SUPPLEMENTARY MATERIAL

Physics of HIV

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1. Details of LAXS data collection and analysis

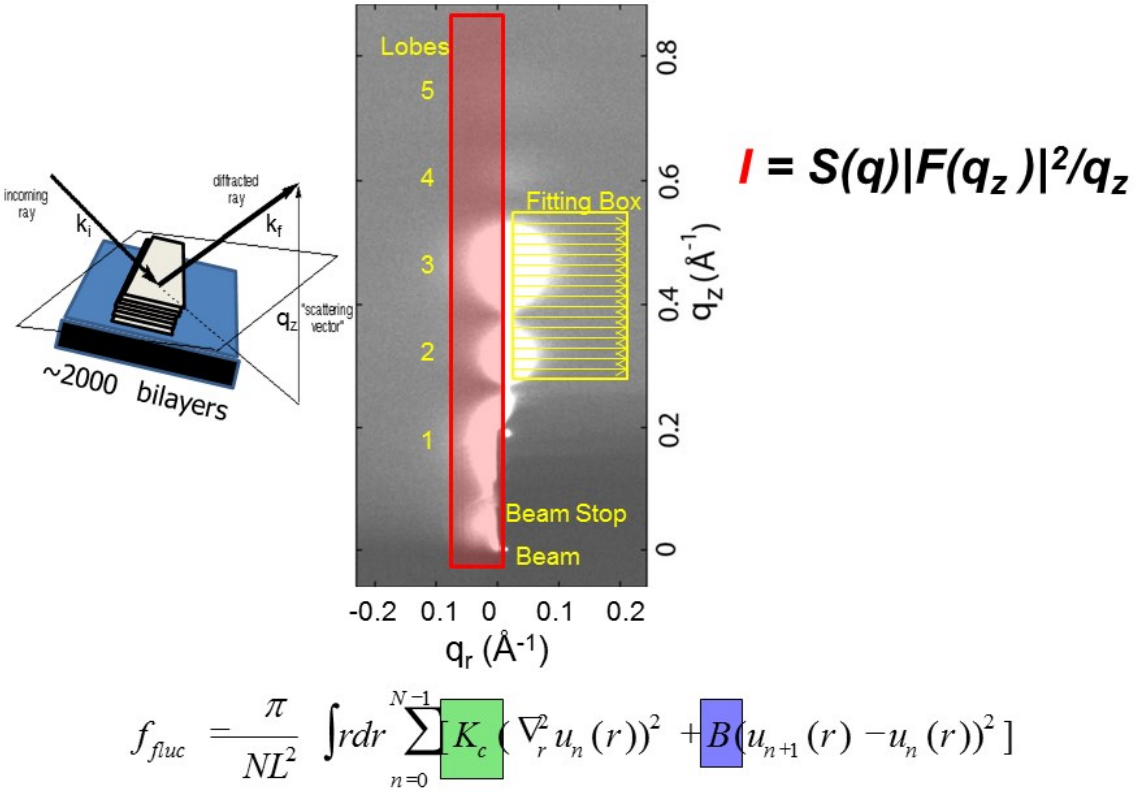
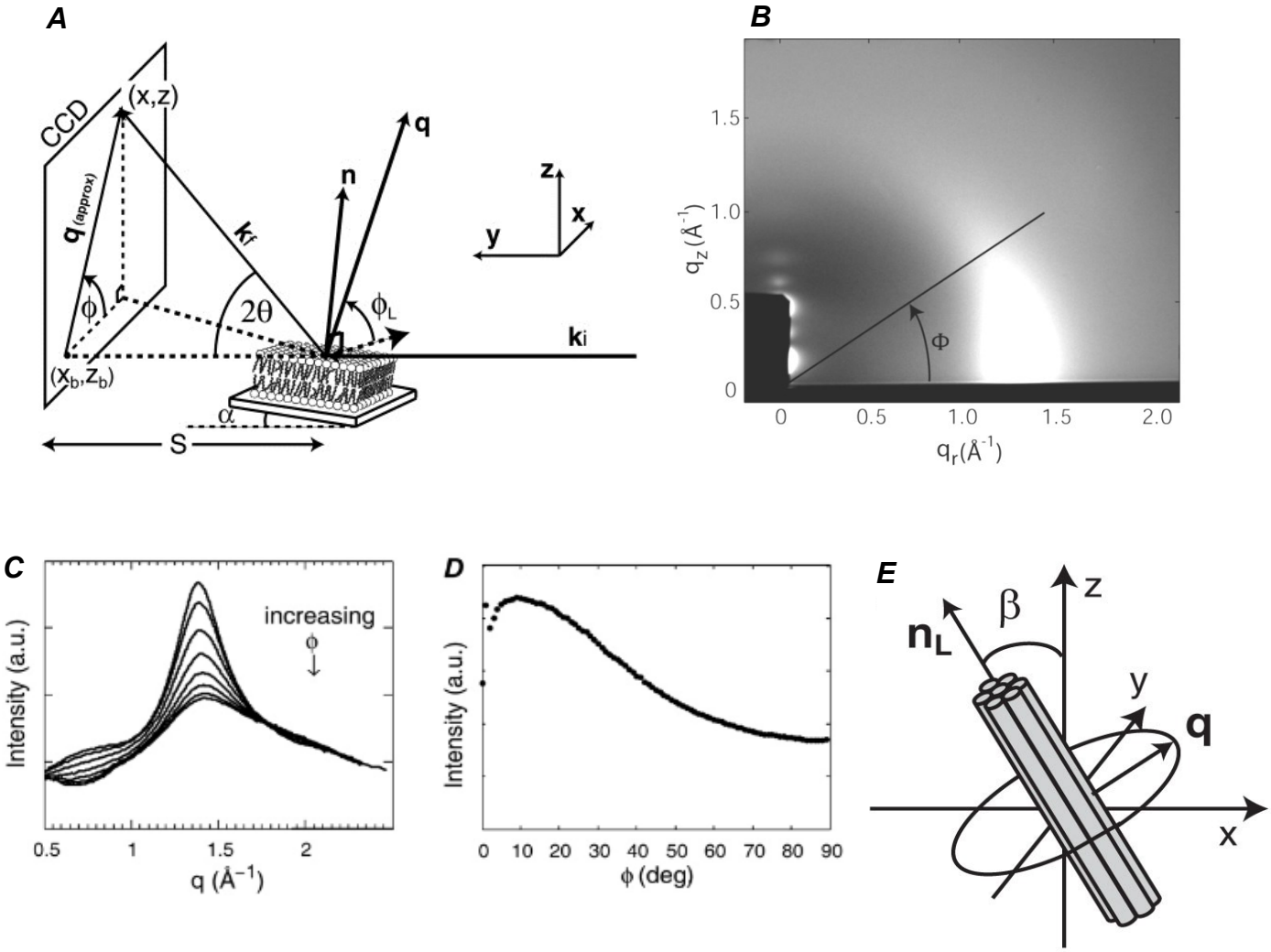


