

Supplement 6 - An analytical model for chain-chain scattering

Supplementary information for:

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

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NOTE: This supplement provides a detailed explanation of the model used to analyze wide angle x-ray scattering data in the above paper. It includes derivations of the formulas used (either previously unpublished or published without derivation). It also includes an in-depth discussion of the possible pitfalls in the approach as applied to model membrane systems.

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S6.1 Introduction

Despite the biological relevance of fluid-phase lipids, much of the work involving wide-angle scattering from model membranes has been focused on gel-phase lipids. For crystalline packing in which the chains are essentially rigid rods, the most important features of the scattering pattern can be predicted by analytical models which consider the chain tilt angle and the dimensions of the crystalline lattice [see (1,2) and the references therein]. For disordered fluid phases, however, there is a distribution of tilt angles and a distribution of interchain packing distances.

An analytical model addressing all of the features of wide-angle scattering from fluid phase lipids would be very complex and require many parameters. Instead, we follow an approach commonly used in the liquid crystal literature for systems of rodlike molecules with fluid-like disorder which was popularized by Leadbetter et al. (3-5). The model focuses on describing the angular distribution of scattering based on the distribution of rod orientations, described by a chain orientational distribution function. The major assumption of the model is that the scatterers are infinitely long rigid rods, and therefore the form factor of each molecule can be neglected. Leadbetter and others are careful to note that this simplistic model does not work equally well in all situations and must be applied and interpreted with caution [for a review see (6)].

We follow the same model used by Leadbetter et al., but the resulting fitting equations in the literature are incorrect. In the following, we explain the model and present a corrected derivation of analytical equations for fitting the x-ray scattering data. We present specific cases where the literature fitting equations predict incorrect behavior, while the new formula predicts the correct behavior. We also address problems with the model as applied to model membrane systems.

S6.2 Overview: assumptions of the model

Prior to Leadbetter, the problem of determining the orientational distribution function and order parameters for systems of rodlike particles from scattering techniques was addressed by a number of researchers (7-10). The general formulas derived were complicated and cumbersome to use. Leadbetter et al. (3-5) popularized a simplified model which has been widely applied to many mesogenic particles (6). Although the model is usually attributed to Leadbetter et al., it was used earlier by Levine and Wilkins (11,12) to fit scattering data from fluid-phase egg lecithin/cholesterol mixtures.

Although we do not know the first source of the model, for simplicity we will refer to the model as the "Leadbetter model." In the model, the system is composed of straight rods of length L separated by a mean distance d_{nn} , with $L \gg d_{nn}$ (in our case $d_{nn} = 2d/\sqrt{3}$, where $d = 2\pi/q_0$). Fig. S6.1 is a schematic for the model, which defines angles and the coordinate system used for the derivation of the analytical equations for the scattering intensity. Note that the x - y - z coordinate system shown in Fig. S6.1 is with respect to the sample and is different than the experimental geometry coordinate system; we define the direction of the membrane normal, \mathbf{n} , as $+\hat{z}$. The figure and the theory are independent of the exact scattering geometry used. Later (Section S6.7) we will relate the sample geometry to the scattering geometry used for experiments on oriented lipid membranes.

The model assumes that the sample is composed of different regions of rods ("grains") with a local director \mathbf{n}_L which makes an angle β with the z -axis. Locally, the rods are assumed to be well-correlated and rotationally symmetric about \mathbf{n}_L . Given these assumptions, scattering occurs only for values of \mathbf{q} that lie on a ring at right angles to \mathbf{n}_L ($\mathbf{q} \cdot \mathbf{n}_L = 0$) with $q = q_0 = 2\pi/d$ (see Fig. S6.1A). Across the sample, \mathbf{n}_L can assume a distribution of orientations, which is described by the chain orientational distribution function, $f(\beta)$. The fraction of rods with a particular orientation is given by $f(\beta) \sin\beta d\beta d\chi$ (see Fig. S6.1C for the definition of angles). For a particular \mathbf{q} , the total scattering is a sum of the scattering contributions from grains with local directors \mathbf{n}_L perpendicular to \mathbf{q} (see Fig. S6.1B). Thus the scattering intensity $I(\phi_L)$ at an angle ϕ_L (see Fig. S6.1D) is a weighted sum of the scattering from grains with tilt angles β in the range: $\phi_L \leq \beta \leq \pi/2$. Note that our sample is rotationally symmetric about the z -axis; that is, the observed scattering intensity depends only on ϕ_L and is independent of the azimuthal angle. The following section develops a formula relating $I(\phi_L)$ to the chain orientational distribution function, $f(\beta)$.

Here we outline the strategy we followed for analysis of our x-ray scattering data based on the model described above:

1. As a starting point derive a formula which relates $I(\phi_L)$ to $f(\beta)$, the chain orientational distribution function [Section S6.3].
2. Assume a particular functional form for $f(\beta)$ with one adjustable parameter m related to the width of the orientational distribution. Insert this $f(\beta)$ into the general formula for $I(\phi_L)$ to determine a closed analytical expression for $I(\phi_L)$ [Section S6.5].
3. Fit the scattering data to obtain $f(\beta)$ with the best-fit value of the adjustable parameter m . Quantities involving average values of β , such as $S_{x\text{-ray}}$, can then be calculated from $f(\beta)$ [Section S6.5].

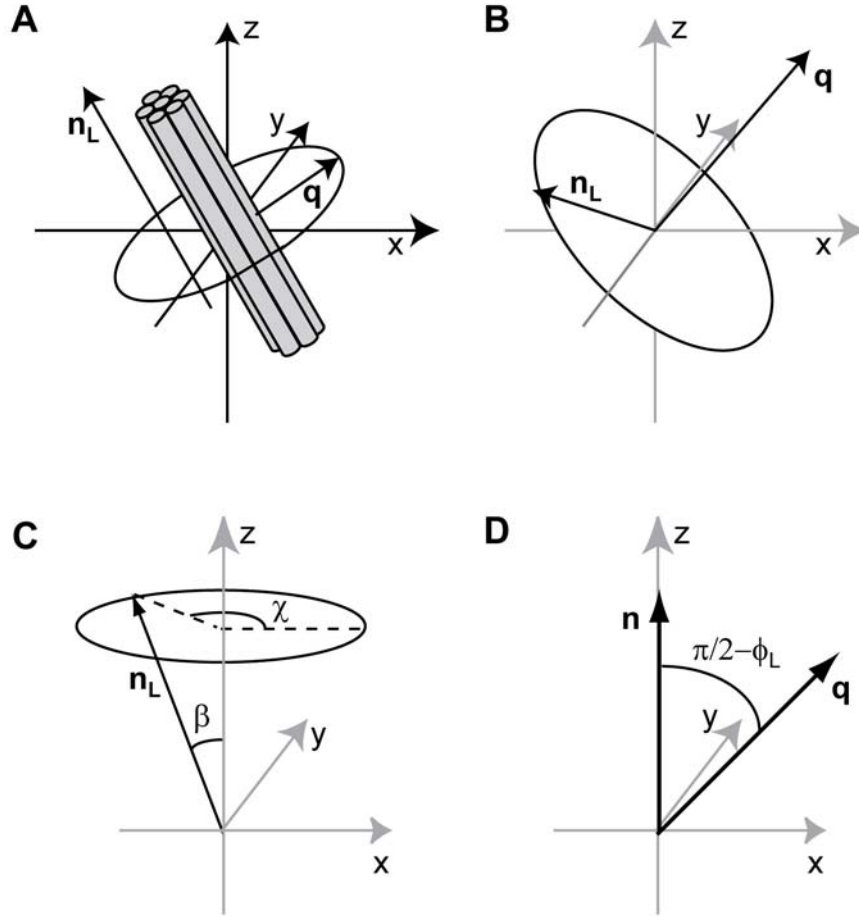


Figure S6.1. (A) In Leadbetter's model, long, thin rods are locally well-aligned along the local director \mathbf{n}_L . For each grain (group of rods) scattering is permitted at right angles to \mathbf{n}_L . (B) The scattering intensity for a given \mathbf{q} is the sum over all grains with directors lying on the ring $\mathbf{q} \cdot \mathbf{n}_L = 0$. (C) The direction of \mathbf{n}_L is described by the polar angle β and the azimuthal angle χ , the angle of the local director \mathbf{n}_L projected on the x - y plane. Across the sample, the local director \mathbf{n}_L can assume a distribution of orientations. (D) The orientation of \mathbf{q} with respect to the sample director \mathbf{n} (in our case the membrane normal) is described by the angle ϕ_L , the angle between the sample plane and \mathbf{q} , or more explicitly the angle complementary to the angle between \mathbf{n} and \mathbf{q} . This figure is modified from Fig. 7 in (13).

