Supplement 3 - Positional ordering

Supplementary information for:

Order parameters and areas in fluid-phase oriented lipid membranes using wide angle x-ray scattering

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S3.1 Introduction

In order to quantify the positional correlation information contained in the WAXS $I(q)$ plots, the peak position (q_0) and the half-width at half-maximum (HWHM) can be plotted as a function of the angle ϕ. This supplement describes our method for determining HWHM and q_0 in comparison to a method which fit the data to a Lorentzian + linear background. Positional ordering information is presented as a function of ϕ for DOPC at different levels of hydration.

S3.2 Calculation of HWHM

For well-behaved peaks with relatively flat baselines, a simple MATLAB function can determine the peak position, and pick out where the peak starts and ends; the half-width can then be calculated automatically. For our WAXS data, the baselines are not flat, and so it is difficult to determine where the peak starts and ends, particularly the high-q end of the peak where water scattering and cutoff of the scattering data make it difficult to determine the baseline. Therefore, we determined the half-width from the low-q half of the peak. The baseline intensity was always taken as $I(q=0.8\ \text{Å}^{-1})$. Fig. S3.1 points out the baseline, q_0, and HWHM for an $I(q)$ plot integrated over a ϕ=5-10˚ sector. Note that the baseline intensity position was somewhat arbitrary. Although this method for finding half-widths is not optimal, it is sufficient for comparing trends in peak intensity and HWHM as a function of ϕ for different sample compositions and temperatures.

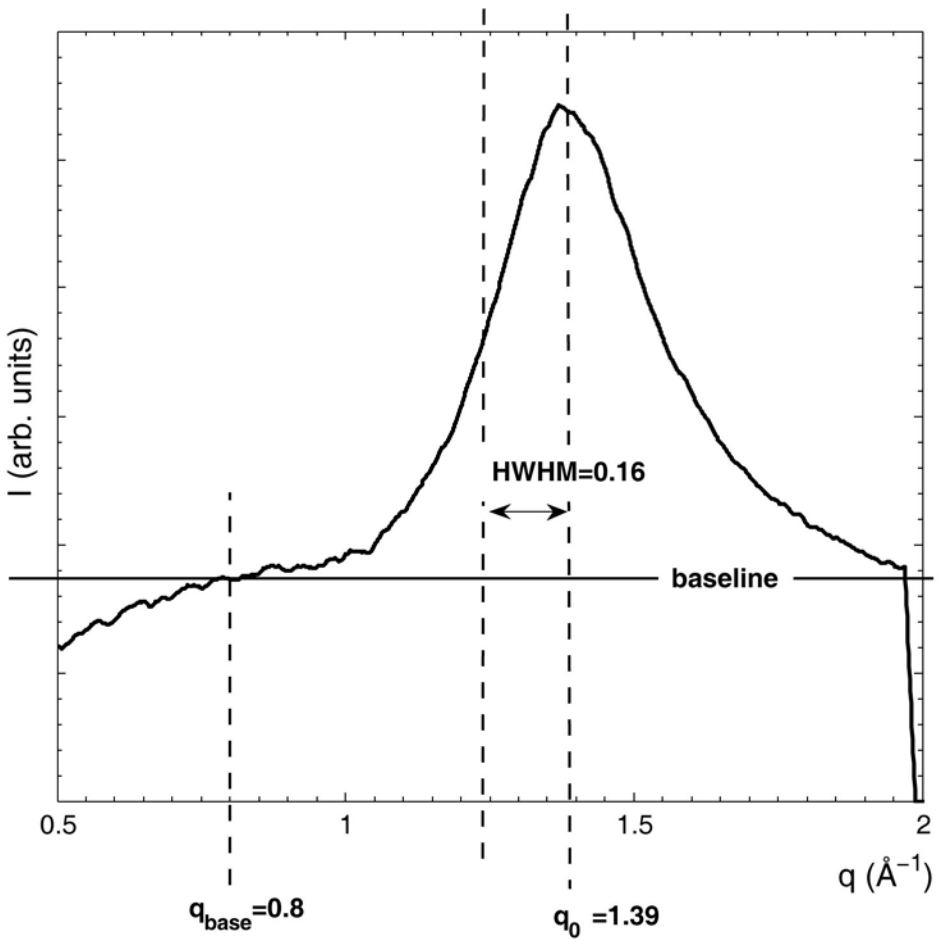

Figure S3.1. $I(q)$ for a ϕ=5-10˚ sector for DOPC (T=25˚C, D=60.0 Å). The baseline, q_0, and HWHM are pointed out on the plot.