Figure S1. LAXS data collection and analysis. Reprinted from [1], Copyright 2004, with permission from Elsevier.

A stack of ~2000 bilayers on a silicon wafer is hydrated within a hydration chamber [2] (not shown), which causes membrane fluctuations near the fully hydrated condition [3, 4]. The wafer is rotated from -1.6 to 7 degrees during the data collection to equally sample all scattered X-rays (30 sec dezingered scans at CHESS or 10 or 20 minute dezingered scans at CMU). Due to the fluctuations, large, nearly spherical "lobes" of diffuse X-ray scattering are produced (numbered in Fig. S1). The scattered X-rays impinge on a CCD detector located 20-40 cm from the sample. These fluctuations are quantitated by measuring the fall-off in lobe intensity in the q_r direction in the yellow fitting box shown [1]. The fitting procedure is a non-linear least squares fit that uses liquid crystal theory and requires our proprietary software, NFIT. Usually 30 iterations are required for convergence. By fitting to the free energy functional (equation at bottom of Fig. S1), the structure factor S(q), the bending modulus K_C and the compression modulus B are obtained. Subsequently, these parameters are fixed and the fit is carried out one additional time, fitting the area under the red slice. The result is the form factor, $F(q_z)$, obtained from the corrected scattering intensity (equation on the right in Fig. S1). q_z in this equation is the

