.4	73.6	73.5	73.9

Table 2.6: Fitting Results for DOPC membranes for the THG (Tat in headgroup) model. $z_{\text{PC}} - z_{\text{CG}} = 3.1 \text{ \AA}$ and $z_{\text{CG}} - z_{\text{HC}} = 1.3 \text{ \AA}$ in all fits.

As shown in Fig. 2.10, the membrane thickness can be defined as the distance D_{PP} between the PC components in the opposing leaflets or the distance D_{HH} between the maxima in the opposing leaflets. D_{HH} is more reliable than D_{PP} because it is a property of the total electron density of a bilayer and, therefore, does not depend strongly on the specific model employed for fitting the data. This point is illustrated in Fig. 2.11, which compares total electron density profiles resulted from best fits with three different Tat widths σ_{Tat} . While positions of Tat were sensitive to values

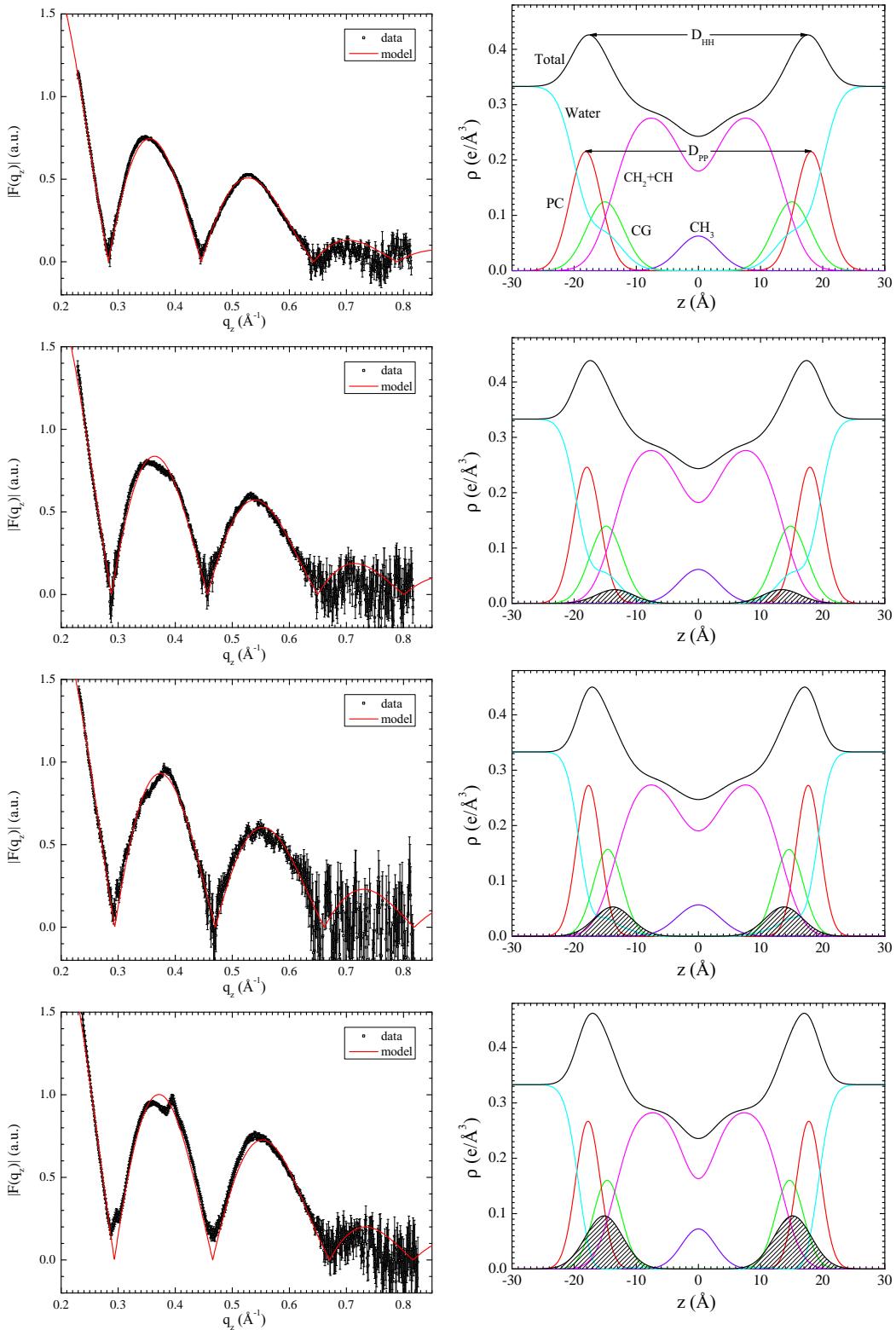


Figure 2.10: The best fits to DOPC form factors (left) and the corresponding electron density profiles (right) with $x_{\text{Tat}} = 0, 0.016, 0.034$, and 0.059 (from top to bottom).

of σ_{Tat} , the total electron density profiles were almost independent of σ_{Tat} . Essentially, other components, namely headgroups, adjusted their widths and positions so that the total electron density profile was about the same. In other words, the model was over parameterized. While the precise values of each parameter was less trustworthy, the total electron density profiles plotted in Fig. 2.11, when Fourier transformed, reproduced the experimental form factors very well and therefore were robust.

In contrast to D_{HH} , D_{PP} is a property that depends on lipid components, which are influenced by how the lipid is parsed and what assumptions and constraints go into the specific model. A disadvantage of using D_{HH} as a measure of the membrane thickness is that D_{HH} is influenced by the electron density of Tat because the total electron density profile includes a contribution from the electron density of Tat. Especially when the mole fraction of Tat in a system becomes large, the Tat electron density contributes significantly to the total electron density profile. If the Tat resided slightly outside of the PC component, the apparent membrane thickness measured by D_{HH} would be larger than D_{PP} . Then, even if the actual bilayer thickness defined by D_{PP} were reduced by the presence of Tat, the effect of thinning might not be obvious. With the above caveat in mind, we report both quantities in what follows since they can be easily calculated from the model.

As described in the previous paragraph, the model parameters were sensitive to specific constraints and assumptions on the model, and as Fig. 2.11 shows, the position of Tat depended on σ_{Tat} . On the other hand, the total electron density profiles were seen to be less sensitive. Figure 2.12 compares the total electron density profiles at different Tat concentrations. Consistent with the form factors shifting to larger q_z as x_{Tat} increased, D_{HH} decreased as x_{Tat} increased. As argued earlier, decrease in D_{HH} does not necessarily indicate decrease in the bilayer thickness, and it could instead be attributed to deeper insertion of Tat into the bilayer. However, compared to the profile of DOPC alone, all three profiles with Tat deviate from the electron density of water at smaller $|z|$ when approached from the water region. This is illustrated in Fig. 2.13 that plots the difference between the total electron density profile of DOPC and those of DOPC with Tat. Negative values of $\Delta\rho = \rho_{\text{DOPC+Tat}} - \rho_{\text{DOPC}}$ indicates that the headgroup, which has excess electron density relative to water, shifted toward the bilayer center as Tat was added to the system, which implies bilayer thinning.

Fitting results for DOPC:DOPE (3:1) and DOPC:DOPE (1:1) are summarized in Table 2.7 and Table 2.8, respectively, and the best fits and corresponding electron

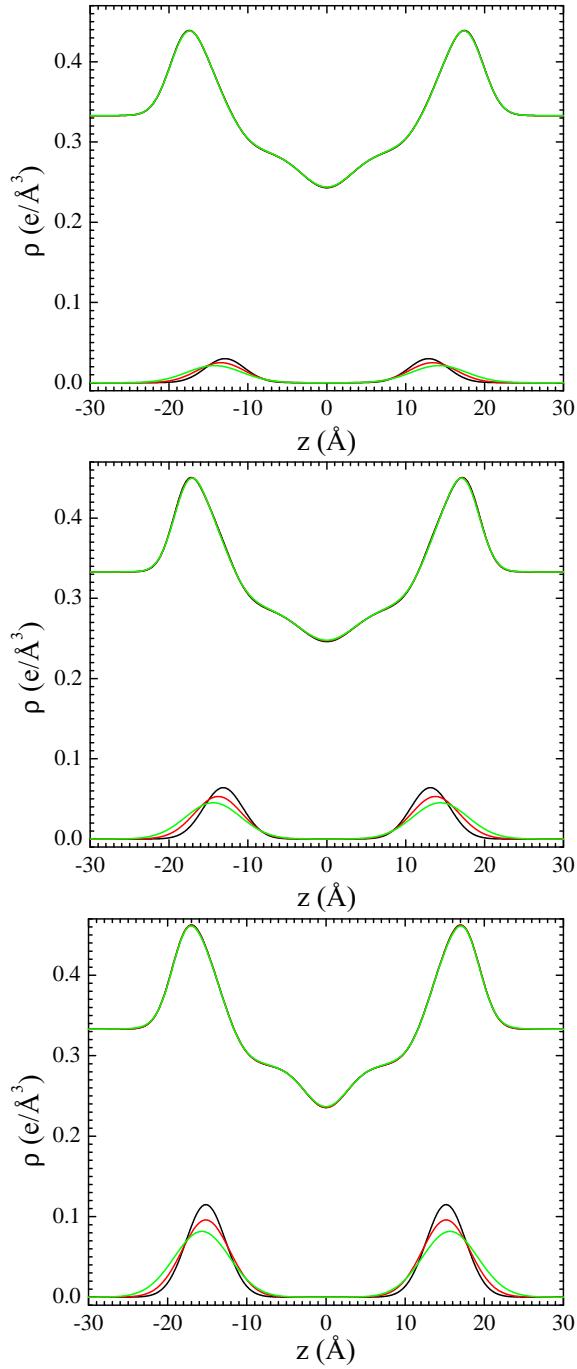


Figure 2.11: Comparison of total electron density profiles corresponding to best fits using different Tat widths σ_{Tat} , 2.5 (red), 3.0 (black), and 3.5 (green). The mole fraction of Tat x_{Tat} was 0.016 (top), 0.034(middle), and 0.059 (bottom). While different values of σ_{Tat} resulted in different positions of Tat, the total electron density profiles were almost identical and independent of σ_{Tat} .

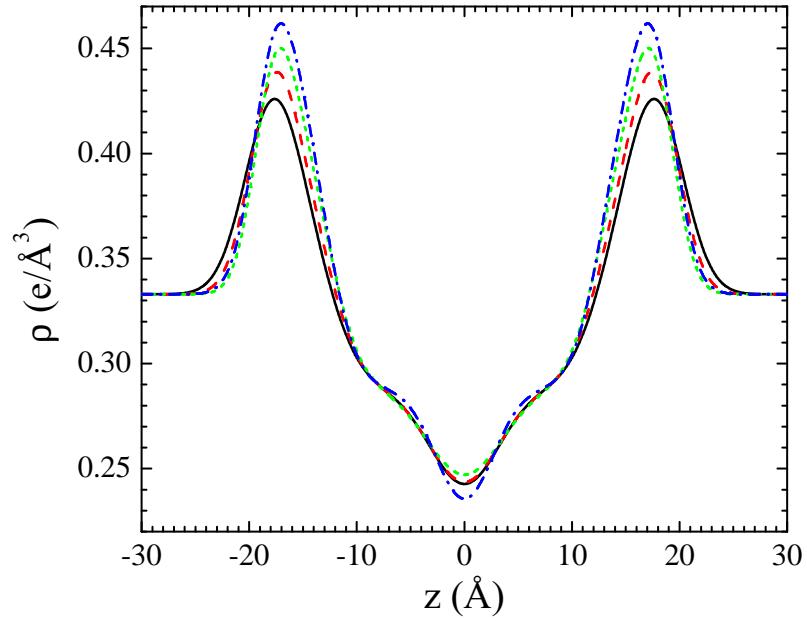


Figure 2.12: Comparison of total electron density profiles at $x_{\text{Tat}} = 0$ (black solid), 0.016 (red dash), 0.034 (green short dash), and 0.059 (blue dash dot).

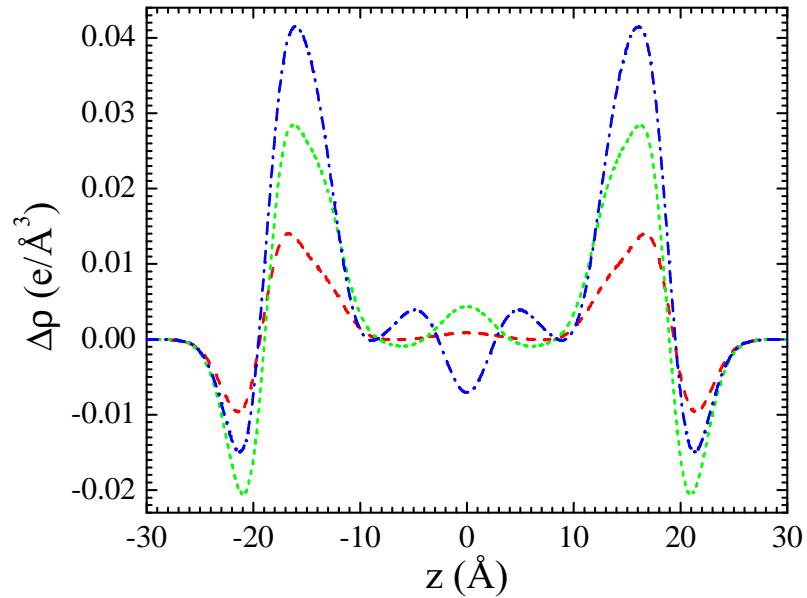


Figure 2.13: Difference between total electron density profiles of DOPC with Tat and that of DOPC. $x_{\text{Tat}} = 0.016$ (red dash), 0.034 (green short dash), and 0.059 (blue dash dot). Positive $\Delta\rho$ means excess electron density due to presence of Tat.

density profiles are shown in Fig. A.1 and Fig. A.2. Figure 2.14 plots total electron density profiles, showing increase of electron density at the headgroup region as Tat concentration increased, similarly to DOPC/Tat systems shown in Fig. 2.12.

x_{Tat}	0	0.016	0.016	0.016	0.034	0.034	0.034	0.059	0.059	0.059
χ^2	924.5	4972	4985	4994	6758	6826	6863	2293	2280	2296
z_{PC}	18.3	18.5	18.5	18.4	18.5	18.4	18.3	18.2	18.2	18.1
σ_{PC}	2.66	2.23	2.26	2.27	2.25	2.31	2.34	2.31	2.19	2.11
z_{CG}	15.2	15.4	15.4	15.3	15.4	15.3	15.2	15.1	15.1	15.0
σ_{CG}	2.92	2.63	2.65	2.69	2.52	2.58	2.63	2.40	2.20	2.01
z_{HC}	13.9	14.1	14.1	14.0	14.1	14.0	13.9	13.8	13.8	13.7
σ_{HC}	2.73	2.70	2.83	2.91	2.86	2.79	2.84	2.25	2.38	2.60
σ_{CH_3}	3.24	2.94	2.97	2.98	2.87	2.90	2.91	2.63	2.61	2.65
z_{Tat}	NA	13.5	14.0	15.0	14.3	14.9	16.0	16.3	16.4	16.9
σ_{Tat}	NA	2.5	3.0	3.5	2.5	3.0	3.5	2.5	3.0	3.5
A_L	70.9	69.8	69.9	70.1	69.5	70.0	70.6	71.3	71.4	71.7

Table 2.7: Fitting Results for DOPC:DOPE (3:1) membranes for the THG model. $z_{\text{PC}} - z_{\text{CG}} = 3.1 \text{ \AA}$ and $z_{\text{CG}} - z_{\text{HC}} = 1.3 \text{ \AA}$ in all fits.

x_{Tat}	0	0.016	0.016	0.016	0.034	0.034	0.034	0.059	0.059	0.059
χ^2	2961	1554	1570	1581	1563	1587	1607	2342	2338	2363
z_{PC}	18.1	18.0	17.9	17.9	17.8	17.7	17.6	17.8	17.8	17.7
σ_{PC}	2.52	2.14	2.17	2.18	1.86	1.92	1.93	2.02	1.97	1.93
z_{CG}	15.0	14.9	14.8	14.8	14.7	14.6	14.5	14.7	14.7	14.6
σ_{CG}	3.00	2.62	2.64	2.66	2.22	2.30	2.31	2.58	2.27	2.14
z_{HC}	13.7	13.6	13.5	13.5	13.4	13.3	13.2	13.4	13.4	13.3
σ_{HC}	3.00	2.69	2.84	2.95	2.65	2.82	3.01	2.47	2.58	2.83
σ_{CH_3}	3.20	3.19	3.22	3.24	3.37	3.43	3.47	2.70	2.70	2.74
z_{Tat}	NA	12.9	13.4	14.2	13.1	13.8	14.4	15.2	15.2	15.7
σ_{Tat}	NA	2.5	3.0	3.5	2.5	3.0	3.5	2.5	3.0	3.5
A_L	71.5	72.4	72.5	72.7	73.6	74.0	74.4	73.6	73.5	73.9

Table 2.8: (Numbers are wrong) Fitting Results for DOPC:DOPE (1:1) membranes for the THG model. $\Delta z_1 = z_{\text{PC}} - z_{\text{CG}}$ and $\Delta z_2 = z_{\text{CG}} - z_{\text{HC}}$.

Figure 2.15 summarizes the results for bilayer thickness as a function of Tat mole fraction x_{Tat} . In all cases, D_{HH} was smaller than D_{PP} , consistent with the results that the value of Tat position z_{Tat} from the bilayer center was smaller than that of PC headgroup position z_{PC} . The CG headgroup also carries high average electron density and is located closer to the bilayer center than the PC headgroup is. Therefore, in

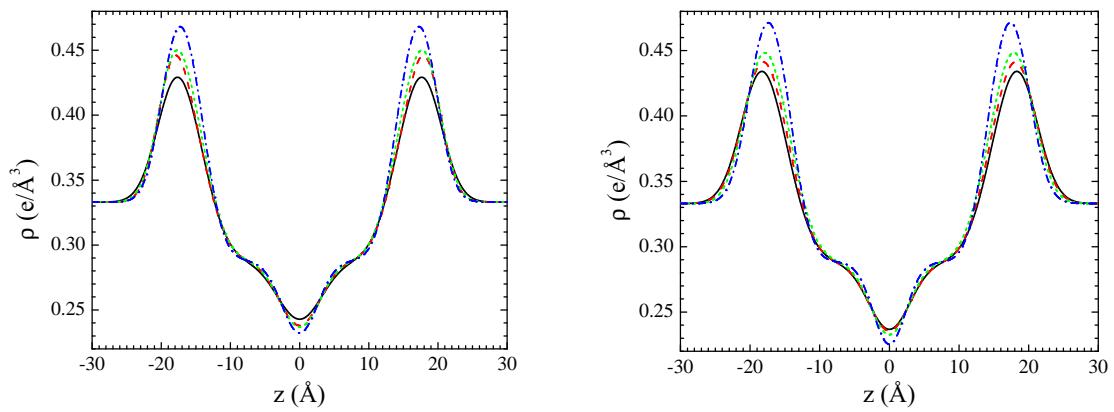


Figure 2.14: Total electron density profiles for DOPC:DOPE (3:1) (left) and DOPC:DOPE (1:1) (right) with Tat mole fraction $x_{\text{Tat}} = 0$ (black solid), 0.016 (red dash), 0.034 (green short dash), and 0.059 (blue dash dot).

general, D_{HH} is smaller than D_{PP} even without presence of Tat. Figure 2.16 compares Tat position to the PC headgroup position, reemphasizing a result that Tat is located inside the PC headgroup. We note, however, that D_{PP} in our models is the average PC-PC distance and not necessarily the same as local bilayer thickness near a Tat peptide. It is reasonable to expect that perturbation of bilayer structure due to Tat is largest near Tat and decays as a function of lateral distance from Tat. In Sec. 2.4.4, we discuss local perturbation of a DOPC bilayer measured in MD simulations. Finally, Fig. 2.17 plots area per lipid as a function of Tat mole fraction. Consistent with bilayer thinning, area per lipid was found to increase in most cases. We could not obtain electron density profiles for DOPC/DOPS (3:1) and the nuclear membrane mimic, due to loss of diffuse scattering by Tats charge neutralization of these negatively charged membranes, which rendered extraction of X-ray form factors unreliable, as described in section 2.4.1.

