Supporting Information

Molecular Dynamics Simulations Reveal the Protective Role of Cholesterol in Beta Amyloid Protein-Induced Membrane Disruptions in Neuronal Membrane Mimics

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S1. Determination of AL Column and nAL Region

In order to study the disruptive effect of a membrane-active protein on the lipid bilayer, an annular lipid (AL) column and the corresponding non-annular lipid (nAL) region were created for each replicate. The AL column is a cylindrically-symmetric column of irregular cross-section inside a simulation box, whereas the nAL region is the complement of the AL column. Hence all the lipids were divided into two exclusive groups, ALs and nALs (See *Materials and Methods*). As shown in **Fig. S1A**, the irregular perimeter of the AL column serves as a 0.5 nm-conformal contour outlining the profile of the protein on the *x*-*y* plane in such a way that each lipid belonging to the AL column is less than 0.5 nm from the nearest atom of the protein. The long cylindrical axis of the AL column is parallel to the *z* direction of the simulation box and extended across the whole simulation box along the *z*-axis as demonstrated in **Fig. S1B**. The nAL region is the whole simulation system but without the AL column as depicted in **Fig. S1 C** and **D**.

Lipids and water molecules were assigned to the AL column by use of an in-house computer program using the following selection criteria: (1) For a PC molecule, it must have at least half of the acyl chain carbons located inside the AL column, (2) For a CHOL molecule, it must have at least half of its atoms inside the AL column, and (3) For a water molecule, it must have at least one of its three atoms inside the AL column. All lipids and water molecules not assigned to the ALs belonged to the nALs.

S2. Estimations of Cross-section Area (*A*) of AL Column and nAL Region

The cross-section area of an irregularly shaped 2D figure was estimated using Monte Carlo integration. First, the figure of interest was placed inside a rectangular box with a given area. For a point randomly selected in the box, the probability of being located inside the irregular-shaped figure is equal to the ratio of the area of the irregular-shaped figure to that of the box. When enough random points are selected in the box, the area of the irregular-shaped figure can be estimated from the fraction of points located inside the figure. Therefore, in calculating the cross-sectional area (A) of the AL column or nAL region, we first created a box with an area A_R on the plane $z = 0$ such that all the water molecules projected onto this plane are inside this box. The area of the region formed by these projected water molecules (A_w) is recognized as the cross-sectional area of the AL column or nAL region. To estimate this area, uniformly-distributed sampling points in the box were generated using a pseudo uniform random-number generator.[1] If the minimum distance between a sampling point and the projected water molecules is less than the critical distance d_c, the sampling point is considered inside the water projection region. In this study, d_c = 0.44 Å according to a calibration procedure described below.

A random snapshot of a simulated system from one of the 16 simulations in this study was chosen and the water density profile of the whole simulation box was calculated through the Gromacs analysis tool g_density[2] as the standard. The critical distance was the distance that minimized the difference between the number density as calculated by g_density and the number density as calculated by our Monte Carlo integration method.

As the number of sampling points n increases, the fraction of sampling points located inside the water projection region converges to the fraction of area of the water projection region in the box.[3] Therefore, we can estimate the cross-sectional area A by **Eq. S1**.

$$A \approx A_w = A_R \cdot \lim_{n\to\infty} \frac{n_W}{n} \tag{S1}$$

Here n is the total number of random sampling points, n_W the number of sampling points inside the water projection region and A_W the area of the water projection region, which is approximately equal to A of the AL column or nAL region. In this work, the total number of random sampling points was close to 3 million to ensure a precision of 0.1% with a 99% confidence interval, according to the worst-case sample size suggested by Fishman.[3]

S3. Calculation of Particle Number ($N_i(z)$)

The particle number $N_i(z)$ denotes the number of particles located inside a segment, or z-slice, for the i^{th} atom type of the AL column or the nAL region, with the central point of the segment being situated at z. The segment has a thickness of Δz (See *Materials and Methods*). This thickness defines the z-resolution of the density profile. Note that the greater the thickness the lower the resolution. However, too small value of the thickness will cause large fluctuation of the density profile along the z-axis. In this study, we chose Δz to be 0.32 nm, and this value achieved a reasonable balance between resolution and fluctuation.

