Comparative Computer Simulation Study of Cholesterol in Hydrated Unary and Binary Lipid Bilayers and in an Anhydrous Crystal

(Supplementary Material)

Elzbieta Plesnar[a], Witold K. Subczynski[b], Marta Pasenkiewicz-Gierula[a]*

[a] Department of Computational Biophysics and Bioinformatics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

[b] Department of Biophysics, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

e-mail: elzbieta.plesnar@uj.edu.pl; subczyn@mcw.edu; marta.pasenkiewicz-gierula@uj.edu.pl,

Corresponding Author: Marta Pasenkiewicz-Gierula, tel: +48-12-664-6518; fax: +48-12-664-6902; e-mail: marta.pasenkiewicz-gierula@uj.edu.pl

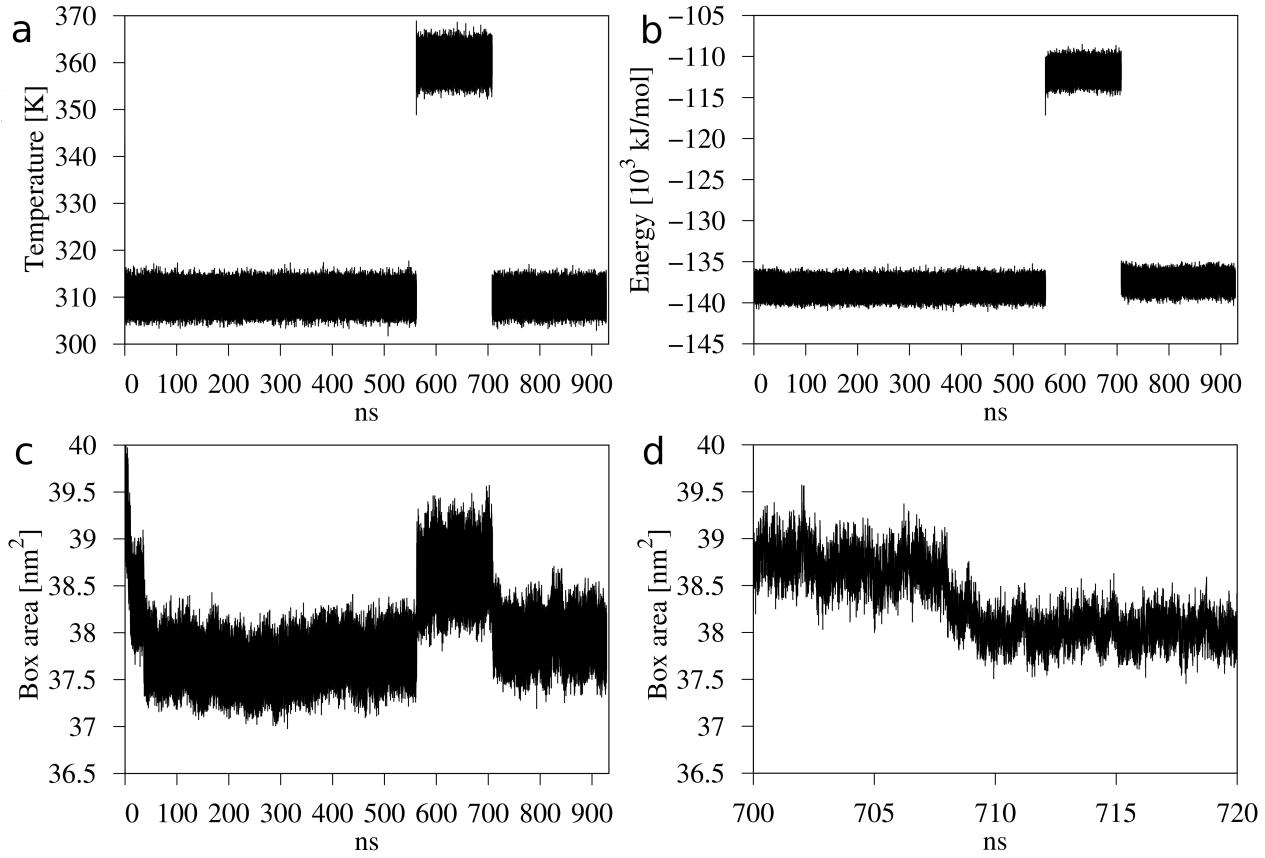

Figure S1. Time profiles of the cholesterol bilayer (model Chol, its initial structure was obtained based on the structure of a unit cell of the anhydrous cholesterol crystal as determined by Shieh et al. [1]; for details, see main text) temperature (a), potential energy (b), and the simulation box surface area (c) from the onset of simulation until 950 ns—the whole simulation time of this bilayer—and also