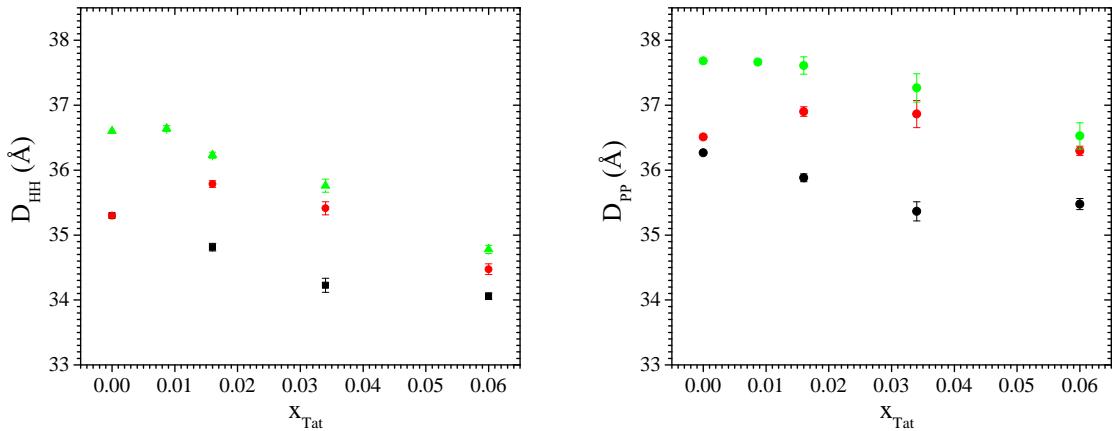


Figure 2.15: Bilayer thickness, D_{HH} (left) and D_{PP} (right) plotted against Tat mole fraction x_{Tat} . Black squares (DOPC), red circles (DOPC:DOPE (3:1)), and green triangles (DOPC:DOPE (1:1)). Error bars are standard deviations from imposing Tat Gaussian widths, $\sigma_{Tat} = 2.5, 3.0$ or 3.5 \AA .

We also studied how the goodness of fit varied as the position of the Tat Gaussian was varied. Figure 2.18 plots χ^2 as a function of the fixed Tat position z_{Tat} . We found that the two models, THG (Tat-in-headgroup region) and THC (Tat-in-hydrocarbon-chain region), resulted in similar electron density profiles, yielding similar χ^2 values when Tat was placed near the hydrocarbon-water interface region. In the THC model, the error function representing the hydrocarbon chain region became wider as Tat was placed closer to the interface region such that the total density profile calculated from

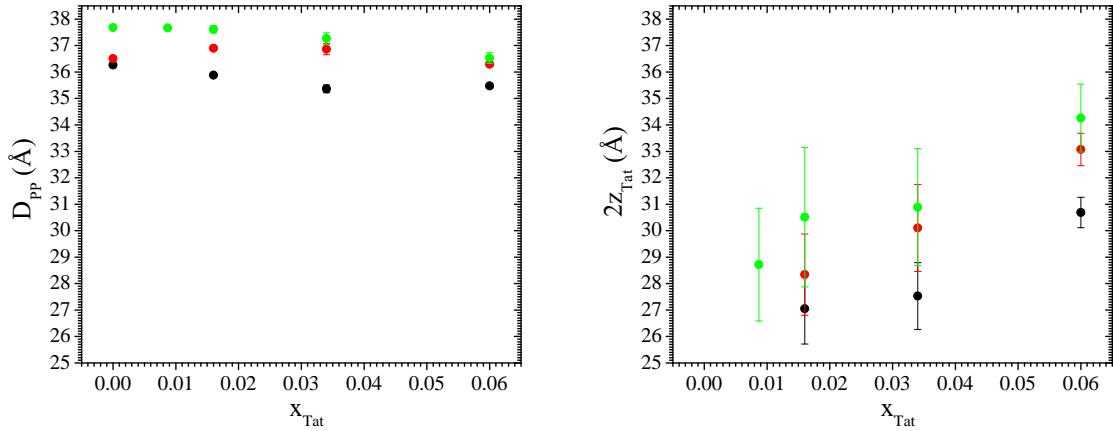


Figure 2.16: Bilayer thickness, D_{PP} (left) and twice Tat position $2z_{\text{Tat}}$ (right) plotted against Tat mole fraction x_{Tat} . Black squares (DOPC), red circles (DOPC:DOPE (3:1)), and green triangles (DOPC:DOPE (1:1)). Error bars are standard deviations from imposing Tat Gaussian widths, $\sigma_{\text{Tat}} = 2.5, 3.0 or 3.5 \AA . The data points of D_{PP} (left) is identical to those in Fig. 2.15, but the left axis is adjusted to facilitate comparison against $2z_{\text{Tat}}$.$

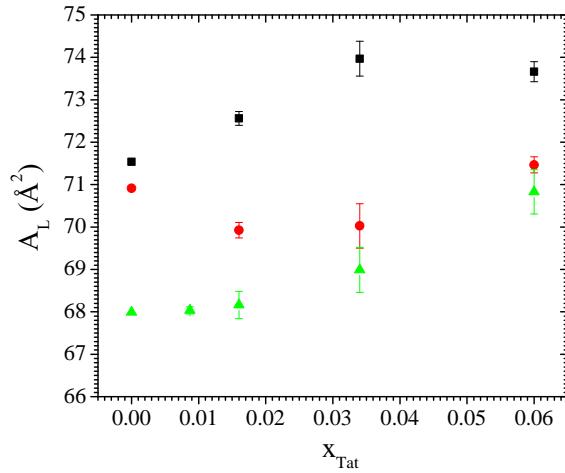


Figure 2.17: Area per lipid plotted against Tat mole fraction x_{Tat} . Black squares (DOPC), red circles (DOPC:DOPE (3:1)), and green triangles (DOPC:DOPE (1:1)). Error bars are standard deviations from imposing Tat Gaussian widths, $\sigma_{\text{Tat}} = 2.5, 3.0 or 3.5 \AA .$

the THC model was very similar to that calculated from the THG model. In general, while the total electron density profile is well determined by our modeling procedures, the values of the parameters for the components are not as well determined as the agreement of the fit to the data may suggest. In many cases, we found multiple local minima in the fitting landscape, including one with Tat closer to the center of the bilayer as shown in Fig. 2.18. χ^2 calculated at these local minima tended to be smaller for larger concentration of Tat. We also found that χ^2 with z_{Tat} in the hydrocarbon chain region and headgroup region was almost equal for the smallest value of x_{Tat} for DOPC:DOPE (1:1) bilayer. The MD simulations performed by Dr. Kun Huang suggested that the interior positions of Tat were artifacts of our model, at least for DOPC bilayers. The simulation results are found in Sec. 2.4.4.

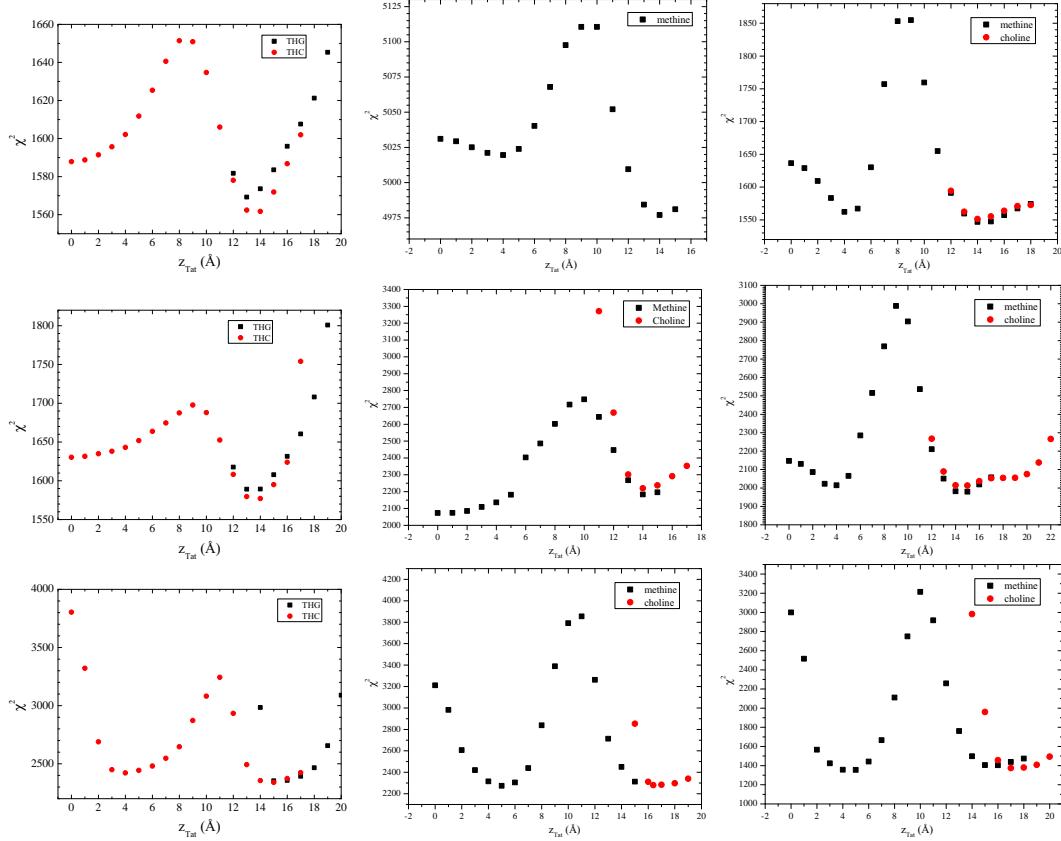


Figure 2.18: χ^2 as a function of z_{Tat} for DOPC, DOPC:DOPE (3:1), and DOPC:DOPE (1:1) (from left to right) with $x_{\text{Tat}} = 0.016, 0.034$, and 0.059 (from top to bottom). $\sigma_{\text{Tat}} = 3.0$. The THG model (black squares) and the THC model (red circles).

As seen from Table 2.6, the widths of the headgroup components became smaller as Tat concentration increased. This decrease seemed somewhat unreasonable; if Tat causes a bilayer to locally become thinner, we would expect that the headgroup components to become wider. Therefore, we also fitted a model with lower bounds on these headgroup widths. Namely, the minimum values of the widths of the headgroup components, PC and CG, were constrained to be greater than or equal to the corresponding values for pure bilayers without Tat. Table 2.9 shows results from fitting the data with lower bounds on the widths of the headgroup components for DOPC/Tat systems. In all cases, both headgroup widths, σ_{PC} and σ_{CG} , resulted in the same value as the value of their corresponding lower bounds. Similarly to fits with unbound widths, $D_{\text{PP}} = 2z_{\text{PC}}$ decreased as Tat concentration increased. The biggest difference between these bound fits and the unbound fits is in Tat position z_{Tat} . Figure 2.19 plots z_{Tat} as a function of Tat mole fraction x_{Tat} for both fits with and without lower bounds. While z_{Tat} increased as x_{Tat} increased for fits without bounds, z_{Tat} stayed more or less constant for fits with the bounds.

x_{Tat}	0	0.016	0.016	0.016	0.034	0.034	0.034	0.059	0.059	0.059
χ^2	2961	1853	1979	2118	2398	2893	3414	3160	4298	5539
z_{PC}	18.1	17.8	17.8	17.8	17.4	17.4	17.4	17.5	17.4	17.3
σ_{PC}	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
z_{CG}	15.0	14.7	14.7	14.7	14.3	14.3	14.3	14.4	14.4	14.3
σ_{CG}	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
z_{HC}	13.7	13.4	13.4	13.4	13.0	13.0	13.0	13.1	13.0	12.9
σ_{HC}	3.0	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
σ_{CH_3}	3.2	3.1	3.1	3.1	3.6	3.6	3.7	2.6	2.6	2.5
z_{Tat}	16.9	16.8	17.0	16.4	16.5	16.7	16.3	16.6	17.1	
σ_{Tat}		2.5	3.0	3.5	2.5	3.0	3.5	2.5	3.0	3.5
A_{L}	71.5	73.5	73.5	73.5	75.6	75.6	75.6	75.0	75.4	75.9

Table 2.9: Fitting Results of the bound THG model for DOPC membranes. $z_{\text{PC}} - z_{\text{CG}} = 3.1$ and $z_{\text{CG}} - z_{\text{HC}} = 1.3$ in all fits.

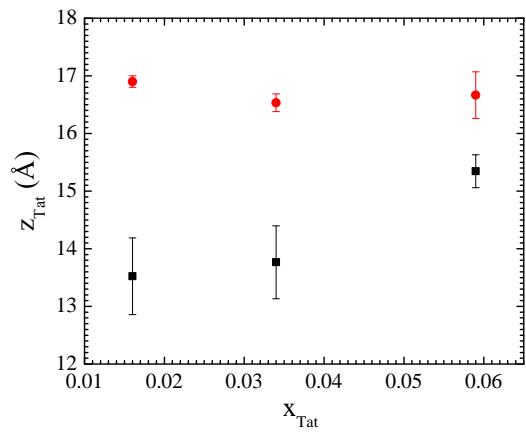


Figure 2.19: z_{Tat} as a function of Tat mole fraction x_{Tat} for fits with lower bounds on the headgroup widths (red circles) and without lower bounds (black squares). Error bars are standard deviations from imposing Tat Gaussian widths, $\sigma_{\text{Tat}} = 2.5, 3.0$ or 3.5 \AA .

2.4.4 Molecular Dynamics Simulations

(Under construction) Due to the slow relaxation in lipid bilayers and limited accuracy of the force field, a good agreement between experimental and MD simulation calculated form factors may be difficult to reach. Consequently, we carried out several constrained simulations at various A_L and z_{Tat} as described in Sec. 2.2.8. We then compared the simulated form factor $F(q_z)$ with the experimentally measured one. Figure 2.20 shows such comparison for a DOPC bilayer. As discussed earlier, the simulated form factor shifted to larger q_z as the area per lipid was increased. From this comparison, we found the simulation at $A_L = 70 \text{ \AA}^2$ to be the best match with the experimental form factor, yielding the lowest χ^2 . However, the form factor for $A_L = 72 \text{ \AA}^2$ matched the experiment better than that for 70 \AA^2 near $q_z = 0.3 \text{ \AA}^{-1}$, which suggests that a better match might lie between 70 and 72 \AA^2 . This case was not investigated further. The electron density profile for the best fit is shown in Fig. 2.21. The comparison for DOPC with $x_{\text{Tat}} = 0.015$ where there is one Tat in each monolayer is shown in Fig. 2.22. The same comparison for DOPC with $x_{\text{Tat}} = 0.03$ is shown in Fig. 2.23.

The best match for DOPC/Tat (128:4) was found when the Tats were constrained at 18 \AA away from the bilayer center (Fig. 2.25). The other best fit results were: DOPC $A_L = 70 \text{ \AA}^2$ and DOPC/Tat(128:2) $A_L = 72 \text{ \AA}^2$, $z_{\text{Tat}} = 18 \text{ \AA}$. It clearly indicates that with increasing Tat concentration, A_L increases. The agreement worsened as Tat was constrained to be closer to the center of the bilayer. When Tats were constrained at 5 \AA away from the bilayer center, we observed a spontaneous formation of water pores in the MD simulation. However, as shown in Fig. 2.25 the corresponding form factor calculated from MD simulations does not match well with experiments.

We summarize our results for how Tat affects the lipid bilayer in Fig. 2.26. The height of Tat, $H_{\text{Tat}} = 8.7 \text{ \AA}$, was the full width at half maximum of the Tat electron density profiles obtained from simulations and the cylindrical radius, $R_{\text{Tat}} = 8.3 \text{ \AA}$, was calculated to give the measured volume. The Z distances from the center of the bilayer were derived from weighted averages of four MD simulations of Tat:DOPC 2:128. The χ^2 obtained by comparison to experiment indicated that the best Z_{Tat} lay between the simulated values of 16 \AA and 18 \AA and the best area/lipid A_L lay between the simulated values of 72 \AA^2 and 74 \AA^2 , so averages were obtained from these four combinations of Z_{Tat} and A_L , weighted inversely with their χ^2 . The average positions, z'_{phos} , of phosphates situated underneath the Tats were calculated by averaging over

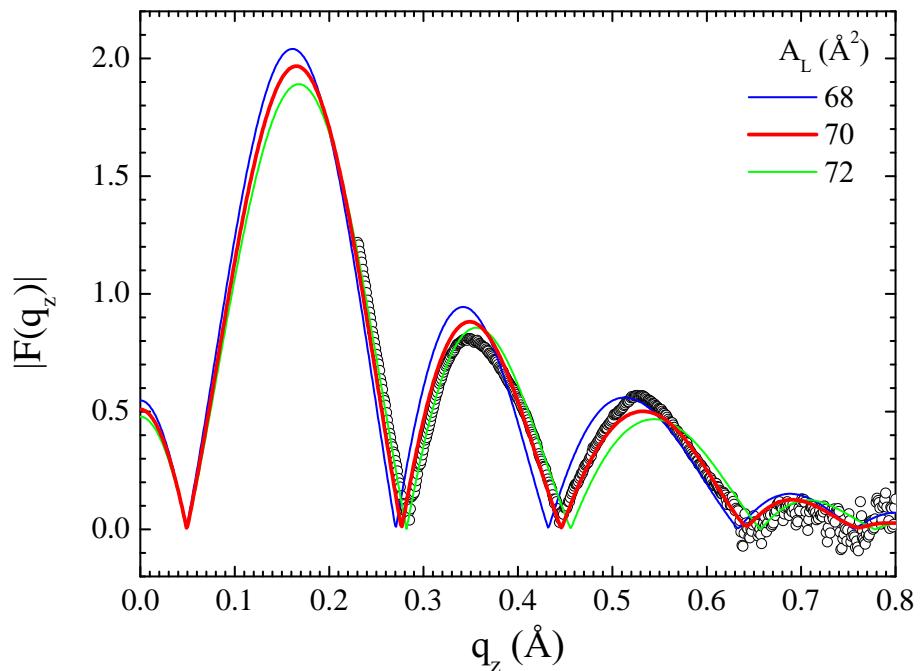


Figure 2.20: MD simulated form factors for DOPC at $A_L = 68 \text{ \AA}^2$ (blue solid line), 70 \AA^2 (red solid line), and 72 \AA^2 (green solid line) compared to the experimental form factor (open circles) scaled vertically to best match the form factor for 70 \AA^2 .

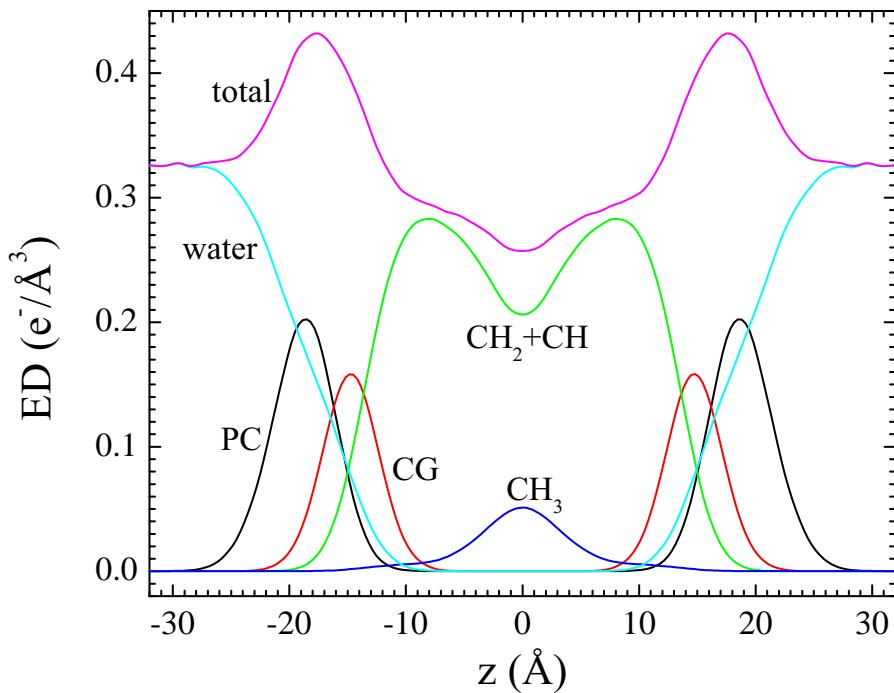


Figure 2.21: The simulated, symmetrized electron density profile for DOPC at $A_L = 70 \text{ \AA}^2$ as a function of the distance away from the bilayer center. Each component profile is labeled with its name: PC (phosphate-choline), CG (carbonyl-glycerol), CH_2+CH (methylene-methine combination), CH_3 (terminal methyl). The sum of all the components is labeled as total.