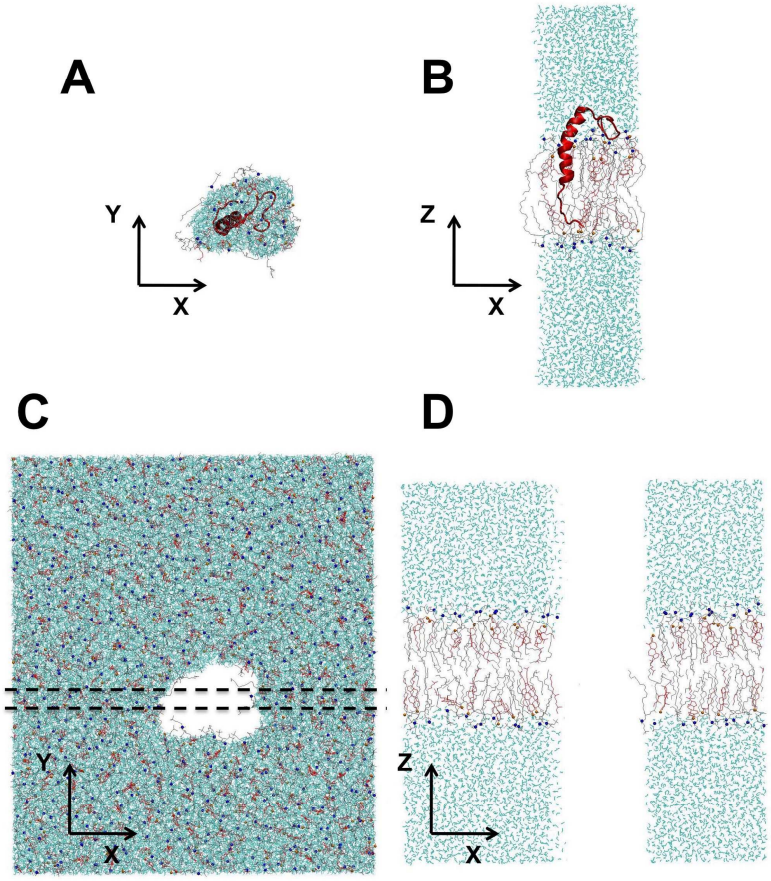

Fig. S1. Demonstration of the construction of an AL column and the nAL region in a simulation system. Top-view (x-y) and side-view (x-z) of an AL column are shown in panels **A** and **B**, respectively. Water molecules (cyan) in the top-view define the cross-section of the AL column. The top-view of the nAL region is shown in panel **C**. Panel **D** shows the side-view of a thin x-z slice marked by two dashed lines in **Panel C** of the nAL column cutting through the void region at y = 5.5 nm with a thickness of 1.8 nm. The protein molecule is highlighted in red in the AL column to demonstrate that the rim of the AL column cross-section is a 0.5nm-conformal contour of the protein profile on the *x-y* plane (see S1). PC and CHOL molecules are labeled in gray and pink, respectively. Phosphorus of PC and oxygen of CHOL are represented by blue and gold spheres, respectively.

S4. Number Density Profiles of Lipid and Water for $A\beta_{40}$ and $A\beta_{42}$ in Cholesterol-depleted Lipid Domains

The number density profiles of water oxygen and PC headgroup phosphorus (P8) calculated in the AL column (black and red, respectively) and the nAL region (dashed black and dashed red, respectively) are shown in **Fig. S2A**. The representative trajectories corresponding to the fully inserted states of $A\beta_{40}$ in PC (A1) and $A\beta_{42}$ in PC (B1), the surface state of $A\beta_{40}$ in PC (A4), and the partially inserted state of $A\beta_{42}$ in PC (B4). The density profiles were averaged over the last 25 ns of the 200 ns-simulations.