the simulation box surface area from the time of 700 ns, when the temperature was lowered back from 360 to 310 K, until 720 ns, when the area reached a stable value of 38.0 ± 0.1 nm^2 (d). The last 200 ns fragments of energy and surface area profiles are shown in Figs 3a and b of the main paper.

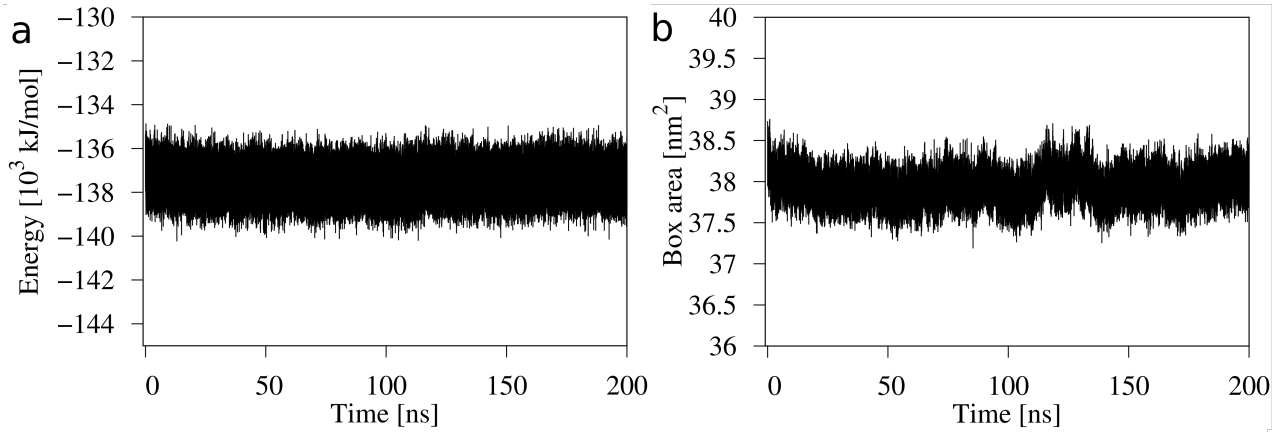

Figure S2. Time profiles of the potential energy (a) and the simulation box surface area (b) of the Chol-2 bilayer for the MD simulation time range 0.5-200 ns. The first 500 ps of the simulation are not included in a, and b because during this time both the potential energy and the surface area were rapidly decreasing and their initial values were off-scale. The Chol-2 bilayer consists of 10 × 10 × 2 Chol molecules. When building the initial structure of the bilayer, Chol molecules were randomly rotated about their long axis (no initial crystal order—in contrast to the Chol bilayer) and were placed in the *x,y*-plane in such a way that both bilayer surfaces were flat and the initial surface area of the simulation box was 56.25 nm² (*cf.*, Figs 2b and d, main text).