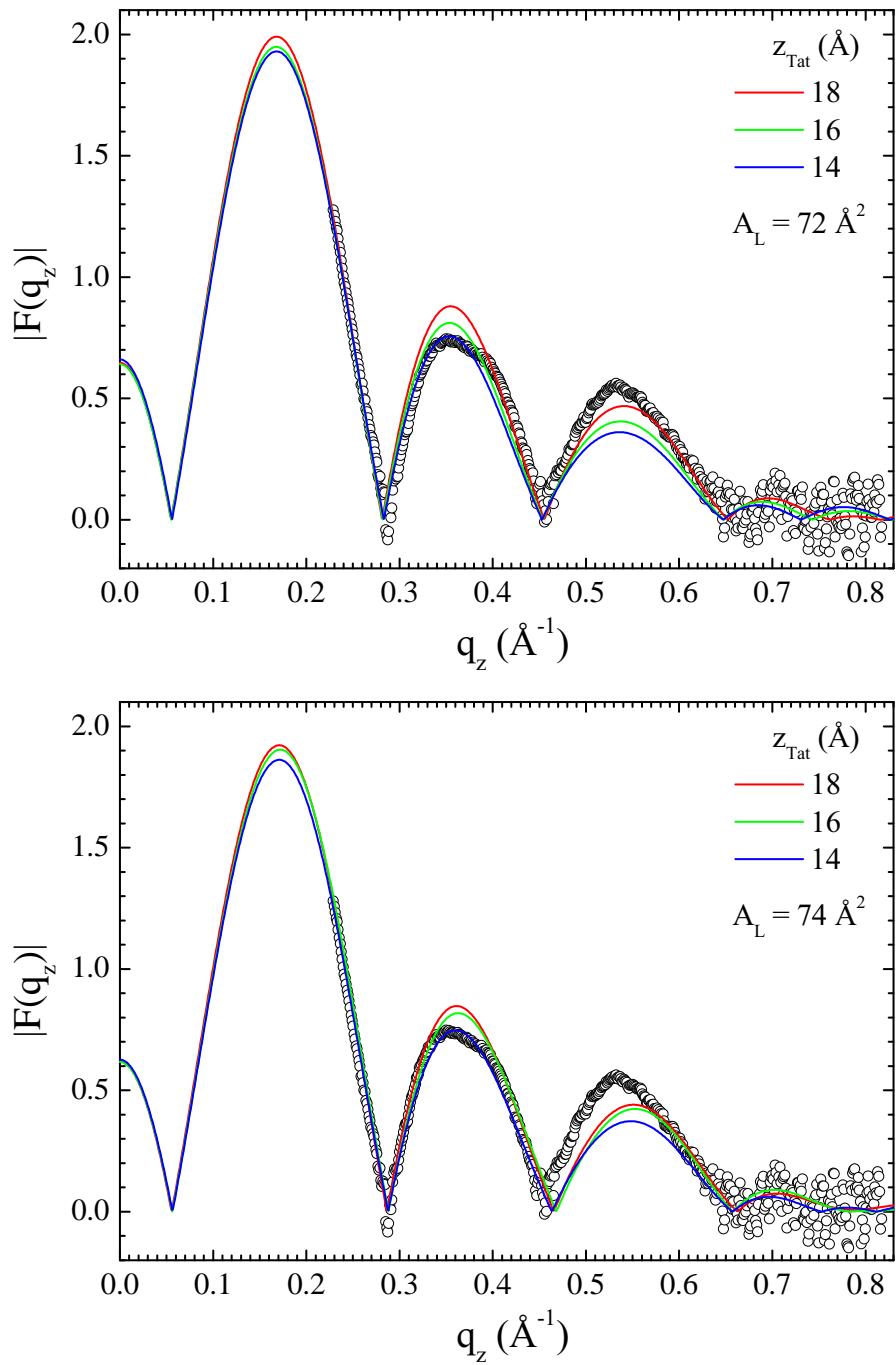


Figure 2.22: MD simulated form factors for DOPC with $x_{\text{Tat}} = 0.015$ at $A_L = 72 \text{ \AA}^2$ (top) and 74 \AA^2 (bottom), with $z_{\text{Tat}} = 18 \text{ \AA}$ (red solid lines), 16 \AA (green solid lines), and 14 \AA (blue solid lines) compared to the experimental form factor (open circles) scaled vertically to best match the form factor for $z_{\text{Tat}} = 18 \text{ \AA}$.

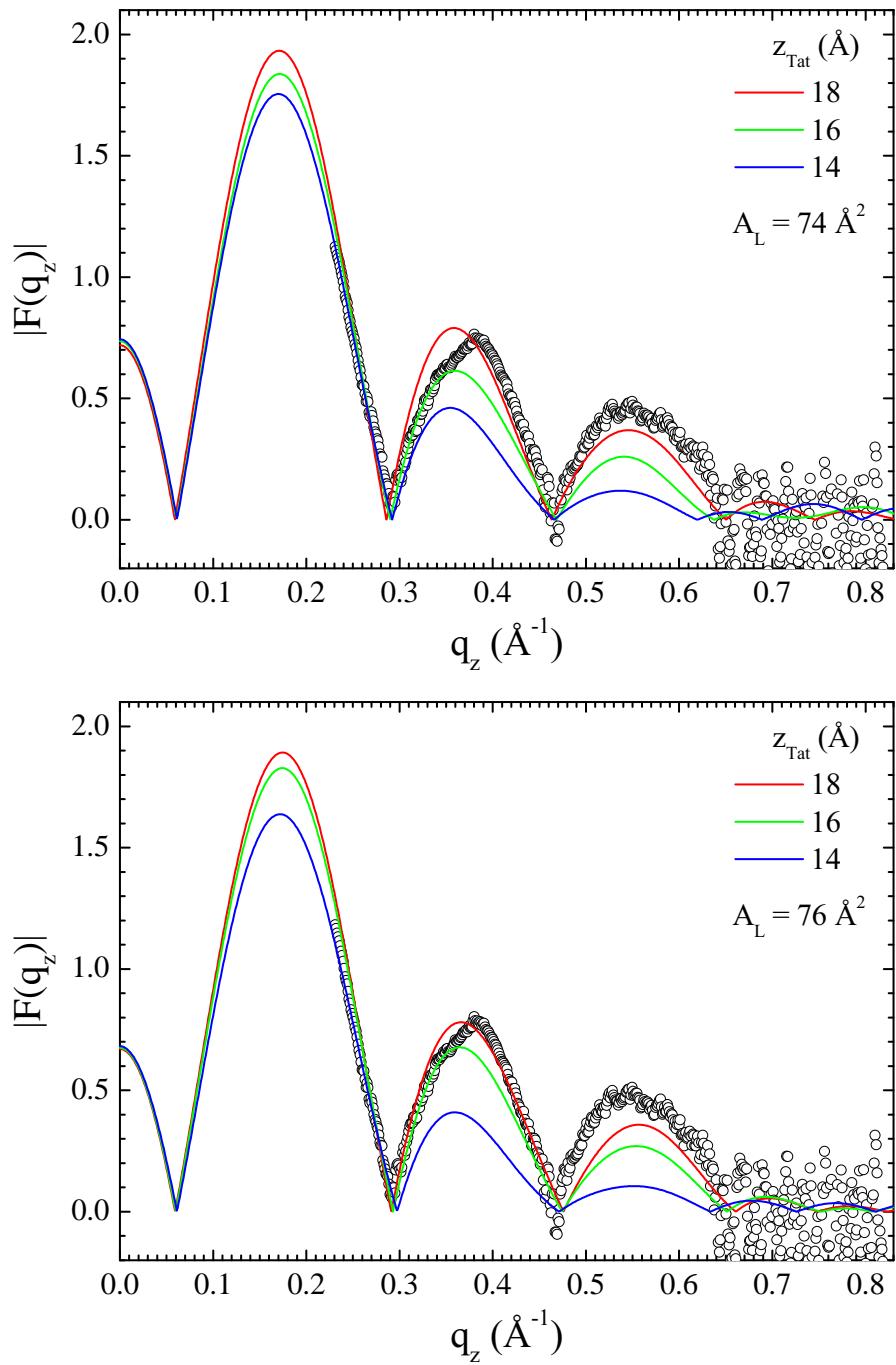


Figure 2.23: MD simulated form factors for DOPC with $x_{\text{Tat}} = 0.030$ at $A_L = 74 \text{ \AA}^2$ (top) and 76 \AA^2 (bottom), with $z_{\text{Tat}} = 18 \text{ \AA}$ (red solid lines), 16 \AA (green solid lines), and 14 \AA (blue solid lines) compared to the experimental form factor (open circles) scaled vertically to best match the form factor for $z_{\text{Tat}} = 18 \text{ \AA}$.

$x_{\text{Tat}} = 0.015$				$x_{\text{Tat}} = 0.030$			
$A_L (\text{\AA}^2)$	$z_{\text{Tat}} (\text{\AA})$	a	χ^2	$A_L (\text{\AA}^2)$	$z_{\text{Tat}} (\text{\AA})$	a	χ^2
70	18	0.621	60.1	70	18	0.621	60.1
70	16	0.568	69.1	70	16	0.568	69.1
70	14	0.439	131	70	14	0.439	131
70	12	0.285	391	70	12	0.285	391
70	10	0.199	440	70	10	0.199	440
70	8	0.196	374	70	8	0.196	374
70	5	0.159	527	70	5	0.159	527
72	18	0.72	18.0	72	18	0.72	18.0
72	16	0.65	24.9	72	16	0.65	24.9
72	14	0.6	31.4	72	14	0.6	31.4
72	12	0.426	104	72	12	0.426	104
72	10	0.219	443	72	10	0.219	443
72	8	0.205	336	72	8	0.205	336
72	5	0.165	448	72	5	0.165	448
74	18	0.722	21.3	74	18	0.722	21.3
74	16	0.704	25.9	74	16	0.704	25.9
74	14	0.631	24.7	74	14	0.631	24.7
74	12	0.412	81.9	74	12	0.412	81.9
74	10	0.312	194	74	10	0.312	194
74	8	0.246	351	74	8	0.246	351
74	5	0.177	427	74	5	0.177	427

Table 2.10: Comparison of the simulated form factors to the experimental form factors.

x_{Tat}	A_L	z_{Tat}	$\langle D_{\text{PP}} \rangle$	D_{PP}	x	Δt	H_{Tat}	R_{Tat}	R_2	z_{phos}	z_{guan}	χ^2
0	70		36.3									
0.015	72	18	35.6	32.8	35.8	3.5	9.2	8.1	15.0	14.7	15.5	18
0.015	72	16	36.1	33.0	36.3	3.3	9.4	8.0	9.0	14.9	14.5	24.9
0.015	74	18	35.0	33.0	35.1	3.3	8.6	8.3	23.9	14.9	16.5	21.3
0.015	74	16	35.0	32.1	35.2	4.2	7.6	8.9	20.4	14.0	13.5	25.9
0.030	74	18	35.3	32.6	NA	3.7	7.6	8.9	NA	14.5	15.5	24.3
0.030	74	16	35.3	31.2	NA	5.1	7.7	8.8	NA	13.1	13.5	40.1
0.030	76	18	34.2	32.0	NA	4.3	7.6	8.9	NA	13.9	16.5	14.8
0.030	76	16	34.9	31.4	NA	4.9	7.8	8.7	NA	13.3	14.5	30.4

Table 2.11: Summary of simulation results. $\langle D_{\text{PP}} \rangle$, phosphorus-phosphorus distance averaged over all lipids; D_{PP} , Tat-perturbed phosphorus atoms; x , thickness away from Tat; Δt , $\langle D_{\text{PP}}^{\text{DOPC}} \rangle - D_{\text{PP}}$; H_{Tat} , Tat height; R_{Tat} , radius of Tat cylinder; R_2 , radius of the calculated in-plane Tat-perturbed region; R_3 , effective radius of the simulation box.

x_{Tat}	A_L	z_{Tat}	$\langle D_{\text{PP}} \rangle$	D_{PP}	Δt	H_{Tat}	R_{Tat}	R_2	z_{phos}	z_{guan}
0.015	72.9	17.1	35.4	32.7	3.6	8.7	8.3	17.1	14.6	15.1
0.030	75.2	17.3	34.8	31.9	4.4	7.7	8.8	NA	13.8	15.4

Table 2.12: Summary of weighted average results. The caption is the same as Table 2.11.

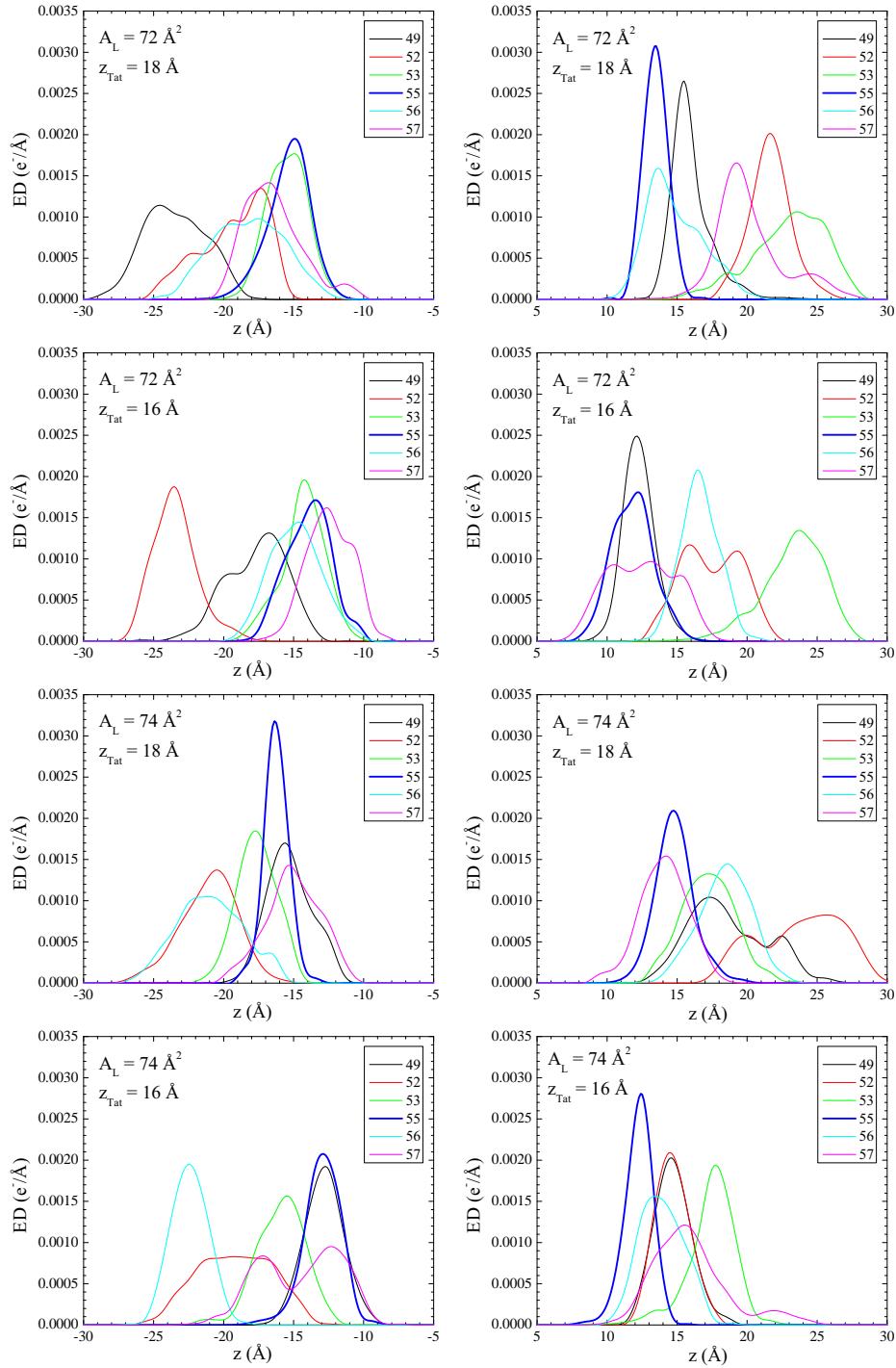


Figure 2.24: Electron density profiles of guanidinium groups from the four best matched simulations for DOPC with $x_{\text{Tat}} = 0.015$ (one Tat on each leaflet). Tat on the lower and upper leaflets are shown on the left and right plots, respectively.

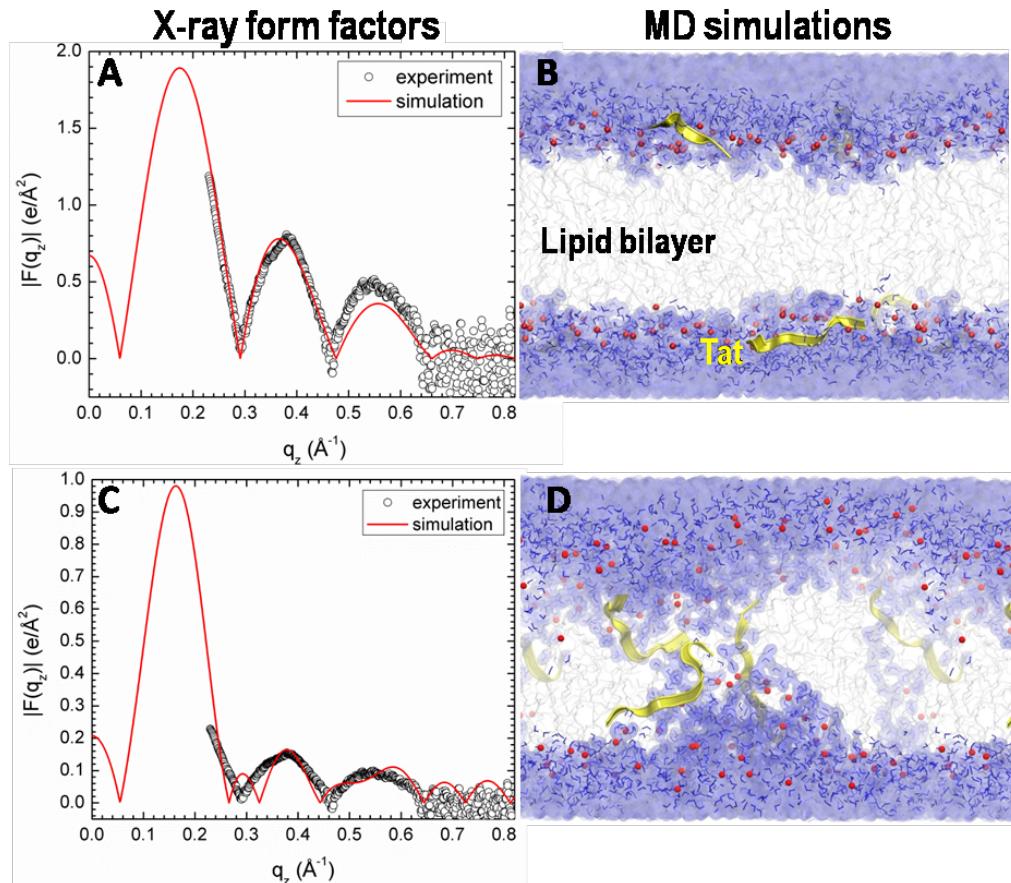


Figure 2.25: MD simulated form factors (red solid lines in A and C) of Tat/(DOPC+Tat), $x_{\text{Tat}}=0.030$, with Tat fixed at $z_{\text{Tat}}=18 \text{\AA}$ (panel A) and 5\AA (panel C) from the bilayer center compared to experimental form factors (open circles) scaled vertically to provide the best fit to the simulations. Corresponding snapshots are shown in Panels B and D in which the lipid chains are represented as grey sticks on a white background, Tats are yellow, phosphate groups are red and water is blue.

the phosphates whose in-plane distance, R , from the center of Tat is smaller than R_{Tat} . The simulation cell extended to 38 Å, far enough to ensure that z_{phos} for most of the lipids is the same as for DOPC. Assuming a simple linear ramp in z_{phos} , Fig. 2.26 then indicates a ring of boundary lipids that extends twice as far in R as Tat itself. Although the guanidinium electron density profile was broad (Fig. not yet included), indicating that some were pointing away from the bilayer relative to the center of Tat, more were pointing towards the bilayer center as indicated in Fig. 2.26.

