

SUPPLEMENTARY MATERIAL

The effect of cholesterol on short- and long-chain monounsaturated lipid bilayers as determined by molecular dynamics simulations and x-ray scattering

N. Kučerka, J. D. Perlmutter, J. Pan, S. Tristram-Nagle, J. Katsaras and J. N. Sachs

The presence of cholesterol crystals

Additional X-ray measurements were conducted in order to ensure that the “lift-off” in the first minimum of thin bilayers scattering data was not an experimental artifact. Non-extruded, multi-lamellar vesicles prepared from 1,2-dimyristoleoyl-*sn*-glycero-phosphatidylcholine (diC14:1PC) and 1,2-dilauroyl-*sn*-glycero-phosphatidylcholine (diC12:0PC) were examined for the presence of cholesterol crystals. Consistent with previous reports (Huang et al., *BBA* 1417 (1999)), we have detected peaks corresponding to crystallized cholesterol at the high cholesterol concentration of 75 mol %, but not at 45 mol %.

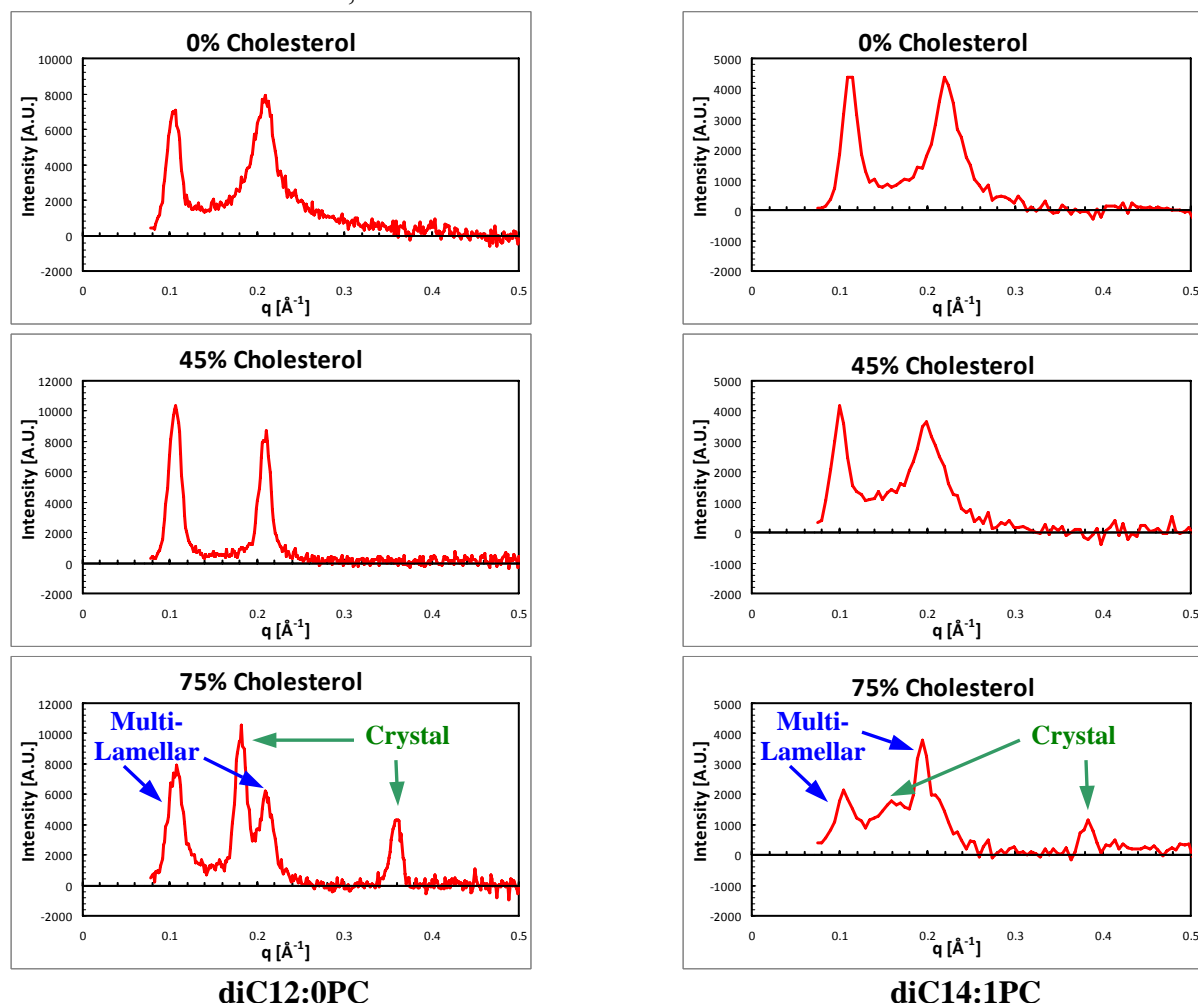


Figure S.1: X-ray scattering intensities from non-extruded mixtures of diC12:0PC (left) and diC14:1PC (right) with cholesterol. Data is averaged over three sample exposures. Cholesterol crystal peaks are clearly seen at high cholesterol concentration (75 mol %), while only multi-lamellar peaks can be observed for lower concentrations.

X-ray scattering from ULVs

X-ray data on unilamellar vesicles (ULVs) were taken at the D-1 station located at the Cornell High Energy Synchrotron Source (CHESS). $\lambda=1.18 \text{ \AA}$ scattered x-rays were collected using a Medoptics charge-coupled device (CCD, 1024 x 1024 pixel array), with linear dimensions of 47.19 μm per square pixel. The sample-to-detector distance was 322 mm, as was determined using a silver behenate standard. Standard 1.5 mm quartz capillaries were used as sample cells. Collected images were “dezingered” and processed for CCD distortion and intensity corrections using calibrated files supplied by CHESS. All data sets were normalized using the incident beam intensity measured through a semitransparent beam stop made out of a 225 μm thick molybdenum foil. The background subtracted images of scattering from the diC22:1PC and diC22:1PC with 40 mol% cholesterol ULVs are shown in Figure S.2.

In this figure, the white pixels correspond to high intensity and black pixels represent smaller intensity values. The red pixels represent intensities of values less than zero. The axis shows corresponding q values. The red rectangle at the bottom left of the image is the semi-transparent molybdenum beam stop. The intensities for the form factor data shown in the main paper were calculated from the radially averaged data.

