Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information:
Influence of different membrane environments on the behavior of cholesterol

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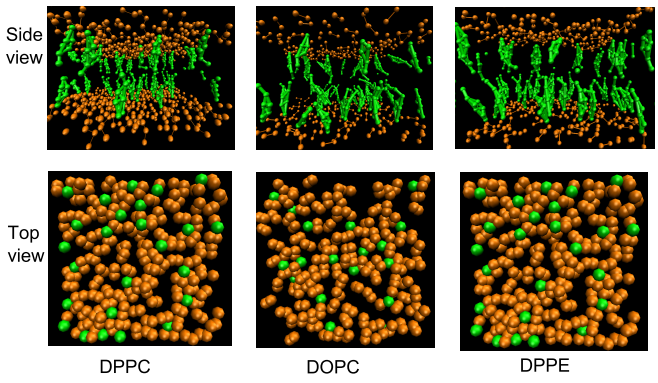

Figure S1: Equilibrium configuration of DPPC, DOPC or DPPE bilayer with 20% cholesterol contents.

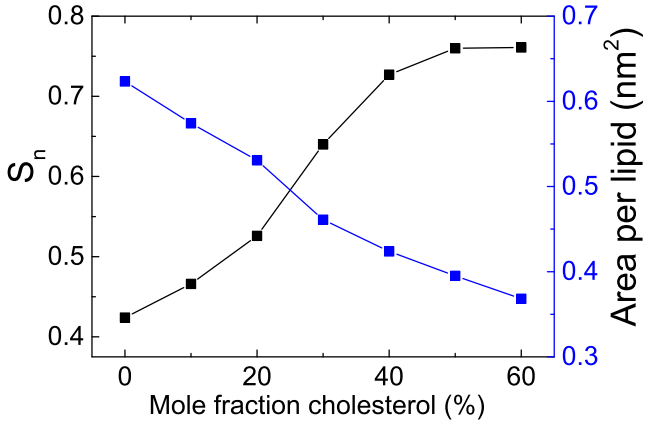

Figure S2: Profile of the order parameter S_n and the area per lipid as functions of mole fraction of cholesterol in DPPC bilayer.

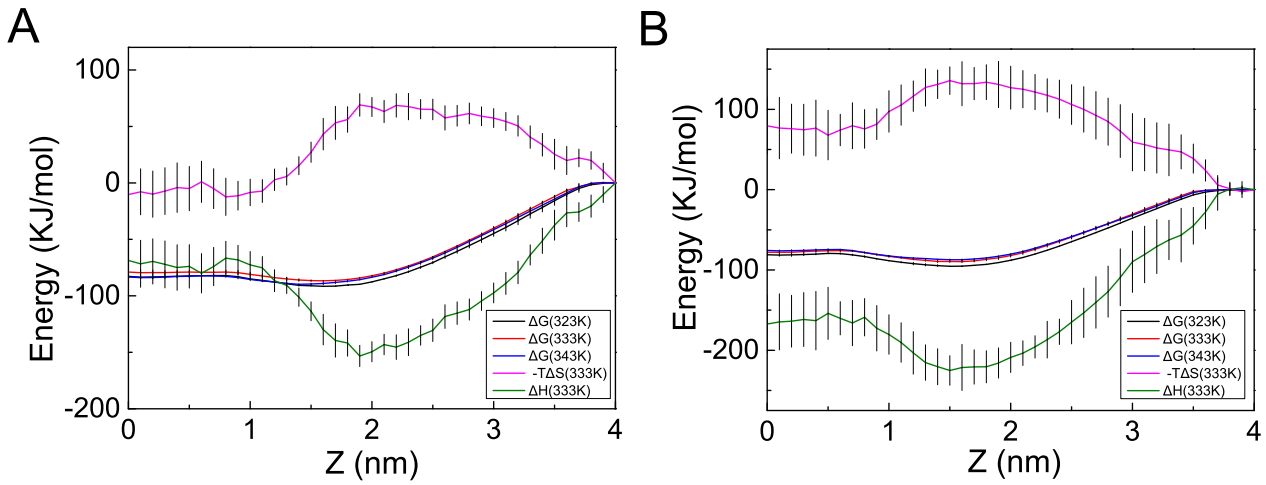

Figure S3: PMFs for cholesterol partitioning in (A) DOPC and (B) DPPE bilayer with three different temperatures and the corresponding entropy and enthalpy contributions for the PMF of 333K.