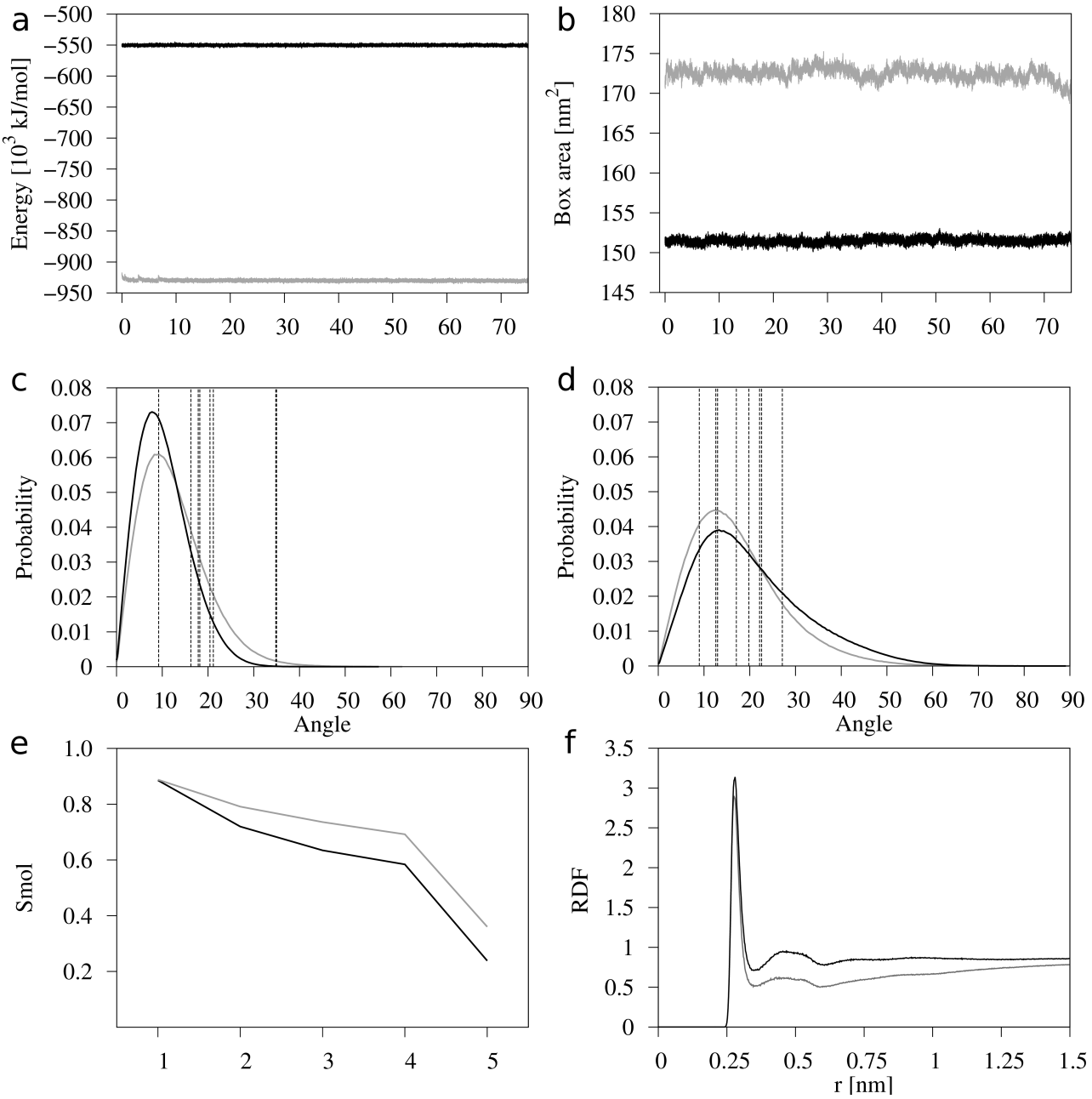

Figure S3. Time profiles of the bilayer potential energy (a) and the simulation box surface area (b) along respective MD trajectories; distributions of tilt angles for Chol rings (c) and chains (d); and the molecular order parameter (Smol) profiles calculated for the Chol chains (e), for the 4Chol (black line) and 4POPC-Chol50 (gray line) bilayers. In (f), radial distribution functions (RDF) of the water oxygen atoms relative to a Chol oxygen atom for the Chol (black line) and POPC-Chol50 (gray line) bilayers, are displayed. The vertical dashed lines in (c) and (d) represent tilts of the ring (c) and chain (d) of each of the eight Chol molecules in the unit cell of the anhydrous Chol crystal [1].