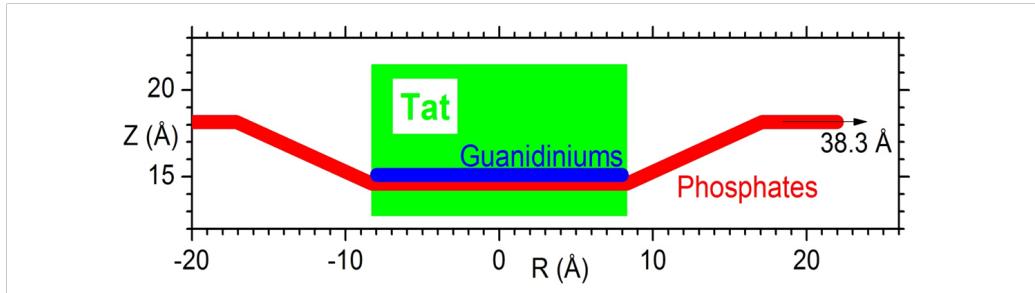


Figure 2.26: Location of Tat in DOPC bilayer. Tat is represented as a cylinder, z is the distance from the bilayer center, and R is the in-plane distance from the center of Tat. The average z of the lipid phosphates as a function of R and the arginine guanidiniums are shown in red and blue, respectively.

2.5 Discussion

Given that 8 of the 11 amino acids in Tat (47-57) are arginines and lysines, one would have suggested 20 years ago that highly charged Tat would partition strongly into solution rather than being associated with lipid bilayers. By contrast, but in agreement with more recent perspectives on arginine partitioning into the interfacial region [73], we find that Tat interacts with lipid bilayers, even with neutral DOPC and DOPC/DOPE mixtures, as well as with negatively charged DOPC/DOPS and nuclear membrane mimic lipid mixtures. This paper presents multiple lines of evidence for a Tat/membrane interaction. Fig. ?? shows that Tat decreases the bending modulus. Although one could argue that such a decrease is only apparent and could instead be due to local changes in membrane spontaneous curvature [74], either interpretation supports a Tat-bilayer interaction. The changes with increasing Tat concentration in the X-ray membrane form factors in Fig. ?? prove that Tat affects membrane structure, and the shift of the zero positions to higher q_z suggests thinning. Thinning

is substantiated by quantitative analysis of the X-ray data and by MD simulations. Fig. 7A shows that the average membrane thickness, as measured by the distance D_{PP} between phosphocholines on opposite surfaces, decreases with increasing Tat concentration. Similar thinning is shown in Fig. 7B for the distance D_{HH} between the maxima in the electron density profiles of opposite surfaces. Compared to D_{PP} , D_{HH} is pulled towards both the carbonyl/glycerol groups and Tat because both have electron densities ($0.4 \text{ e}/\text{\AA}^3$) greater than water ($0.33 \text{ e}/\text{\AA}^3$) or hydrocarbon ($0.3 \text{ e}/\text{\AA}^3$). Although the thinning shown in Figs. 7A and 7B is not large, it obviously requires interaction of Tat with the bilayers. Fig. 7C shows that A_L increases with increasing Tat concentration, by both model fitting and MD simulations.

It is of considerable interest to learn where Tat resides, on average, in the membrane, as this would establish a base position from which translocation would be initiated. We have combined our two main methods, MD simulations and X-ray scattering, to address this question. In general, Tats locate at the bilayer/water interface as indicated in Section 3.2, and they are close to the phosphocholine headgroup region by comparing the simulated 2ZTat in Fig. 7.D with 7.A. Although the SDP modeling of the X-ray data obtains excellent fits to the experimental form factors for a model with Tat deep in the hydrocarbon interior (see Fig. S5), the corresponding MD simulation (shown in Fig. 4.C) eliminates this spurious result. Fig. 7D also shows that modeling gives smaller values for z_{Tat} than the simulation. The modeling result is supportive of the original simulation result of Herce and Garcia that Tat resides closer to the bilayer center than do the phosphocholine groups [45]. That is a base position that would be a possibly important precursor to translocation, as would the larger A_L .

Several groups have carried out calculations and MD simulations showing that the cost of moving an arginine group from water to the bilayer center is 12-26 kcal/mol [73, 75-77] or 6-7 kcal/mol if side-chain snorkeling to the surface is taken into account [78]. This is not inconsistent with our result that Tat interacts with the membrane because, as is well known, the bilayer is not just a hydrocarbon slab, but has interfacial headgroup regions where Tat can reside. It has been suggested that the free energy cost for charged amino acids entering the headgroup region is similar to that for partitioning into octanol, about an order of magnitude smaller free energy cost than partitioning into cyclohexane [79-81]. Simulations suggest that the free energy is smaller for an arginine residing in the interfacial region than in water,

roughly by 3 kcal/mole, depending upon the lipid [73, 81]. Our results therefore appear energetically reasonable.

One concern with diffraction experiments on samples consisting of adjacent bilayers in a stack or in a multilamellar vesicle is that the samples have to be partially dried to obtain conventional diffraction data. But then there is no pure water layer between adjacent bilayers, so a hydrophilic peptide is forced into the interfacial, partially hydrophilic region of the lipid bilayer. In contrast, by using diffuse scattering, we obtained structure from experimental samples that had a range of lamellar D spacings (see Fig. 2 caption) that were considerably larger than the thickness of the bilayer in Fig. 7A, thereby providing an ample pure water space, typically greater than 20Å. The result that $2z_{\text{Tat}}$ shown in Fig. 7D is so much smaller than our repeat spacings shows that Tat preferentially associates with the membrane rather than dissociating into water.

Tat also increases the mosaic spread observed by X-ray and neutron scattering as shown in Figs. S1-3; this is a much larger scale disordering of the stack of bilayers.

We analyzed the secondary structures of Tats from MD simulations using the Define Secondary Structure of Proteins (DSSP) program [82]. Data from the MD simulation which has the best fit to experimental X-ray form factors show that Tat contains neither α -nor β -helix structures. It appears that the membrane does not influence the conformation of solubilized Tat.

Given our structural and elastic moduli results, we now compare to other experiments in the literature. In 2008, the Wong group implicated Tats ability to induce saddle-splay curvature with a potential role of bidentate hydrogen bonding as key [35]. Rhodamine-tagged Tat only entered GUVs when the PE headgroup was included with PS and PC lipids (PS/PC/PE, 20:40:40), indicating that hydrogen-bonding, and/or curvature-promoting lipids are required for Tat translocation. In PS/PE (20:80) lipids, they found Tat caused a highly curved cubic phase using X-ray diffraction [35]. In our experiments, there was little effect of adding DOPE to DOPC at either a 3:1 or 1:1 mole ratio on decrease in the bending modulus, bilayer thinning, or Tats outward movement with increasing concentration. Our two results are not inconsistent, however, since curvature-promotion appears not to be required for Tats ability to lower the energy required to bend nor to locate Tat in the bilayer, both of which may be important for Tat translocation. Yet Tat does translocate across membranes in their experiments only with PE in the membrane, so the ability to

induce saddle-splay curvature may also be required for Tat translocation. An X-ray, neutron and AFM study reported thickening upon initial Tat binding, in contradiction to our result in Fig. 7B that shows thinning [83]. We suggest that this difference was caused by their using stiff gel phase DPPC lipid that did not allow bound Tat to perturb the bilayer. Using a variety of techniques, including high sensitivity isothermal titration calorimetry and ^2H - and ^{31}P -NMR, Ziegler *et al.* [?] presented evidence that the lipid bilayer remains intact upon Tat binding and our results confirm this. Finally, we compare our structural results to those obtained by solid state NMR, although at a lower hydration level than in our sample. Su *et al.* [42] found that Tat lies parallel to the bilayer surface in the headgroup region of DMPC/DMPG (8:7) bilayers, similar to our cartoon in Fig. 9.

2.6 Conclusion

Although a recent MD simulation using umbrella sampling [84] found that the free energy required for R_9C to traverse a membrane was smaller if a water pore was present, we could not directly test the existence of a transient water pore from our X-ray scattering experiment. This is because, even with a water pore, the translocation process still requires crossing a free energy barrier which is a non-equilibrium process. X-ray form factors measure an equilibrium state. If the form factors obtained from water pore structures agreed well with experiments, it would indicate that the pore structure was thermodynamically stable. This may be the case for some antimicrobial peptides, but certainly not for cell-penetrating peptides. Finding a kinetically competent pathway for the interesting phenomenon of translocation of highly charged Tat through hydrophobic membranes is difficult. An energetically passive translocation likely occurs very seldom on an MD simulation time scale, and it probably happens quickly, so it would not significantly change the average structure of the membrane in which it occurs. Although our results in this paper do not reveal a kinetically competent pathway, they do show that Tat is drawn to the surface of the membrane, and is therefore ready for translocation at a region of local thinning. And they show that these interactions tend to soften (Fig. 2) the membrane and increase the area per lipid A_L , thereby likely reducing the energy barrier for passive translocation.

Appendices

Appendix A

A.1 More results from chapter 2

A.2 Mosaic Spread for NFIT analysis

First we calculate how mosaic spread affects the structure factor $S(q)$. Next we discuss two experimental methods. Third, we discuss the updated NFIT program. Fourth, we show the results.

A.2.1 Mosaic Spread: Calculation

In this section, an analytical framework for dealing with mosaic spread is developed. A sample of oriented stacks of bilayers consists of many small domains, within which layers are registered in an array. An ideal domain is a domain where the layers are parallel to the substrate, whose surface is in the sample xy -plane, so the orientation \mathbf{n} of an ideal domain is perpendicular to the substrate as shown in Fig. A.3. In general, the orientation \mathbf{n}' of a domain is tilted from that of an ideal domain by some angle α . Then, we consider a mosaic spread distribution function, $P(\alpha)$, representing a probability of finding a domain with a tilt α . We assume that the sample is symmetric about the substrate normal, so that the distribution $P(\alpha)$ does not depend on the azimuthal angle, β . The normalization condition on $P(\alpha)$ is

$$1 = \int_0^{2\pi} d\beta \int_0^{\frac{\pi}{2}} d\alpha \sin \alpha P(\alpha). \quad (\text{A.1})$$

The object of this section is to derive the X-ray scattering structure factor including the distribution function $P(\alpha)$.

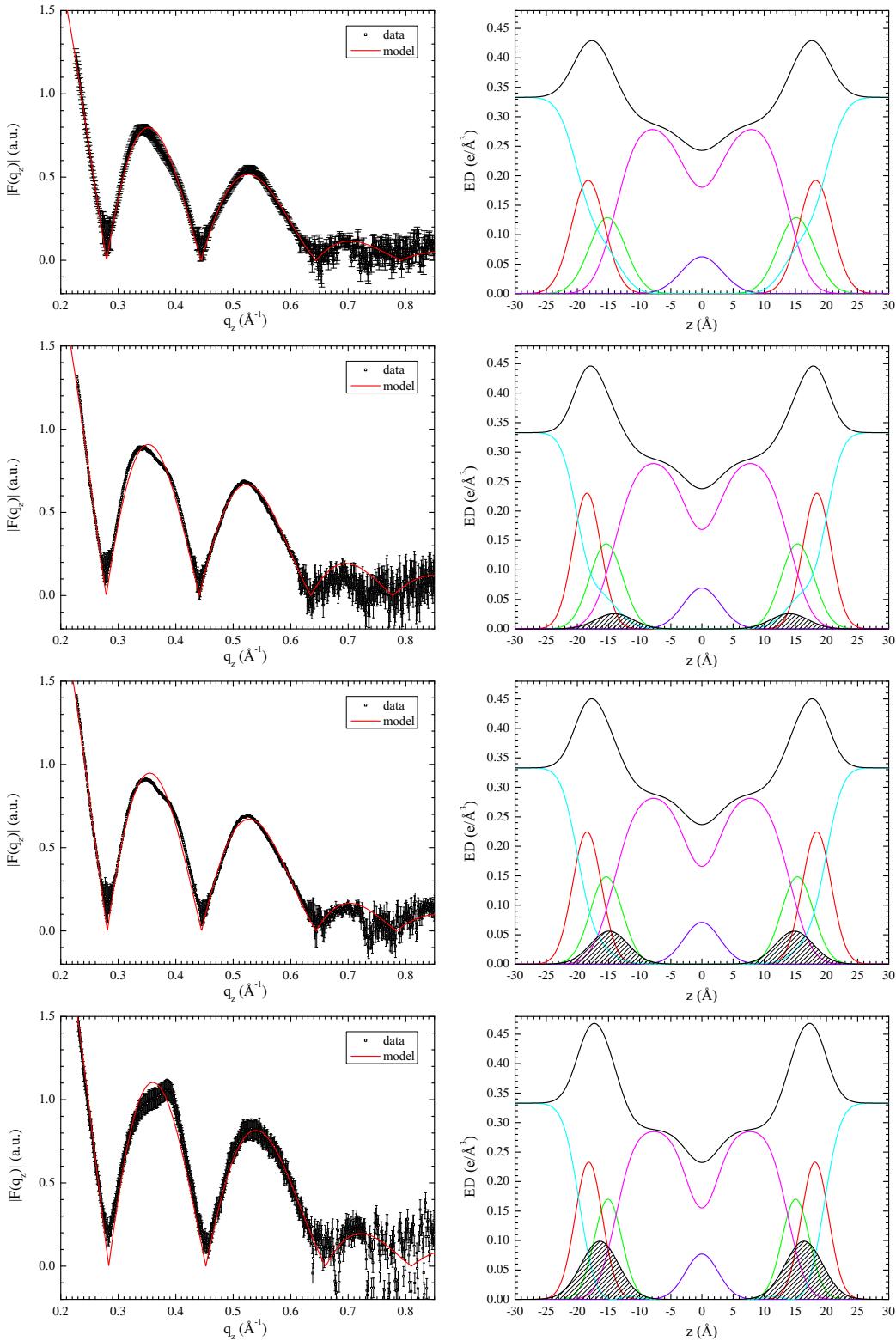


Figure A.1: The best fits to DOPC:DOPE (3:1) form factors (left) and the corresponding electron density profiles (right) with $x_{\text{Tat}} = 0, 0.016, 0.034$, and 0.059 (from top to bottom).

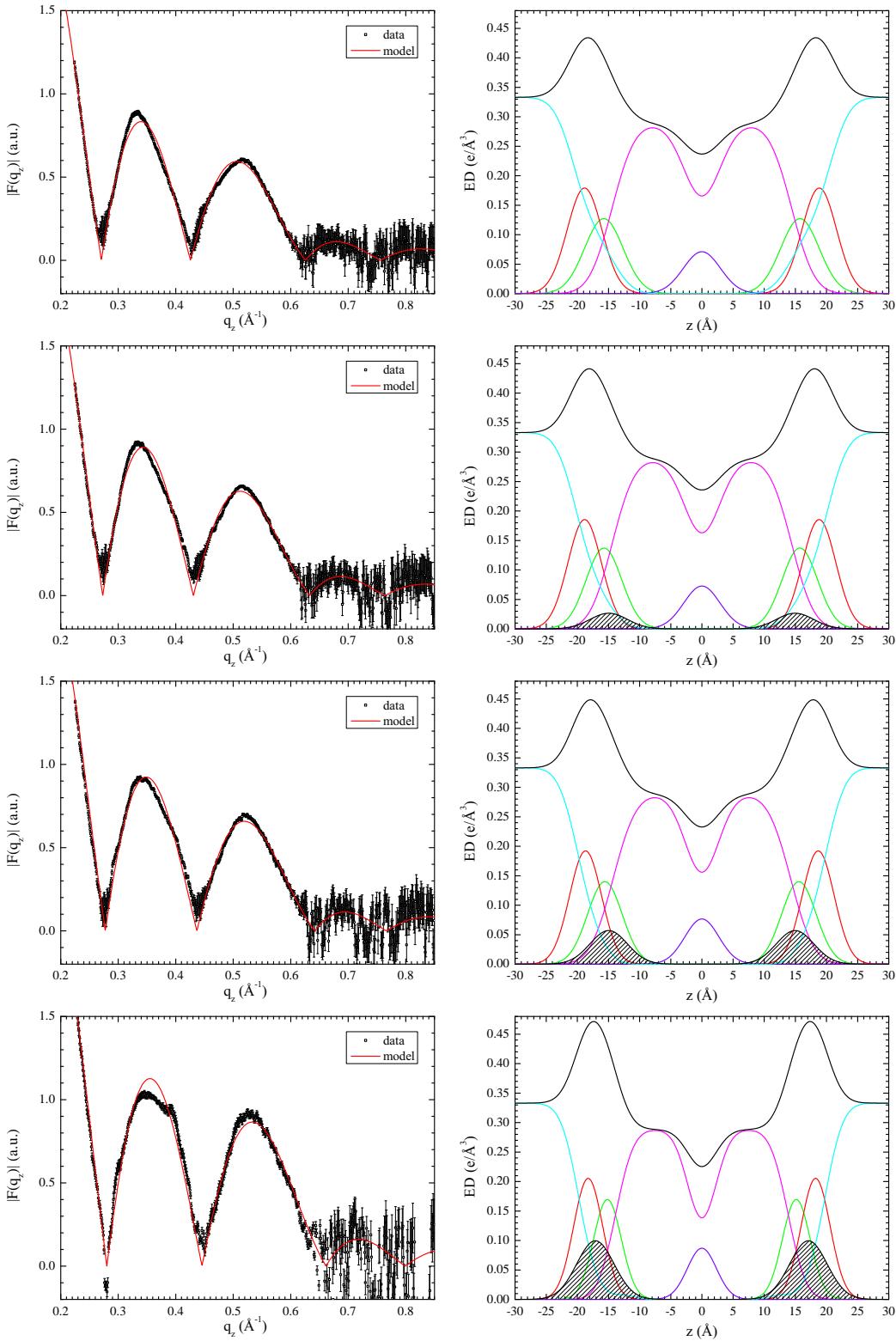


Figure A.2: The best fits to DOPC:DOPE (1:1) form factors (left) and the corresponding electron density profiles (right) with $x_{\text{Tat}} = 0, 0.016, 0.034$, and 0.059 (from top to bottom).

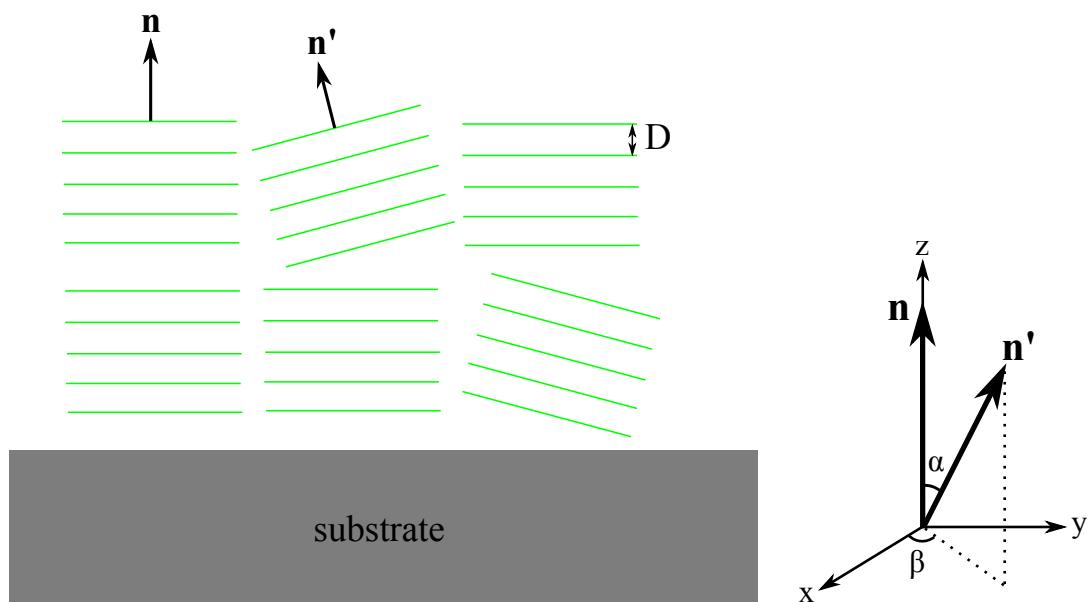


Figure A.3: Two dimensional view of mosaic spread (left) and notations used in this section (right). The stacking direction of an ideal domain is \mathbf{n} and that of a tilted domain \mathbf{n}' . The deviation of \mathbf{n}' from \mathbf{n} denoted as α quantifies the degree of misorientation of a domain. The x , y , and z -axes are the sample coordinates.