S6.3 Corrected calculation of $I(\phi_L)$

We first present a corrected calculation for the scattering intensity for the system described above (the limit of infinitely straight rods which form differently aligned regions). As discussed above, scattering only occurs for $\mathbf{q} \perp \mathbf{n}_L$ and $q=q_0 \approx 2\pi/d$. The scattering observed on the detector from a grain with local director \mathbf{n}_L is given by:

$$I(\mathbf{q}) = I_T \frac{V^{2/3}}{2\pi q_0} \delta(\mathbf{q} \cdot \mathbf{n}_L V^{1/3}) \delta(|\mathbf{q}| - q_0) V^{1/3} \quad (\text{S6.1})$$

where I_T is the total scattered intensity, V is the volume, and q_0 is the radius of the scattering ring in reciprocal space ($q_0 \approx 2\pi/d$). Using the geometry defined in Fig. S6.1, the scattering wavevector \mathbf{q} is given by $\mathbf{q} = q_0(\cos \phi_L \hat{\mathbf{x}} + \sin \phi_L \hat{\mathbf{z}})$ and the local director \mathbf{n}_L is given by $\mathbf{n}_L = \sin \beta \cos \chi \hat{\mathbf{x}} + \sin \beta \sin \chi \hat{\mathbf{y}} + \cos \beta \hat{\mathbf{z}}$. Because the sample is rotationally symmetric with respect to the z -axis, the exact value of q_x or q_y in our calculation does not matter; we could have used $\mathbf{q} = q_0(\cos \phi_L \hat{\mathbf{y}} + \sin \phi_L \hat{\mathbf{z}})$. We just need to allow for all possible values of $q_r = \sqrt{q_x^2 + q_y^2}$.

To obtain the total scattering from the collection of rodlike particles, we must integrate $I(\mathbf{q})$ over all possible local directors \mathbf{n}_L , weighting by the fraction of rods with a particular orientation, $f(\beta) \sin \beta d\beta d\chi$, where $f(\beta)$ is referred to as the chain orientational distribution function. The scattering is given by:

$$I(q, \phi_L) = \int_{\beta=0}^{\pi/2} \int_{\chi=0}^{2\pi} I_T \frac{2V^{2/3}}{q_0} \delta\left(qV^{1/3}(\cos \phi_L \sin \beta \cos \chi + \sin \phi_L \cos \beta)\right) \times \delta\left((q - q_0)V^{1/3}\right) f(\beta) \sin \beta d\beta d\chi \quad (\text{S6.2})$$

Scattering is only possible for $q=q_0$. Integrating across the peak gives:

$$I(\phi_L) = \int_{q=0}^{\infty} I(q, \phi_L) q^2 dq \quad (\text{S6.3})$$

$$= \int_{\beta=0}^{\pi/2} \int_{\chi=0}^{2\pi} 2I_T \delta(\cos \phi_L \sin \beta \cos \chi + \sin \phi_L \cos \beta) f(\beta) \sin \beta d\beta d\chi$$

We now perform the following substitution:

$$\begin{aligned} u &= \cos \phi_L \sin \beta \cos \chi + \sin \phi_L \cos \beta \\ du &= -\cos \phi_L \sin \beta \sin \chi d\chi \\ \chi = \pm\pi &\rightarrow u = \sin(\phi_L - \beta) \\ \chi = 0 &\rightarrow u = \sin(\phi_L + \beta) \end{aligned} \quad (\text{S6.4})$$

By inserting the results of Eq. S6.4 into Eq. S6.3, we obtain for the scattering:

$$I(\phi_L) = \int_{\beta=0}^{\pi/2} 2I_T f(\beta) \sin \beta d\beta \times \int_{u=\sin(\phi_L-\beta)}^{\sin(\phi_L+\beta)} \frac{2\delta(u) du}{\cos \phi_L \sin \beta \sin \chi} \quad (\text{S6.5})$$

Note that the integral with respect to u can only be non-zero when $\beta \geq \phi_L$. The value of this integral is then determined solely by its value at $u=0$ for which,

$$u = \cos \phi_L \sin \beta \cos \chi + \sin \phi_L \cos \beta = 0 \rightarrow |\sin \chi| = \sqrt{1 - \left(\frac{\tan \phi_L}{\tan \beta}\right)^2} \quad (\text{S6.6})$$

By integrating over u in Eq. S6.5, we obtain finally for the scattering as a function of ϕ_L :

$$I(\phi_L) = \int_{\beta=\phi_L}^{\beta=\pi/2} \frac{4I_T f(\beta) d\beta}{\cos \phi_L \sqrt{1 - \left(\frac{\tan \phi_L}{\tan \beta}\right)^2}} \quad (\text{S6.7})$$

Finally, simplifying and combining constants gives:

$$I(\phi_L) = C \int_{\beta=\phi_L}^{\beta=\pi/2} \frac{\sec \phi_L f(\beta) \tan \beta d\beta}{\sqrt{\tan^2 \beta - \tan^2 \phi_L}} \quad (\text{S6.8})$$

where C is a constant, which is proportional to the amount of sample, the incident beam intensity, and the length of the exposure. This is our starting point for x-ray analysis in the strategy outlined at the end of Section S6.2. Eq. S6.8 appears without derivation in (13) and has been attributed (14) to earlier work by Kratky (15). Note, the equation can also be re-arranged to give the form,

$$I(\phi_L) = C \int_{\beta=\phi_L}^{\beta=\pi/2} \frac{f(\beta) \sin \beta d\beta}{\sqrt{\sin^2 \beta - \sin^2 \phi_L}} \quad (\text{S6.9})$$

S6.4 Problems with Leadbetter's formula for $I(\phi_L)$

Leadbetter and Norris (3) have derived a different formula for $I(\phi_L)$ using the same model and assumptions as described in Section S6.2. Like the derivation presented in Section S6.3, Leadbetter and Norris evaluated $I(\phi_L)$ by summing the scattering intensity from all grains oriented at right-angles to the scattering vector. However, Leadbetter and Norris incorrectly assumed that the integrated scattering intensity of a given grain is independent of the grain's orientation. As a result of the incorrect assumption, they obtained the following formula (referred to in the following discussion as the "Leadbetter formula"):

$$I_{\text{Leadbetter}}(\phi_L) = C \int_{\beta=\phi}^{\beta=\pi/2} \frac{\sec^2 \phi_L f(\beta) \sin \beta d\beta}{\sqrt{\tan^2 \beta - \tan^2 \phi_L}}. \quad (\text{S6.10})$$

Compared with the new corrected formula (Eq. S6.8), the Leadbetter formula (Eq. S6.10) is missing a factor of $\cos \phi_L / \cos \beta$. Within the context of Leadbetter and Norris's derivation, this factor is needed to account for the dependence of integrated scattering intensity on relative grain orientation. Because Leadbetter and Norris did not include this factor in their derivation, Eq. S6.10 is incorrect. In contrast, by explicitly evaluating the delta function in the derivation of Eq. S6.8, the effect of grain orientation on scattering intensity was automatically included.

Although Eq. S6.10 is often attributed to Leadbetter et al. (3-5), it appears earlier in Levine's thesis (11). Levine and Wilkins characterized chain orientational order in egg lecithin/cholesterol mixtures using Eq. S6.10 with different forms for $f(\beta)$ (11,12).

The most straightforward way of showing that the Leadbetter formula is incorrect is to consider explicit cases where the formula fails. Two situations where Eq. S6.10 fails and Eq. S6.8 gives the correct behavior are:

1. The Leadbetter formula results in an incorrect invariant. This was recently pointed out without explicit proof in (14).
2. The Leadbetter formula fails when all the rods are oriented at $\beta=\pi/2$.

The particular case of all rods oriented at $\beta=\pi/2$ is a very unlikely situation, especially for model membrane systems, which would mean all the chains were lying flat in the plane of the membrane.

By showing cases where the Leadbetter formula fails, our purpose is not to claim that there is anything wrong with the main conclusions made from the liquid crystal research done over the past 30 years using Eq. S6.10. In most realistic situations with rods oriented at modest values of β (closer to 0 than $\pi/2$), the Leadbetter formula is not unreasonable, especially if one is only interested in trends in the orientational distribution, or equivalently trends in the order parameters.

In some work, the average of grain orientations \mathbf{n}_L over the azimuthal angle χ is neglected by assuming $I(\phi_L)=f(\beta)$ (16). This is equivalent to assuming that scattering from a grain oriented with angle β will only occur at the angle $\phi_L=\beta$. Again, if the main purpose of the work is to focus on trends in order parameters, this assumption should not drastically change the conclusions of the work. We used the corrected formula (Eq. S6.8) because it seems to correctly predict $I(\phi_L)$ for the model described in Section S6.2.