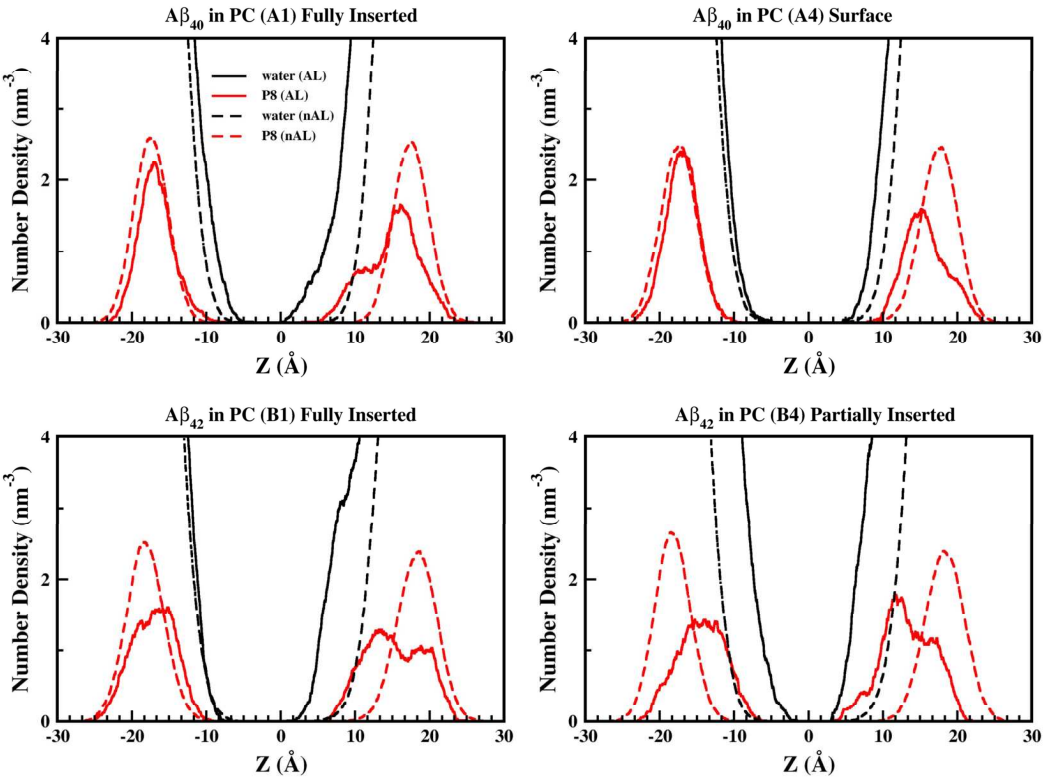

Fig. S2A. Representative density profile of PC headgroup and water for $A\beta_{40}$ and $A\beta_{42}$ in the AL column and the nAL region of the PC bilayers in the absence of cholesterol.

S5. Comparison of the Number Density Profiles of Lipid and Water in the nAL Region of the Cholesterol-depleted Lipid domains and in Pure PC Bilayers

The number density profiles of water oxygen and PC headgroup phosphorus (P8) calculated in the nAL region (dashed black and dashed red, respectively) and in the pure PC bilayers (dashed green and dashed brown) are shown in **Fig. S2B**. See S4 above for more details.

