# Structure of Supported DPPC/Cholesterol Bilayers Studied via X-ray Reflectivity: Electronic Supplementary Information

## 1 Details of the Fitting Model

The structure of the bilayers was modeled by approximating the electron density profile of the surface as a series of $m$ uniform density slabs. Each slab was described by three parameters, its electron density, $N_i$, its thickness, $D_i$, and the interfacial width between the slab and the slab above, $\sigma_i$. The topmost slab was then transitioned to a semi-infinite plane of water. The electron density of the model profile is then given by

$$\rho(z) = \frac{1}{2} \sum_{i=1}^{i=m} \delta N_i \left\{ 1 + \text{erf} \left[ \frac{(z - z_i)}{\sqrt{2}\sigma_i} \right] \right\} \tag{1}$$

Here $z$ is the distance normal to the substrate surface. We define $\delta N_i = N_i - N_{i-1}$ with the outermost density, $N_0$, taken to be water. The positions of the slab interfaces are given by $z_i = \sum_{j=i}^{j=m-1} D_j$ and $z_m = 0$. We define the error function by

$$\text{erf}\,(z) = \frac{2}{\sqrt{\pi}} \int_0^z e^{-t^2} dt \tag{2}$$

While each slab requires three parameters, the total number of free parameters was significantly reduced according to the following constraints: A) Since the water overlayer is infinite in extent, there is no interfacial width or thickness required to describe that layer and the density can be fixed to that of bulk water. B) The three layers employed to model the substrate were independently determined from an x-ray reflectivity measurement of an uncoated silicon substrate in water and not varied during the fits to the supported bilayers. For the bare substrate fits, the value of the transition layer within the substrate was fixed to the same values used by Steinrück et. al.[1], who originally recognized that this layer was essential for high quality fits at large $q$ for organic overlayers on silicon substrates. C) The electron density of the thin water layer directly adjacent to the substrate was fixed to that of water. D) The electron density of the distal and proximal head groups were fixed to be equal. E) The electron density of the distal and proximal acyl chain groups were fixed to be equal. F) The roughness of all components within a given leaflet were fixed to be equal. G) The roughness of the transition from the methyl region to the outer leaflet was taken as equal to the roughness of the inner leaflet. H) The thickness of the head group region was fixed at 0.70 nm. I) The thickness of the methyl dip region was fixed at 0.20 nm J) The density of the methyl dip region was approximated as zero. The thicknesses for the head group and methyl group regions were held fixed at values comparable to literature values for these region

widths for bulk multilayers[2] as it was found that allowing these parameters to vary did not significantly improve the fits.

The fixed parameters and their values are given in tab. 1. Fitting parameters for the model are given below in tab. 2. The mapping of the fitting parameters into the the full slab model is detailed in tab. 3.

Table 1: List of fixed parameters

| parameter | description | value |
|---|---|---|
| $D_{\text{interface}}$ | thickness of Si/SiO$_x$ interface | 0.15 nm |
| $D_{\text{SiOx}}$ | SiOx layer thickness | 0.999 nm |
| $D_W$ | water layer at substrate interface | 0.33 nm |
| $D_H$ | head group thickness | 0.7 nm |
| $D_M$ | methyl overlap region thickness | 0.2 nm |
| $N_{\text{Si}}$ | Silicon substrate electron density | 702.5 $e^-$/nm$^3$ |
| $N_{\text{interface}}$ | Si/SiO$_x$ transition region density | 560 $e^-$/nm$^3$ |
| $N_{\text{SiOx}}$ | SiO$_x$ electron density | 692.0 $e^-$/nm$^3$ |
| $N_M$ | electron density of methyl overlap region | 0 $e^-$/nm$^3$ |
| $N_W$ | Water electron density | 334.2 $e^-$/nm$^3$ |
| $\sigma_{\text{Si}}$ | roughness of Si interface | 0.15 nm |
| $\sigma_{\text{interface}}$ | roughness of Si/SiO$_x$ transition region | 0.15 nm |
| $\sigma_{\text{SiOx}}$ | roughness of SiO$_x$/water interface | 0.3 nm |

Table 2: List of fitting parameters

| parameter | description |
|---|---|
| $D_A$ | acyl chain thickness |
| $\delta D_A$ | outer acyl chain expansion |
| $N_H$ | head group electron density |
| $N_A$ | acyl chain electron density |
| $\sigma$ | proximal leaflet interfacial roughness |
| $\delta\sigma$ | roughness expansion of distal leaflet |