S6.4.1 Calculation of the invariant

The invariant is (Ref. 17, p.28-29):

$$\text{Invariant} = \int_V I(\mathbf{q}) d\mathbf{q} \quad (\text{S6.11})$$

Equation S6.11 states that the total scattering in all of \mathbf{q} space for all sample orientations is a conserved quantity. The most familiar form of Eq. S6.11 is the case of a powder sample, in which there is complete rotational symmetry:

$$\text{Invariant} = \int I(q) q^2 dq . \quad (\text{S6.12})$$

In our case, there is rotational symmetry about the z -axis only, and the invariant is (Ref. 18, p. 243):

$$\text{Invariant} = \int \int_{\phi_L, q} I(q, \phi_L) q^2 \sin\left(\frac{\pi}{2} - \phi_L\right) dq d\phi_L = \int \int_{\phi, q} I(q, \phi_L) q^2 \cos \phi_L dq d\phi_L . \quad (\text{S6.13})$$

Since the model assumes that scattering occurs at only one value of $q=2\pi/d$, the equation we need to test is:

$$\text{Const} = \int_{\phi_L=0}^{\phi_L=\pi/2} I(\phi_L) \cos \phi_L d\phi_L \quad (\text{S6.14})$$

Note that Deutsch (19) and Davidson et al. (6) assume that the following equation should hold:

$$Const = \int_{\phi_L=0}^{\phi_L=\pi/2} I(\phi_L) d\phi_L \quad (S6.15)$$

Davidson et al. (6) state: "As already implicitly noted [by Deutsch], no solid angle correction...needs to be made to calculate the integrated intensity because it is directly included in $I(\phi_L)$ by the solid angle of the detector itself." This statement is false because $I(\phi_L)$ is supposed to represent only the scattering measured by the detector for a particular orientation of the sample. In our case and the case of Leadbetter's model, sample orientations are averaged with respect to rotation about the z -axis. Unlike a powder sample for which the $\cos\phi_L$ term in the invariant calculation is unnecessary, our samples are not rotationally averaged with respect to the x - y axes. The solid angle must be taken into account when calculating the total scattering over all sample orientations (18).

The following calculation will show that Eq. S6.14 is not generally true for Leadbetter's formula. We wish to solve the following integral:

$$\int_{\phi_L=0}^{\phi_L=\pi/2} I_{\text{Leadbetter}}(\phi_L) \cos\phi_L d\phi_L = \int_{\phi_L=0}^{\phi_L=\pi/2} \cos\phi_L d\phi_L \int_{\beta=\phi_L}^{\beta=\pi/2} \frac{\sec^2\phi_L f(\beta) \sin\beta d\beta}{\sqrt{\tan^2\beta - \tan^2\phi_L}}. \quad (S6.16)$$

Switching the order of integration gives:

$$\int_{\phi_L=0}^{\phi_L=\pi/2} I_{\text{Leadbetter}}(\phi_L) \cos\phi_L d\phi_L = \int_{\beta=0}^{\beta=\pi/2} f(\beta) \sin\beta \int_{\phi_L=0}^{\phi_L=\beta} \frac{d\phi_L}{\cos\phi_L \sqrt{\tan^2\beta - \tan^2\phi_L}} d\beta. \quad (S6.17)$$

The inner integral can be simplified by making the following substitution:

$$\sin\phi_L = \sin\beta \sin\Omega \quad \text{where } \Omega = 0 \text{ to } \frac{\pi}{2} \quad (S6.18)$$

$$d\phi_L = \frac{\sin\beta \cos\Omega d\Omega}{\cos\phi_L} = \frac{\sin\beta \cos\Omega d\Omega}{\sqrt{1 - \sin^2\beta \sin^2\Omega}}$$

$$\begin{aligned} \int_{\phi_L=0}^{\phi_L=\beta} \frac{d\phi_L}{\cos\phi_L \sqrt{\tan^2\beta - \tan^2\phi_L}} &= \int_{\phi_L=0}^{\phi_L=\beta} \frac{\cos\beta d\phi_L}{\sqrt{\sin^2\beta - \sin^2\phi_L}} \\ &= \int_{\Omega=0}^{\Omega=\pi/2} \frac{\cos\beta \sin\beta \cos\Omega d\Omega}{\sqrt{1 - \sin^2\beta \sin^2\Omega} \times \sqrt{\sin^2\beta - \sin^2\beta \sin^2\Omega}} \\ &= \int_{\Omega=0}^{\Omega=\pi/2} \frac{\cos\beta d\Omega}{\sqrt{1 - \sin^2\beta \sin^2\Omega}} \\ &= \cos\beta K(\sin\beta) \end{aligned}$$

where K is a complete elliptic integral of the first kind. Inserting the results of S6.18 into Eq. S6.17, we obtain using Leadbetter's equation for $I(\phi_L)$:

$$\int_{\phi_L=0}^{\phi_L=\pi/2} I_{\text{Leadbetter}}(\phi_L) \cos\phi_L d\phi_L = \int_{\phi_L=0}^{\phi_L=\pi/2} f(\beta) \sin\beta \cos\beta K(\sin\beta) d\beta, \quad (S6.19)$$

which clearly depends upon the distribution of orientations, $f(\beta)$. Thus, Leadbetter's model does not satisfy the scattering invariant.

We now show that the corrected formula (Eq. S6.8) does satisfy the requirement for the invariant (Eq. S6.14). Substituting Eq. S6.8 into Eq. S6.14 gives:

$$\begin{aligned} \int_{\phi_L=0}^{\phi_L=\pi/2} I(\phi_L) \cos \phi_L d\phi_L &= \int_{\phi_L=0}^{\phi_L=\pi/2} \int_{\beta=\phi}^{\beta=\pi/2} \frac{\sec \phi_L f(\beta) \tan \beta d\beta}{\sqrt{\tan^2 \beta - \tan^2 \phi_L}} \cos \phi_L d\phi_L \\ &= \int_{\phi_L=0}^{\phi_L=\pi/2} \int_{\beta=\phi}^{\beta=\pi/2} \frac{f(\beta) \tan \beta d\beta}{\sqrt{\tan^2 \beta - \tan^2 \phi_L}} d\phi_L \end{aligned} \quad (\text{S6.20})$$

Switching the order of integration and pulling out a $\sin \beta$ in order to make use of the normalization condition for $f(\beta)$, we obtain:

$$\int_{\phi_L=0}^{\phi_L=\pi/2} I(\phi_L) \cos \phi_L d\phi_L = \int_{\beta=0}^{\beta=\pi/2} f(\beta) \sin \beta d\beta \int_{\phi_L=0}^{\phi_L=\beta} \frac{1}{\cos \beta \sqrt{\tan^2 \beta - \tan^2 \phi_L}} d\phi_L \quad (\text{S6.21})$$

The integral over ϕ_L can be simplified as follows to a form where clever substitution will help solve the integral:

$$\begin{aligned} &\int_{\phi_L=0}^{\phi_L=\beta} \frac{1}{\cos \beta \sqrt{\tan^2 \beta - \tan^2 \phi_L}} d\phi_L \\ &= \int_{\phi_L=0}^{\phi_L=\beta} \frac{1}{\cos \beta \sqrt{\tan^2 \beta - \tan^2 \phi_L}} \times \frac{\cos \phi_L \cos \beta}{\sqrt{\cos^2 \phi_L \cos^2 \beta}} d\phi_L \\ &= \int_{\phi_L=0}^{\phi_L=\beta} \frac{\cos \phi_L}{\sqrt{\sin^2 \beta \cos^2 \phi_L - \sin^2 \phi_L \cos^2 \beta}} d\phi_L \\ &= \int_{\phi_L=0}^{\phi_L=\beta} \frac{\cos \phi_L}{\sqrt{\sin^2 \beta (1 - \sin^2 \phi_L) - \sin^2 \phi_L \cos^2 \beta}} d\phi_L \\ &= \int_{\phi_L=0}^{\phi_L=\beta} \frac{\cos \phi_L}{\sqrt{\sin^2 \beta - \sin^2 \phi_L}} d\phi_L \end{aligned} \quad (\text{S6.22})$$

We now make the following substitutions to solve the integral over ϕ_L :

$$\begin{aligned} \sin \phi_L &= \sin \beta \sin \psi \quad \text{where } \psi = 0 \text{ to } \frac{\pi}{2} \\ \cos \phi_L d\phi_L &= \sin \beta \cos \psi d\psi \\ \sqrt{\sin^2 \beta - \sin^2 \phi_L} &= \sqrt{\sin^2 \beta - \sin^2 \beta \sin^2 \psi} = \sin \beta \cos \psi \\ \int_{\phi_L=0}^{\phi_L=\beta} \frac{1}{\cos \beta \sqrt{\tan^2 \beta - \tan^2 \phi_L}} d\phi_L &= \int_{\phi_L=0}^{\phi_L=\beta} \frac{\cos \phi_L}{\sqrt{\sin^2 \beta - \sin^2 \phi_L}} d\phi_L \\ &= \int_{\psi=0}^{\psi=\pi/2} \frac{\sin \beta \cos \psi}{\sin \beta \cos \psi} d\psi = \frac{\pi}{2} \end{aligned} \quad (\text{S6.23})$$

Inserting the results of Eq. S6.23 into Eq. S6.21, we obtain for the corrected formula for $I(\phi_L)$:

$$\int_{\phi_L=0}^{\phi_L=\pi/2} I(\phi_L) \cos \phi_L d\phi_L = \int_{\beta=0}^{\beta=\pi/2} f(\beta) \sin \beta d\beta \int_{\phi_L=0}^{\phi_L=\beta} \frac{1}{\cos \beta \sqrt{\tan^2 \beta - \tan^2 \phi_L}} d\phi_L \quad (\text{S6.24})$$

$$= \frac{1}{4\pi} \times \frac{\pi}{2} = \frac{1}{8}.$$

The correct equation for the invariant (Eq. S6.14) is indeed a constant for the corrected formula (Eq. S6.8), while Leadbetter's formula (Eq. S6.10) does not result in the correct invariant calculation. This suggests an error in Leadbetter's formula.