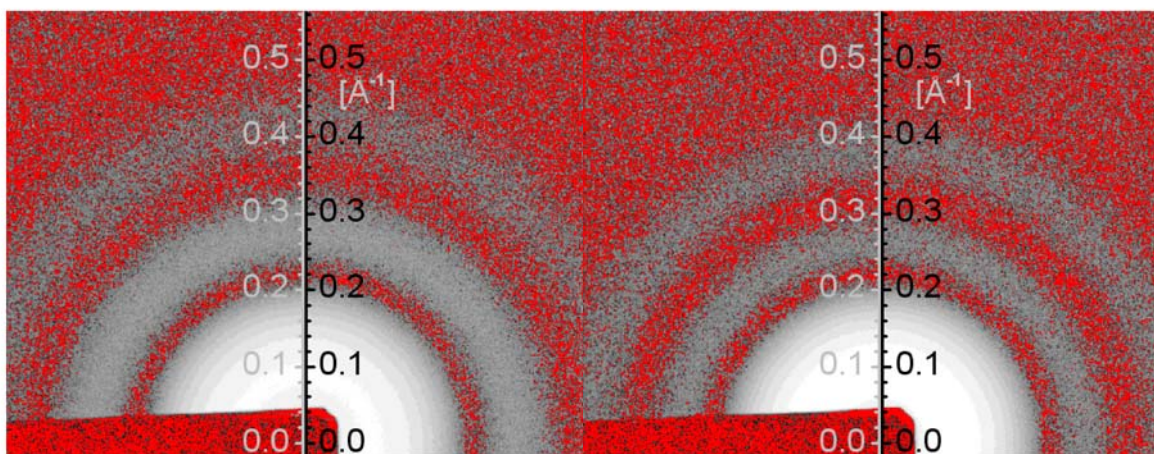


Figure S.2: 2D CCD x-ray scattering data of ULV bilayers composed of diC22:1PC (left) and diC22:1PC with 40% cholesterol (right).

X-ray scattering from ORIs

This section presents the primary x-ray diffuse data of oriented (ORI), hydrated samples for both diC14:1PC and diC14:1PC with 40% cholesterol which were collected using the RUH3R rotating anode at Carnegie Mellon University, as described in the main paper. Both images are the sum of two or more 5 minute scans near full hydration. The D-spacing was 51 Å for pure lipid multilayers (left panel) and 58 Å for bilayers with cholesterol (right panel). In this figure, the scale at the right correlates intensity with pixel color. The two axes show q values. The beam is depicted by the white oval near the bottom of the image and the horizontal line above the beam is the edge of the semi-transparent molybdenum beam stop. The intensity for the form factor data shown in the main paper was collected in the q_z direction using a swath of width 0.07 \AA^{-1} in q_r direction. The edge of this swath was placed at $q_r = 0.03 \text{ \AA}^{-1}$ in order to avoid convolution with the beam and reflectivity from the silicon substrate that occurs at $q_r = 0 \text{ \AA}^{-1}$.

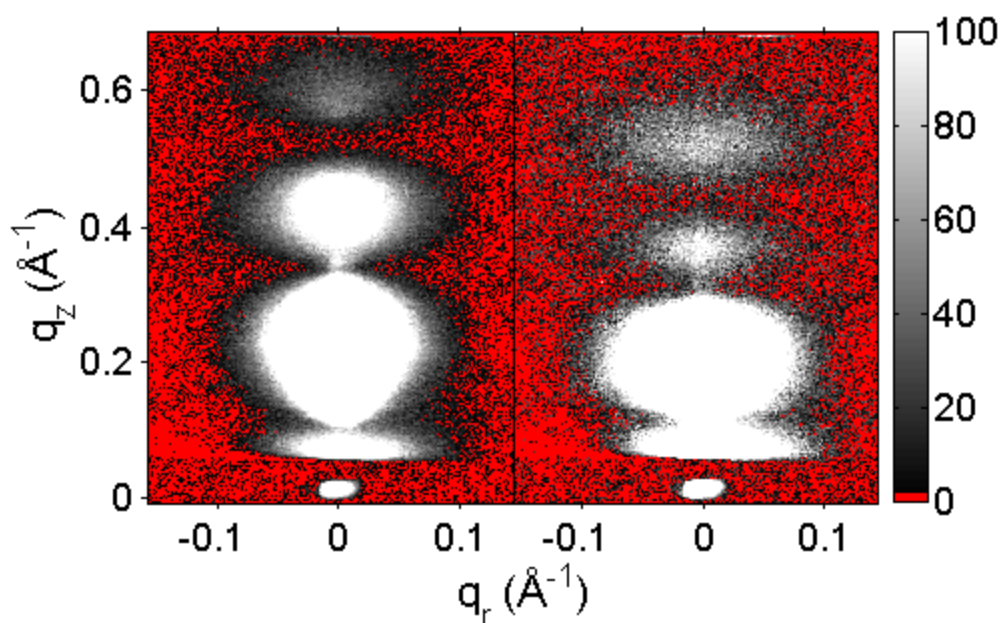


Figure S.3: 2D CCD x-ray scattering data of fully hydrated, oriented lipid samples composed of diC14:1PC (left) and diC14:1PC with 40% cholesterol (right).