Table 3: Mapping of fit parameters to slab parameters

| layer no. | Material | $N$ | $D$ | $\sigma$ |
|---|---|---|---|---|
| 0 | water | $N_W$ | | |
| 1 | distal head group | $N_H$ | $D_H$ | $\sigma + \delta\sigma$ |
| 2 | distal acyl chain | $N_A$ | $D_A + \delta D_A$ | $\sigma + \delta\sigma$ |
| 3 | methyl overlap region | $N_M$ | $D_M$ | $\sigma + \delta\sigma$ |
| 4 | proximal acyl chain | $N_A$ | $D_A$ | $\sigma$ |
| 5 | proximal head group | $N_H$ | $D_H$ | $\sigma$ |
| 6 | proximal water | $N_W$ | $D_W$ | $\sigma$ |
| 7 | $SiO_x$ | $N_{SiOx}$ | $D_{SiOx}$ | $\sigma_{SiOx}$ |
| 8 | $Si/SiO_x$ interface | $N_{interface}$ | $D_{interface}$ | $\sigma_{interface}$ |
| 9 | Silicon substrate | $N_{Si}$ | | $\sigma_{Si}$ |

## 2  Fits to bare substrates

In order to characterize the profile of the bare substrate, reflectivity measurements were performed on a silicon substrate prepared in an identical manner to the substrates used for the bilayer measurements and similarly submerged in water within the sample chamber. It is known that bare silicon will form a thin oxide layer whose growth is limited to of order of one nm. We have modeled the scattering from the substrate interface using the form given Steinrück *et. al.*[1] which includes bulk Si terminated with a thin oxide layer and a thin transition region between the Si and the oxide. They showed in their work that while it is possible to fit just the bare substrate without this transition region, fits to substrates with overlayers were inaccurate without including it. We included this interfacial region using the same parameters as were used in their work: an electron density of $N_e = 560$ e$^-$/nm$^3$, an interfacial width of 0.15 nm and an interfacial roughness on both sides of 0.15 nm. Fitting just the thickness and density of the silicon oxide layer then gave values of $N_e = 693(3)$ e$^-$/nm$^3$, $d$=1.00(2) nm and $\sigma$=0.302(5) nm. The final fit to the data is shown below in fig. 1.
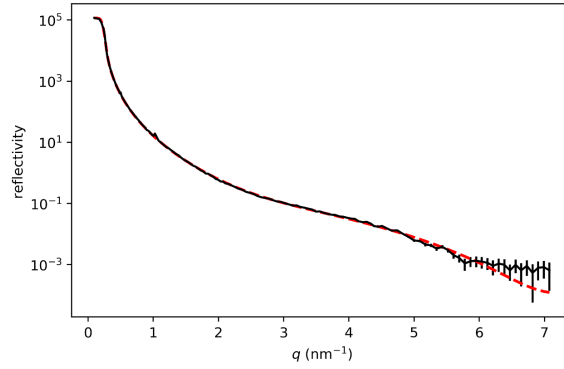
Figure 1: Fit to bare substrate in water

# 3 Fits for all temperatures and cholesterol concentrations

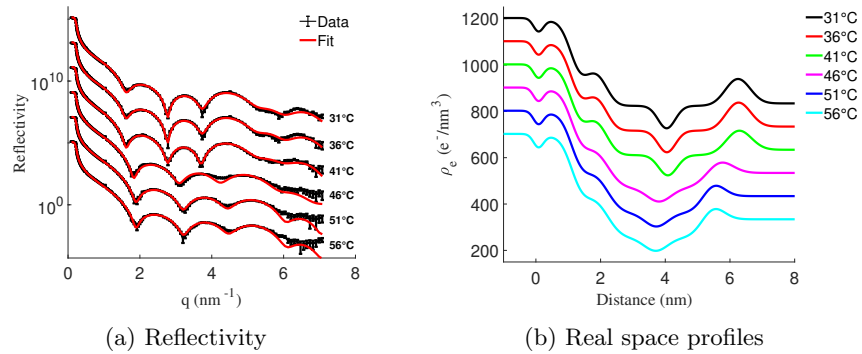Figs. 2-6 show the fits and corresponding real space profiles for all the measured data.



(a) Reflectivity

(b) Real space profiles

Figure 2: Reflectivity with fits and corresponding real space profiles for 0 mol% data.

(a) Reflectivity

(b) Real space profiles
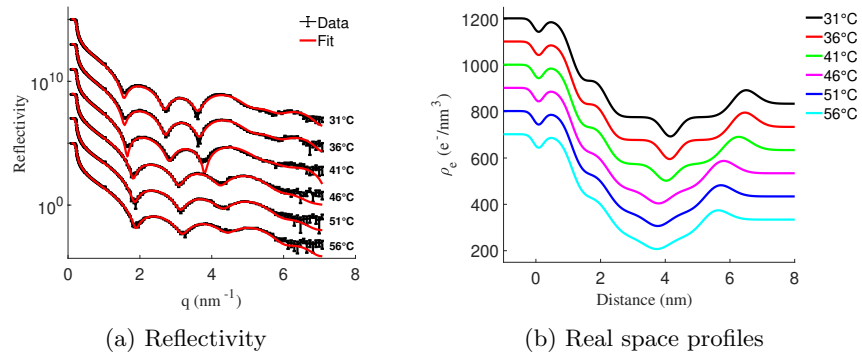
Figure 3: Reflectivity with fits and corresponding real space profiles for 10 mol% data.



