CHOLESTEROL TRANSLOCATION IN A PHOSPHOLIPID MEMBRANE

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SUPPLEMENTARY MATERIAL

Simulation details

Molecular dynamics simulations are performed with the GROMACS 4.5 package (1). The cutoff for the non-bonded Lennard-Jones interaction is 0.9 nm. The Coulomb interaction is calculated with the particle mesh Ewald (PME) method. In PME the real-space contribution is obtained with a cutoff of 0.9 nm and the reciprocal-space contribution is calculated using the fast Fourier transform technique with a grid spacing of 0.16. We use the Nose-Hover scheme to maintain the temperature at 323K. DPPC, CHOL and water molecules are coupled independently with the thermostat using a time constant of 0.2 ps. We also use the Parrinello-Rahman scheme for semi-isotropic coupling in the *x-y* and *z* directions. The coupling constant is taken to be 2.5 ps and the pressure in the system is 1 bar.

We create the DPPC bilayer by placing DPPC molecules on a grid and then randomly replace a fraction of DPPC molecules by CHOL molecules. The size of the MD box is chosen to give a reasonable initial value for the average area per lipid. Subsequently, we add water molecules randomly and equilibrate the entire system for one hundred nanoseconds.

Since CHOL flip-flop is a rare event in an MD simulation, we have also used the accelerated all-atom parallel replica MD approach to study the flip-flop phenomenon. In a parallel replica simulation, we run five parallel replicas on five sets of identical processors. Whenever the difference between the maxima and minima of the *z* coordinate of the hydroxyl group of a CHOL molecule in a replica exceeds 3 nm (bilayer thickness

is 4-5 nm), we record that as a "rare event" and advance the clock by five times the simulated time of the rare event.

Calculations of self-diffusion coefficients and stress profiles

The lateral self-diffusion coefficients for DPPC and CHOL molecules are obtained from the slopes of mean square displacements in the *x-y* plane as a function of time. The mean square displacements are averaged over 500 time frames over a time period of 100 ns.
The Irving-Kirkwood contour method is used to calculate stress profiles normal to the DPPC bilayer (*z* direction). We divide the system into slabs of length 0.2 nm in the *z* direction and calculate stresses by averaging over 5000 frames spanning 50 ns.

Area Per Molecule

We use the method suggested by Hosfaβ et al. (2) to calculate the area per DPPC molecule, A_{DPPC}, and the area per CHOL molecule, A_{CHOL}. We first calculate the bilayer height,

$$h = \frac{V_{box} - N_W V_W}{A_{box}} \qquad (S1)$$

where V_{box} and A_{box} are respectively the volume and area of the simulation box, and V_W is the volume of a water molecule. Following Hosfaβ et al., we set $V_W = 0.0312$ nm^3 (N_W = 14620) and calculate the volume of a DPPC molecule in the UL:

$$V_{DPPC}^{UL} = \frac{1}{N_{DPPC}^{UL}} \left[\frac{V_{box} - N_W V_W}{2} - N_{CHOL}^{UL} V_{CHOL} \right] \qquad (S2)$$

where N_{DPPC}^{UL} is half the total number of DPPC molecules and N_{CHOL}^{UL} is the number of CHOL molecules in the upper leaflet, which varies with time. Hosfaβ et al. suggest a value of 0.593 nm^3 for V_{CHOL}, the crystalline volume of a CHOL molecule. The relation,

$$A_{DPPC}^{UL} \frac{h}{2} = V_{DPPC}^{UL} \qquad (S3)$$

gives A_{DPPC}^{UL}, and A_{CHOL}^{UL} is obtained from

$$A_{CHOL}^{UL} = \frac{1}{N_{CHOL}^{UL}} \left[A_{box} - A_{DPPC}^{UL} N_{DPPC}^{UL} \right] \qquad (S4)$$

We follow the same procedure to calculate the areas for DPPC and CHOL molecules in the lower leaflet.

We find that A_{CHOL} = 0.28 nm^2 at t = 100 ns and 15 μs. At 15 μs, the CHOL concentration in the UL and LL are 25% and 34% respectively. Hosfaβ et al. report A_{CHOL} = 0.28 nm^2 at 25% CHOL concentration and 0.27 nm^2 at 40% CHOL concentration. At t = 15 μs the corresponding A_{DPPC} are 0.57 nm^2 and 0.52 nm^2. Hosfaβ et al. report A_{DPPC} = 0.55 nm^2 at 25% CHOL concentration and 0.54 nm^2 at 40% CHOL concentration.