Counting of number of cholesterol molecules. We partition the curved membrane into small square lattice on the horizontal plane, then we use the data of all phospholipid's coordinates and combine the least-square method to fit a planar plane for each lattice. Whether cholesterol is in the inner leaflet or the outer leaflet is determined by the relative location of cholesterol's headgroup from the planar plane. If cholesterol's location changes

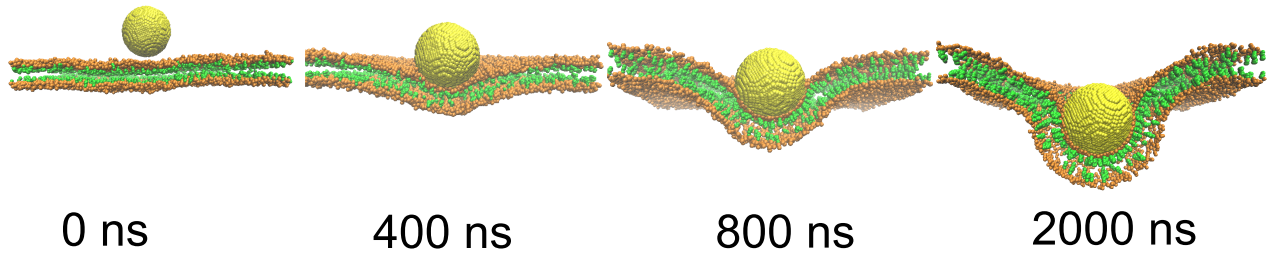

Figure S4: Time sequences of the nanoparticle interacting with the membrane.

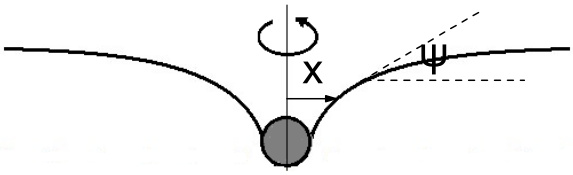

Figure S5: Schematic graph of the curved membrane.

from one side of planer plane to the other side and stays for a time in the new location ($\geq$ 50 ns), one flip-flop incident of cholesterol will be counted. In addition, we know that in the rotationally symmetric cases, the mean curvature of membrane is expressed as[1, 2]: $H = \frac{\sin\psi(x)}{x} + \cos\psi(x)\frac{\mathrm{d}\psi}{\mathrm{d}x}$ (see Fig. S3). Here x is the distance to the symmetric axis and ψ is the angle between tangent to the membrane's contour and the horizontal direction. Then we also use the discrete method to estimate approximately the mean curvature by calculating x and $\psi(x)$. In this way, we distinguish the three major different portions according the mean curvature: H$\approx$2/R, H$>$0 and H$\leq$0 portion. To estimate the phospholipid headgroup's size, the coordinates of lipid's headgroup beads (the former four beads of the Martini's lipid) are used to fit the planar plane for the inner leaflet and the outer leaflet separately. Then the phospholipid headgroup's size is estimated by diving area of the planar plane by the total numbers of lipids in certain portions.

Additional discussion of the influence of the redistribution of cholesterol. We have shown in the main text that the free energy of the system will decrease due to the redistribution of cholesterol. And when $\alpha_1 H$ is rather small, it can be seen that the effective bending

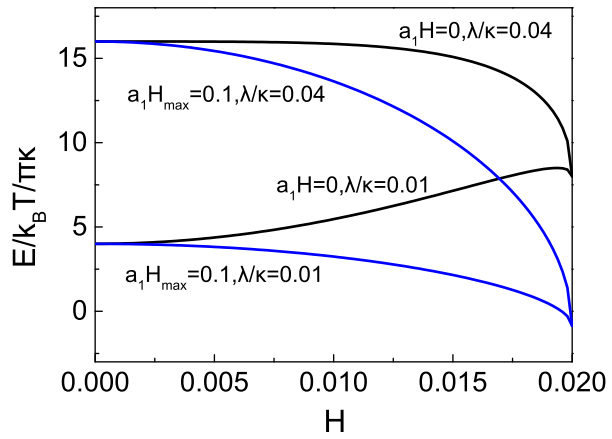

Figure S6: Reduced energy of the budding domain as a function of curvature.