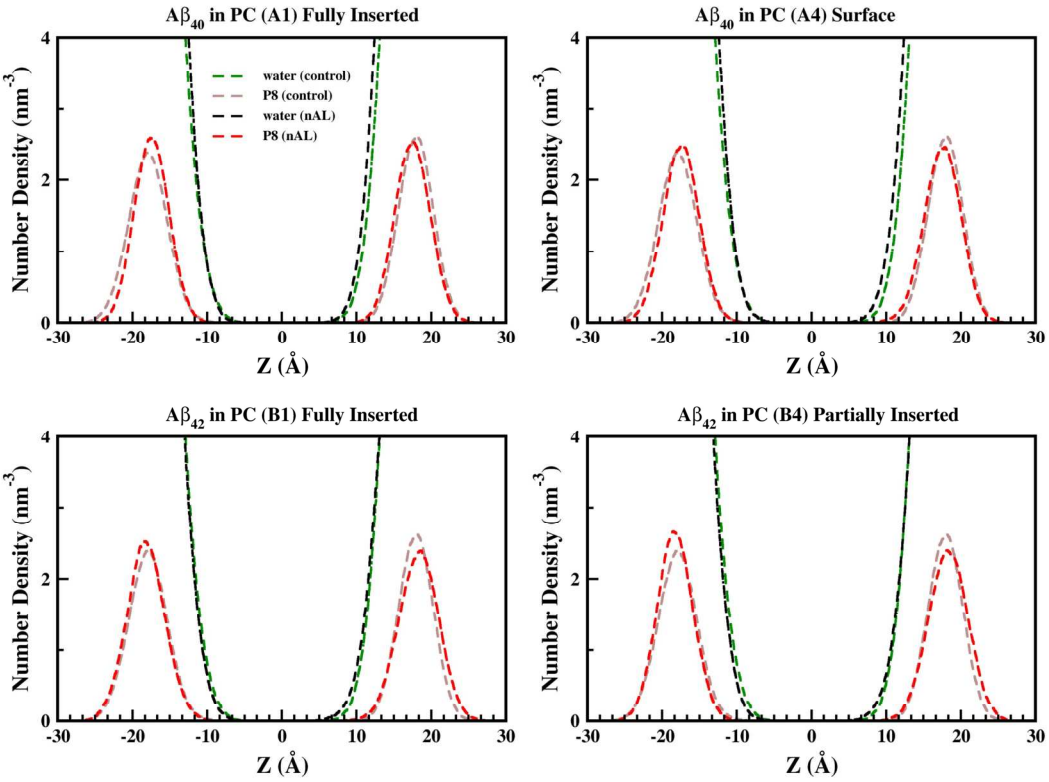

Fig. S2B. Representative density profile of PC headgroup and water in the nAL region of the cholesterol-depleted lipid domains and in pure PC bilayers (control).

S6. Number Density Profiles of Lipid and Water for Aβ$_{40}$ and Aβ$_{42}$ in Cholesterol-enriched Lipid Domains.

The number density profiles of water oxygen, PC headgroup phosphorus (P8) and 3-β hydroxyl polar oxygen (O6) of cholesterol calculated in the AL column (black, red and blue, respectively) and (dashed black, dashed red and dashed blue) are shown in **Fig. S3A**. The representative trajectories corresponding to the fully inserted states of Aβ$_{40}$ in PC/CHOL (C1) and Aβ$_{42}$ in PC/CHOL (D1 and D3), and partially inserted state of Aβ$_{40}$ in PC/CHOL (C4). See S4 above for more details.