S6.4.2 Leadbetter's formula and the corrected form in two cases

In order to further test the validity of Leadbetter's original equation and the modified form, the following discussion will consider two cases for the distribution of rods, or chains in the sample: 1) a sample where all the rods have the same tilt angle $\beta = \beta_0$; and 2) a sample where all rods are tilted at the angle $\beta = \pi/2$. Both Leadbetter's original formula and the corrected form give plausible results for the first case (when $\beta_0 < \pi/2$), but Leadbetter's original formula does not give a reasonable result for the second case.

S6.4.2.1 Case 1: Rods all have $\beta = \beta_0$.

In this case, the distribution function takes on the following form:

$$f(\beta) = C_0 \delta(\beta - \beta_0), \quad (\text{S6.25})$$

where the constant C_0 is found by satisfying:

$$\int_{\beta=0}^{\pi/2} C_0 \delta(\beta - \beta_0) \sin \beta d\beta = \frac{1}{4\pi} \quad (\text{S6.26})$$

Solving for C_0 we obtain finally for $f(\beta)$:

$$f(\beta) = \frac{1}{4\pi \sin \beta_0} \delta(\beta - \beta_0), \quad (\text{S6.27})$$

The corrected formula (Eq. S6.8) becomes with the above $f(\beta)$:

$$\begin{aligned}
I(\phi_L) &= C \int_{\beta=\phi_L}^{\beta=\pi/2} \frac{1}{4\pi \sin \beta_0} \delta(\beta - \beta_0) \frac{\sec \phi_L \tan \beta d\beta}{\sqrt{\tan^2 \beta - \tan^2 \phi_L}} \quad (\text{S6.28}) \\
&= 0 \quad \text{for } \phi_L > \beta_0 \\
&= \text{Const} \times \frac{1}{\sin \beta_0} \frac{1}{\cos \phi_L} \frac{\tan \beta_0}{\sqrt{\tan^2 \beta_0 - \tan^2 \phi_L}} \quad \text{for } 0 \leq \phi_L \leq \beta_0 \\
&= \text{Const} \times \frac{1}{\cos \phi_L} \frac{\cos \beta_0}{\sqrt{\tan^2 \beta_0 - \tan^2 \phi_L}} \\
&= \text{Const} \times \frac{1}{\cos \phi_L} \frac{1}{\sqrt{\sin^2 \beta_0 - \cos^2 \beta_0 \tan^2 \phi_L}} \\
&= \text{Const} \times \frac{1}{\sqrt{\sin^2 \beta_0 \cos^2 \phi_L - \cos^2 \beta_0 \sin^2 \phi_L}} \\
&= \text{Const} \times \frac{1}{\sqrt{\sin^2 \beta_0 (1 - \sin^2 \phi_L) - \cos^2 \beta_0 \sin^2 \phi_L}} \\
&= \text{Const} \times \frac{1}{\sqrt{\sin^2 \beta_0 - \sin^2 \phi_L}}
\end{aligned}$$

In summary for $I(\phi_L)$ for the corrected formula in the case of all rods oriented with $\beta=\beta_0$ we obtain:

$$\begin{aligned}
I(\phi_L) &= 0 \quad \text{for } \phi_L > \beta_0 \\
&= \text{Const} \times \frac{1}{\sqrt{\sin^2 \beta_0 - \sin^2 \phi_L}} \quad \text{for } 0 \leq \phi_L \leq \beta_0 \quad (\text{S6.29})
\end{aligned}$$

With the distribution function described by Eq. S6.27, the Leadbetter formula (Eq. S6.10) becomes:

$$\begin{aligned}
I_{\text{Leadbetter}}(\phi_L) &= C \int_{\beta=\phi}^{\beta=\pi/2} \frac{1}{4\pi \sin \beta_0} \delta(\beta - \beta_0) \frac{\sec^2 \phi_L \sin \beta d\beta}{\sqrt{\tan^2 \beta - \tan^2 \phi_L}} \quad (\text{S6.30}) \\
&= 0 \quad \text{for } \phi_L > \beta_0 \\
&= \text{Const} \times \frac{1}{\sin \beta_0} \frac{1}{\cos^2 \phi_L} \frac{\sin \beta_0 d\beta}{\sqrt{\tan^2 \beta_0 - \tan^2 \phi_L}} \quad \text{for } 0 \leq \phi_L \leq \beta_0 \\
&= \text{Const} \times \frac{\cos \beta_0}{\cos \phi_L} \frac{1}{\sqrt{\sin^2 \beta_0 - \sin^2 \phi_L}}
\end{aligned}$$

In the last line the same steps as shown in Eq. S6.28 were followed. In summary for $I(\phi_L)$ for the Leadbetter formula in the case of all rods oriented with $\beta=\beta_0$ we obtain:

$$\begin{aligned}
I_{\text{Leadbetter}}(\phi_L) &= 0 \quad \text{for } \phi_L > \beta_0 \\
&= \text{Const} \times \frac{\cos \beta_0}{\cos \phi_L} \frac{1}{\sqrt{\sin^2 \beta_0 - \sin^2 \phi_L}} \quad \text{for } 0 \leq \phi_L \leq \beta_0 \quad (\text{S6.31})
\end{aligned}$$

Figure S6.2 shows plots of the results of Eq. S6.29 and Eq. S6.31. Both plots are reasonable for $\beta < \pi/2$.

S6.4.2.2 Case 2: Rods all have $\beta = \pi/2$

The case of all rods with orientation $\beta = \pi/2$ is a special case of the one described in the previous section. For any given grain direction ($\chi, \beta = \pi/2$), scattering is concentrated on a circle through the poles (+z and -z axes). Since there is rotational symmetry about the z-axis, $I(\phi_L)d\phi_L$ should be proportional to $1/A_{\text{ring}}$, where A_{ring} is the area of a ring in reciprocal space with ϕ_L between ϕ_L and $\phi_L + d\phi_L$: $A_{\text{ring}} = 2\pi \sin(\pi/2 - \phi_L)d\phi_L = 2\pi \cos \phi_L d\phi_L$. The scattering intensity is given by:

$$I(\phi_L) \propto \frac{1}{A_{\text{ring}}} \propto \frac{1}{\cos \phi_L}. \quad (\text{S6.32})$$

Another way to think about this case is that the total intensity for any range $d\phi_L$ should be proportional to the size of the angular range. That is,

$$\int_{\phi_L}^{\phi_L + d\phi_L} I(\phi_L) \cos \phi_L d\phi_L \sim d\phi_L. \quad (\text{S6.33})$$

$I(\phi_L) \propto 1/\cos \phi_L$ is the scaling factor needed to produce the same intensity integrated over any range $d\phi_L$.

For the corrected formula (Eq. S6.8), Eq. S6.29 with $\beta_0 = \pi/2$ gives the expected result:

$$I(\phi_L) = \text{Const} \times \frac{1}{\sqrt{\sin^2 \frac{\pi}{2} - \sin^2 \phi_L}} = \text{Const} \times \frac{1}{\cos \phi_L}. \quad (\text{S6.34})$$

For the Leadbetter formula (Eq. S6.10), Eq. S6.31 with $\beta_0 = \pi/2$ gives:

$$I_{\text{Leadbetter}}(\phi_L) = \text{Const} \times \frac{\cos \frac{\pi}{2}}{\cos \phi_L} \frac{1}{\sqrt{\sin^2 \frac{\pi}{2} - \sin^2 \phi_L}} = 0 \quad (\text{S6.35})$$

The Leadbetter formula does not yield a reasonable result in the case of rods all oriented with $\beta_0 = \pi/2$. In our system, this corresponds to the case of all the chains lying in the plane of the membrane, an unphysical situation. In fact, this is an unlikely situation for all the systems studied in the liquid crystal literature with the Leadbetter formula, and so the distribution functions found using the Leadbetter formula are probably still reasonable, especially since much of the work in the literature was concerned with trends. Figure S6.2 summarizes the results of the last two sections with a plot of $I(\phi_L)$ with three specific values of β_0 .

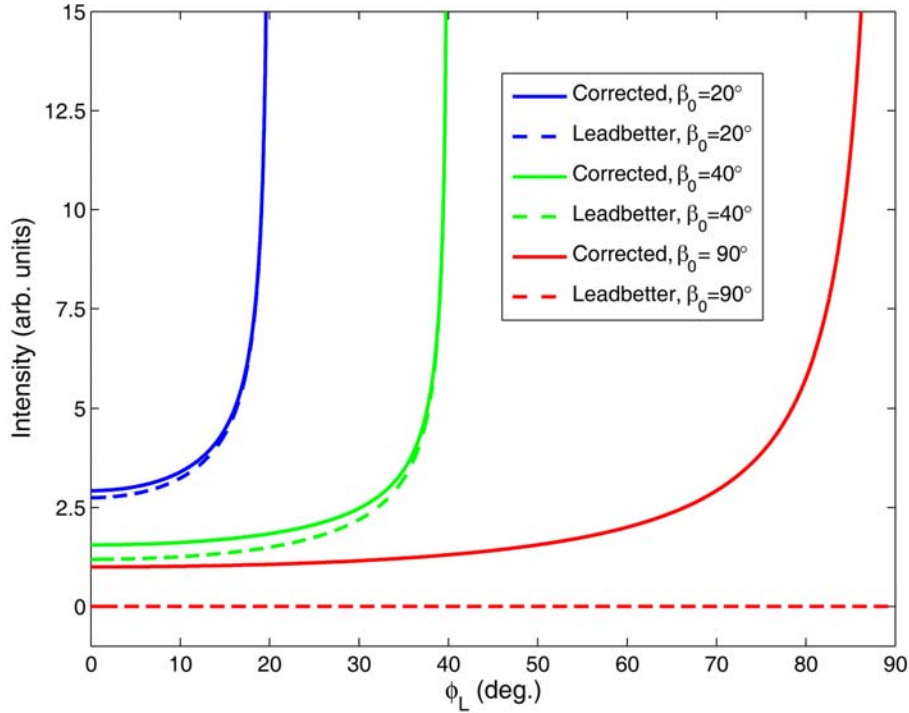


Figure S6.2. Plot of $I(\phi_L)$ in the case that all rods are oriented with angle $\beta=\beta_0$ for three values of β_0 : 20° , 40° , and 90° . For this case, both the Leadbetter formula (Eq. S6.31) and the corrected formula (S6.29) give reasonable results for $\beta_0 < 90^\circ$, while the Leadbetter result is unreasonable for $\beta_0 = 90^\circ$.

