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Supplemental Information

Shape Transformations of Lipid Bilayers Following Rapid Cholesterol Uptake

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Supplementary Information

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1 Condensing effect

In this section, we derive the relation between the area change, cholesterol mole fraction, and area fraction for a DOPC-Cholesterol binary mixture bilayer. The total area of a DOPC-Cholesterol bilayer can be written as

$$A(\chi) = a(\chi)n_{\text{tot}} = \frac{a(\chi)n_{\text{DOPC}}}{1 - \chi},$$

where $\chi = n_{\text{Chol}}/n_{\text{tot}}$ is the mole fraction of cholesterol, $a(\chi)$ is the average area per molecule in the binary mixture membrane, $n_{\text{tot}} = n_{\text{Chol}} + n_{\text{DOPC}}$, and n_{DOPC} , and n_{Chol} are the number of DOPC and cholesterol molecules. Since during the first stage of shape changes (membrane expansion), the number of DOPC molecules remains constant, we have

$$\Delta a = \frac{A - A_0}{A_0} = \frac{a(\chi)}{a(0)} \frac{1}{1 - \chi} - 1. \quad (1)$$

Note that $a(0)$ is the area per molecule of a pure DOPC bilayer. Also by definition, the cholesterol area fraction can be written in terms of cholesterol mole fraction

$$\phi = \frac{A_{\text{chol}}}{A_{\text{tot}}} = \frac{n_{\text{chol}}a_{\text{chol}}}{n_{\text{tot}}a(\chi)} = \frac{a_{\text{chol}}}{a(\chi)}\chi, \quad (2)$$

or

$$\chi = \frac{a(\chi)}{a_{\text{chol}}}\phi.$$

Given equations 1 and 2, one can obtain the relation between ϕ , and Δa , if the average area per molecule as a function of cholesterol mole fraction $a(\chi)$ is known. It is well known that the area per molecule of DOPC molecules in a DOPC-Chol membrane decreases as the ratio of cholesterol content increases [1, 2], which results in condensing of binary mixture bilayer.

Following the experimental data and method given in reference [2] for DOPC SLB patches, we use an exponential fit for the change of area per molecule of DOPC-Cholesterol bilayer as a function of cholesterol mole fraction

$$a(\chi) = p_1 + p_2 e^{-p_3 \chi}, \quad (3)$$

where $p_1 = 36.5 \text{ \AA}$, $p_2 = 31.1 \text{ \AA}$, $p_3 = 2.2$. Assuming that the a_{chol} remains constant [1], we have $a_{\text{chol}} = a(1) = 39.9 \text{ \AA}$, and $a_{\text{DOPC}} = a(0) = 67.6 \text{ \AA}$. Thus, given the above equation and equations 1 and 2, the cholesterol area fraction can be calculated from the experimentally measured area variations.

As shown in Fig. S1 (blue curve), the area per molecule decreases as χ increases. We briefly discuss next the origin of this condensing effect. By definition,

$$a(\chi) = (1 - \chi)a_{\text{DOPC}} + \chi a_{\text{chol}} \quad (4)$$

Because $a_{\text{DOPC}} > a_{\text{chol}}$, even if the area per DOPC molecule, a_{DOPC} was independent of χ , there would be an overall condensing effect. By calculating $a_{\text{DOPC}}(\chi)$ from equations 3 and 4 and plotting it in Fig. S1, we show that there is a significant additional effect as a result of intrinsic area decrease of DOPC molecules in the presence of cholesterol.

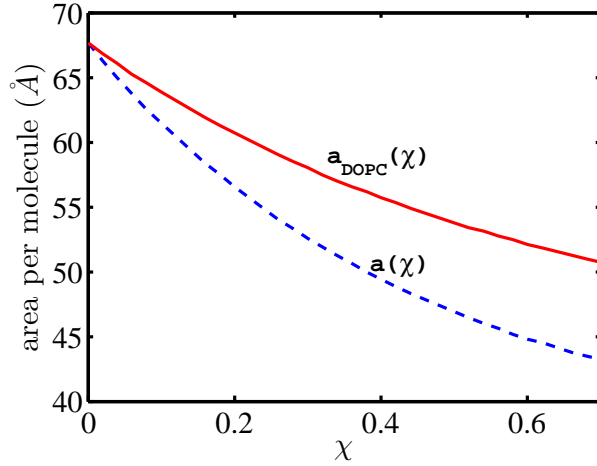


Figure 1: Average area per molecule of a DOPC-Chol bilayer taken from reference [2] (blue curve), and the calculated average area of DOPC molecules accounting for the cholesterol condensing (red curve).