(a) Reflectivity

(b) Real space profiles
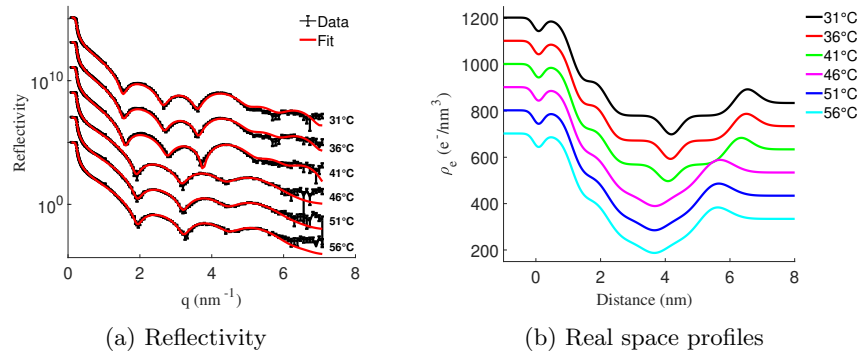
Figure 4: Reflectivity with fits and corresponding real space profiles for 20 mol% data.



(a) Reflectivity

(b) Real space profiles
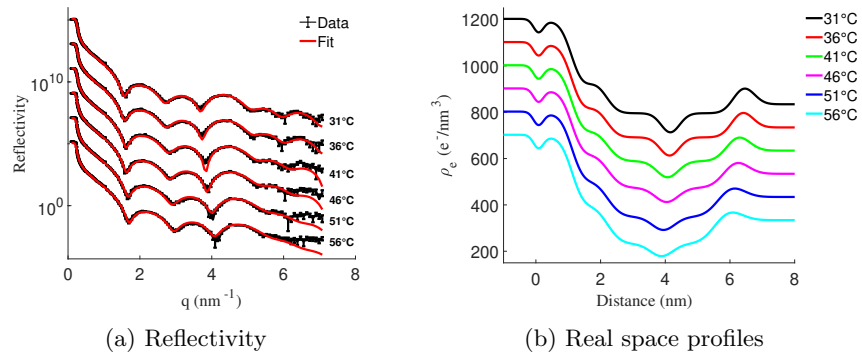
Figure 5: Reflectivity with fits and corresponding real space profiles for 33 mol% data.

(a) Reflectivity



(b) Real space profiles
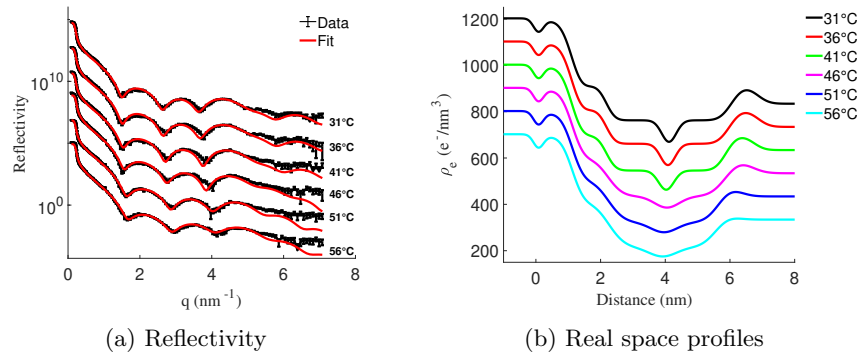
Figure 6: Reflectivity with fits and corresponding real space profiles for 40 mol% data.

# 4 Spot to spot sample variations

The figures below show the reflectivity and best fit real space profiles for samples at each cholesterol concentration measured at two non-overlapping spots on the same sample.
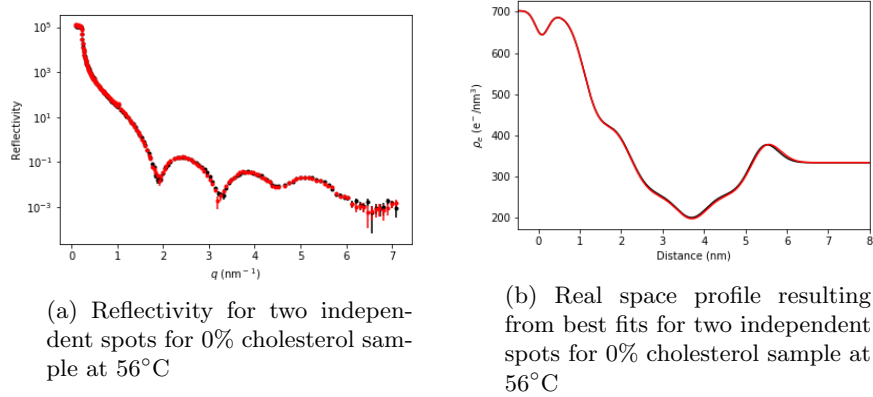