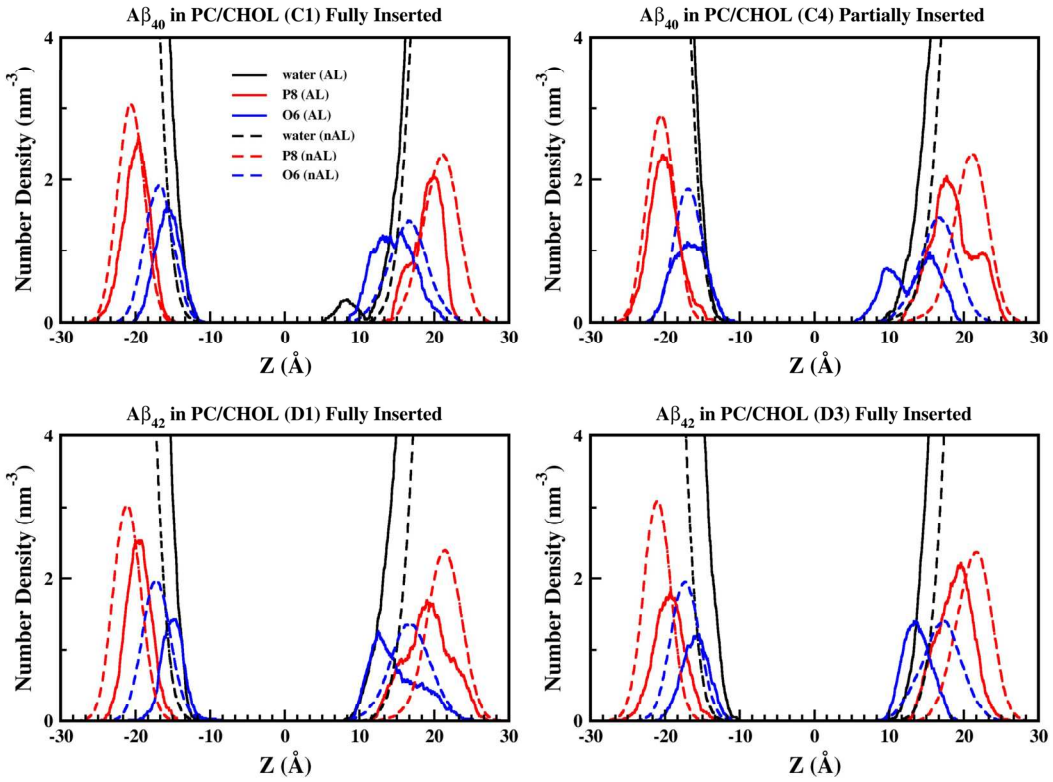

Fig. S3A. Representative density profile of PC headgroup and water for Aβ$_{40}$ and Aβ$_{42}$ in the AL column and the nAL regions of the PC/CHOL bilayers.

S7. Comparison of the Number Density Profiles of Lipid and Water in the nAL Region of the Cholesterol-enriched Lipid Domains and in the Pure PC/CHOL Bilayers

The number density profiles of water oxygen, PC headgroup phosphorus (P8), and 3-β hydroxyl polar oxygen (O6) of cholesterol calculated in the nAL region (dashed black, dashed red and dashed blue, respectively) and in the pure PC/CHOL bilayers (dashed green, dashed brown and dashed purple, respectively) are shown in **Fig. S3B**. See S6 above for more details.