S3.3 Attempted fits to a Lorentzian plus linear background

Spaar and Salditt (1) subtracted out non-chain scattering by fitting their $I(q)$ data for specified ϕ ranges (sector plots) to a Lorentzian plus linear background. To produce $I(\phi)$ plots, the scattering under the Lorentzians was then integrated and plotted as a function of ϕ. This method of extracting the lipid scattering provides a simple way of obtaining the peak position and the HWHM as a function of ϕ, since these values are simply fitting parameters for the Lorentzian.

Fig. S3.2 shows a fit of our WAXS data for DOPC to a Lorentzian plus linear background, given by

$$I(q) = c_0 + c_1 q + \frac{I_{\max}}{1 + \left(\frac{q - q_0}{q_w} \right)^2}, \qquad \text{(S3.1)}$$

where c_0 is the y-intercept and c_1 is the slope of the linear background, $I_{\max}$ is the peak height of the Lorentzian, q_0 is the peak center, and q_w is the HWHM. For this sample at small ϕ, q_0=1.39 Å^{-1} and q_w=HWHM=0.16 Å^{-1}, which agrees exactly with the HWHM and q_0 as obtained differently in Fig. S3.1. This sample is a best case scenario, where the water background is very low. Although the fit looks reasonable, this method does not work for images with large water backgrounds. Samples with more highly ordered chains (samples in the Lo phase) have little scattering at the larger ϕ angles. In some cases, the peak in the $I(q)$ plot at large ϕ disappears, making a fit to a Lorentzian plus linear background unreasonable. Spaar and Salditt (1) only used their fitting procedure on samples in the Ld phase with D spacings ~10 Å less than full hydration conditions. Another problem with the fits is that the slope and y-intercept for the linear backgrounds change substantially for the different ϕ ranges, as shown in the legends in Fig. S3.2. Because of these problems, we chose to produce $I(\phi)$ plots according to the method outlined in Section C of the Materials and Methods in the paper.