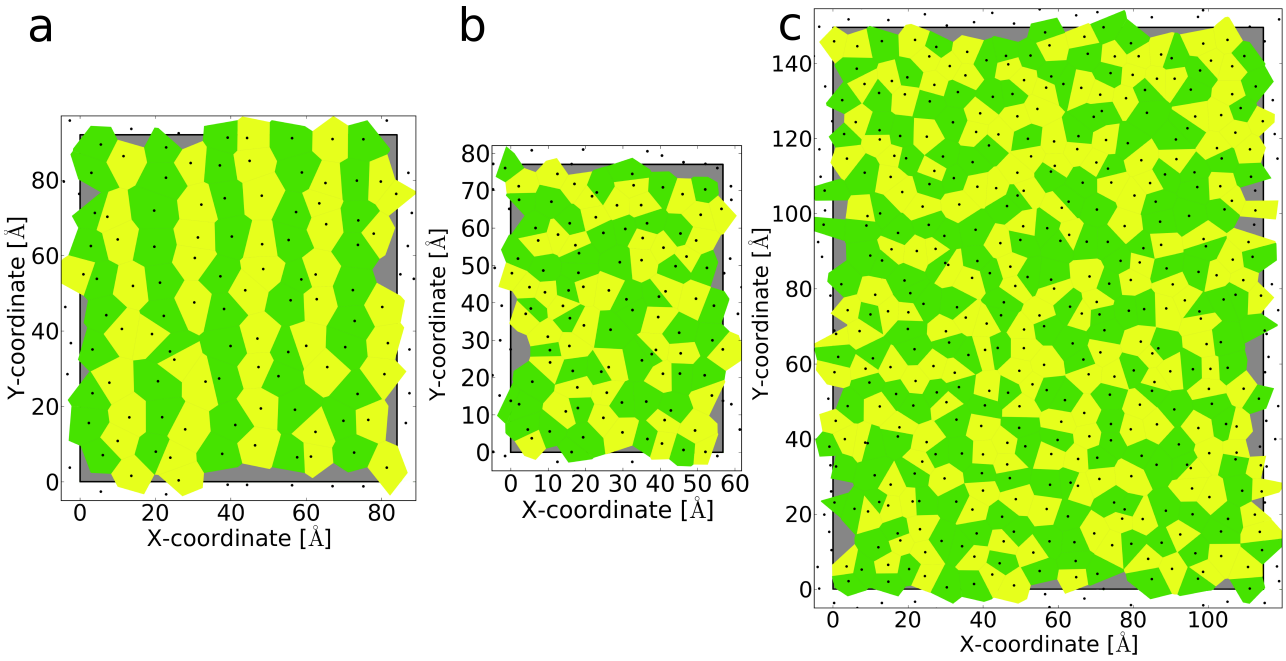

Figure S4. Top views of the POPC-Chol50 bilayers (the ratio of the actual sizes of the bilayers is preserved). Snapshots of the initial (a) and final (b, c) lateral distributions (based on Voronoi tessellation) of Chol and POPC molecules in the POPC-Chol50 (a, b) and the 4POPC-Chol50 (c) bilayers. The areas occupied by Chol and POPC molecules are marked yellow and green, respectively. The black dots indicate the center-of-mass of each molecule. The 4POPC-Chol50 bilayer was constructed from the POPC-Chol50 bilayer by replicating the last frame of its 200-ns MD simulation over periodic boundaries, and was simulated for the next 75 ns. Thus, the initial and final distributions are separated by 200 ns (POPC-Chol50) or 275 ns (4POPC-Chol50). In the course of MD simulation, the Chol and POPC molecules changed their initial positions nevertheless their homogenous distribution in the bilayers is preserved.