S6.5 Analytical form for scattering assuming the Maier-Saupe distribution function

The Leadbetter formula (Eq. S6.10) has been inverted numerically (3) or analytically (19) to obtain $f(\beta)$ from the measured $I(\phi_L)$. However, the inversion can be complicated and cumbersome to use. Instead, we chose to follow a commonly used approach in which an analytical expression for the scattering is derived by assuming a particular form for the distribution function $f(\beta)$, which has an adjustable parameter m related to the width of the distribution. This expression can then be fit to the experimental data to obtain $f(\beta)$, from which order parameters and other quantities can be calculated (see Ref. 6 for a review of this approach).

The Maier-Saupe orientational distribution function has been applied in analysis of x-ray data to a number of nematic and smectic liquid crystalline systems (3,6,16,20,21). Jähnig (22) has theoretically described the lipid gel to fluid transition using a Maier-Saupe mean-field approach. Because of the wide applicability of Maier-Saupe theory, we chose to assume the Maier-Saupe distribution function for the lipid chains. The Maier-Saupe distribution results from a mean-field model; it ignores the details of molecular structure and angular correlations between neighboring rods (23-26).

S6.5.1 Maier-Saupe distribution function

The final normalized form for the Maier-Saupe orientational distribution function is (23-26):

$$f(\beta) = \frac{1}{Z} \exp(m \cos^2 \beta) \quad (\text{S6.36})$$

where m is a parameter which can take on any positive value and the normalization constant Z can be solved for with the normalization condition:

$$\int_{\beta=0}^{\pi/2} f(\beta) \sin \beta d\beta = \frac{1}{4\pi} \quad (\text{S6.37})$$

$$\int_{\beta=0}^{\pi/2} \frac{1}{Z} \exp(m \cos^2 \beta) \sin \beta d\beta = \frac{1}{4\pi}$$

Substituting $x = \cos \beta$ gives:

$$Z = 4\pi \int_0^1 \exp(mx^2) dx. \quad (\text{S6.38})$$

Note in general:

$$\int \exp(au^2) du = \frac{\sqrt{\pi}}{2\sqrt{a}} \operatorname{erfi}(u\sqrt{a}) \quad (\text{S6.39})$$

and

$$\operatorname{erfi}(v) = \frac{2}{\sqrt{\pi}} \exp(v^2) D(v),$$

where erfi is the imaginary error function and D is Dawson's integral. Using Eq. S6.39 in Eq. S6.38, we get for Z :

$$Z = 4\pi \frac{\sqrt{\pi}}{2\sqrt{m}} \operatorname{erfi}(\sqrt{m}) \quad (\text{S6.40})$$

$$= 4\pi \frac{\exp(m)}{\sqrt{m}} D(\sqrt{m})$$

Fig. S6.3 plots the Maier-Saupe distribution function, $f(\beta)$, as well as $f(\beta)\sin\beta$ for two cases: $m=3.69$ and $m=30.6$. For larger m , the distribution is narrower, meaning that the rods are more likely to be oriented in a narrow range around $\beta=0$.

We have assumed that the distribution is centered about the angle $\beta_t=0$ (the same direction as the membrane normal); in the liquid-crystal literature, this type of sample is referred to as smectic A. In smectic C liquid crystals, the distribution is centered around a nonzero angle β_t . For this situation, in Eq. S6.36, β is replaced by $\beta-\beta_t$ (see Ref. 3 for further complications involving smectic C samples). We had no reason to believe that the samples we studied had an average tilt angle other than $\beta_t=0$, and so we did not include this complication in the analysis.

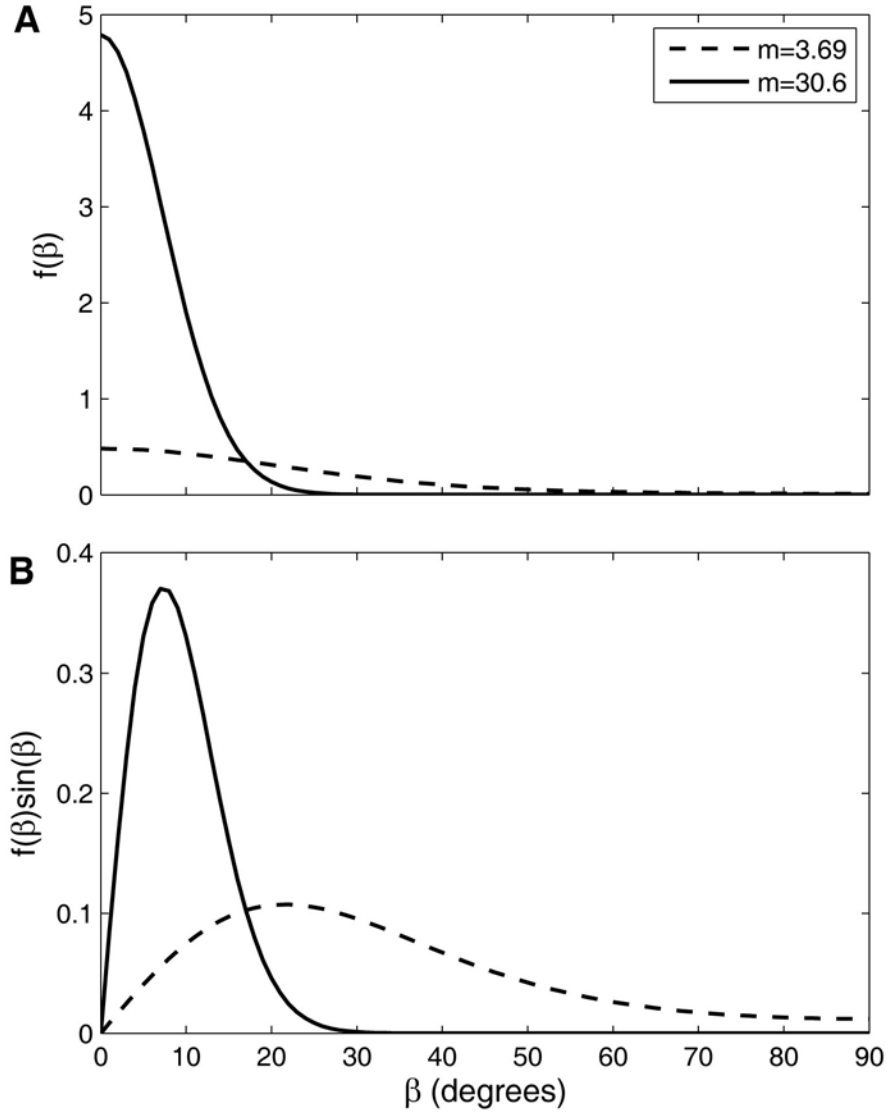


Figure S6.3. (A) Plot of Eq. S6.36, the Maier-Saupe orientational distribution function, $f(\beta)$, for two different values of m . (B) Plot of $f(\beta)\sin\beta$ for the same two values of m . Note that $f(\beta)\sin\beta$ is proportional to the fraction of rods oriented at the angle β .