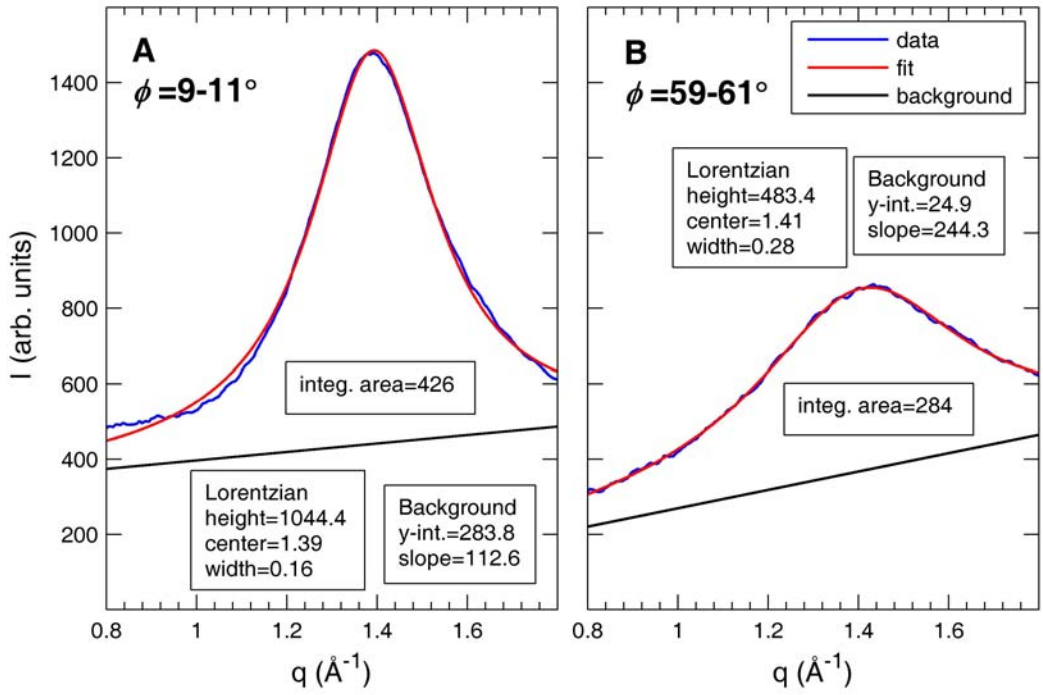

Figure S3.2. $I(q)$ for DOPC (T=25˚C, D=60.0 Å, G-1,Oct. 2006) for the following sectors: (*A*) ϕ=9-11˚ and (*B*) ϕ=59-61˚. Data are plotted in blue, and the results of a fit to Eq. S3.1 are plotted in red. The black line corresponds to the linear background. The fitting parameters and the integrated area under the Lorentzian (fit-background) are shown.

S3.4 DOPC positional ordering as a function of ϕ

Fig. S3.3 shows positional order information for DOPC at different levels of hydration. Fig. S3.3*A* shows the scattering maximum, q_0, as a function of the angle ϕ. Note that for the fully hydrated sample (D=63.3 Å), q_0 increases rapidly to a maximum of 1.7 Å^{-1}. This sample was flooded with excess water. At larger ϕ the high-q water scattering, which is isotropic, begins to overwhelm the relatively weaker lipid scattering. Therefore, the value of q_0 no longer corresponds to the maximum in the lipid scattering at large ϕ.

Fig. S3.3*B* shows the half width at half maximum (HWHM) as a function of ϕ. Spaar and Salditt (1) interpreted the increase in HWHM(ϕ) as an indication that the chain-chain scattering was less well correlated at larger ϕ. The steep rise in the HWHM(ϕ) for the D=63.3 Å plot is again due to water. If we only compare the data at low ϕ, where the complications due to water scattering are not an issue, the position of the scattering maximum, q_0 (~1.39 Å^{-1}), and the HWHM (~0.16 Å^{-1}) are relatively insensitive to hydration level for lamellar repeats of 51.1 Å to 63.3 Å.

Figs. S3.3*C* and *D* convert the $q_0(\phi)$ and HWHM(ϕ) information from reciprocal space to real space for DOPC (D =60.0 Å). The WAXS spacing (d=2π/q_0) is shown in Fig. S3.3*C*. The decrease in d as a function of ϕ is likely due to drift of q_0 to higher values as water scattering becomes relatively more prominent. The correlation length (ξ=1/HWHM) as a function of ϕ for DOPC at 25˚C is compared to Spaar and Salditt's (1) data for DMPC at 45˚C. Both samples are in the Ld phase. The DOPC $\xi(\phi)$ data is systematically larger than the DMPC data. Spaar and Salditt (1) compared several Ld phase lipids, and did not report such a large difference. The difference may be a result in the different methods used to calculate HWHM as described in the previous two sections, although these methods were in agreement for our DOPC sample. Kaganer et al. (2) point out that in the monolayer literature some groups report the correlation length as 1/HWHM (assumes Lorentzian lineshape) while others use the Scherrer equation (3), which is larger by a factor of 0.94π. Fig. S3.3*D* shows ξ=1/HWHM in order to compare with Spaar and Salditt's data.