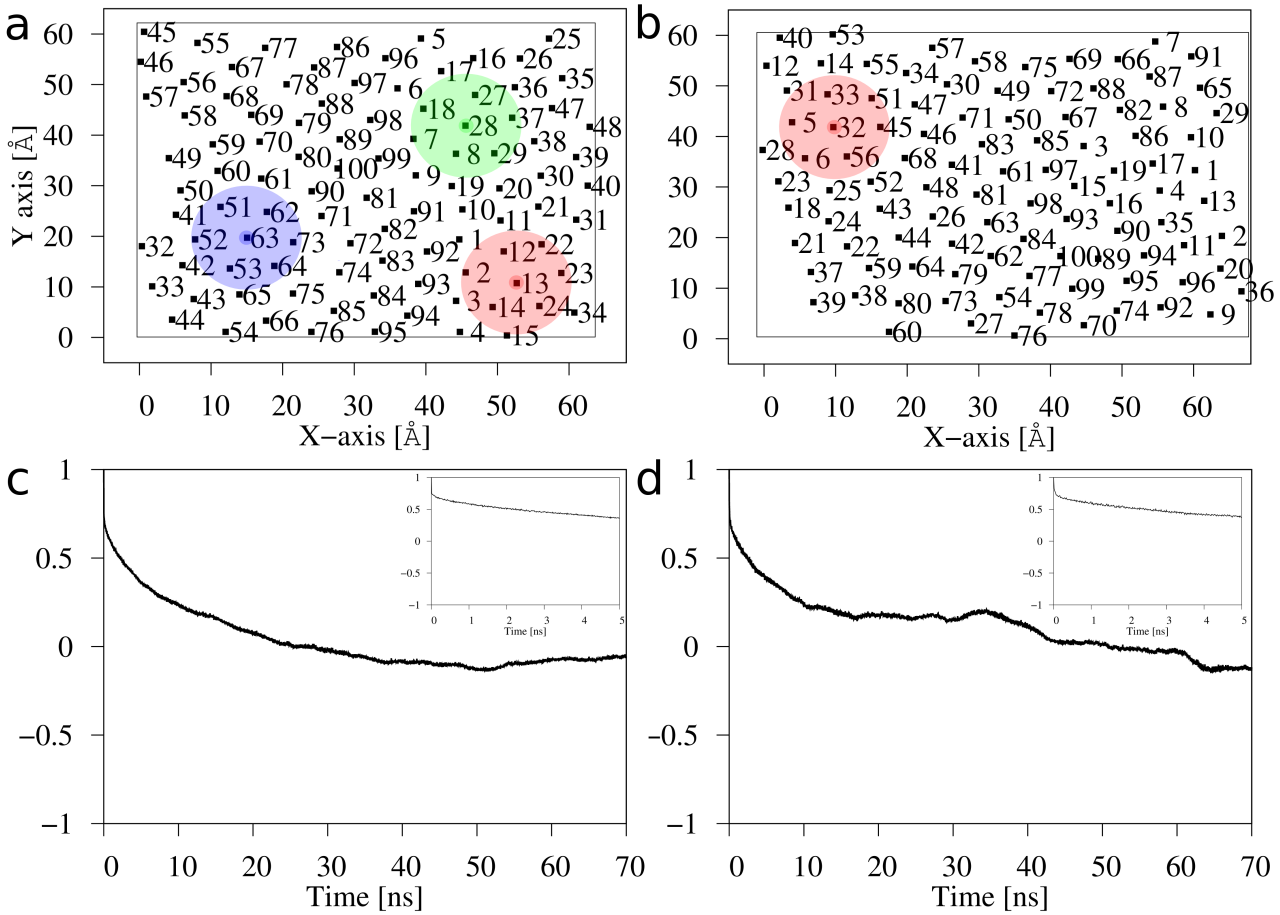

Figure S5. Collectivity of parallel rotation of neighboring Chol molecules in the Chol-2 and Chol bilayers. To check whether the rotation of neighboring Chol molecules is correlated in the Chol-2 bilayer, three Chol molecules located in different parts of the Chol-2 bilayer were chosen (a). For each of these three molecules, a time profile (70 ns, sampled with 70 ps time steps) of the cosine of the change ($\Delta\beta$) in the angle (β) between the projection of the C13-C18 bond on the *x,y*-plane and that of each of its six nearest neighbors, relative to the initial β_0, was calculated. The initial value of $\cos(\Delta\beta)$ is thus 1. Each profile was averaged over 1000 initial times and over six pairs. The average of the three profiles is shown in (c). The same calculation was performed for a selected Chol molecule in the Chol bilayer (b). The time profile of $\cos(\Delta\beta)$, averaged over 1000 initial times and over six pairs is shown in (d). The curves in (c) and (d) indicate that the parallel rotation of a Chol molecule in the Chol-2 and Chol bilayers is rather poorly correlated with that of its near-neighbors and also that the initial decays of the correlations are rapid.