Using the distribution function, we can calculate quantities involving average values of the chain tilt angle β . For a function $X(\beta)$, the average value can be calculated as follows:

$$\langle X(\beta) \rangle = \frac{\int_{\beta=0}^{\beta=\pi/2} X(\beta) f(\beta) \sin \beta d\beta}{\int_{\beta=0}^{\beta=\pi/2} f(\beta) \sin \beta d\beta} \quad (\text{S6.41})$$

The molecular order parameter $S_{x\text{-ray}}$ (usually referred to simply as S in the liquid crystal literature) is a common quantity used to describe the average orientational order in the system. $S_{x\text{-ray}}$ is calculated as follows:

$$S_{x\text{-ray}} = \frac{1}{2} \left(3 \langle \cos^2 \beta \rangle - 1 \right) = \frac{\int_{\beta=0}^{\beta=\pi/2} \frac{1}{2} (3 \cos^2 \beta - 1) f(\beta) \sin \beta d\beta}{\int_{\beta=0}^{\beta=\pi/2} f(\beta) \sin \beta d\beta} \quad (\text{S6.42})$$

For the Maier-Saupe distribution,

$$\begin{aligned} S_{x\text{-ray}} &= \frac{\int_{\beta=0}^{\beta=\pi/2} \frac{1}{2} (3 \cos^2 \beta - 1) f(\beta) \sin \beta d\beta}{\int_{\beta=0}^{\beta=\pi/2} f(\beta) \sin \beta d\beta} \quad (\text{S6.43}) \\ &= \frac{\int_{\beta=0}^{\beta=\pi/2} \frac{1}{2} (3 \cos^2 \beta - 1) \exp(m \cos^2 \beta) \sin \beta d\beta}{\int_{\beta=0}^{\beta=\pi/2} \exp(m \cos^2 \beta) \sin \beta d\beta} \\ &= \frac{\int_{x=0}^1 \frac{1}{2} (3x^2 - 1) \exp(mx^2) dx}{\int_{x=0}^1 \exp(mx^2) dx} \\ &= \frac{3}{4m} \left(\frac{\sqrt{m}}{D(\sqrt{m})} - 1 \right) - \frac{1}{2} \end{aligned}$$

S6.5.2 Calculation of $I(\phi_L)$ using the Maier-Saupe distribution function

Kelkar and Paranjpe (21) derived a simple analytical equation for $I(\phi_L)$ using the Leadbetter formula (S6.10) and the Maier-Saupe distribution. The following derivation uses the corrected formula (Eq. S6.8). Inserting the Maier-Saupe orientational distribution function (Eq. S6.36) into the corrected formula (Eq. S6.8) gives:

$$I(\phi_L) = \frac{C}{Z} \int_{\beta=\phi_L}^{\beta=\pi/2} \frac{\exp(m \cos^2 \beta) \tan \beta d\beta}{\cos \phi_L \sqrt{\tan^2 \beta - \tan^2 \phi_L}} \quad (\text{S6.44})$$

The integral in Eq. S6.44 can be solved by converting to a more convenient form:

$$\begin{aligned}
I(\phi_L) &= \frac{C}{Z} \int_{\beta=\phi_L}^{\beta=\pi/2} \frac{\exp(m \cos^2 \beta) \tan \beta d\beta}{\cos \phi_L \sqrt{\tan^2 \beta - \tan^2 \phi_L}} \times \frac{\cos \beta}{\cos \beta} \\
&= \frac{C}{Z} \int_{\beta=\phi_L}^{\beta=\pi/2} \frac{\exp(m \cos^2 \beta) \sin \beta d\beta}{\cos \phi_L \sqrt{\sin^2 \beta - \tan^2 \phi_L \cos^2 \beta}} \\
&= \frac{C}{Z} \int_{\beta=\phi_L}^{\beta=\pi/2} \frac{\exp(m \cos^2 \beta) \sin \beta d\beta}{\cos \phi_L \sqrt{1 - (\tan^2 \phi_L + 1) \cos^2 \beta}} \\
&= \frac{C}{Z} \int_{\beta=\phi_L}^{\beta=\pi/2} \frac{\exp(m \cos^2 \beta) \sin \beta d\beta}{\cos \phi_L \sqrt{1 - \frac{\cos^2 \beta}{\cos^2 \phi_L}}}
\end{aligned} \tag{S6.45}$$

The following substitutions can simplify the above equation:

$$\cos \beta = \cos \phi_L \cos \psi \tag{S6.46}$$

$$\psi = 0 \leftrightarrow \beta = \phi_L; \quad \psi = \frac{\pi}{2} \leftrightarrow \beta = \frac{\pi}{2}$$

$$\sqrt{1 - \frac{\cos^2 \beta}{\cos^2 \phi_L}} = \sqrt{1 - \cos^2 \psi} = \sin \psi$$

$$d(\cos \beta) = -\sin \beta d\beta = -\cos \phi_L \sin \psi d\psi$$

Inserting the above substitutions into Eq. S6.45 and simplifying gives:

$$I(\phi_L) = \frac{C}{Z} \int_{\psi=0}^{\psi=\pi/2} \exp(m \cos^2 \phi_L \cos^2 \psi) d\psi \tag{S6.47}$$

We now convert Eq. S6.47 into a form which can be found in an integral table by using the double angle formula $\cos^2 A = (\cos 2A + 1)/2$. Letting $a = m \cos^2 \phi_L$, Eq. S6.47 becomes:

$$\begin{aligned}
I(\phi_L) &= \frac{C}{Z} \int_{\psi=0}^{\psi=\pi/2} \exp\left(a \left(\frac{\cos 2\psi + 1}{2}\right)\right) d\psi \\
&= \frac{C}{Z} \exp\left(\frac{a}{2}\right) \int_{\psi=0}^{\psi=\pi/2} \exp\left(\frac{a \cos 2\psi}{2}\right) d\psi \\
&= \frac{C}{Z} \exp\left(\frac{a}{2}\right) \int_{y=0}^{y=\pi} \exp\left(\frac{a \cos y}{2}\right) \frac{dy}{2} \\
&= \frac{C}{Z} \exp\left(\frac{a}{2}\right) \int_{y=0}^{y=2\pi} \exp\left(\frac{a \cos y}{2}\right) \frac{dy}{4} \\
&= \frac{C}{Z} \exp\left(\frac{a}{2}\right) \int_{y=0}^{y=2\pi} \exp\left(\frac{a \sin y}{2}\right) \frac{dy}{4}
\end{aligned} \tag{S6.48}$$

where in the last three lines we have substituted $y = 2\psi$, changed the upper limit of integration from π to 2π , and changed a cosine to a sine. The general form for this integral can be found in an integral table:

$$I_0(x) = \frac{1}{2\pi} \int_{Q=0}^{2\pi} \exp(x \sin Q) dQ \quad (\text{S6.49})$$

where $I_0(x)$ is a modified Bessel function of the first kind (27). Re-substituting $a = m \cos^2 \phi_L$, we obtain

$$I(\phi_L) = \frac{C}{Z} \times \exp\left(\frac{m \cos^2 \phi_L}{2}\right) \times \frac{\pi}{2} I_0\left(\frac{m \cos^2 \phi_L}{2}\right) \quad (\text{S6.50})$$

Inserting normalization constant Z (Eq. S6.40) into Eq. S6.50 results in the final equation for $I(\phi_L)$ assuming the Maier-Saupe distribution:

$$I(\phi_L) = \frac{C}{8} \times \frac{\sqrt{m}}{\exp(m)D(\sqrt{m})} \times \exp\left(\frac{m \cos^2 \phi_L}{2}\right) \times I_0\left(\frac{m \cos^2 \phi_L}{2}\right) \quad (\text{S6.51})$$

We used Eq. S6.51 as a starting point for the data fitting equations. Eq. S6.51, in a different but equivalent form, is also given without derivation in (14).

S6.6 Discussion of assumptions and simplifications in the analytical model

Fitting Eq. S6.51 to experimental $I(\phi_L)$ data is a simple way of obtaining the chain orientational distribution function, from which average values involving β , such as the molecular order parameter $S_{x\text{-ray}}$, can be calculated. However, this approach makes several major assumptions, and so we cannot expect that the $f(\beta)$ found is the true orientational distribution function for the system.

We first consider problems independent of the exact form of the distribution function chosen (problems with the Leadbetter model and resulting corrected formula, Eq. S6.8). The following two assumptions can introduce opposite errors in $f(\beta)$ (3,6):

1. The model assumes that the scattering intensity comes from a cluster of interfering particles. This assumption will result in a distribution function sharper than the true $f(\beta)$ or equivalently will tend to overestimate the order parameter.
2. The model assumes that the rods are infinitely long and thus neglects the form factor of the rods. The scattering due to a rod of finite length will contribute to the width of $I(\phi_L)$. Neglecting this effect results in a too broad distribution function, and thus a systematic underestimate of the order parameter.

For highly oriented phases (i.e. the lipid gel phase), the second effect of neglecting the form factor for a finite-sized rod is very important.

Fig. S6.4 shows the scattering expected for rods of length L with perfect orientation $\beta=0^\circ$ and packed into a hexagonal lattice.