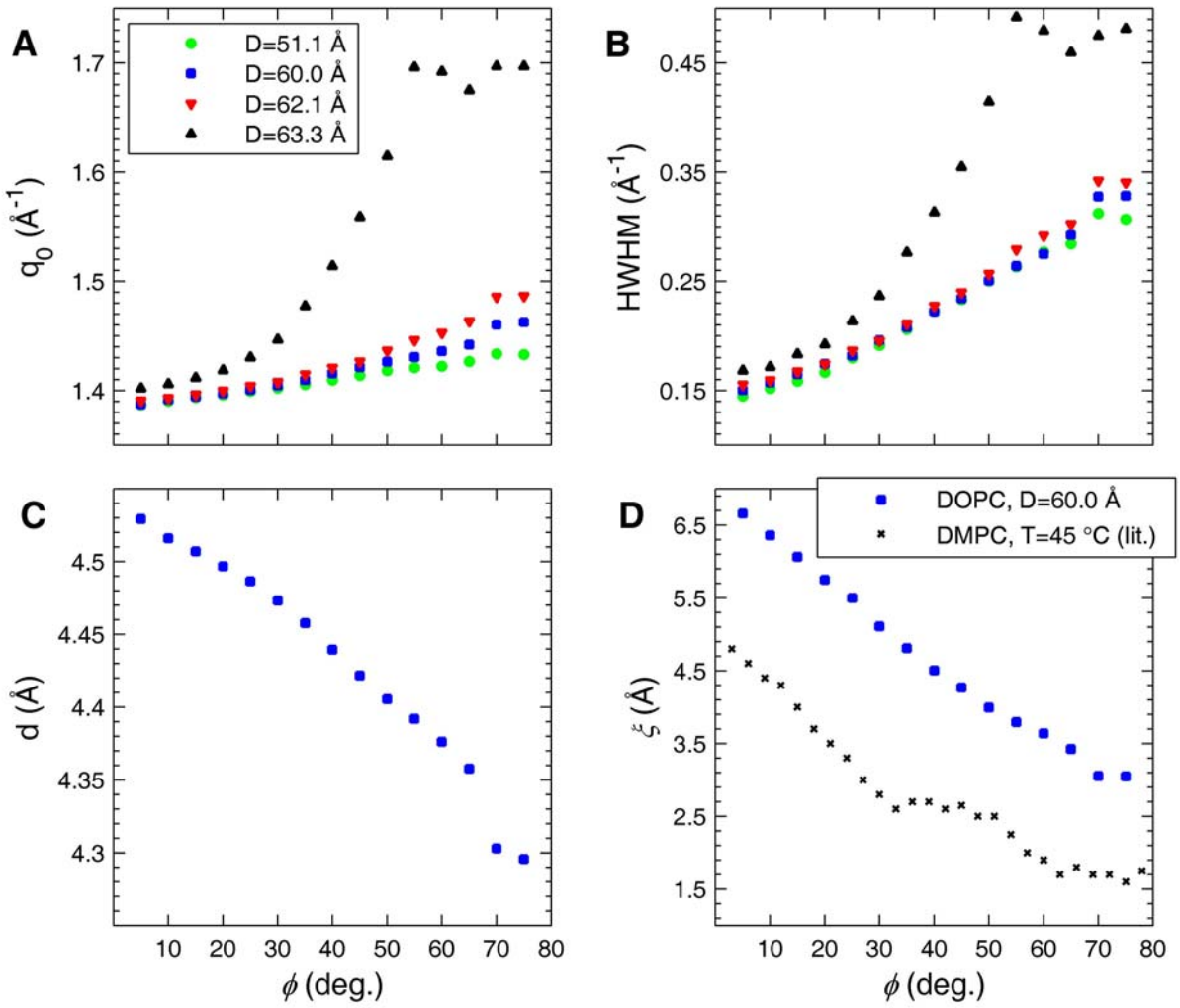

Figure S3.3. Lateral positional information from the wide-angle data for DOPC at different levels of hydration (see legend at top left). Plots (*A*) and (*B*) show the information in reciprocal space: (*A*) peak position, $q_0(\phi)$ and (*B*) HWHM(ϕ). Plots (*C*) and (*D*) convert the information to real-space for the *D*=60.0 Å data: (*C*) chain-chain spacing, $d(\phi)=2\pi/ q_0(\phi)$ and (*D*) correlation length, $\xi(\phi)=1/\text{HWHM}(\phi)$. Spaar and Salditt's $\xi(\phi)$ data for DMPC at 45˚C are shown in plot (*D*) for comparison.

REFERENCES

1. Spaar, A., and T. Salditt. 2003. Short range order of hydrocarbon chains in fluid phospholipid bilayers studied by x-ray diffraction from highly oriented membranes. Biophys. J. 85:1576-1584.
2. Kaganer, V. M., H. Möhwald, and P. Dutta. 1999. Structure and phase transitions in Langmuir monolayers. Rev. Mod. Phys. 71:779-819.
3. Guinier, A. 1963. X-ray Diffraction in Crystals, Imperfect Crystals, and Amorphous Bodies. W. H. Freeman and Company, San Francisco.