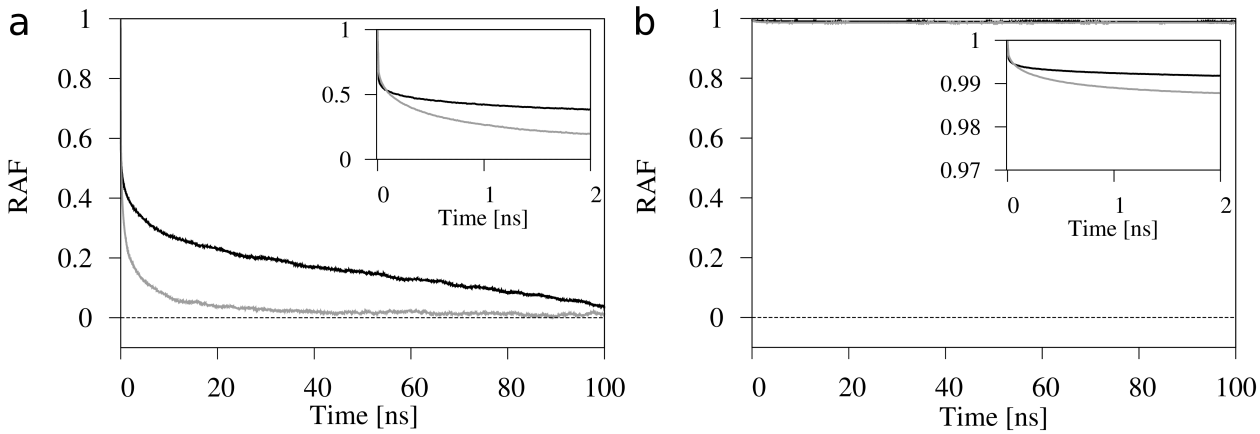

Figure S6. Analysis of the wobble-in-cone type of motion [2, 3] of Chol C3-C17 vector. **"**Precession" (a) assessed by calculating RAF for the projection of the C3-C17 vector on the *x,y*-plane, and perpendicular rotation (b) assessed by calculating RAF for the of the projection of the C3-C17 vector on the normal (this reflects that the rotation takes place relative to the initial Chol tilt) in the Chol-2 (black line) and POPC-Chol50 (gray line) bilayers (for details, see the main text). In (a) and (b), the insets show decays of the RAFs within the first 2 ns. In wobbling motion, transient precession and perpendicular rotations cannot be separated because changes in θ are coupled with changes in φ (for definition of θ and φ angles, see main text, or legends to Fig. S7), so "pure precession" (changes of φ for a constant θ value) cannot be monitored. The RAF for the perpendicular rotation decays from 1.0 to 0.990 ± 0.004 in the POPC-Chol50 bilayer and to 0.989 ± 0.003 in the Chol bilayer—this means that wobbling of Chol molecules in both bilayers is limited to the angular amplitude of 8.1°. The inset in (b) shows that RAF for the POPC-Chol50 bilayer decays to a constant (0.99 ± 0.004) within ~2 ns, thus, perpendicular rotation of Chol in the POPC-Chol50 bilayer is fast. Initially, rapid decays of RAFs in (a) and in (b) are coupled but later on, the rate of the decays in (a) becomes much slower. This indicates that population of the whole range of φ angles takes a considerably longer time than population of the accessible range of θ angles. For the POPC-Chol50 bilayer, the RAF in (a) decays to 0.02 ± 0.06 within less than 50 ns, and then retains the average value of 0.01 ± 0.08 for the next 50 ns of the analysis time; for the Chol-2 bilayer, the RAF decays to 0.04 ± 0.2 within ~100 ns (it reaches zero after next 20 ns, results not shown). The above numbers mean that, on average, the C3-C17 vector of a Chol molecule freely wobbles within the cone of the semi cone angle of 8.1° and the cone axis defined by the initial tilt of the Chol molecule, and that wobbling is faster in the POPC-Chol50 than in the Chol-2 bilayer.

