SUPPLEMENTARY MATERIAL

Asymmetric distribution of cholesterol in unilamellar vesicles of monounsaturated phospholipids

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STRUCTURAL MODEL

The data in the present study are of sufficient quality that they can be used in an analysis capable of determining a bilayer's structural properties using advanced models. However, the focus here is on the determination of bilayer asymmetry, rather than the structural details of the bilayer itself. The model used consists of two positive amplitude Gaussians for the headgroup region, while the terminal methyl trough is described by a single negative Gaussian. The smooth transition between the hydrophilic and hydrophobic regions is achieved through the use of error functions (see Figure S1).

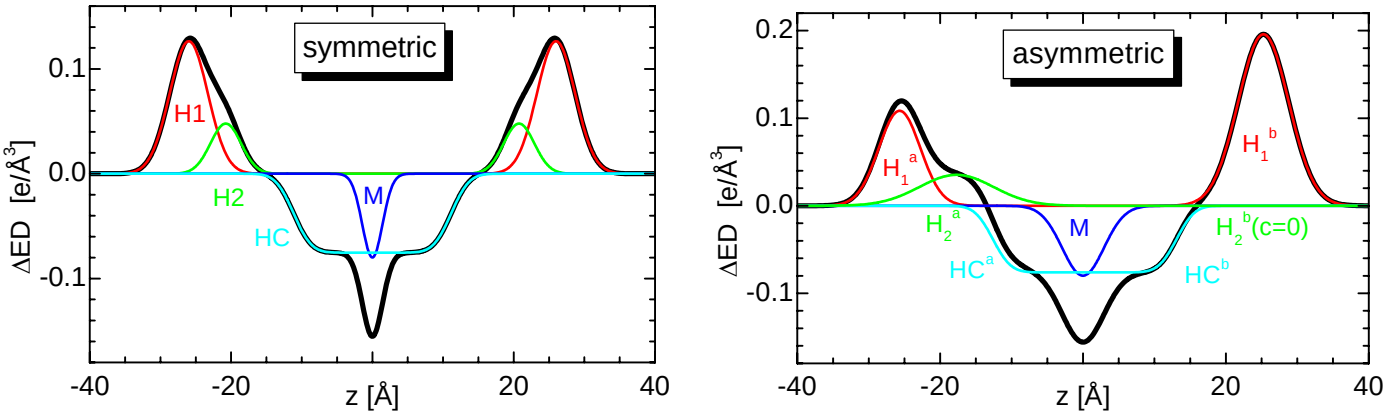

FIGURE S1: Model of bilayer electron density profile consisting of two headgroup Gaussians (H1 and H2), terminal methyl trough Gaussian (M), and the hydrocarbon chain Error functions (HC).

Each Gaussian (i.e. H1, H2, M) is described by three parameters namely, its position (z_0), width (σ) and height (c) as follows:

$$G(z) = \frac{1}{\sqrt{2\pi}}\left(c^a \exp\left[-\frac{\left(z + z_0^a\right)^2}{2\left(\sigma^a\right)^2} \right] + c^b \exp\left[-\frac{\left(z + z_0^b\right)^2}{2\left(\sigma^b\right)^2} \right] \right), \quad \text{(S1)}$$

where a and b emphasize that there are two sides to a bilayer. Writing the equation in this manner simplifies the modeling of symmetric ($a = b$, left panel) and asymmetric ($a \neq b$, right panel) bilayers. Similarly, the number of parameters for the central methyl Gaussian is reduced by requiring $z_0 = 0$.

As was previously shown,[1] the hydrocarbon chain region (HC) is well represented using the sum of two mirrored Error functions i.e.,

$$HC(z) = c\left[erf\left(z, z_0^a, \sigma^a\right) - erf\left(z, z_0^b, \sigma^b\right) \right], \quad \text{(S2)}$$

where erf is the classical Error function that has a step centered at z_0 , a width of σ, and is written as

$$erf\left(z, z_0, \sigma\right) = \frac{2}{\sqrt{\pi}} \int_0^{\frac{z - z_{0i}}{\sqrt{2}\sigma}} \exp\left[-x^2 \right] dx . \quad \text{(S3)}$$

The water subtracted electron density (i.e. bilayer minus water) is then obtained through the summation of the three Gaussians and the Error functions

$$\Delta ED(z) = G_{H1}(z) + G_{H2}(z) + G_M(z) + HC(z) . \quad \text{(S4)}$$

REFERENCES

(1) Kučerka, N., Nagle, J. F., Sachs, J. N., Feller, S. E., Pencer, J., Jackson, A., and Katsaras, J.; *Biophys.J.* **2008**, *95*, 2356-2367.