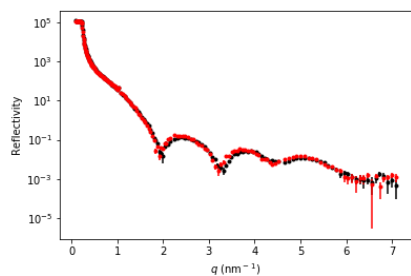(a) Reflectivity for two independent spots for 0% cholesterol sample at 56°C



(b) Real space profile resulting from best fits for two independent spots for 0% cholesterol sample at 56°C
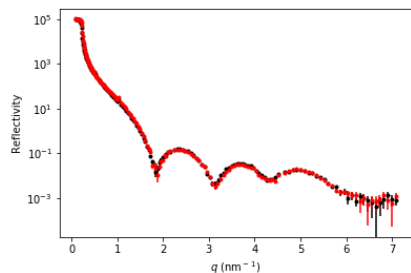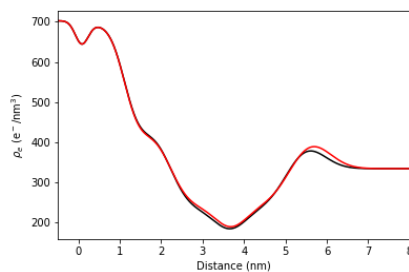
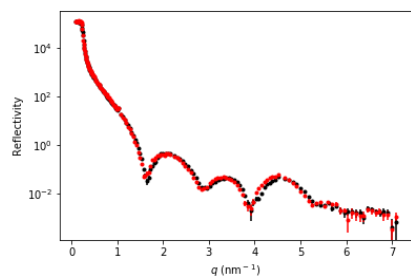(a) Reflectivity for two independent spots for 10% cholesterol sample at 51°C
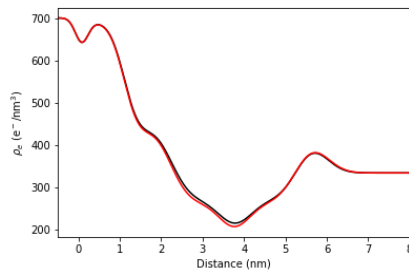


(b) Real space profile resulting from best fits for two independent spots for 10% cholesterol sample at 51°C
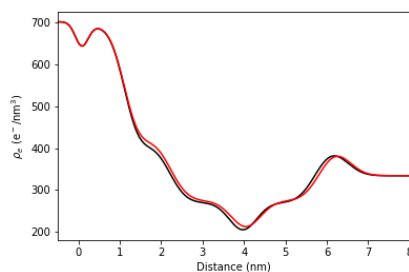


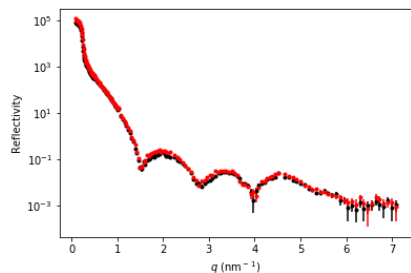(a) Reflectivity for two independent spots for 20% cholesterol sample at 46°C



(b) Real space profile resulting from best fits for two independent spots for 20% cholesterol sample at 46°C
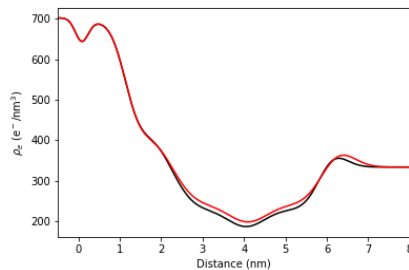


(a) Reflectivity for two independent spots for 33% cholesterol sample at 41°C



(b) Real space profile resulting from best fits for two independent spots for 33% cholesterol sample at 41°C

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(a) Reflectivity for two independent spots for 40% cholesterol sample at $46°$C



(b) Real space profile resulting from best fits for two independent spots for 40% cholesterol sample at $46°$C

# 5  References

[1] H. G. Steinrück, *et. al.*, ACS Nano, 2014, **8**, 12676-12681.

[2] J. F. Nagle, P. Cognet, F. G. Dupuy and S. Tristram-Nagle, *Chemistry and Physics of Lipids*, 2019, **218**, 168-177.