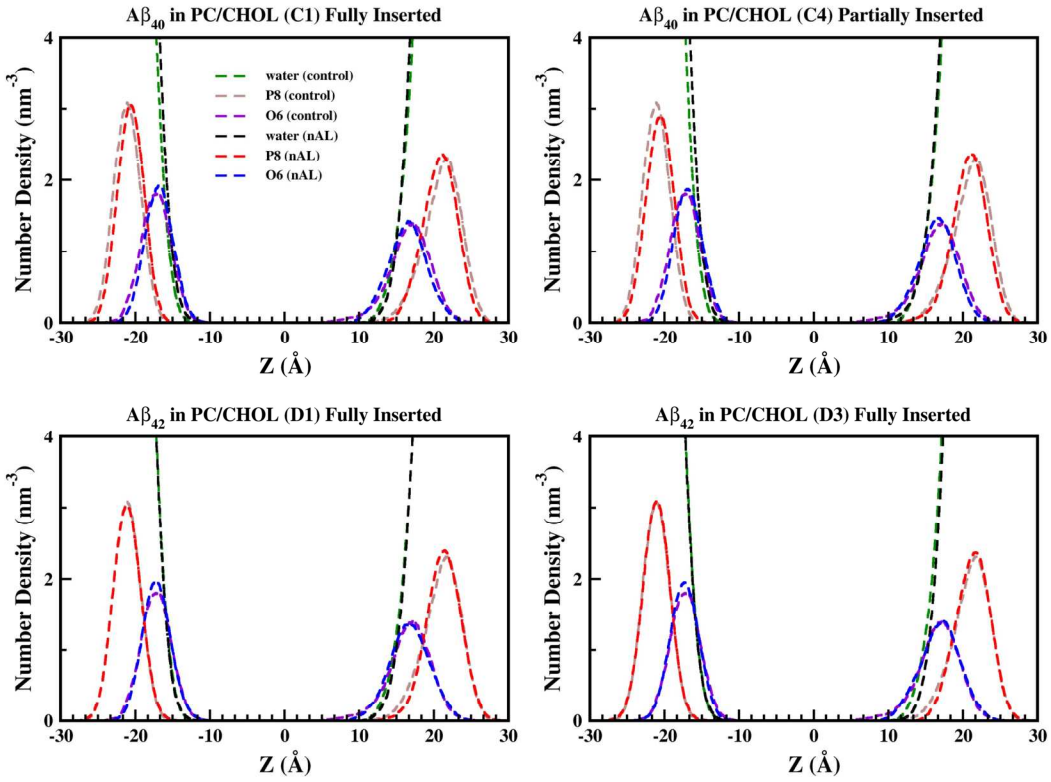

Fig. S3B. Representative density profile of PC headgroup and water in the nAL region of the cholesterol-enriched domain and in pure PC/CHOL bilayers.

S8. Comparison of the order parameter of PC acyl chains in the nAL region of cholesterol-depleted lipid domains and in the Pure PC bilayers.

Fig. S4A shows the order parameters of the *sn*-1 and *sn*-2 chains for the nAL region (black triangle and blue circle, respectively) and the pure PC bilayer (green triangle and green circle, respectively) in the upper and lower layers as a function of the carbon number position. The representative plots correspond to the fully inserted states of $A\beta_{40}$ in PC (A1) and $A\beta_{42}$ in PC (B1) and the surface state of $A\beta_{40}$ in PC (A4) and partially inserted state of $A\beta_{42}$ in PC (B4). The order parameters were calculated over the last 25 ns of the 200 ns-simulations.

