

## Supplementary Material

# Optimization of Giant Unilamellar Vesicle Electroformation for Phosphatidylcholine/Sphingomyelin/Cholesterol Ternary Mixtures

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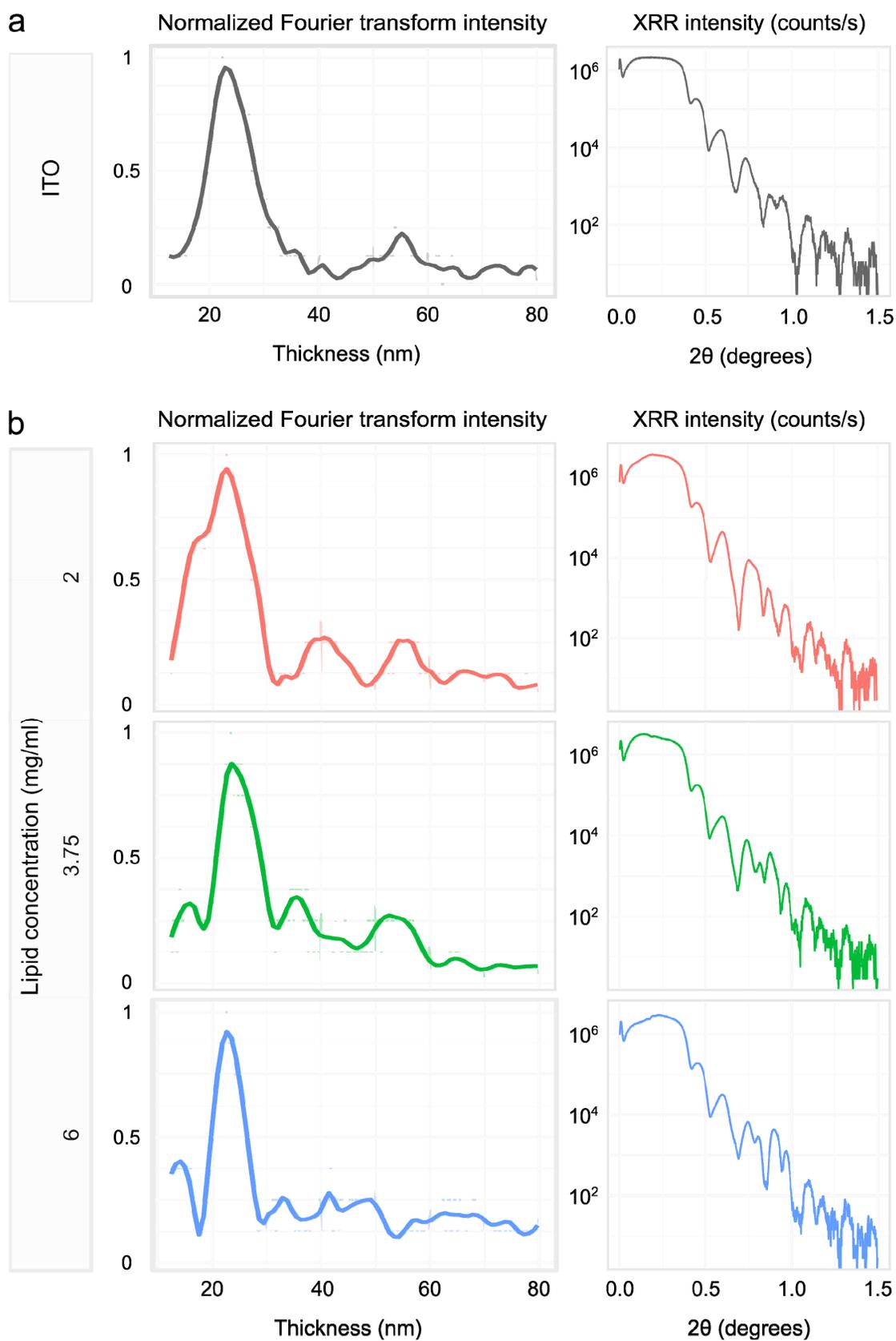
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Figure S1 shows thicknesses obtained using XRR measurements. The right column shows the raw data, and the left one the normalized Fourier transform intensity. Curves for the ITO sample show a dominant peak centered at  $\sim 25 \mu\text{m}$ , with an additional smaller peak at  $\sim 55 \mu\text{m}$  (Figure S1a).

Figure S1b compares the thicknesses measurements between films made using three different lipid film concentrations and a Chol/(POPC+SM) mixing ratio of 0.75. The results for the 2 mg/ml concentration show an additional hump around  $20 \mu\text{m}$ . This is the dominant peak when we account the ITO signal and its value is in agreement with the AFM measurements. The middle concentration mostly restores the symmetrical shape of the main ITO peak, with a dominant lipid film peak occurring at  $\sim 35 \mu\text{m}$ . The highest measured concentration seems to be the least homogenous, but an increase in thickness compared to lower concentrations is evident from the wide peak appearing at  $\sim 70 \mu\text{m}$ . The results are in line with the trend of increasing thickness with increasing lipid concentration suggested by the AFM results in the main text.



**Figure S1.** XRR analysis of spin-coated lipid films thicknesses. The right column shows the raw data, and the left one the normalized Fourier transform intensity. **(a)** Measurements for an ITO sample. **(b)** Measurements for different lipid concentrations using a Chol/(POPC+SM) mixing ratio of 0.75.