Order parameter

We calculate the order parameter tensor $\tilde{S}$ defined as:

$$\tilde{S}_{ij} = \frac{1}{2} < 3\cos\theta_i \cos\theta_j - \delta_{ij} > \quad , \tag{S5}$$

where θ_i is the angle between the ith "molecular vector" and the bilayer normal. The "molecular vector" for the ith CH_2 unit has z component from C_{i-1} to C_{i+1} and the y component in the plane containing C_{i-1}, C_i, and C_{i+1}.The brackets denote an ensemble average. From the diagonal elements of $\tilde{S}$, we obtain the deuterium order parameter S_{CD}

$$S_{CD} = \frac{2}{3} S_{xx} + \frac{1}{3} S_{yy} \quad , \tag{S6}$$

Bending Free Energy

The bending free energy is defined as,

$$G_{bend} = \frac{K_b}{2} \int dx_1 dx_2 (\kappa_1(x_1,x_2) + \kappa_2(x_1,x_2))^2 \quad , \tag{S7}$$

where K_b is the bending rigidity. κ_1 and κ_2 are principal curvatures, defined in terms of the eigenvalues of the curvature matrix:

$$\mathrm{K} = \begin{bmatrix} \kappa_{11} & \kappa_{12} \\ \kappa_{21} & \kappa_{22} \end{bmatrix}, \tag{S8}$$

where $\kappa_{ij} = \dfrac{\partial^2 h}{\partial x_i \partial x_j}$ and h is the height of the membrane.

Curvature plays an important role in the structure and mechanical behavior of biomembranes (3). We calculated the curvature energy of the UL and LL at intervals of 2.5 μs. We divided the *x*-*y* plane into pixels of size 1.5 nm. This grid size was chosen to ensure that each pixel was occupied. The height of the membrane was determined from the *z* coordinate of either the N4 or P8 atom of DPPC molecules. The calculations involved averaging over 21 frames spanning 4 ns.

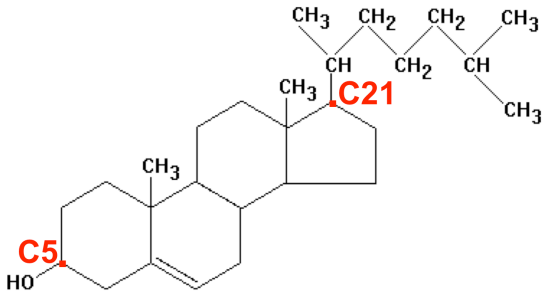

Figure S1: Structure of a cholesterol molecule with the C5 and C21 atoms highlighted. These atoms are used to define the tilt angle.

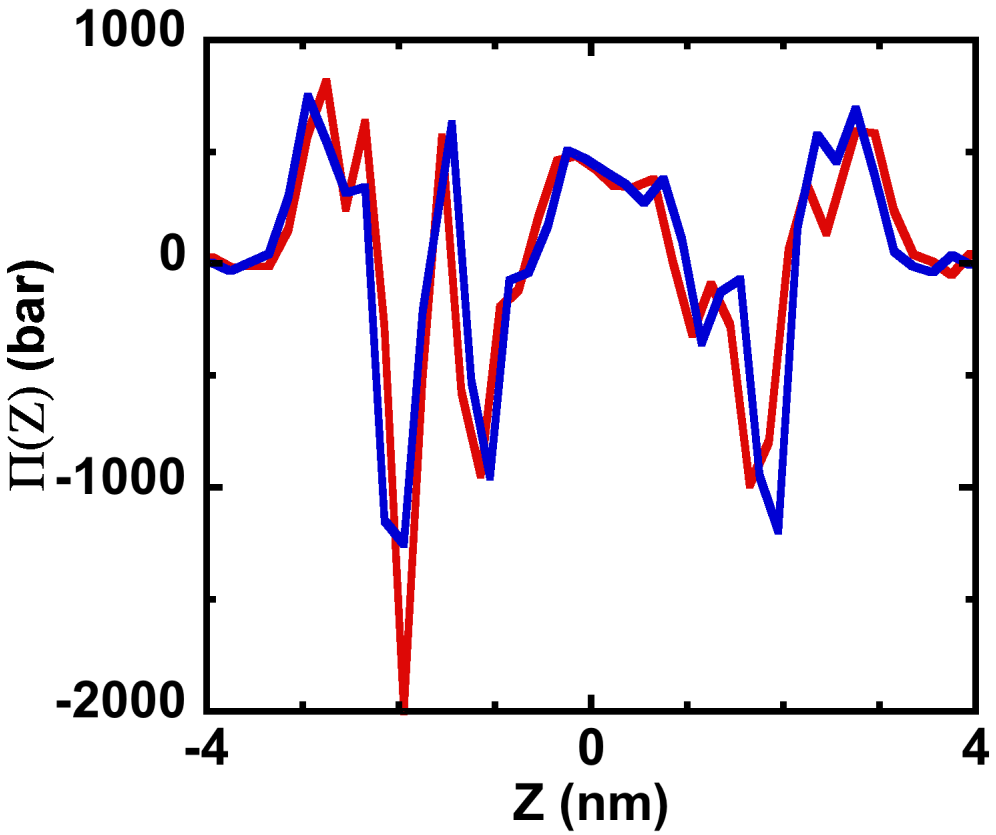

Figure S2: Stress, Π(z), profiles across the bilayer at time t = 5 μs (red) and 15 μs (blue).