First, let us consider a two dimensional example. Our sample consists of two identical domains except a tilt α shown in Fig. A.4. Then, the sample structure factor $S^{\text{sam}}(\mathbf{q})$ is a superposition of the structure factor $S(\mathbf{q})$ of the ideal domain and $S(\mathbf{q}')$ of the tilted domain,

$$S^{\text{sam}}(\mathbf{q}) = S(q_x, q_z) + S(q'_x, q'_z). \quad (\text{A.2})$$

To express $S(q'_x, q'_z)$ in terms of the sample q -space (q_x, q_z) , we write q'_x and q'_z in terms of q_x , q_z , and α ,

$$\begin{aligned} q'_x &= \mathbf{q} \cdot \hat{\mathbf{x}}' = q \cos\left(\frac{\pi}{2} - \theta + \alpha\right) \\ q'_z &= \mathbf{q} \cdot \hat{\mathbf{z}}' = q \sin\left(\frac{\pi}{2} - \theta + \alpha\right) \\ q_x &= q \cos(\pi/2 - \theta) \\ q_z &= q \sin(\pi/2 - \theta) \end{aligned} \quad (\text{A.3})$$

where $q = |\mathbf{q}|$. Eq. (A.2) and (A.3) give the structure factor of a sample consisting of the two domains. With a continuous distribution of \mathbf{n}' , we integrate over the angle α with each structure factor modulated by the distribution function $P(\alpha)$,

$$S_M(\mathbf{q}) = S_M(q, \theta) = \int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} d\alpha S(q'_x, q'_z) P(\alpha), \quad (\text{A.4})$$

Variables q and θ are used in the above equation to make a connection with the three dimensional case, where the spherical coordinates are convenient, which we discuss now.

For a three dimensional sample, the basic idea is the same as the two dimensional case. In the three dimensional case, we also rotate the vector \mathbf{n}' about the z -axis by an angle β after the rotation about the y -axis by an angle α , so all we need to do is to apply appropriate rotation matrices to the sample xyz -axes which define the domain coordinates $x'y'z'$.

The rotation matrix for rotating a vector about the y -axis is given by

$$R_y = \begin{pmatrix} \cos \alpha & 0 & \sin \alpha \\ 0 & 1 & 0 \\ -\sin \alpha & 0 & \cos \alpha \end{pmatrix} \quad (\text{A.5})$$

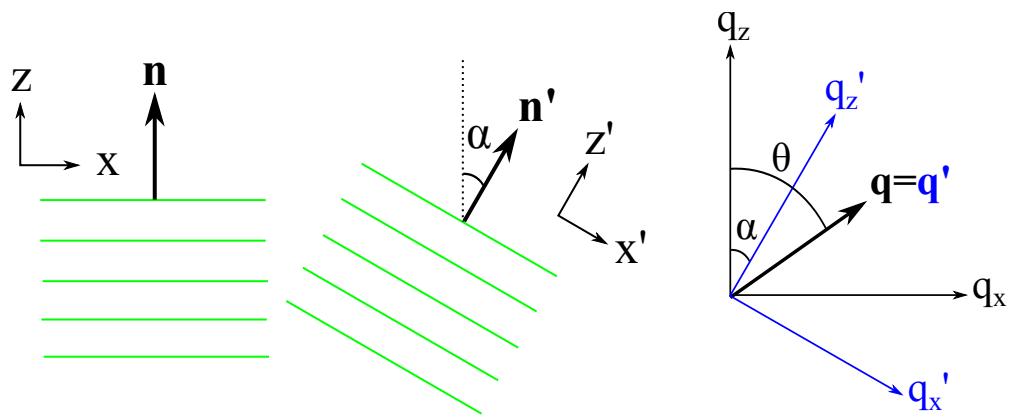


Figure A.4: Example of a two dimensional sample consisting of an ideal and tilted domains. $\mathbf{q} = (q_x, q_z)$ is the sample q -space and $\mathbf{q}' = (q'_x, q'_z)$ is the domain q -space. The two q -spaces are related by a rotation of α about the y -axis, which is into the page.

and for rotating about the z -axis

$$R_z = \begin{pmatrix} \cos \beta & -\sin \beta & 0 \\ \sin \beta & \cos \beta & 0 \\ 0 & 0 & 1 \end{pmatrix}. \quad (\text{A.6})$$

Then, what we want is

$$\hat{\mathbf{x}}' = R_z R_y \begin{pmatrix} 1 \\ 0 \\ 0 \end{pmatrix} = \begin{pmatrix} \cos \alpha \cos \beta \\ \cos \alpha \sin \beta \\ -\sin \alpha \end{pmatrix} \quad (\text{A.7})$$

$$\hat{\mathbf{y}}' = R_z R_y \begin{pmatrix} 0 \\ 1 \\ 0 \end{pmatrix} = \begin{pmatrix} -\sin \beta \\ \cos \beta \\ 0 \end{pmatrix} \quad (\text{A.8})$$

$$\hat{\mathbf{z}}' = R_z R_y \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix} = \begin{pmatrix} \sin \alpha \cos \beta \\ \sin \alpha \sin \beta \\ \cos \alpha \end{pmatrix}. \quad (\text{A.9})$$

The domain q -space, (q'_x, q'_y, q'_z) , in terms of the sample q -space (q_x, q_y, q_z) is given by

$$q'_x = \mathbf{q} \cdot \hat{\mathbf{x}}' = q_x \cos \alpha \cos \beta + q_y \cos \alpha \sin \beta - q_z \sin \alpha, \quad (\text{A.10})$$

$$q'_y = \mathbf{q} \cdot \hat{\mathbf{y}}' = -q_x \sin \beta + q_y \cos \beta, \quad (\text{A.11})$$

$$q'_z = \mathbf{q} \cdot \hat{\mathbf{z}}' = q_x \sin \alpha \cos \beta + q_y \sin \alpha \sin \beta + q_z \cos \alpha. \quad (\text{A.12})$$

The transformation expressed in the spherical coordinates is

$$\cos \theta' = \frac{q'_z}{q} = \sin \theta \sin \alpha \cos(\phi - \beta) + \cos \theta \cos \alpha, \quad (\text{A.13})$$

$$\tan \phi' = \frac{q'_y}{q'_x} = \frac{\sin \theta \sin(\phi - \beta)}{\sin \theta \cos \alpha \cos(\phi - \beta) - \cos \theta \sin \alpha}. \quad (\text{A.14})$$

Summing over all the domains, we get for the mosaic spread modified structure factor

$$S_M(q, \theta, \phi) = \int_0^{2\pi} d\beta \int_0^{\frac{\pi}{2}} d\alpha S(q, \theta', \phi') P(\alpha) \quad (\text{A.15})$$

with Eq. (A.13) and Eq. (A.14).

To test these equations, let us apply them to the simple case of a stack of rigid layers with their normals parallel to the z -axis in spherical coordinates. The structure factor is then

$$S(q, \theta, \phi) = \frac{\delta(q - \frac{2\pi h}{D})}{q^2} \delta(\cos \theta - 1) \delta(\phi) \quad (\text{A.16})$$

where $\delta(x)$ is the Dirac delta function. From Eq. (A.14), $\delta(\phi')$ is equivalent to $\delta(\beta - \phi)$. Setting $\beta = \phi$ in Eq. (A.13) gives $\cos \theta' = \cos(\alpha - \theta)$. Then, the mosaic spread modified structure factor $S_M(\mathbf{q})$ is

$$\begin{aligned} S_M(q, \theta, \phi) &= \int d\alpha \int d\beta \frac{\delta(q - \frac{2\pi h}{D})}{q^2} \delta(\cos \theta' - 1) \delta(\beta - \phi) P(\alpha) \\ &= \frac{\delta(q - \frac{2\pi h}{D})}{q^2} \int d\alpha \delta(\cos[\alpha - \theta] - 1) P(\alpha) \\ &= \frac{\delta(q - \frac{2\pi h}{D})}{q^2} P(\theta). \end{aligned} \quad (\text{A.17})$$

Eq. (A.17) describes hemispherical shells with radii of $2\pi h/D$ in the sample q -space. As will be described in the next section, a 2D detector records cross sections of these shells, which give rise to mosaic arcs along $q = 2\pi h/D$.

The structure factor of thermally fluctuating layers is not simple delta functions and gives rise to diffuse scattering. Analysis of the diffuse scattering from a sample with mosaic spread requires Eq. (A.15).

A.2.2 Mosaic Spread: Near Equivalence of Two Methods

In this section, we discuss experimental procedures to probe appropriate q -space to measure the mosaic spread distribution, $P(\alpha)$. In our setup, the angle of incidence between the beam and substrate, denoted by ω , can be varied. A conventional method to measure $P(\alpha)$ is a rocking scan, where one measures the integrated intensity of a given Bragg peak as a function of ω with a fixed detector position. Another method that takes an advantage of an area detector [111] measures the intensity as a function of χ on a two dimensional detector (see Fig. A.5). This method has been used to quantify complete pole figures for thin films with fiber texture (isotropic in-plane orientation) [112]. First, we want to compare the two methods mentioned above and determine their relationship.

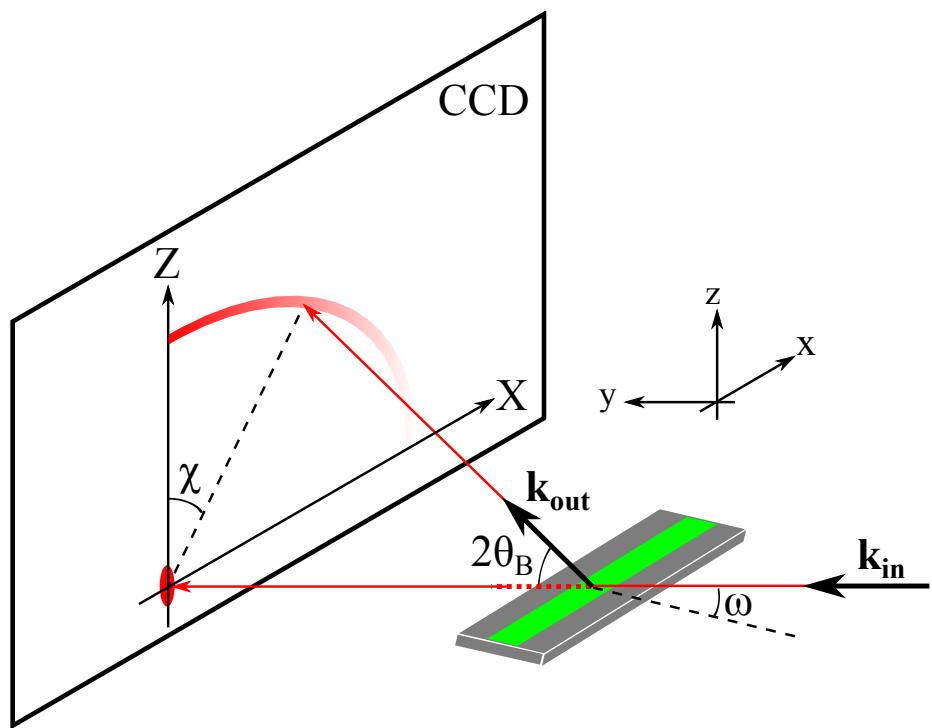


Figure A.5: Notations used in this section. The arc originating from the Z -axis is the mosaic arc due to the mosaic spread distribution.

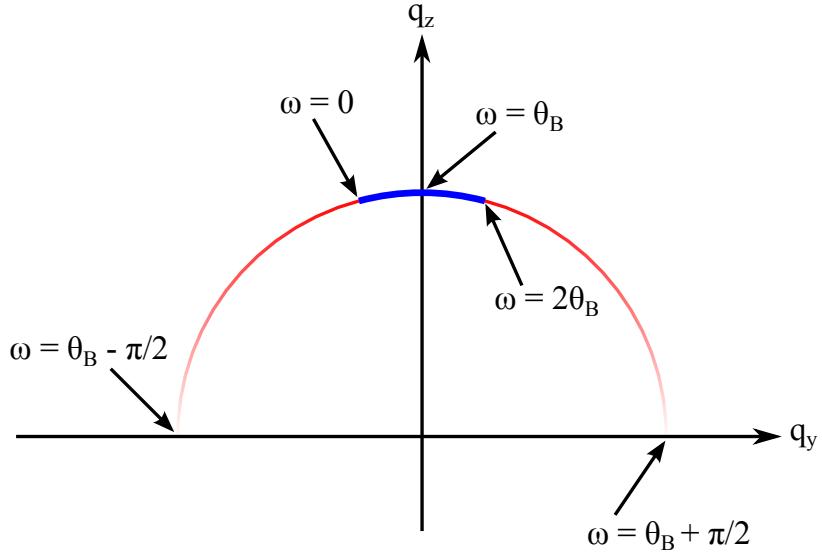


Figure A.6: Rocking scan trace in q -space.

Eq. (3.14) expressed in terms of the coordinates defined in Fig. A.5 is

$$\begin{aligned} q_x &= q \cos \theta \sin \chi \\ q_y &= q (-\sin \theta \cos \omega + \cos \theta \cos \chi \sin \omega) \\ q_z &= q (\sin \theta \sin \omega + \cos \theta \cos \chi \cos \omega). \end{aligned} \quad (\text{A.18})$$

For a rocking scan focused on a particular order, $\chi = 0$ and $\theta = \theta_B$ while ω is varied about θ_B , where θ_B is the Bragg angle. Then,

$$\begin{aligned} q_x &= 0 \\ q_y &= q_B \sin(\omega - \theta_B) \\ q_z &= q_B \cos(\omega - \theta_B), \end{aligned} \quad (\text{A.19})$$

which shows that this scan traces a part of the circular path in the $q_x = 0$ plane as shown in Fig. A.6. As Fig. A.6 shows, however, the rocking scan only probes a small fraction of the entire distribution, limited by $2\theta_B$. As discussed in section 3.3.3, beyond $\omega = 2\theta_B$, the substrate blocks scattering. On the other hand, the ring analysis takes advantage of a two dimensional detector and can probe a substantially wider range of the distribution in principle: approximately $\pm 45^\circ$ at $\omega = \theta_B$. This method is now described.

In the ring method, we set $\omega = \theta_B$ and scan on the detector along $\theta = \theta_B$ as a function of χ . Then, Eq. (A.18) becomes

$$\begin{aligned} q_x &= q \cos \theta_B \sin \chi \\ q_y &= q \sin \theta_B \cos \theta_B (\cos \chi - 1) \\ q_z &= q(\sin^2 \theta_B + \cos^2 \theta_B \cos \chi), \end{aligned} \quad (\text{A.20})$$

where $q = 4\pi \sin \theta_B / \lambda$. For small θ_B , Eq. (A.20) reduces to

$$\begin{aligned} q_x &\approx q \sin \chi \\ q_y &\approx 0 \\ q_z &\approx q \cos \chi. \end{aligned} \quad (\text{A.21})$$

For a sharp Bragg peak, this ring method gives the same mosaic intensity $I(\chi, \theta_B)$ in Eq. (A.21) as the rocking method mosaic intensity $I(\omega - \theta_B)$ in Eq. (A.19) because the mosaic distribution $P(\alpha)$ is in-plane isotropic. Differences occur when diffuse scattering is added. The diffuse scattering intensity is much broader and weaker than the Bragg peaks. In the ring method, it can be estimated as the average from two rings offset on either side from θ_B and subtracted from the θ_B ring.

A.2.3 NFIT

The original NFIT program was written by Dr. Yufeng Liu and described in his thesis. It was used in the Nagle lab, with small updates for data handling, from 2003 until recently. A newer version has been implemented by Michael Jablin that calculates the theoretical structure factor using cylindrical domains appropriate for in-plane correlations [48] rather than rectangular domains appropriate for coherence domains. All these versions approximated the effect of mosaic spread roughly by averaging only in the q_r direction at fixed q_z which means that mosaic rings are approximated as mosaic lines or spikes. The subsequent development described here and not yet adopted by the Nagle lab calculates the structure factor $S(q_r, q_z)$ with rotational symmetry about the z -axis, which eliminates the ϕ' dependence in Eq. (A.15). The program interpolates $S(q_r, q_z)$ in terms of the spherical coordinates q and θ with $\phi = 0$ to perform the double integration in Eq. (A.15). After the mosaic spread

integration, the program performs the q_y integration described in section 2.2.6. For this integration, the calculated S_M is interpolated in terms of q_x , q_y , and q_z .

Note: if the structure factor defined in the Cartesian coordinates is desired (for a case of square domains instead of circular ones), Eq. (A.10 – A.12) can be used instead of Eq. (A.13) and (A.14).

While it is an improvement, the new program also is an approximation because it does not include the unknown form factor $|F(q_z)|$. The mosaic spread integration mixes up intensity at different q_z values, so the separation of $|F(q_z)|$ from $S(\mathbf{q})$ is in principle impossible. One way to deal with this issue would be to combine the SDP program, which determines $|F(q_z)|$, with the NFIT program, but that will end up with too many non-linear parameters. Another possibility is to limit the fitting range to regions close to the meridian. For a small range of integration, it is not unreasonable to assume that the form factor is approximately constant as can be seen from Eq. (A.12) with small q_x , q_y , and α . Therefore, the analysis developed in this appendix ignores the form factor.

A.3 More results from LAXS models

h	k	Model $F(h, k)$							Data $ F(h, k) $	σ_F
		Fit1	Fit2	Fit3	Fit4	Fit5	Fit6	Fit7		
1	-1	-74.0	-71.6	-39.4	-78.4	-77.1	-79.1	-79.8	86.3	3.7
1	0	-94.3	-89.2	-63.1	-98.6	-100.0	-99.6	-100.1	100.0	0.5
1	1	23.7	19.9	19.9	23.9	25.2	24.1	24.2	43.1	2.6
1	2	-6.0	-2.3	-8.3	-6.0	-6.9	-5.9	-6.0	0.0	3.9
1	3	0.3	-3.7	6.9	1.4	2.0	1.5	1.4	8.8	0.2
2	-2	-17.2	-20.2	-28.5	-19.7	-20.4	-20.1	-20.1	18.0	0.6
2	-1	-62.2	-59.1	-53.9	-67.9	-66.5	-65.7	-66.9	76.0	0.4
2	0	-32.1	-31.9	-30.8	-33.2	-33.0	-33.0	-33.1	28.7	0.2
2	1	31.8	30.2	32.3	31.5	31.5	32.1	32.0	39.5	0.4
2	2	-25.0	-24.2	-22.9	-24.0	-23.9	-24.3	-24.3	24.6	0.3
2	3	15.0	15.0	14.8	14.9	14.9	14.9	14.9	14.6	0.1
2	4	-6.1	-5.2	-12.0	-8.6	-8.9	-8.6	-8.5	9.2	0.2
2	5	1.1	-2.4	10.2	6.6	7.0	6.8	6.6	5.6	0.7
2	6	0.1	5.5	-4.0	-7.2	-7.1	-7.0	-7.0	4.1	0.3
3	-2	34.2	33.3	29.9	40.3	40.6	39.9	40.1	33.2	0.8
3	-1	39.4	39.1	27.6	45.5	44.9	44.0	44.4	45.9	0.4
3	0	-3.2	-4.3	-2.3	-4.3	-4.0	-4.1	-4.2	13.2	0.5
3	1	-9.4	-6.9	-11.2	-9.2	-9.6	-9.8	-9.5	0.0	7.1
3	2	14.1	12.4	15.0	14.0	14.3	14.5	14.3	10.2	0.2
3	3	-12.9	-13.7	-12.5	-13.1	-13.1	-13.2	-13.1	13.6	0.2
3	4	8.6	11.7	9.0	9.5	9.4	9.2	9.3	13.0	0.2
3	5	-4.1	-7.9	-7.1	-6.0	-5.9	-5.6	-5.7	9.6	0.1
3	6	1.1	3.6	5.4	3.9	3.9	3.6	3.7	5.6	0.4
4	-3	-18.1	-18.9	-18.0	-20.4	-21.7	-22.6	-21.6	23.0	0.6
4	-2	-48.5	-45.2	-23.9	-53.5	-53.2	-53.5	-53.0	42.8	0.5
4	-1	-17.8	-19.9	-7.8	-19.4	-19.0	-18.7	-18.7	22.6	0.9
4	0	11.3	14.3	7.8	12.7	12.6	12.7	12.6	16.2	0.1
4	1	-2.8	-7.8	-1.0	-4.1	-3.7	-3.7	-3.8	7.2	0.6
4	2	-4.0	1.6	-5.4	-2.9	-3.3	-3.5	-3.3	9.9	0.3
4	3	7.1	3.2	7.8	6.3	6.5	6.7	6.5	0.0	2.1
4	4	-6.5	-5.7	-6.8	-6.4	-6.3	-6.4	-6.4	3.0	0.3
4	5	4.2	6.1	5.0	4.7	4.4	4.3	4.4	4.1	0.2
4	6	-1.8	-4.9	-3.8	-2.8	-2.5	-2.3	-2.5	2.5	1.1