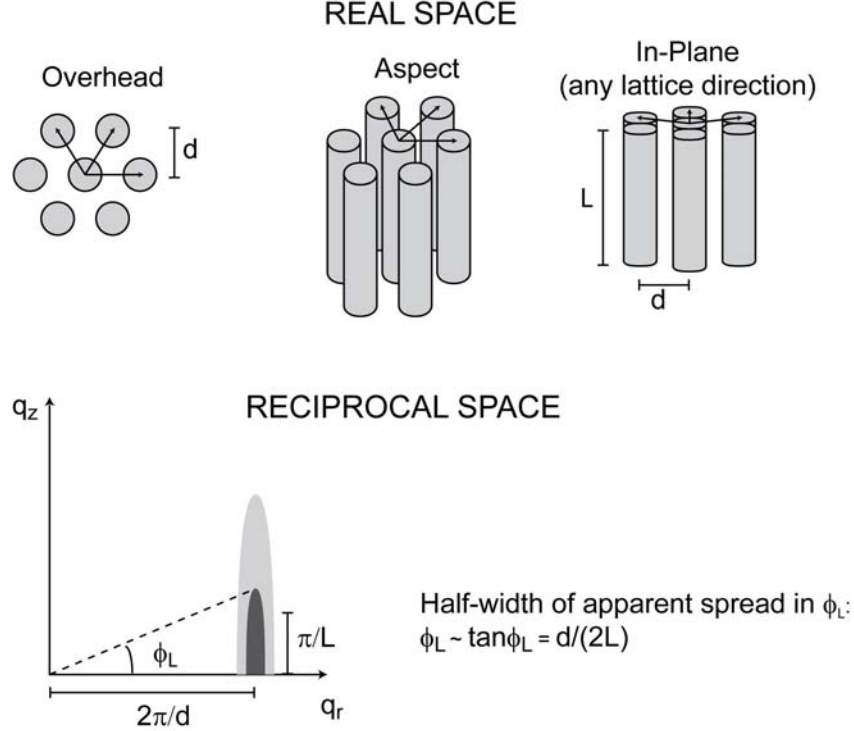


Figure S6.4. The top panel shows the perfect hexagonal packing of rods of length L all oriented with $\beta=0$. The rods form rows with spacing d . The bottom panel shows the scattering expected from such a system assuming rotational symmetry about the z -axis. The first diffraction spot will occur at $q_r = \sqrt{q_x^2 + q_y^2} = 2\pi/d$. In the q_z direction, the spot has a half-maximum intensity value at $q_z \approx \pi/L$. The apparent angular spread of the intensity is given by $\Delta\phi_L \approx d/(2L)$.

In general the scattering intensity from the sample depicted in Fig. S6.4 will depend on the Fourier transform of the electron density:

$$A(\mathbf{q}) = \int \rho(\mathbf{r}) \exp(-i\mathbf{q} \cdot \mathbf{r}) d^3r \quad (\text{S6.52})$$

We can describe the electron density of the rod by a rectangular function:

$$\begin{aligned} \rho(z) &= \text{Const} && \text{for } |z| < L/2 \\ &= 0 && \text{for } |z| > L/2 \end{aligned} \quad (\text{S6.53})$$

Since we assumed a perfect hexagonal lattice, scattering is only allowed for distinct values of q_r , the smallest value being $q_r = \sqrt{q_x^2 + q_y^2} = 2\pi/d$. The scattering in the q_z direction depends on the Fourier transform for the rectangular function (Ref. 28, p. 359):

$$A(q_z) = \int \rho(z) \exp(-iq_z z) dz = L \frac{\sin\left(\frac{q_z L}{2}\right)}{\left(\frac{q_z L}{2}\right)} = L \text{sinc}\left(\frac{q_z L}{2}\right) \quad (\text{S6.54})$$

where the sinc function is defined as:

$$\begin{aligned} \text{sinc}(t) &= \frac{\sin t}{t} & t \neq 0 \\ &= 1 & t = 0 \end{aligned} \quad (\text{S6.55})$$

The scattering intensity is proportional to the square of $A(q_z)$:

$$I(q_z) \propto [A(q_z)]^2 \propto \left[\frac{\sin\left(\frac{q_z L}{2}\right)}{\left(\frac{q_z L}{2}\right)} \right]^2 = \text{sinc}^2\left(\frac{q_z L}{2}\right) \quad (\text{S6.56})$$

Figure S6.5 shows plots of $\text{sinc}(q_z L/2)$ and $\text{sinc}^2(q_z L/2)$. The half-maximum value of $I(q_z) \propto \text{sinc}^2(q_z L/2)$ is near π/L . The apparent angular spread is given by $\Delta\phi_L \approx d/(2L)$, where $\Delta\phi_L$ refers to the half-width at half-maximum (see Fig. S6.4).

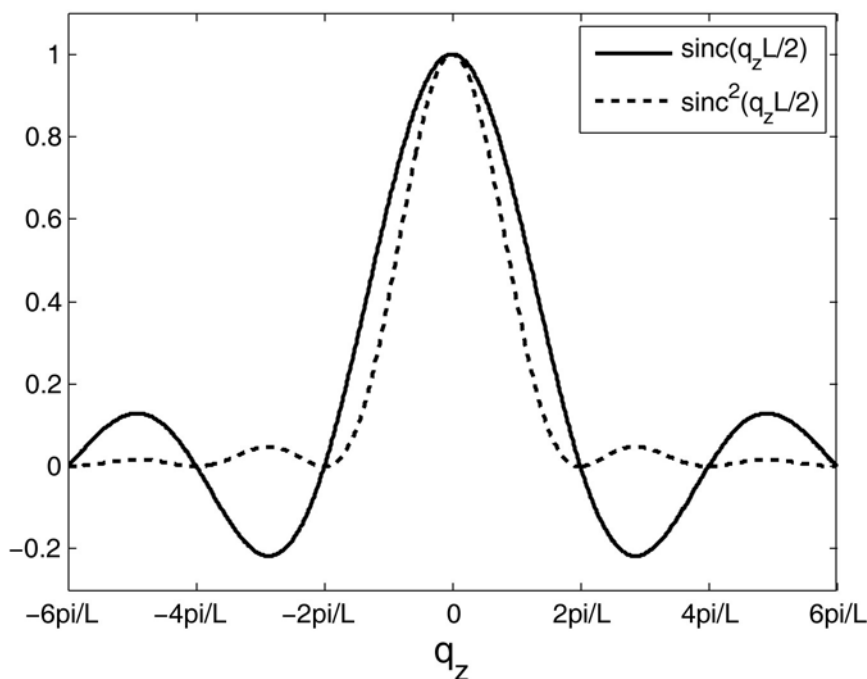


Figure S6.5. Plots of $\text{sinc}(q_z L/2)$ and $\text{sinc}^2(q_z L/2)$. For a rod of length L with electron density described by a rectangular function along the z -axis, the Fourier transform $A(q_z) \propto \text{sinc}(q_z L/2)$ and $I(q_z) \propto \text{sinc}^2(q_z L/2)$.

We can provide a lower bound to the effect of the finite length of the lipid acyl chains on the angular spread of scattering using the measured length of a DPPC chain. The effective length depends on the phase that the chain is in (gel, Ld, or Lo). Table S6.1 shows calculations for $\Delta\phi_L$ using L from the literature for DPPC chains in different phases.

Table S6.1. Apparent angular spread of scattering for finite rods with different effective lengths, L .

Phase	$d_{\text{rod}}=d_{\text{cc}}$ (Å)	L^* (Å)	$\Delta\phi_L \approx d_{\text{rod}}/(2L)$
gel	~ 4.2	18.75 (DPPC, all trans)	0.11 rad=6.4°
Ld	~ 4.5	11.6 (DPPC, 52°C)	0.19 rad=11°
Lo	~ 4.6	14.9 (DPPC + 33% Chol, 52°C)	0.15 rad=8.8°

*The effective acyl chain lengths (L) come from (29).

According to Table S6.1, by neglecting the finite length of the rods, the Leadbetter model would overestimate the angular distribution of scattering due to a distribution of chain orientations by ~ 6 -11°. For more ordered samples, we can expect the model will begin to break down due to the neglect of the finite length of the rods because this effect, and not the orientational disorder, begins to dominate the ϕ -width. Leadbetter and Norris (3) found that the model begins to break down for order parameters ($S_{\text{x-ray}}$) greater than 0.8. de Vries (8) has considered the effect of neglecting the finite size of the rods on the orientational distribution function and on the order parameters calculated from the x-ray diffraction data from liquid crystals. He concluded that although the errors on the calculated orientational distribution function can be large, the errors on the chain order parameters are not as large and the order parameters from the x-ray data are reasonable approximations. Since we are mainly interested in trends in order parameters as a function of temperature and composition, approximate values are sufficient.

For model membrane gel phases, the chains are well-ordered and the angular spread of scattering will be dominated by the apparent width $\Delta\phi_L \approx d/(2L)$ due to the finite size of the acyl chains, an effect which the Leadbetter model does not consider (see Ref. 13 for a model which explicitly considers the form factor for scattering from a rodlike polymer system). In liquid phases (Ld or Lo), we do expect chain orientational disorder, which the Leadbetter approach is designed to model. Therefore, the model is more appropriate for liquid-phase lipids. Because chain tilts, lipid areas, etc. can be obtained from the gel phase diffraction data with simple models, this is not a major limitation [see (1,2) and the references therein]. The value of the Leadbetter model as applied to model membrane systems is that it provides a way of quantitatively analyzing the WAXS data from liquid-phase samples.

In many ways, the gel phase seems to be a better match to the assumptions of the model. For example, in the gel phase the acyl chains' conformation more closely resemble rods. Chains in the fluid phase are conformationally disordered. The chains in the liquid phase are often modeled with two divisions, one closer to the headgroup region and one closer to the middle of the bilayer. The methylene segments toward the middle of the bilayer have significantly more disorder than the segments closer to the surface (30-32). In liquid phases, a model consisting of rigid rods with orientation described by the single angle β is clearly a simplification. Levine and Wilkins (12) point out that if we think of the scattering domains as segments of chains, then application of such a simple model is less problematic.