Lorentz polarization factor. The form factor obtained is then used to fit via the Fourier transform to a model of an electron density profile that uses Gaussians and error functions for the various membrane components. The computer program that carries out this fitting was written by Dr. Norbert Kučerka and is called the Scattering Density Profile (SDP) method [5]. One important input to the SDP program is the lipid molecular volume, which is measured using an Anton-Paar 5000M densitometer. Densities are measured for individual membrane mimics, mimics containing peptides and for pure peptides. When electrons are counted, then the Y-axis can be expressed as absolute electron density (e/Å^3). The headgroup molecular volume is estimated by adding fractional volumes of a PC lipid [6], a PE lipid [7], a PG lipid [8], a PS lipid [9] and PI lipids [10]. The constraints used in the SDP fitting were the Gaussian width of the methyl trough (2-5 Å) and the distance between the carbonyl group position and the hydrocarbon edge (D_C) (1.3 Å). The SDP program yields many structural parameters, including bilayer thickness (D_{HH}, $2D_C$) and area/lipid A_L.

2. Details of WAXS data collection and analysis



F $$S_{xray} = \frac{1}{2}(3\,\langle cos^2\beta\rangle - 1)$$

Figure S2. WAXS data collection and analysis. A. Scattering geometry, B. WAXS scattering from a well-ordered sample with background subtracted, C. WAXS intensity as a function of φ angle in 10 degree increments starting at the equator, D. Continuous WAXS intensity as a function of φ angle integrated from 1.2 to 2.2 $Å^{-1}$ in q_r, E. Chain scattering model used to fit to WAXS intensity in D., F. Equation for determination of S_{xray}. Reprinted from [11], Copyright 2008, with permission from Elsevier.

In order to obtain WAXS data, the same sample that was hydrated in LAXS is then X-rayed with the CCD detector close (~10-15 cm) to the sample. Instead of rotating the wafer continuously as in LAXS, two pictures are taken: $\alpha = +0.5$ degrees and $\alpha = -0.5$ degrees. Both are dezingered, 30 second scans (CHESS), or dezingered 10 minute scans (CMU), which are then subtracted from each other. This procedure removes all extraneous scatter due to the mylar chamber windows and shadows. The scattering geometry is shown in Fig. S2A. The chain-chain correlation appears as strong diffuse scatter emanating upwards from the equator in a radial fashion around the φ angle; an example is shown in Fig. S2B. The fall-off in this diffuse intensity around the φ angle yields information about chain order; a steep fall-off, such as shown in Fig. S2B, indicates well-ordered chains, while a more continuous fall-off indicates less ordered chains. In order to carry out the analysis which quantitates the chain orientational order, a sector plot is first made by integrating in 10 degree pie sectors the WAXS intensity starting at the equator. An example of the resulting plot is shown in Fig. S2C. The q_r position of the maximum intensity is used to calculate the interchain d-spacing as $2\pi / q_r = d$. The sector plot is also used to determine the q_r range over which the WAXS intensity will be integrated, which is usually from ~1.2 to ~2.2 $Å^{-1}$. The WAXS intensity is then integrated as a function of φ over the chosen q_r range resulting in the intensity plot shown in Fig. S2D. In the chain scattering model shown in Fig. S2E, long thin rods are locally well aligned along the local director n_L, with orientation described by the angle β. For each grain (group of rods), scattering is permitted only at right angles to n_L. While acyl chains from lipids in the fluid phase are not long cylinders as shown, this model allows the cylinders to tilt (β) to approximate chain disorder. From the fit of the intensity data as a function of φ angle to the liquid crystal theory [11], we obtain S_{xray} using the equation in Fig. S2F, as well as the RMSE (root mean square error), which indicates the goodness of the fit. The order parameter for hydrocarbon chains obtained with WAXS (S_{xray}), although quantitatively lower than S_{CD} from NMR experiments, is able to detect different acyl chain order states in fluid lipid phases as previously shown [11, 12]. The fitting is accomplished with a Matlab computer program written by Dr. Thalia Mills and Dr. Gil Toombes. Many more details about this WAXS analysis can be found in Ref. [11] and in the six Supplementary Material sections published in the Biophysical Journal in 2008.

SUPPORTING REFERENCES

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