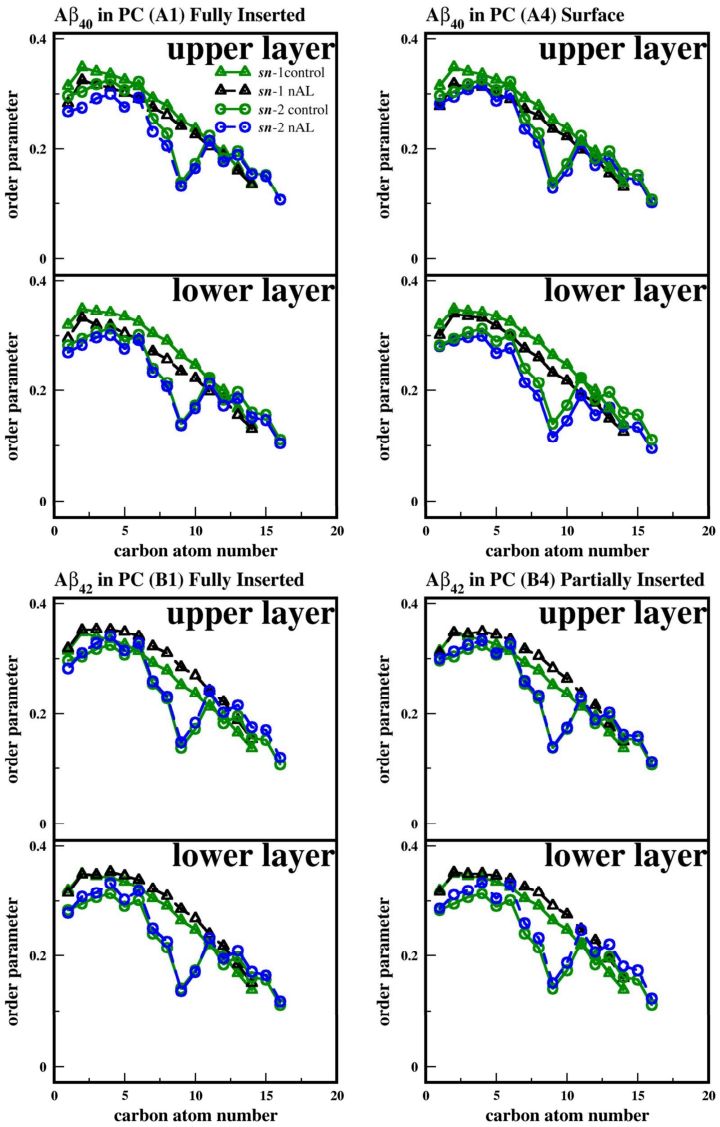

Fig. S4A. Order Parameter of Acyl Chains in nAL and Pure PC bilayers

S9. Comparison of the order parameter of PC acyl chains in the nAL region of cholesterol-enriched lipid domains and in the Pure PC/CHOL bilayers.

Fig. S4B shows the order parameters of the *sn*-1 and *sn*-2 chains for the nAL region (black triangle and blue circle, respectively) and the pure PC bilayer (green triangle and green circle, respectively) in the upper and lower layers as a function of the carbon number position. The representative plots correspond to the fully inserted states of $A\beta_{40}$ in PC/CHOL (C1), $A\beta_{42}$ in PC/CHOL (D1 and D3) and partially inserted state of $A\beta_{40}$ in PC/CHOL (C4). The order parameters were calculated over the last 25 ns of the 200-ns simulations.