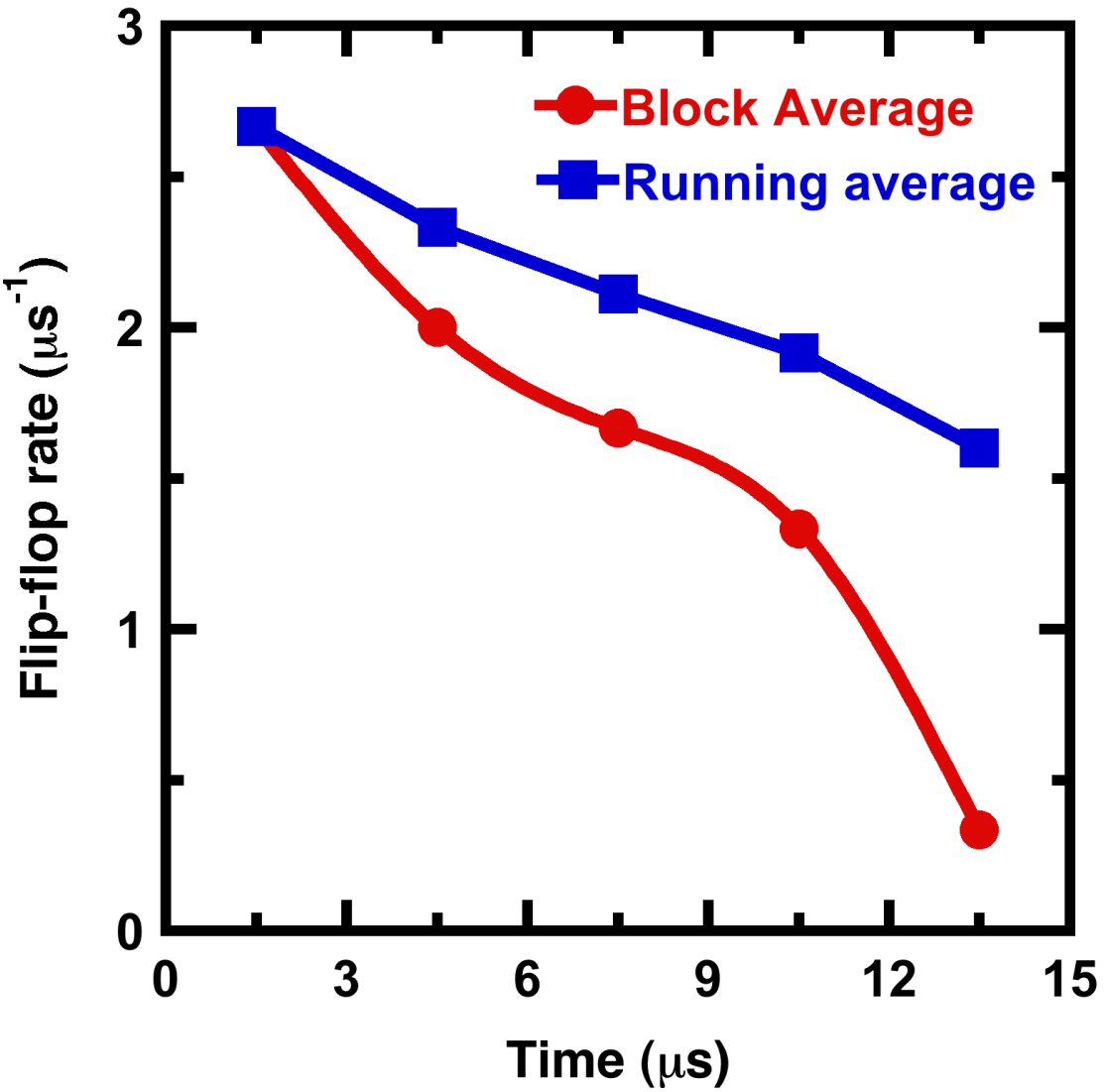

Figure S3: CHOL flip-flop rates measured in inverse microseconds versus time. The rates are calculated using block (red circles) and running (blue squares) averages. The block size is 3 μs. Continuous lines are drawn through the data points to guide the eye. The flip-flop rates decrease by an order of magnitude (red curve) with time.

REFERENCES

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