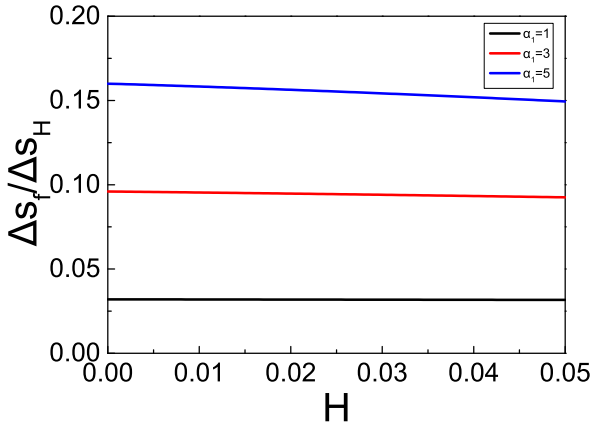

Figure S7: The ratio of $\Delta s_f/\Delta s_H$ as a function of curvature at different values of α_1.

modulus decreases due to the redistribution of cholesterol. When $\alpha_1 H$ is not very small, the free energy decrease should be even larger and its influence to the membrane deformation process can be more prominent. As discussed in the main text, we consider a domain with L as 200 nm and take the representative value of f as 0.2, κ as 15 and A as 0.60. As shown in Fig. S6, we find that if we don't consider the influence of cholesterol's redistribution (that is, α_1=0), only when λ/κ acquires an enough value ($\lambda/\kappa = 0.04$), the system's energy will decrease along with the enlargement of the bud's curvature H, so the domain will bud. If the influence of cholesterol's redistribution is also considered, it will further facilitate the budding of the domain. As shown in Fig. S6, when $\alpha_1 H$ takes the value of 0.1, we can

find that for the case of $\lambda/\kappa = 0.04$, the system's energy decreases more rapidly along with the enlargement of the bud's curvature H. And for the case of $\alpha_1 H$= 0.1 and $\lambda/\kappa = 0.01$, the system's energy changes from increasing to decreasing along with the enlargement of curvature H, so the critical ratio of λ/κ for budding is decreased.

The redistribution of cholesterol will change the local mole fractions of cholesterol. In turn, the change of mole fraction of cholesterol will influence the area of per phospholipid. But as discussed in the main text, low levels of cholesterol inserts into the interstitial space under phospholipid headgroup and only has little effect on the size of primary phospholipid. So we have omitted this effect in the main text. In most cases, curvature indeed plays the dominant roles in changing the area of per phospholipid's headgroup. But the change of mole fraction of cholesterol still has small influence in certain condition. The change of mole fraction of cholesterol for one leaflet due to the redistribution of cholesterol can be expressed as:

$$\Delta f = \frac{f\frac{\exp(\alpha_1 H)}{\exp(\alpha_1 H)+\exp(-\alpha_1 H)}}{f\frac{\exp(-\alpha_1 H)}{\exp(\alpha_1 H)+\exp(-\alpha_1 H)} + \frac{1-f}{2}} - f. \tag{S1}$$

As shown in the main text, the area per lipid for 30% DPPC bilayer enlarges about 6% than that of pure DPPC bilayer. So here, we roughly consider that a 1% variation of mole fraction of cholesterol will change the area per phospholipid by 0.2% (6%/30). Then the area change induced by changing mole fraction of cholesterol (due to the redistribution of cholesterol) is expressed as $\Delta s_f = \frac{\Delta f}{1\%}0.2\%$. The area change induced by curvature is $\Delta s_H = s - s_0 = zH$ (take z as 1 nm). Taking initial mole fraction of cholesterol $f = 20\%$ as an example, the ratio of $\Delta s_f/\Delta s_H$ is shown in Fig. S7. We can find that if α_1 is rather small, the ratio of $\Delta s_f/\Delta s_H$ is very small, so the influence of the change of mole fraction of cholesterol can be omitted indeed. And if α_1 takes a larger value (α_1=3, 5), the ratio of $\Delta s_f/\Delta s_H$ is about within 0.1-0.15, so the change of mole fraction of cholesterol indeed weaken the effect of membrane curvature, but the curvature is still the major factor which influences the area per phospholipid's headgroup.

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[1] H. J. Deuling and W. Helfrich, *J. Phys.* (Paris), 1976, **37**, 1335.

[2] Z. C. Ou-Yang and W. Helfrich, *Phys. Rev. Lett.*, 1987, **59**, 2486.