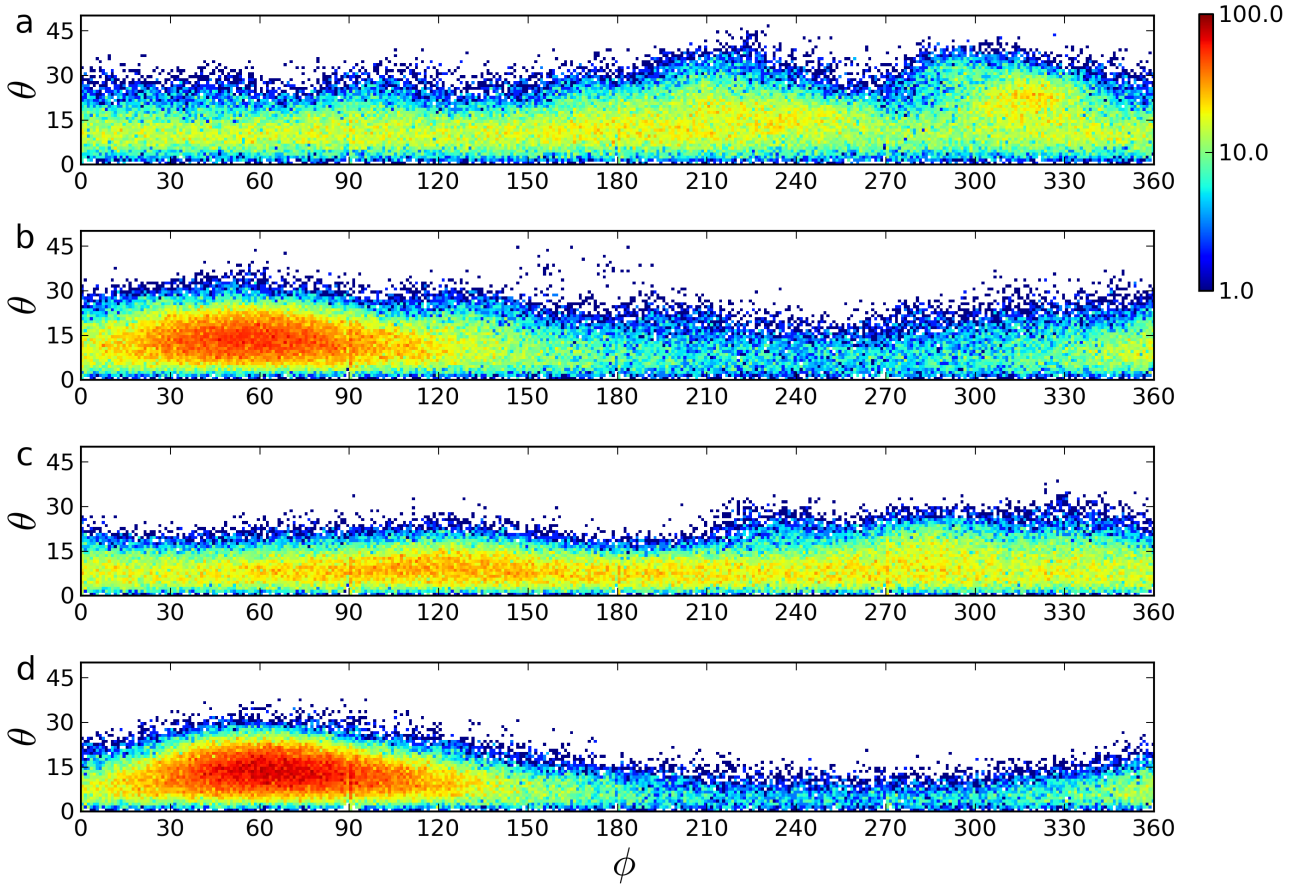

Figure S7. Simultaneous populations of angles by wobbling Chol molecules during 100 ns sampled with a 1 ps time step for two representative Chol molecules (fast and slow) in the POPC-Chol50 (a, b) and Chol (c, d) bilayers. The fast moving molecules are represented in (a, c), and the slow moving molecules in (b, d). The φ angle is between the projection of the C3-C17 vector on the *x,y*-plane and the *x*-axis, and the θ angle is between the C3-C17 vector and the bilayer normal. The color scale on the right-hand side codes logarithm of the number of cases when a given angle was populated during the analysis time. The white color indicates zero cases.

References

1. Shieh, H. S.; Hoard, L. G.; Nordman, C. E., *Acta Crystallogr. B* **1981,** 37, 1538-1543.
2. Kinosita, K.; Kawato, S.; Ikegami, A., Theory of Fluorescence Polarization Decay in Membranes. *Biophys. J.* **1977**, 20, 289-305.
3. Kinosita, K.; Ikegami, A.; Kawato, S., On the Wobbling-in-Cone Analysis of Fluorescence Anisotropy Decay. *Biophys. J.* **1982**, 37, 461-464.