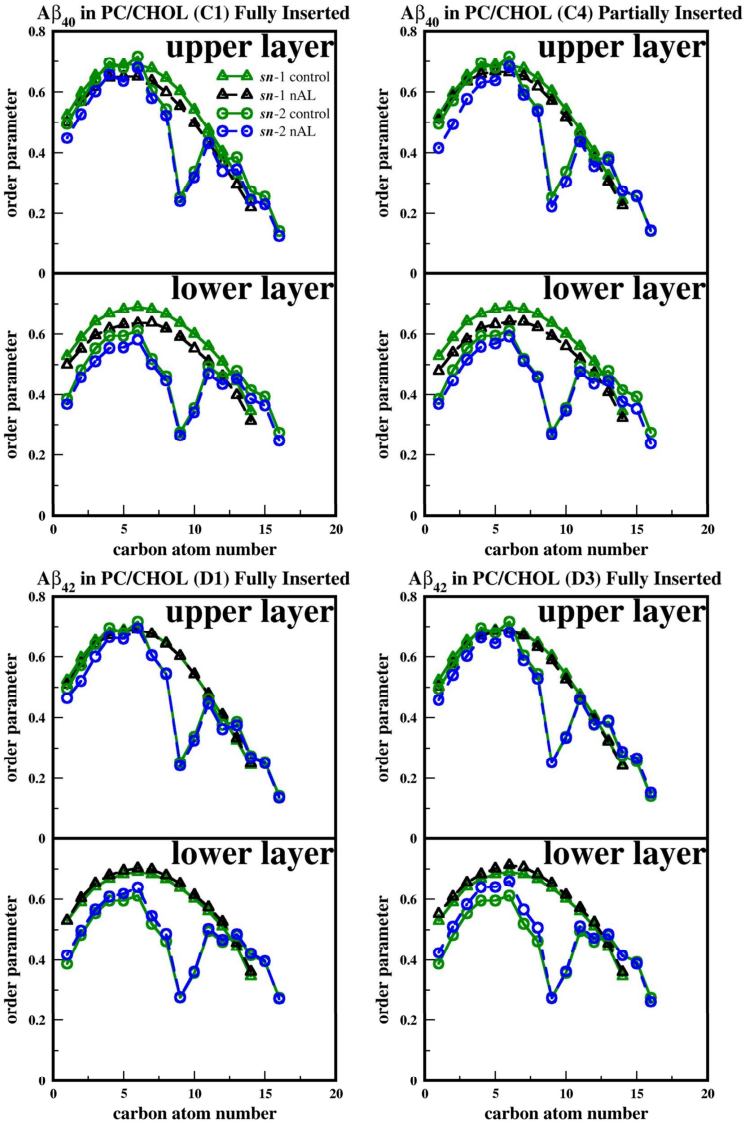

Fig. S4B. Order Parameter of Acyl Chains in nAL and Pure PC/CHOL bilayers

S10. Local Compositions of Cholesterol in the AL and nAL regions of Cholesterol-depleted and Cholesterol-enriched domains.

Fig. S5 shows the local compositions of cholesterol (X_{CHOL}) calculated in the AL (green) and nAL (red) regions of some representative replicates for $A\beta_{40}$ (C1 and C2) and $A\beta_{42}$ (D1 and D3). The averaged values of X_{CHOL} of the representative replicates for the last 25 ns of the simulations are shown in Table S1 below.

Table S1. Calculated X_{CHOL} for four representative replicates during the entire 200 ns simulations. Both the averages and the standard deviations are shown.

Replicate	AL	nAL
C1	0.436 ± 0.023	0.399 ± 0.001
C2	0.413 ± 0.030	0.400 ± 0.001
D1	0.369 ± 0.020	0.401 ± 0.001
D3	0.360 ± 0.026	0.402 ± 0.001

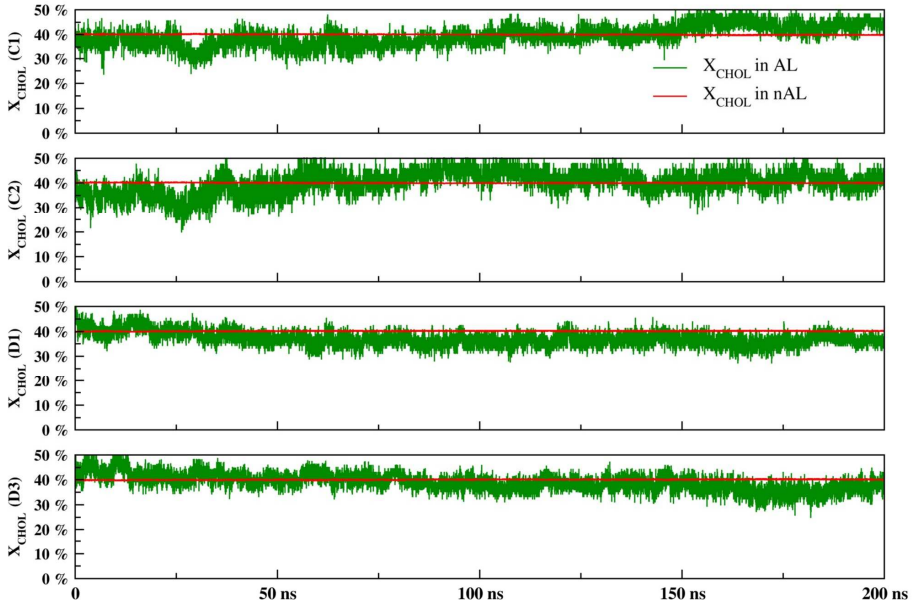

Fig. S5. Time evolution of X_{CHOL} for some representative replicates.

References:

(1) Press, W. H.; Flannery, B. P.; Teukolsky, S. A.; Vetterling, W. T. Numerical Recipes in C: The Art of Scientific Computing, 2nd Edition; Cambridge University Press, 1992; pp 282.

(2) Van Der Spoel, D.; Lindahl, E.; Hess, B.; Groenhof, G.; Mark, A. E.; Berendsen, H. J. *J. Comput. Chem.* 2005, *26*, 1701-1718.

(3) Fishman, G. S. *Monte Carlo: Concepts, Algorithms, and Applications*; Springer-Verlag: New York, 1997.