Table A.1: Form factors for $h = 1$ to 4

h	k	Model $F(h, k)$							Data $ F(h, k) $	σ_F
		Fit1	Fit2	Fit3	Fit4	Fit5	Fit6	Fit7		
5	-3	-18.2	-17.8	-26.6	-16.2	-16.4	-17.7	-17.3	15.6	0.6
5	-2	-21.1	-21.4	-19.3	-19.3	-19.3	-19.6	-19.4	16.3	0.2
5	-1	1.8	1.9	4.4	2.0	2.0	2.2	2.2	7.5	0.2
5	0	4.7	4.8	6.4	4.3	4.6	4.5	4.3	6.5	0.1
5	1	-6.1	-8.3	-8.2	-6.1	-6.4	-6.3	-6.1	6.4	0.2
6	-4	-1.9	-1.8	6.9	2.2	2.2	-3.0	-2.8	5.9	0.2
6	-3	-4.3	-4.0	7.8	6.6	6.7	-5.9	-5.9	5.9	0.2
6	-2	-1.4	-1.7	1.5	2.7	2.8	-1.7	-1.8	3.8	0.3
6	-1	0.8	1.1	-2.7	-2.0	-2.2	1.1	1.1	3.4	0.3
6	0	-0.2	-0.5	0.8	0.7	0.7	-0.3	-0.3	3.4	0.1
6	1	-0.2	0.1	1.5	0.6	0.8	-0.2	-0.2	3.9	0.1
6	2	0.3	0.3	-2.0	-1.2	-1.5	0.3	0.3	0.0	0.9
6	3	-0.2	-0.5	0.5	1.0	1.2	-0.2	-0.2	3.5	0.1
6	4	-0.1	0.6	1.5	-0.2	-0.1	0.0	0.0	3.4	0.1
7	-4	-12.8	-12.0	-13.9	-9.8	-9.7	-9.6	-9.6	10.0	0.1
7	-3	-12.8	-13.0	-7.5	-9.6	-9.6	-9.2	-9.4	8.1	0.2
7	-2	1.1	0.9	3.0	0.9	1.0	1.1	1.1	4.2	0.9
7	-1	2.2	2.5	1.8	1.5	1.7	1.7	1.7	3.6	0.2
7	0	-2.4	-3.8	-3.1	-1.8	-2.1	-2.2	-2.2	2.8	0.1
8	0	-0.8	0.1	-1.0	-0.4	0.1	-0.4	-0.4	0.0	0.9
9	-5	-5.6	-5.2	2.5	-0.7	-7.3	-8.7	-8.0	6.1	0.5
9	-4	-5.5	-5.6	1.1	-0.6	-6.6	-8.0	-7.4	5.6	0.5
9	-3	0.5	0.3	-0.7	0.1	0.7	1.1	1.0	0.0	3.3
9	-2	0.9	1.2	-0.2	0.1	1.0	1.4	1.2	3.0	0.4
9	-1	-1.0	-1.7	0.7	-0.1	-1.3	-1.9	-1.7	0.0	1.7
9	0	0.4	1.7	-0.4	0.1	0.6	1.0	0.9	2.2	0.6

Table A.2: Form factors for $h = 5$ to 9

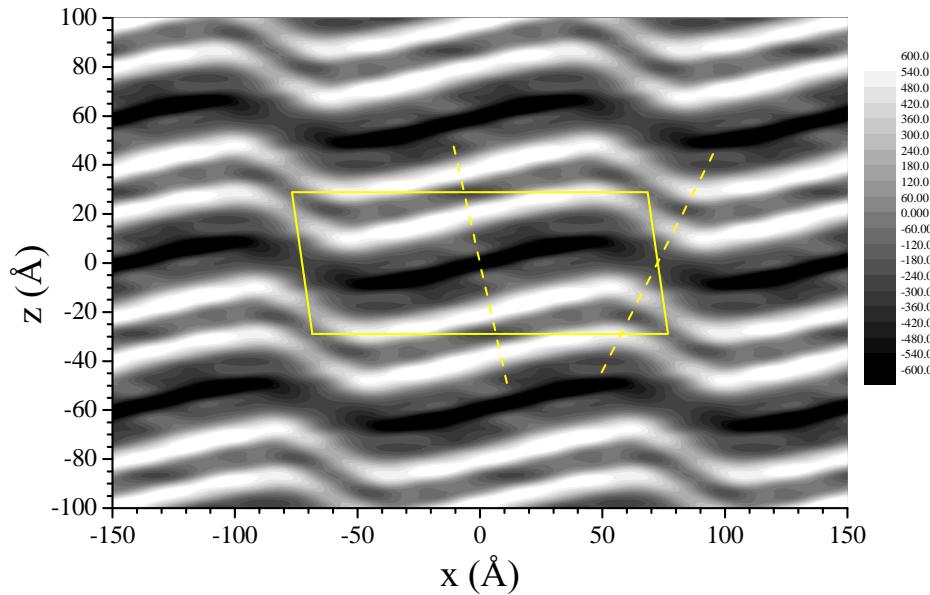


Figure A.7: Two dimensional electron density profile from Fit1.

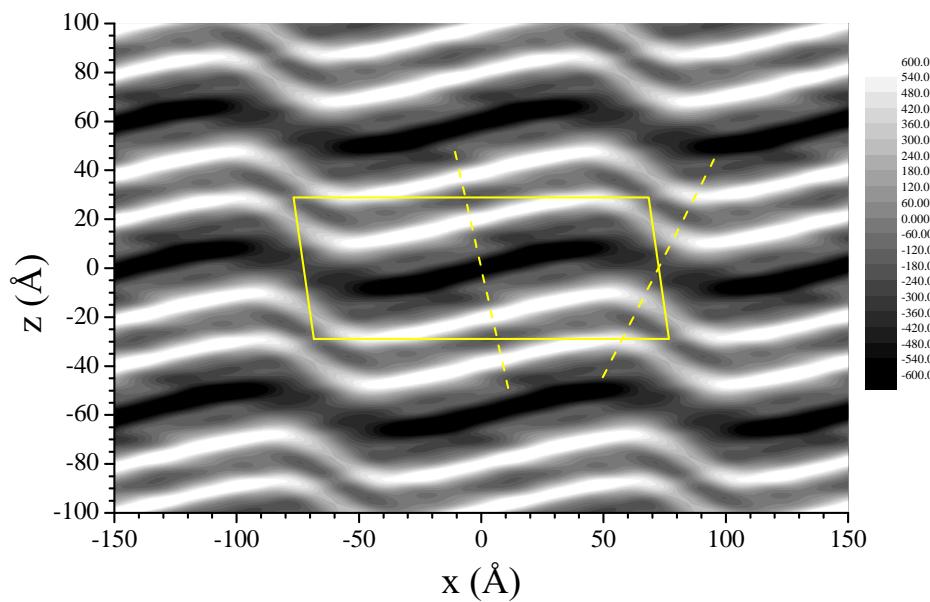


Figure A.8: Two dimensional electron density profile from Fit3.

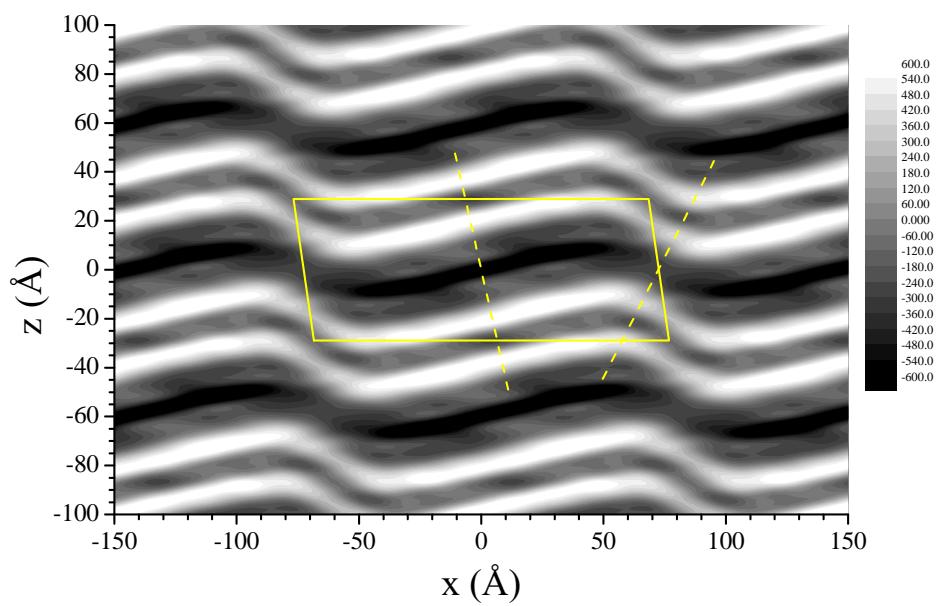


Figure A.9: Two dimensional electron density profile from Fit7.

A.4 Derivation of the contour part of the form factor

In this section, we derive F_C . The ripple profile, $u(x)$ is given by

$$u(x) = \begin{cases} -\frac{A}{\lambda_r - x_0} \left(x + \frac{\lambda_r}{2} \right) & \text{for } -\frac{\lambda_r}{2} \leq x < -\frac{x_0}{2} \\ \frac{A}{x_0} x & \text{for } -\frac{x_0}{2} \leq x \leq \frac{x_0}{2} \\ -\frac{A}{\lambda_r - x_0} \left(x - \frac{\lambda_r}{2} \right) & \text{for } \frac{x_0}{2} < x \leq \frac{\lambda_r}{2} \end{cases} \quad (\text{A.22})$$

The contour part of the form factor is the Fourier transform of the contour function, $C(x, z)$,

$$F_C(\mathbf{q}) = \frac{1}{\lambda_r} \int_{-\frac{\lambda_r}{2}}^{\frac{\lambda_r}{2}} dx \int_{-\frac{D}{2}}^{\frac{D}{2}} dz C(x, z) e^{iq_z z} e^{iq_x x}$$

As discussed in section X, the modulated models allow the electron density to modulate along the ripple direction, x . This means

$$C(x, z) = \begin{cases} f_1 \delta[z - u(x)] & \text{for } -\frac{\lambda_r}{2} \leq x < -\frac{x_0}{2} \\ \delta[z - u(x)] & \text{for } -\frac{x_0}{2} < x < \frac{x_0}{2} \\ f_1 \delta[z - u(x)] & \text{for } \frac{x_0}{2} \leq x < \frac{\lambda_r}{2} \\ + f_2 \delta\left(x + \frac{x_0}{2}\right) \delta\left(z + \frac{A}{2}\right) + f_2 \delta\left(x - \frac{x_0}{2}\right) \delta\left(z - \frac{A}{2}\right). \end{cases} \quad (\text{A.23})$$

The contribution from the minor arm is

$$\begin{aligned} & \frac{1}{\lambda_r} \int_{-\frac{\lambda_r}{2}}^{-\frac{x_0}{2}} dx e^{iq_x x} e^{iq_z u(x)} + \int_{\frac{x_0}{2}}^{\frac{\lambda_r}{2}} dx e^{iq_x x} e^{iq_z u(x)} \\ &= \frac{1}{\lambda_r} \int_{\frac{x_0}{2}}^{\frac{\lambda_r}{2}} dx e^{-i[q_x x - q_z \frac{A}{\lambda_r - x_0} (x - \frac{\lambda_r}{2})]} + \int_{\frac{x_0}{2}}^{\frac{\lambda_r}{2}} dx e^{i[q_x x - q_z \frac{A}{\lambda_r - x_0} (x - \frac{\lambda_r}{2})]} \\ &= \frac{2}{\lambda_r} \int_{\frac{x_0}{2}}^{\frac{\lambda_r}{2}} \cos \left[\left(q_x - q_z \frac{A}{\lambda_r - x_0} \right) x + q_z \frac{A}{\lambda_r - x_0} \frac{\lambda_r}{2} \right] \end{aligned} \quad (\text{A.24})$$

Using a trigonometric identity,

$$\sin u - \sin v = 2 \cos[(u + v)/2] \sin[(u - v)/2],$$

and defining

$$\omega(\mathbf{q}) = \frac{1}{2} (q_x x_0 + q_z A), \quad (\text{A.25})$$

we further simplify Eq. (A.24),

$$\begin{aligned} &= \frac{2}{\lambda_r} \frac{\lambda_r - x_0}{\frac{1}{2} q_x \lambda_r - \omega} \cos \left[\frac{1}{2} \left(\frac{1}{2} q_x \lambda_r + \omega \right) \right] \sin \left[\frac{1}{2} \left(\frac{1}{2} q_x \lambda_r - \omega \right) \right] \\ &= \frac{1}{\lambda_r} \frac{\lambda_r - x_0}{\frac{1}{2} q_x \lambda_r - \omega} \cos \left[\frac{1}{2} \left(\frac{1}{2} q_x \lambda_r + \omega \right) \right] \frac{\sin \left(\frac{1}{2} q_x \lambda_r - \omega \right)}{\cos \left[\frac{1}{2} \left(\frac{1}{2} q_x \lambda_r - \omega \right) \right]} \\ &= \frac{\lambda_r - x_0}{\lambda_r} \frac{\cos \left[\frac{1}{2} \left(\frac{1}{2} q_x \lambda_r + \omega \right) \right]}{\cos \left[\frac{1}{2} \left(\frac{1}{2} q_x \lambda_r - \omega \right) \right]} \frac{\sin \left(\frac{1}{2} q_x \lambda_r - \omega \right)}{\frac{1}{2} q_x \lambda_r - \omega}. \end{aligned} \quad (\text{A.26})$$

Similarly, we calculate the contribution from the major arm,

$$\begin{aligned} \frac{1}{\lambda_r} \int_{-\frac{x_0}{2}}^{\frac{x_0}{2}} dx e^{i \left(\frac{q_z A}{x_0} + q_x \right) x} &= \frac{2}{\lambda_r} \int_0^{\frac{x_0}{2}} dx \cos \left(\frac{q_z A}{x_0} + q_x \right) x \\ &= \frac{x_0 \sin \omega}{\lambda_r \omega} \end{aligned} \quad (\text{A.27})$$

The contribution from the kink region is

$$\begin{aligned} &\frac{1}{\lambda_r} \iint dx dz \left[\delta \left(x + \frac{x_0}{2} \right) \delta \left(z + \frac{A}{2} \right) + \delta \left(x - \frac{x_0}{2} \right) \delta \left(z - \frac{A}{2} \right) \right] e^{iq_x x} e^{iq_z z} \\ &= \frac{2}{\lambda_r} \cos \omega. \end{aligned} \quad (\text{A.28})$$

Therefore,

$$\begin{aligned} F_C(\mathbf{q}) &= \frac{x_0 \sin \omega}{\lambda_r \omega} + f_1 \frac{\lambda_r - x_0}{\lambda_r} \frac{\cos \left[\frac{1}{2} \left(\frac{1}{2} q_x \lambda_r + \omega \right) \right]}{\cos \left[\frac{1}{2} \left(\frac{1}{2} q_x \lambda_r - \omega \right) \right]} \frac{\sin \left(\frac{1}{2} q_x \lambda_r - \omega \right)}{\frac{1}{2} q_x \lambda_r - \omega} \\ &\quad + \frac{2f_2}{\lambda_r} \cos \omega \end{aligned} \quad (\text{A.29})$$

some additional models. We write the form factor as

$$F(\mathbf{q}) = F_C^M(\mathbf{q}) F_T^M(\mathbf{q}) + f_1 F_C^m(\mathbf{q}) F_T^m(\mathbf{q}) + f_2 F_C^k(\mathbf{q}) F_T^k(\mathbf{q}) \quad (\text{A.30})$$

such that

$$F_C^M = \frac{x_0}{\lambda_r} \frac{\sin \omega}{\omega} \quad (\text{A.31})$$

$$F_C^m = \frac{\lambda_r - x_0}{\lambda_r} \frac{\cos \left[\frac{1}{2} \left(\frac{1}{2} q_x \lambda_r + \omega \right) \right]}{\cos \left[\frac{1}{2} \left(\frac{1}{2} q_x \lambda_r - \omega \right) \right]} \frac{\sin \left(\frac{1}{2} q_x \lambda_r - \omega \right)}{\frac{1}{2} q_x \lambda_r - \omega} \quad (\text{A.32})$$

$$F_C^k = \frac{2}{\lambda_r} \cos \omega. \quad (\text{A.33})$$

A.5 Rotation of a Two-Dimensional Function

Let us consider rotating a function, $f(x, z)$ in two dimensions by an angle, ψ , in the counterclockwise direction (see Fig. X). This is easily achieved by rotating the coordinate system by ψ in the clockwise direction. Let rotated coordinates be x' and z' . A point in the original coordinates, (x, z) , is written as (x', z') in the new coordinates. More specifically, the point P is written as $\mathbf{P} = x\hat{\mathbf{x}} + z\hat{\mathbf{z}} = x'\hat{\mathbf{x}}' + z'\hat{\mathbf{z}}'$. $\hat{\mathbf{x}}$ and $\hat{\mathbf{z}}$ in the $x'z'$ coordinate system are written as

$$\hat{\mathbf{x}} = \cos \psi \hat{\mathbf{x}}' + \sin \psi \hat{\mathbf{z}}' \quad (\text{A.34})$$

$$\hat{\mathbf{z}} = -\sin \psi \hat{\mathbf{x}}' + \cos \psi \hat{\mathbf{z}}'. \quad (\text{A.35})$$

Pluggin these in $\mathbf{P} = x\hat{\mathbf{x}} + z\hat{\mathbf{z}}$ leads to

$$x' = x \cos \psi - z \sin \psi \quad (\text{A.36})$$

$$z' = z \cos \psi + x \sin \psi, \quad (\text{A.37})$$

the inverse of which is

$$x = x' \cos \psi + z' \sin \psi \quad (\text{A.38})$$

$$z = -x' \sin \psi + z' \cos \psi. \quad (\text{A.39})$$

Using the latter equations, $f(x, z)$ can be expressed in terms of x' and z' . The resulting function $f(x', z')$ is the rotated version of $f(x, z)$.

As an example, let us consider a Dirac delta function located at $(x, z) = (0, Z_{\text{H}})$, that is, $f(x, z) = \delta(x)\delta(z - Z_{\text{H}})$. After the rotation by ψ , it becomes

$$\begin{aligned} f(x, z) &\rightarrow \delta(x \cos \psi + z \sin \psi)\delta(-x \sin \psi + z \cos \psi - Z_{\text{H}}) \\ &= \frac{\delta(x + z \tan \psi)}{|\cos \psi|} \frac{\delta(-x \sin \psi \cos \psi + z \cos^2 \psi - Z_{\text{H}} \cos \psi)}{1/|\cos \psi|} \\ &= \delta(x + z \tan \psi)\delta(z \tan \psi \sin \psi \cos \psi + z \cos^2 \psi - Z_{\text{H}} \cos \psi) \\ &= \delta(x + z \tan \psi)\delta(z - Z_{\text{H}} \cos \psi), \end{aligned}$$

which is a part of the expression for $T_{\psi}(x, z)$ in the simple delta function model.