An improvement of the model would be to consider the rods as flexible [for examples of modification of Maier-Saupe mean-field theory for the situation of flexible rods see (22,33)]. Such a modification would require more fitting parameters. Since our data are well-fit by using a simple Maier-Saupe distribution for rigid rods, we could not distinguish between the simple model and a more complicated model on the basis of how well they fit the data. However, we

may be able to re-interpret the fitting parameters in the context of a more complicated, and more realistic, model.

In our approach, we constrained ourselves to using the Maier-Saupe orientational distribution function. Assuming a form for $f(\beta)$ is convenient because it allows us to fit our data to a closed-form equation using a basic least-squares fitting routine. However, there are other simple models for the chain-orientational distribution function which may be more appropriate for our system; the most appropriate form for $f(\beta)$ may depend on the lipid, temperature, and hydration level. For example, Levine and Wilkins (11,12) found that they needed to assume different functional forms for the orientational distribution functions in order to fit their egg lecithin data at different levels of hydration. However, all of our data (including different hydration levels) was well-fit by the Maier-Saupe distribution. While the Maier-Saupe distribution function works well to describe the scattering from many liquid crystalline systems, other models are sometimes more appropriate (34,35). As long as our focus is on trends in chain orientational order, the exact form of the chain orientational distribution function should not play a major role as long as it fits the data reasonably.

S6.7 Relationship between the sample geometry and the experimental scattering geometry: the $\phi_L \approx \phi$ approximation

Fig. S6.6 shows the experimental scattering geometry and shows how the angle ϕ_L is related to the angle ϕ . Again, the x - y - z coordinates in Fig. S6.1 (sample geometry) and Fig. S6.6 (experimental geometry) are different. The angle ϕ is the angle on the detector from the x -axis, while ϕ_L is the angle between \mathbf{q} and the sample plane, more specifically the angle complementary to the angle between the membrane normal \mathbf{n} and \mathbf{q} . In the preceding sections, we have derived an equation relating the scattering intensity to ϕ_L . The derivation of $I(\phi_L)$ is independent of the exact experimental scattering geometry used, and so Eq. S6.8 can be used for a variety of experimental setups. However, to produce the $I(\phi)$ plots, we integrated over constant ϕ , not constant ϕ_L . We make the approximation $\phi_L \approx \phi$. Leadbetter et al. (3-5) always implicitly made this approximation. As we will show below, this approximation is valid for our experiments, but the approximation begins to break down for scattering at wider angles. This is not a limitation of the model, as $I(\phi_L)$ instead of $I(\phi)$ can be calculated from the detector images (13).

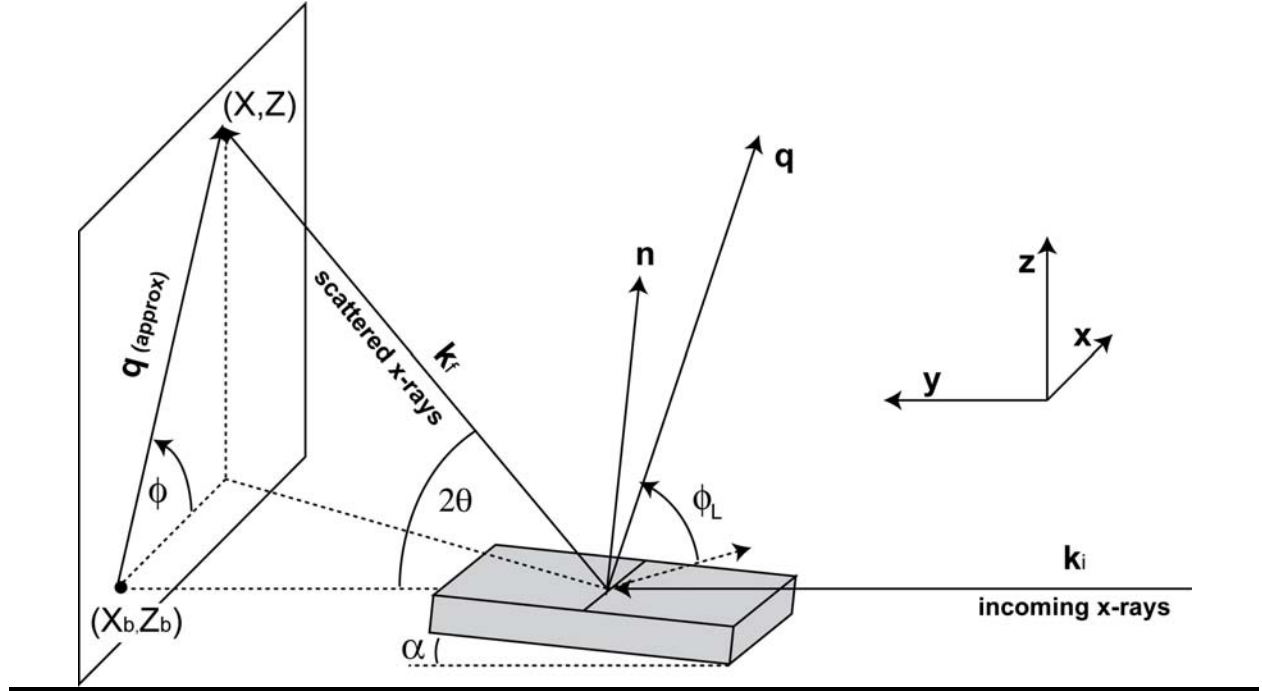


Figure S6.6. Experimental scattering geometry. The distance between the sample and detector is S .

The exact relations between the different angles and vectors shown in Fig. 2 are (assuming $X_b=Z_b=0$):

$$\tan(2\theta) = \frac{\sqrt{X^2 + Z^2}}{S} \quad (\text{S6.57})$$

$$\cos \phi = \frac{X}{\sqrt{X^2 + Z^2}}$$

$$q = |\mathbf{q}| = \left(\frac{4\pi}{\lambda}\right) \sin \theta$$

$$k = |\mathbf{k}| = 2\pi / \lambda$$

$$\mathbf{k}_i = k \hat{\mathbf{y}}$$

$$\mathbf{k}_r = k(\sin 2\theta \cos \phi \hat{\mathbf{x}} + \cos 2\theta \hat{\mathbf{y}} + \sin 2\theta \sin \phi \hat{\mathbf{z}})$$

$$\mathbf{q} = q(\cos \theta \cos \phi \hat{\mathbf{x}} - \sin \theta \hat{\mathbf{y}} + \cos \theta \sin \phi \hat{\mathbf{z}})$$

$$\hat{\mathbf{n}} = -\sin \alpha \hat{\mathbf{y}} + \cos \alpha \hat{\mathbf{z}}$$

$$\sin \phi_L = \hat{\mathbf{n}} \cdot \frac{\mathbf{q}}{q} = \sin \phi \cos \alpha \cos \theta + \sin \alpha \sin \theta$$

In our experiments α is small ($\sim 0.15^\circ$), and so the equation relating $\sin \phi_L$ to $\sin \phi$ simplifies to:

$$\sin \phi_L = \sin \phi \cos \theta . \quad (\text{S6.58})$$

For both the February and October 2006 setups, $\theta \sim 8^\circ$ ($\cos \theta = 0.99$) for $q = 1.4 \text{ \AA}^{-1}$. Table S6.2 relates ϕ_L to ϕ using Eq. S6.58 with $\cos \theta = 0.99$. Since even for the most orientationally disordered samples, the region of change in the $I(\phi)$ plot is over by $\phi \approx 60^\circ$, the approximation $\phi_L \approx \phi$ introduces negligible error. Note also that we cannot record on our detector the full range (0-90°) of ϕ_L for a single sample orientation. Again, this is not important because the data do not change much for ϕ (or ϕ_L) $> 60^\circ$.

Table S6.2. Relationship between ϕ and ϕ_L using Eq. S6.58 with $\cos \theta = 0.99$

ϕ (degrees)	ϕ_L (degrees)
0	0
10	9.9
20	19.8
30	29.7
40	39.5
50	49.3
60	59.0
70	68.5
80	77.2
90	81.9

S6.8 Remark on the calculation of $I(\phi)$

For simplicity, Leadbetter's model assumes that the scattering peak has no width in reciprocal space; that is, scattering occurs only for $q_0 = 2\pi/d$. However, the chain-chain scattering occurs over a range of q values as discussed by Haase et al. (36). Clearly, given the definition of the invariant (Eq. S6.13), $I(\phi)$ should formally be defined as,

$$I(\phi) = \int_{q_1}^{q_2} I(\phi, q) q^2 dq . \quad (\text{S6.59})$$

However, when the radial width of the scattering peak is not too large compared to peak position ($\Delta q / q_0 < 1$), the exact integration measure ($dq, q^2 dq$, etc.) has only a small effect on the resulting integral. Experimentally, we measured the angular dependence of the scattering by integrating the data over the peak:

$$I(\phi) = \int_{q_1=0.8}^{q_2=1.8} I(\phi, q) dq . \quad (\text{S6.60})$$

The range of $q = 0.8 - 1.8 \text{ \AA}^{-1}$ was chosen to be the widest possible considering experimental limitations. We compared the results obtained using Eq. S6.60 and Eq. S6.59 and found the differences in the fitting results were negligible. We decided to use Eq. S6.60 because Eq. S6.59 is more sensitive to noise.

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