A.6 Derivation of the transbilayer part of the form factor in the 2G hybrid model

In this section, we derive the trasbilayer part of the form factor calculated from the 2G hybrid model discussed in section X. Defining $z' = -x \sin \psi + z \cos \psi$, the Fourier transform of a Gaussian function along the line tilted from z -axis by ψ is

$$\begin{aligned} & \iint dz dx \rho_{\text{Hi}} \exp \left\{ -\frac{(z' - Z_{\text{Hi}})^2}{2\sigma_{\text{Hi}}^2} \right\} \delta(x \cos \psi + z \sin \psi) e^{iq_x x} e^{iq_z z} \\ &= \frac{1}{\cos \psi} \int_{-\frac{D}{2}}^{\frac{D}{2}} dz \rho_{\text{Hi}} \exp \left\{ -\frac{(z - Z_{\text{Hi}} \cos \psi)^2}{2\sigma_{\text{Hi}}^2 \cos^2 \psi} + i(q_z - q_x \tan \psi) z \right\} \\ & \approx \rho_{\text{Hi}} \sqrt{2\pi} \sigma_{\text{Hi}} \exp \left\{ i\alpha Z_{\text{Hi}} - \frac{1}{2} \alpha^2 \sigma_{\text{Hi}}^2 \right\} \end{aligned} \quad (\text{A.40})$$

with $\alpha = q_z \cos \psi - q_x \sin \psi$. Using Eq. (A.40) and adding the other side of the bilayer and the terminal methyl term, we get

$$F_{\text{G}} = \sqrt{2\pi} \left[-\rho_{\text{M}} \sigma_{\text{M}} \exp \left\{ -\frac{1}{2} \alpha^2 \sigma_{\text{M}}^2 \right\} + \sum_{i=1}^{\text{1 or 2}} 2\rho_{\text{Hi}} \sigma_{\text{Hi}} \cos(\alpha Z_{\text{Hi}}) \exp \left\{ -\frac{1}{2} \alpha^2 \sigma_{\text{Hi}}^2 \right\} \right]. \quad (\text{A.41})$$

The strip part of the model in the minus fluid convention is

$$\rho_{\text{S}}(z) = \begin{cases} -\Delta\rho & \text{for } 0 \leq z < Z_{\text{CH}_2} \cos \psi, \\ 0 & \text{for } Z_{\text{W}} \cos \psi \leq z \leq D/2, \end{cases} \quad (\text{A.42})$$

where $\Delta\rho = \rho_{\text{W}} - \rho_{\text{CH}_2}$. Then, the corresponding Fourier transform is

$$\begin{aligned} F_{\text{S}} &= \iint dz dx e^{iq_x x} e^{iq_z z} \rho_{\text{S}}(z) \delta(x \cos \psi + z \sin \psi) \\ &= \frac{2}{\cos \psi} \int_0^{Z_{\text{CH}_2} \cos \psi} dz \cos \left(\frac{\alpha}{\cos \psi} z \right) (-\Delta\rho) \\ &= -2\Delta\rho \frac{\sin(\alpha Z_{\text{CH}_2})}{\alpha}. \end{aligned} \quad (\text{A.43})$$

The bridging part of the model in the minus fluid convention is

$$\rho_B(x, z) = \frac{\Delta\rho}{2} \cos\left[\frac{-\pi}{\Delta Z_H}(z' - Z_W)\right] - \frac{\Delta\rho}{2} \quad (\text{A.44})$$

for $Z_{CH_2} \cos \psi < z < Z_W \cos \psi$, and 0 otherwise. Here, $\Delta Z_H = Z_W - Z_{CH_2}$. Then, for the strip part of the form factor, we have

$$\begin{aligned} F_B &= \iint dz dx e^{iq_x x} e^{iq_z z} \delta(x \cos \psi + z \sin \psi) \rho_B(x, z) \\ &= \frac{\Delta\rho}{\cos \psi} \int_{Z_{CH_2} \cos \psi}^{Z_W \cos \psi} dz \cos\left(\alpha \frac{z}{\cos \psi}\right) \left\{ \cos\left[-\frac{\pi}{\Delta Z_H} \left(\frac{z}{\cos \psi} - Z_W\right)\right] - 1 \right\} \\ &= \Delta\rho \left\{ \frac{\Delta Z_H \sin\left[\frac{\pi(-u+Z_W)}{\Delta Z_H} + \alpha u\right]}{-2\pi + 2\alpha \Delta Z_H} + \frac{\Delta Z_H \sin\left[\frac{\pi(u-Z_W)}{\Delta Z_H} + \alpha u\right]}{2\pi + 2\alpha \Delta Z_H} - \frac{\sin(\alpha u)}{\alpha} \right\} \Big|_{Z_{CH_2}}^{Z_W} \\ &= -\frac{\Delta\rho}{\alpha} [\sin(\alpha Z_W) - \sin(\alpha Z_{CH_2})] \\ &\quad + \frac{\Delta\rho}{2} \left(\frac{1}{\alpha + \frac{\pi}{\Delta Z_H}} + \frac{1}{\alpha - \frac{\pi}{\Delta Z_H}} \right) [\sin(\alpha Z_W) + \sin(\alpha Z_{CH_2})]. \end{aligned} \quad (\text{A.45})$$

Because our X-ray scattering intensity was measured in a relative scale, an overall scaling factor was necessary for a non linear least square fitting procedure. This means that $\Delta\rho$ can be absorbed in the scaling factor. Doing so means that the values of ρ_{Hi} and ρ_M resulting from a fitting procedure are relative to $\Delta\rho$. One way to have these parameters in the absolute scale is to integrate the bilayer electron density over the lipid volume and equate the result to the total number of electrons in the lipid, which can easily be calculated from the chemical formula. For the ripple phase study in this thesis, the absolute values of the electron density were not of importance, so the discussion was omitted in the main text.

A.7 Correction due to refractive index

q_z needs to be corrected for index of refraction [50].

Let θ' and λ' be the true scattering angle and wavelength within the sample. The wavelength by an energy analyzer, λ , and the scattering angle calculated from a position on a CCD detector, θ are apparent. The correction is not necessary in the horizontal direction. The Snell's law in Fig. X gives

$$n \cos \theta = n' \cos \theta' \quad (\text{A.46})$$

$$n\lambda = n'\lambda'. \quad (\text{A.47})$$

For low angle X-ray scattering, the momentum transfer along z direction is

$$q_z = \frac{4\pi \sin \theta'}{\lambda'} \quad (\text{A.48})$$

$$= \frac{4\pi n'}{n\lambda} \sin \theta' \quad (\text{A.49})$$

$$= \frac{4\pi n'}{n\lambda} \sqrt{1 - \cos^2 \theta'} \quad (\text{A.50})$$

$$= \frac{4\pi n'}{n\lambda} \sqrt{1 - \left(\frac{n}{n'} \cos \theta\right)^2}. \quad (\text{A.51})$$

The apparent scattering angle, θ , is directly related to the vertical pixel position, p_z , by

$$\theta = \frac{1}{2} \tan^{-1} \left(\frac{p_z}{S} \right), \quad (\text{A.52})$$

where S is the sample-to-detector distance. The typical units of S and p_z are in mm. In our experimental setup, $n = 1$ and $n' = 0.9999978$ for lipids at $\lambda = 1.18 \text{ \AA}$. $S = 359.7 \text{ mm}$.

A.8 Thin Rod Model of the ripple phase

The thin rod model will be applied to the ripple phase WAXS. In this model, electron density of lipid chains are described as delta functions and lipid head groups are assumed not to contribute to scattering. Since the molecular packing of the major side of ripple phase is hypothesized to be gel-like, the model may be adequate. First, we will study diffraction from chains packed in gel phase manner whose system size is infinite but whose packing plane make an angle ξ with the xy plane. This infinite case is adequate for indexing the ripple Bragg peaks while it ignores the peak broadening effect. The system will later be truncated along the ripple direction to see the effect of the finite size on peak broadening. Finally, in-plane powder will be taken into account to derive a peak intensity pattern.

First, let us calculate the positions of the diffraction peaks from a two dimensional orthorhombic lattice whose plane makes an angle ξ with respect to the xy plane and extends to infinity. As a unit cell, we will take a parallelepipedon containing two rods, one located at the origin and the other located at the center (Fig. A.10). The lattice vectors are $\mathbf{a}_1 = a_1 \cos \xi \hat{\mathbf{x}} + a_1 \sin \xi \hat{\mathbf{z}}$ and $\mathbf{a}_2 = a_2 \hat{\mathbf{y}}$. **There are other choices for how the lattice is oriented with respect to the ripple direction, which should be considered as well.** Then, the Laue conditions are given by

$$2\pi h = \mathbf{q} \cdot \mathbf{a}_1 = (a_1 \cos \xi) q_x + (a_1 \sin \xi) q_z \quad (\text{A.53})$$

$$2\pi k = \mathbf{q} \cdot \mathbf{a}_2 = a_2 q_y, \quad (\text{A.54})$$

with h and k being zero or integer. Let us define the chain tilt angle θ to be the angle between the stacking z direction and the chain direction. We also define ϕ to represent the direction into which chains are tilted. In other words, θ and ϕ are usual spherical coordinates with respect to the ripple x , y , and z axes, not the local bilayer Cartesian axes. With this choice of coordinates, chains are tilted with respect to the local bilayer normal if $\theta = 0$. $\theta = \xi$ and $\phi = \pi$ gives chains parallel to the local bilayer normal, or $\theta_t = 0$. **It would be good to work out the relation between θ and θ_t , θ_t being the chain tilt with respect to the local bilayer normal.**

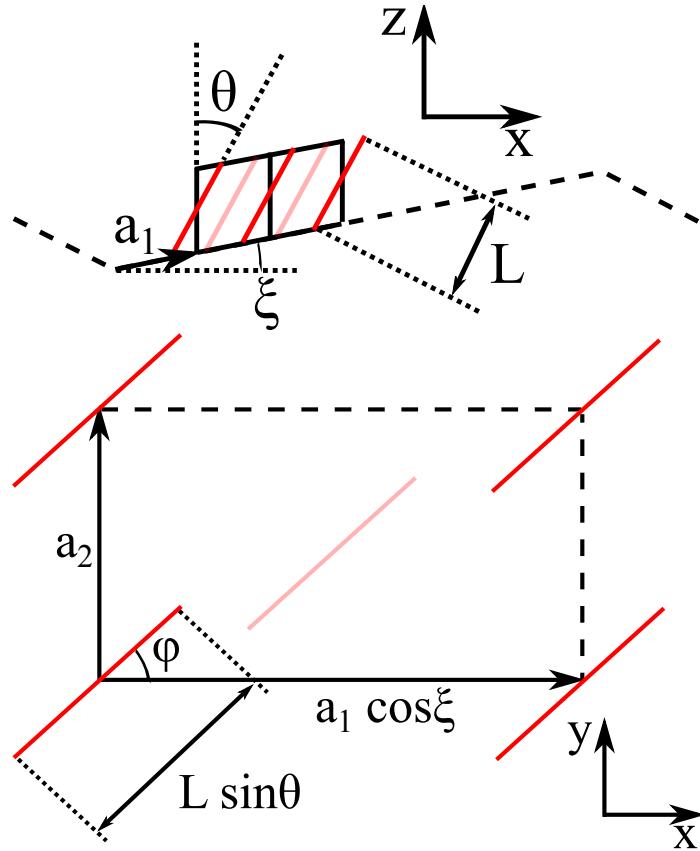


Figure A.10: Unit cell for chain packing in the major arm. (top) Projection of the unit cell in the xz -plane. The unit cell is taken as a parallelepipedon shown by black solid lines, each unit cell containing two chains. Chains located at the center of the unit cell are drawn as opaque red lines while chains at the lattice points are drawn as solid red lines. The dash line indicates the mid-plane of a rippling bilayer. Chains are tilted with respect to the stacking z direction by θ and the major arm is tilted with respect to the ripple x direction by ξ . The chain length is denoted by L . \mathbf{a}_1 and \mathbf{a}_2 are orthorhombic unit cell vectors. (bottom) Projection of the unit cell in the xy -plane. $\phi = 0$ means chains are tilted in the xz plane and $\phi = \pi/2$ means chains are titled into the direction perpendicular to the ripple direction.

The electron density, assuming a delta function for each chain, is given by

$$\rho(\mathbf{r}) = \delta(x - \alpha z, y - \beta z) + \quad (\text{A.55})$$

$$\delta \left[x - \frac{a_1 \cos \xi}{2} - \alpha \left(z - \frac{a_1 \sin \xi}{2} \right), y - \frac{a_2}{2} - \beta \left(z - \frac{a_1 \sin \xi}{2} \right) \right], \quad (\text{A.56})$$

where $\alpha = \tan \theta \cos \phi$ and $\beta = \tan \theta \sin \phi$. The first rod extends for

$$-L/2 \sin \theta \cos \phi \leq x \leq L/2 \sin \theta \cos \phi \quad (\text{A.57})$$

$$-L/2 \sin \theta \sin \phi \leq y \leq L/2 \sin \theta \sin \phi \quad (\text{A.58})$$

$$-L/2 \cos \theta \leq z \leq L/2 \cos \theta, \quad (\text{A.59})$$

and the second rod for

$$-L/2 \sin \theta \cos \phi + a_1/2 \cos \xi \leq x \leq L/2 \sin \theta \cos \phi + a_1/2 \cos \xi \quad (\text{A.60})$$

$$-L/2 \sin \theta \sin \phi + a_2/2 \leq y \leq L/2 \sin \theta \sin \phi + a_2/2 \quad (\text{A.61})$$

$$-L/2 \cos \theta + a_1/2 \sin \xi \leq z \leq L/2 \cos \theta + a_1/2 \sin \xi. \quad (\text{A.62})$$

Then, the form factor is given by

$$F(\mathbf{q}) = \int dx \int dy \int dz \rho(\mathbf{r}) e^{i\mathbf{q} \cdot \mathbf{r}} \quad (\text{A.63})$$

$$\begin{aligned} &= \int_{-\frac{L}{2} \cos \theta}^{\frac{L}{2} \sin \theta} dz e^{i(\alpha q_x + \beta q_y + q_z)z} + \\ &\int_{-\frac{L}{2} \cos \theta + \frac{a_1}{2} \sin \xi}^{\frac{L}{2} \cos \theta + \frac{a_1}{2} \sin \xi} dz e^{\frac{i}{2}[q_x(a_1 \cos \xi - \alpha a_1 \sin \xi) + q_y(a_2 - \beta a_1 \sin \xi)]} e^{i(\alpha q_x + \beta q_y + q_z)z} \\ &= \left[1 + e^{\frac{i}{2}(a_1 \cos \xi q_x + a_1 \sin \xi q_z + a_2 q_y)} \right] \frac{2}{\gamma} \sin \left(\frac{\gamma L \cos \theta}{2} \right) \\ &= [1 + e^{i\pi(h+k)}] \frac{2}{\gamma} \sin \left(\frac{\gamma L \cos \theta}{2} \right), \end{aligned} \quad (\text{A.64})$$

where $\gamma = \alpha q_x + \beta q_y + q_z$. Eq. A.64 shows that peaks with $h+k$ being odd is extinct. For $h+k$ even, we have

$$F(\mathbf{q}) = \frac{4}{\gamma} \sin \left(\frac{\gamma L \cos \theta}{2} \right). \quad (\text{A.65})$$

For (20) peak, $q_y = 0$ and $4\pi = a_1 \cos \xi q_x + a_1 \sin \xi q_z$. The second equation can be rewritten to give

$$q_z = -\frac{1}{\tan \xi} q_x + \frac{4\pi}{a_1 \sin \xi} \quad (\text{A.66})$$

which defines a straight line in $q_x q_z$ -plane along which (20) Bragg rod appears. Eq. A.65 has a peak at $\gamma = 0$. Hence, the maximum intensity of (20) peak is at q_x and q_z that satisfy Laue conditions and $\gamma = 0$. This gives three equations and three unknowns. Explicitly written, we have

$$q_y = 0 \quad (\text{A.67})$$

$$4\pi = a_1 \cos \xi q_x + a_1 \sin \xi q_z \quad (\text{A.68})$$

$$0 = \tan \theta \cos \phi q_x + q_z \quad (\text{A.69})$$

Solving these, we get

$$q_x = \frac{4\pi}{a_1 \cos \xi (1 - \tan \theta_t \cos \phi \tan \xi)} \quad (\text{A.70})$$

$$q_z = \frac{-4\pi \tan \theta_t \cos \phi}{a_1 \cos \xi (1 - \tan \theta_t \cos \phi \tan \xi)} \quad (\text{A.71})$$

For $\phi = \pi/2$, we have $q_x = 4\pi/(a_1 \cos \xi)$ and $q_z = 0$, so one would expect to see a peak on the equator, the case of which is similar to $L_{\beta I}$ phase in gel phase. To get back to ordinary gel phase, ξ should be set equal to zero.

For any (hk) line, we again have three equations and three unknowns as

$$2\pi h = q_x a_1 \cos \xi + q_z a_1 \sin \xi \quad (\text{A.72})$$

$$2\pi k = q_y a_2 \quad (\text{A.73})$$

$$0 = q_x \tan \theta_t \cos \phi + \frac{2\pi k}{a_2} \tan \theta_t \sin \phi + q_z \quad (\text{A.74})$$

Solving for q_x , q_y , and q_z , we obtain

$$q_x = \frac{2\pi(h + ka\beta \sin \xi)}{a_1 \cos \xi(1 - \alpha \tan \xi)} \quad (\text{A.75})$$

$$q_y = \frac{2\pi k}{a_2} \quad (\text{A.76})$$

$$q_z = \frac{-2\pi(h\alpha + ka\beta \cos \xi)}{a_1 \cos \xi(1 - \alpha \tan \xi)}, \quad (\text{A.77})$$

where $a = a_1/a_2$.

Bibliography

- [1] PF Fahey and WW Webb. Lateral diffusion in phospholipid bilayer membranes and multilamellar liquid crystals. *Biochemistry*, 17(15):3046–3053, 1978.
- [2] A. Tardieu, Vittorio Luzzati, and F.C. Reman. Structure and polymorphism of the hydrocarbon chains of lipids: A study of lecithin-water phases. *Journal of Molecular Biology*, 75(4):711 – 733, 1973.
- [3] Elizabeth J Luna and Harden M McConnell. The intermediate monoclinic phase of phosphatidylcholines. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 466(3):381–392, 1977.
- [4] Bruce R. Copeland and Harden M. McConnel. The rippled structure in bi-layer membranes of phosphatidylcholine and binary mixtures of phosphatidyl-choline and cholesterol. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 599(1):95 – 109, 1980.
- [5] D Ruppel and E Sackmann. On defects in different phases of two-dimensional lipid bilayers. *Journal de Physique*, 44(9):1025–1034, 1983.
- [6] JAN Zasadzinski and MB Schneider. Ripple wavelength, amplitude, and configuration in lyotropic liquid crystals as a function of effective headgroup size. *Journal de Physique*, 48(11):2001–2011, 1987.
- [7] JA Zasadzinski, J Schneir, J Gurley, V Elings, and PK Hansma. Scanning tunneling microscopy of freeze-fracture replicas of biomembranes. *Science*, 239(4843):1013–1015, 1988.
- [8] Daniel C. Wack and Watt W. Webb. Synchrotron x-ray study of the modulated lamellar phase $p\beta'$ in the lecithin-water system. *Phys. Rev. A*, 40:2712–2730, Sep 1989.

- [9] RJ Wittebort, CF Schmidt, and RG Griffin. Solid-state carbon-13 nuclear magnetic resonance of the lecithin gel to liquid-crystalline phase transition. *Biochemistry*, 20(14):4223–4228, 1981.
- [10] Marilyn B Schneider, WINSTON K Chan, and Watt W Webb. Fast diffusion along defects and corrugations in phospholipid p beta, liquid crystals. *Biophysical journal*, 43(2):157–165, 1983.
- [11] GS Smith, EB Sirota, CR Safinya, and Noel A Clark. Structure of the 1 β phases in a hydrated phosphatidylcholine multimembrane. *Physical review letters*, 60(9):813, 1988.
- [12] Rainer Fischer, Mariola Fotin-Mleczek, Hansjrg Hufnagel, and Roland Brock. Break on through to the other sidebiophysics and cell biology shed light on cell-penetrating peptides. *ChemBioChem*, 6(12):2126–2142, 2005.
- [13] Alain Joliot and Alain Prochiantz. Transduction peptides: from technology to physiology. *Nat Cell Biol*, 6(3), 2004.
- [14] Maria Lindgren, Mattias Hillbrink, Alain Prochiantz, and lo Langel. Cell-penetrating peptides. *Trends in Pharmacological Sciences*, 21(3):99 – 103, 2000.
- [15] Alan D. Frankel and Carl O. Pabo. Cellular uptake of the tat protein from human immunodeficiency virus. *Cell*, 55(6):1189 – 1193, 1988.
- [16] Maurice Green and Paul M. Loewenstein. Autonomous functional domains of chemically synthesized human immunodeficiency virus tat trans-activator protein. *Cell*, 55(6):1179 – 1188, 1988.
- [17] Eric Viks, Priscille Brodin, and Bernard Lebleu. Hiv-1 tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus. *Journal of Biological Chemistry*, 272(25):16010–16017, 1997.
- [18] Gohar Ter-Avetisyan, Gisela Tnnemann, Danny Nowak, Matthias Nitschke, Andreas Herrmann, Marek Drab, and M. Cristina Cardoso. Cell entry of arginine-rich peptides is independent of endocytosis. *Journal of Biological Chemistry*, 284(6):3370–3378, 2009.

- [19] Gisela Tnnemann, Robert M. Martin, Simone Haupt, Christoph Patsch, Frank Edenhofer, and M. Cristina Cardoso. Cargo-dependent mode of uptake and bioavailability of tat-containing proteins and peptides in living cells. *The FASEB Journal*, 20(11):1775–1784, 2006.
- [20] Andr Ziegler, Pierluigi Nervi, Markus Drrenberger, and Joachim Seelig. The cationic cell-penetrating peptide cpptat derived from the hiv-1 protein tat is rapidly transported into living fibroblasts: optical, biophysical, and metabolic evidence. *Biochemistry*, 44(1):138–148, 2005. PMID: 15628854.
- [21] J. S. Wadia, R. V. Stan, and S. F. Dowdy. Transducible tat-ha fusogenic peptide enhances escape of tat-fusion proteins after lipid raft macropinocytosis. *Nature Medicine*, 10(3):310–315, 2004.
- [22] I. M. Kaplan, J. S. Wadia, and S. F. Dowdy. Cationic tat peptide transduction domain enters cells by macropinocytosis. *Journal of Controlled Release*, 102(1):247–253, 2005.
- [23] David A Mann and Alan D Frankel. Endocytosis and targeting of exogenous hiv-1 tat protein. *The EMBO journal*, 10(7):1733, 1991.
- [24] Jean Philippe Richard, Kamran Melikov, Hilary Brooks, Paul Prevot, Bernard Lebleu, and Leonid V Chernomordik. Cellular uptake of unconjugated tat peptide involves clathrin-dependent endocytosis and heparan sulfate receptors. *Journal of Biological Chemistry*, 280(15):15300–15306, 2005.
- [25] Simon W Jones, Richard Christison, Ken Bundell, Catherine J Voyce, Sarah Brockbank, Peter Newham, and Mark A Lindsay. Characterisation of cell-penetrating peptide-mediated peptide delivery. *British journal of pharmacology*, 145(8):1093–1102, 2005.
- [26] Agnès Vendeville, Fabienne Rayne, Anne Bonhoure, Nadir Bettache, Philippe Montcourrier, and Bruno Beaumelle. Hiv-1 tat enters t cells using coated pits before translocating from acidified endosomes and eliciting biological responses. *Molecular biology of the cell*, 15(5):2347–2360, 2004.
- [27] Christina Foerg, Urs Ziegler, Jimena Fernandez-Carneado, Ernest Giralt, Robert Rennert, Annette G Beck-Sickinger, and Hans P Merkle. Decoding the

- entry of two novel cell-penetrating peptides in hela cells: lipid raft-mediated endocytosis and endosomal escape. *Biochemistry*, 44(1):72–81, 2005.
- [28] Antonio Fittipaldi and Mauro Giacca. Transcellular protein transduction using the tat protein of hiv-1. *Advanced drug delivery reviews*, 57(4):597–608, 2005.
- [29] Ying Liu, Melina Jones, Cynthia M Hingtgen, Guojun Bu, Nick Laribee, Rudolph E Tanzi, Robert D Moir, Avindra Nath, and Johnny J He. Uptake of hiv-1 tat protein mediated by low-density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands. *Nature medicine*, 6(12):1380–1387, 2000.
- [30] Vladimir P Torchilin, Ram Rammohan, Volkmar Weissig, and Tatyana S Levchenko. Tat peptide on the surface of liposomes affords their efficient intracellular delivery even at low temperature and in the presence of metabolic inhibitors. *Proceedings of the National Academy of Sciences*, 98(15):8786–8791, 2001.
- [31] Vladimir P Torchilin, Tatyana S Levchenko, Ram Rammohan, Natalia Volodina, Brigitte Papahadjopoulos-Sternberg, and Gerard GM D’Souza. Cell transfection in vitro and in vivo with nontoxic tat peptide-liposome–dna complexes. *Proceedings of the National Academy of Sciences*, 100(4):1972–1977, 2003.
- [32] Carsten Rudolph, Christian Plank, James Lausier, Ulrike Schillinger, Rainer H Müller, and Joseph Rosenecker. Oligomers of the arginine-rich motif of the hiv-1 tat protein are capable of transferring plasmid dna into cells. *Journal of Biological Chemistry*, 278(13):11411–11418, 2003.
- [33] Ashok Chauhan, Akshay Tikoo, Arvinder K Kapur, and Mahavir Singh. The taming of the cell penetrating domain of the hiv tat: myths and realities. *Journal of Controlled Release*, 117(2):148–162, 2007.
- [34] JM Sabatier, E Vives, K Mabrouk, ABDELAZIZ Benjouad, H Rochat, A Duval, B Hue, and ELMOSTAFA Bahraoui. Evidence for neurotoxic activity of tat from human immunodeficiency virus type 1. *Journal of virology*, 65(2):961–967, 1991.

- [35] A. Mishra, V. D. Gordon, L. H. Yang, R. Coridan, and G. C. L. Wong. Hiv tat forms pores in membranes by inducing saddle-splay curvature: Potential role of bidentate hydrogen bonding. *Angewandte Chemie-International Edition*, 47(16):2986–2989, 2008.
- [36] S. T. Yang, E. Zaitseva, L. V. Chernomordik, and K. Melikov. Cell-penetrating peptide induces leaky fusion of liposomes containing late endosome-specific anionic lipid. *Biophysical Journal*, 99(8):2525–2533, 2010.
- [37] P. E. G. Thoren, D. Persson, E. K. Esbjorner, M. Goksor, P. Lincoln, and B. Norden. Membrane binding and translocation of cell-penetrating peptides. *Biochemistry*, 43(12):3471–3489, 2004.
- [38] SD Krämer and H Wunderli-Allenspach. No entry for tat (44–57) into liposomes and intact mdck cells: novel approach to study membrane permeation of cell-penetrating peptides. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1609(2):161–169, 2003.
- [39] C. Ciobanasu, J. P. Siebrasse, and U. Kubitscheck. Cell-penetrating hiv1 tat peptides can generate pores in model membranes. *Biophysical Journal*, 99(1):153–62, 2010.
- [40] Philip A Gurnev, Sung-Tae Yang, Kamran C Melikov, Leonid V Chernomordik, and Sergey M Bezrukov. Cationic cell-penetrating peptide binds to planar lipid bilayers containing negatively charged lipids but does not induce conductive pores. *Biophysical journal*, 104(9):1933–1939, 2013.
- [41] H. D. Herce, A. E. Garcia, J. Litt, R. S. Kane, P. Martin, N. Enrique, A. Rebolledo, and V. Milesi. Arginine-rich peptides destabilize the plasma membrane, consistent with a pore formation translocation mechanism of cell-penetrating peptides. *Biophysical Journal*, 97(7):1917–1925, 2009.
- [42] Y. C. Su, A. J. Waring, P. Ruchala, and M. Hong. Membrane-bound dynamic structure of an arginine-rich cell-penetrating peptide, the protein transduction domain of hiv tat, from solid-state nmr. *Biochemistry*, 49(29):6009–6020, 2010.

- [43] S. Shojania and J. D. O’Neil. Hiv-1 tat is a natively unfolded protein - the solution conformation and dynamics of reduced hiv-1 tat-(1-72) by nmr spectroscopy. *Journal of Biological Chemistry*, 281(13):8347–8356, 2006.
- [44] P. Bayer, M. Kraft, A. Ejchart, M. Westendorp, R. Frank, and P. Rosch. Structural studies of hiv-1 tat protein. *Journal of Molecular Biology*, 247(4):529–535, 1995.
- [45] H. D. Herce and A. E. Garcia. Molecular dynamics simulations suggest a mechanism for translocation of the hiv-1 tat peptide across lipid membranes. *Proceedings of the National Academy of Sciences of the United States of America*, 104(52):20805–20810, 2007.
- [46] S. Yesylevskyy, S. J. Marrink, and A. E. Mark. Alternative mechanisms for the interaction of the cell-penetrating peptides penetratin and the tat peptide with lipid bilayers. *Biophysical Journal*, 97(1):40–49, 2009.
- [47] SL Barna, MW Tate, SM Gruner, and EF Eikenberry. Calibration procedures for charge-coupled device x-ray detectors. *Review of Scientific Instruments*, 70(7):2927–2934, 1999.
- [48] Y. Lyatskaya, Y. F. Liu, S. Tristram-Nagle, J. Katsaras, and J. F. Nagle. Method for obtaining structure and interactions from oriented lipid bilayers. *Physical Review E*, 63(1):0119071–0119079, 2001.
- [49] Y. F. Liu and J. F. Nagle. Diffuse scattering provides material parameters and electron density profiles of biomembranes. *Physical Review E*, 69(4):040901–040904(R), 2004.
- [50] Yufeng Liu. *NEW METHOD TO OBTAIN STRUCTURE OF BIOMEMBRANES USING DIFFUSE -RAY SCATTERING: APPLICATION TO FLUID PHASE DOPC LIPID BILAYERS*. PhD thesis, Carnegie Mellon University, 2003.
- [51] John F Nagle and Stephanie Tristram-Nagle. Structure of lipid bilayers. *Biochimica et Biophysica Acta (BBA)-Reviews on Biomembranes*, 1469(3):159–195, 2000.

- [52] 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.
- [53] 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.
- [54] 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.
- [55] <http://seal.web.cern.ch/seal/documents/minuit/mnusersguide.pdf>.
- [56] <http://lcgapp.cern.ch/project/cls/work-packages/mathlibs/minuit/index.html>.
- [57] 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.
- [58] 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.
- [59] 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.
- [60] 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.
- [61] W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey, and M. L. Klein. Comparison of simple potential functions for simulating liquid water. *Journal of Chemical Physics*, 79(2):926–935, 1983.

- [62] Norbert Kuerka, John Katsaras, and JohnF. Nagle. Comparing membrane simulations to scattering experiments: Introducing the simtoexp software. *Journal of Membrane Biology*, 235(1):43–50, 2010.
- [63] Shuichi Miyamoto and Peter A Kollman. Settle: an analytical version of the shake and rattle algorithm for rigid water models. *Journal of computational chemistry*, 13(8):952–962, 1992.
- [64] B. Hess, H. Bekker, H. J. C. Berendsen, and J. G. E. M. Fraaije. Lincs: A linear constraint solver for molecular simulations. *J Comput Chem*, 18(12):1463–1472, 1997.
- [65] Tom Darden, Darrin York, and Lee Pedersen. Particle mesh ewald: An $n \log(n)$ method for ewald sums in large systems. *The Journal of chemical physics*, 98(12):10089–10092, 1993.
- [66] Giovanni Bussi, Davide Donadio, and Michele Parrinello. Canonical sampling through velocity rescaling. *The Journal of chemical physics*, 126(1):014101, 2007.
- [67] Michele Parrinello and Aneesur Rahman. Polymorphic transitions in single crystals: A new molecular dynamics method. *Journal of Applied physics*, 52(12):7182–7190, 1981.
- [68] Norbert Kučerka, Yufeng Liu, Nanjun Chu, Horia I Petrache, Stephanie Tristram-Nagle, and John F Nagle. Structure of fully hydrated fluid phase DMPC and DLPC lipid bilayers using X-ray scattering from oriented multilamellar arrays and from unilamellar vesicles. *Biophysical journal*, 88(4):2626–2637, 2005.
- [69] Norbert Kučerka, Stephanie Tristram-Nagle, and John F Nagle. Closer look at structure of fully hydrated fluid phase dppc bilayers. *Biophysical journal*, 90(11):L83–L85, 2006.
- [70] Norbert Kučerka, Stephanie Tristram-Nagle, and John F Nagle. Structure of fully hydrated fluid phase lipid bilayers with monounsaturated chains. *The Journal of membrane biology*, 208(3):193–202, 2005.

- [71] Stephanie Tristram-Nagle, Chao-Ping Yang, and John F Nagle. Thermodynamic studies of purple membrane. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 854(1):58–66, 1986.
- [72] <http://www.basic.northwestern.edu/biotools/proteinCalc.html>.
- [73] A. C. V. Johansson and E. Lindahl. The role of lipid composition for insertion and stabilization of amino acids in membranes. *Journal of Chemical Physics*, 130(18), 2009.
- [74] S. Tristram-Nagle and J. F. Nagle. Hiv-1 fusion peptide decreases bending energy and promotes curved fusion intermediates. *Biophysical Journal*, 93(6):2048–2055, 2007.
- [75] L. B. Li, I. Vorobyov, and T. W. Allen. Potential of mean force and pk(a) profile calculation for a lipid membrane-exposed arginine side chain. *Journal of Physical Chemistry B*, 112(32):9574–9587, 2008.
- [76] I. Vorobyov, L. B. Li, and T. W. Allen. Assessing atomistic and coarse-grained force fields for protein-lipid interactions: The formidable challenge of an ionizable side chain in a membrane. *Journal of Physical Chemistry B*, 112(32):9588–9602, 2008.
- [77] J. L. MacCallum, W. F. D. Bennett, and D. P. Tieleman. Distribution of amino acids in a lipid bilayer from computer simulations. *Biophysical Journal*, 94(9):3393–3404, 2008.
- [78] E. V. Schow, J. A. Freites, P. Cheng, A. Bernsel, G. von Heijne, S. H. White, and D. J. Tobias. Arginine in membranes: The connection between molecular dynamics simulations and translocon-mediated insertion experiments. *Journal of Membrane Biology*, 239(1-2):35–48, 2011.
- [79] W. C. Wimley, T. P. Creamer, and S. H. White. Solvation energies of amino acid side chains and backbone in a family of host-guest pentapeptides. *Biochemistry*, 35(16):5109–5124, 1996.
- [80] W. C. Wimley and S. H. White. Experimentally determined hydrophobicity scale for proteins at membrane interfaces. *Nature Structural Biology*, 3(10):842–848, 1996.

- [81] B. Roux. Lonely arginine seeks friendly environment. *Journal of General Physiology*, 130(2):233–236, 2007.
- [82] W. Kabsch and C. Sander. Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers*, 22(12):2577–637, 1983.
- [83] D. Choi, J. H. Moon, H. Kim, B. J. Sung, M. W. Kim, G. Y. Tae, S. K. Satija, B. Akgun, C. J. Yu, H. W. Lee, D. R. Lee, J. M. Henderson, J. W. Kwong, K. L. Lam, K. Y. C. Lee, and K. Shin. Insertion mechanism of cell-penetrating peptides into supported phospholipid membranes revealed by x-ray and neutron reflection. *Soft Matter*, 8(32):8294–8297, 2012.
- [84] K. Huang and A. E. Garcia. Free energy of translocating an arginine-rich cell-penetrating peptide across a lipid bilayer suggests pore formation. *Biophysical Journal*, 104(2):412–420, 2013.
- [85] Martin J. Janiak, Donald M. Small, and G. Graham Shipley. Nature of the thermal pretransition of synthetic phospholipids: dimyristoyl- and dipalmitoyl-lecithin. *Biochemistry*, 15(21):4575–4580, 1976.
- [86] Martin J Janiak, Donald M Small, and G Graham Shipley. Temperature and compositional dependence of the structure of hydrated dimyristoyl lecithin. *Journal of Biological Chemistry*, 254(13):6068–6078, 1979.
- [87] Haruhiko Yao, Sinzi Matuoka, Boris Tenchov, and Ichiro Hatta. Metastable ripple phase of fully hydrated dipalmitoylphosphatidylcholine as studied by small angle x-ray scattering. *Biophysical journal*, 59(1):252–255, 1991.
- [88] W J Sun, S Tristram-Nagle, R M Suter, and J F Nagle. Structure of the ripple phase in lecithin bilayers. *Proceedings of the National Academy of Sciences*, 93(14):7008–7012, 1996.
- [89] Beth A Cunningham, Ari-David Brown, David H Wolfe, W Patrick Williams, and Anthony Brain. Ripple phase formation in phosphatidylcholine: Effect of acyl chain relative length, position, and unsaturation. *Physical Review E*, 58(3):3662, 1998.

- [90] Kell Mortensen, Walter Pfeiffer, Erich Sackmann, and Wolfgang Knoll. Structural properties of a phosphatidylcholine-cholesterol system as studied by small-angle neutron scattering: ripple structure and phase diagram. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 945(2):221–245, 1988.
- [91] Jeremy P Bradshaw, Michael S Edenborough, Philip JH Sizer, and Anthony Watts. Observation of rippled dioleoylphosphatidylcholine bilayers by neutron diffraction. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 987(1):111–114, 1989.
- [92] JT Woodward IV and JA Zasadzinski. Amplitude, wave form, and temperature dependence of bilayer ripples in the p β phase. *Physical Review E*, 53(4):R3044, 1996.
- [93] PC Mason, BD Gaulin, RM Epand, GD Wignall, and JS Lin. Small angle neutron scattering and calorimetric studies of large unilamellar vesicles of the phospholipid dipalmitoylphosphatidylcholine. *Physical Review E*, 59(3):3361, 1999.
- [94] M. P. Hentschel and F. Rustichelli. Structure of the ripple phase P'_β in hydrated phosphatidylcholine multimembranes. *Phys. Rev. Lett.*, 66:903–906, Feb 1991.
- [95] John Katsaras, Stephanie Tristram-Nagle, Yufeng Liu, RL Headrick, E Fontes, PC Mason, and John F Nagle. Clarification of the ripple phase of lecithin bilayers using fully hydrated, aligned samples. *Physical Review E*, 61(5):5668, 2000.
- [96] Kheya Sengupta, V. A. Raghunathan, and John Katsaras. Structure of the ripple phase of phospholipid multibilayers. *Phys. Rev. E*, 68:031710, Sep 2003.
- [97] Li Li and Ji-Xin Cheng. Coexisting stripe-and patch-shaped domains in giant unilamellar vesicles. *Biochemistry*, 45(39):11819–11826, 2006.
- [98] Alex H. de Vries, Serge Yefimov, Alan E. Mark, and Siewert J. Marrink. Molecular structure of the lecithin ripple phase. *Proceedings of the National Academy of Sciences of the United States of America*, 102(15):5392–5396, 2005.
- [99] Olaf Lenz and Friederike Schmid. Structure of symmetric and asymmetric “ripple” phases in lipid bilayers. *Phys. Rev. Lett.*, 98:058104, Jan 2007.

- [100] C-M Chen, TC Lubensky, and FC MacKintosh. Phase transitions and modulated phases in lipid bilayers. *Physical Review E*, 51(1):504, 1995.
- [101] J. Katsaras and V. A. Raghunathan. Molecular chirality and the ripple phase of phosphatidylcholine multibilayers. *Phys. Rev. Lett.*, 74:2022–2025, Mar 1995.
- [102] S. A. Tristram-Nagle. Preparation of oriented, fully hydrated lipid samples for structure determination using x-ray scattering. *Methods Mol Biol*, 400:63–75, 2007.
- [103] http://henke.lbl.gov/optical_constants.
- [104] 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.
- [105] Thalia T Mills, Gilman ES Toombes, Stephanie Tristram-Nagle, Detlef-M Smilgies, Gerald W Feigenson, and John F Nagle. Order parameters and areas in fluid-phase oriented lipid membranes using wide angle x-ray scattering. *Biophysical journal*, 95(2):669–681, 2008.
- [106] George H Vineyard. Grazing-incidence diffraction and the distorted-wave approximation for the study of surfaces. *Physical Review B*, 26(8):4146, 1982.
- [107] CE Miller, J Majewski, EB Watkins, DJ Mulder, T Gog, and TL Kuhl. Probing the local order of single phospholipid membranes using grazing incidence x-ray diffraction. *Physical review letters*, 100(5):058103, 2008.
- [108] S Tristram-Nagle, R Zhang, RM Suter, CR Worthington, WJ Sun, and JF Nagle. Measurement of chain tilt angle in fully hydrated bilayers of gel phase lecithins. *Biophysical journal*, 64(4):1097–1109, 1993.
- [109] Bertram Eugene Warren. *X-ray Diffraction*. Courier Dover Publications, 1969.
- [110] W-J Sun, RM Suter, MA Knewton, CR Worthington, S Tristram-Nagle, R Zhang, and JF Nagle. Order and disorder in fully hydrated unoriented bilayers of gel-phase dipalmitoylphosphatidylcholine. *Physical Review E*, 49(5):4665, 1994.

- [111] Alejandro B. Rodriguez-Navarro. Registering pole figures using an X-ray single-crystal diffractometer equipped with an area detector. *Journal of Applied Crystallography*, 40(3):631–634, Jun 2007.
- [112] Jessy L. Baker, Leslie H. Jimison, Stefan Mannsfeld, Steven Volkman, Shong Yin, Vivek Subramanian, Alberto Salleo, A. Paul Alivisatos, and Michael F. Toney. Quantification of thin film crystallographic orientation using x-ray diffraction with an area detector. *Langmuir*, 26(11):9146–9151, 2010. PMID: 20361783.