2 M β CD lipid removal from CSLB

Supported lipid bilayers do not change shape when exposed to M β CD, except for very high concentrations, when we see formation of holes (Fig. S2). These observations suggest that the underlying support has a stabilization effect on the membrane.

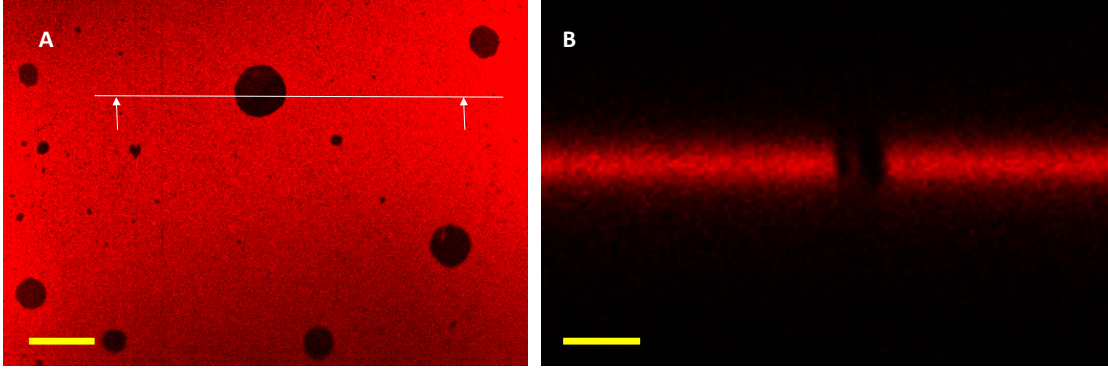


Figure 2: Lipid removal by $M\beta CD$ at high concentrations. (a) Top view and (b) side view images (white line) of a CSLB, 30 minutes after exposure to 50mg/mL $M\beta CD$.

3 Shrinking of SLB patches

Our experimental results show that the newly expanded membrane in the SLB patches following exposure to $M\beta CD$ -Chol is unstable. It disintegrates shortly after expansion, leaving the SLB patch at its original shape. While we do not precisely understand the exact mechanism of disintegration, we propose that the newly expanded patch membrane is more susceptible to DOPC removal by empty cyclodextrins because its adhesion to the substrate is compromised. One possibility, that we explore in this section is that the newly formed patch membrane does not adhere to a bare glass substrate as the original portion of the patch but onto a layer of cyclodextrin molecules which adsorb from the solution to the glass. $M\beta CD$ molecules have a hydrophilic exterior that facilitates their adsorption to the hydrophilic glass substrate, thus changing its wettability and charge.

To explore this hypothesis, we prepare SLB patches on two different coverslips. Both are hydrophilised by plasma treatment but one, which we refer to as 'treated', is additionally incubated with $M\beta CD$ (50mg/mL) for one minute, which is about the time that the membrane expansion occurs at high concentrations of cholesterol solution. Intact patches form initially on both substrates. However when exposed to highly concentrated 50mg/ml $M\beta CD$ solution, the patches disintegrate- presumably due to the rapid extraction of the DOPC lipids- by fragmenting into clumps of brightly fluorescent lipids, which gradually disappear from the substrate (see images on Fig. S3-a and b). Patches on $M\beta CD$ pre-treated substrates appear much more unstable. They disintegrate and disappear from the substrate about 4 times faster, compared to patches on untreated substrates. This is further confirmed by measuring the decrease of the fluorescent signal from a given membrane area inside both patches (Fig. S3-c).

To capture the dynamics of the SLB patch expansion and shrinkage upon $M\beta CD$ -Chol exposure we can write the total change of area A_{new} in terms of cholesterol adsorption area, A_{chol} , and phospholipid removal area, A_{removal} , as:

$$\dot{A}_{\text{new}} = \dot{A}_{\text{chol}} - \dot{A}_{\text{removal}}.$$

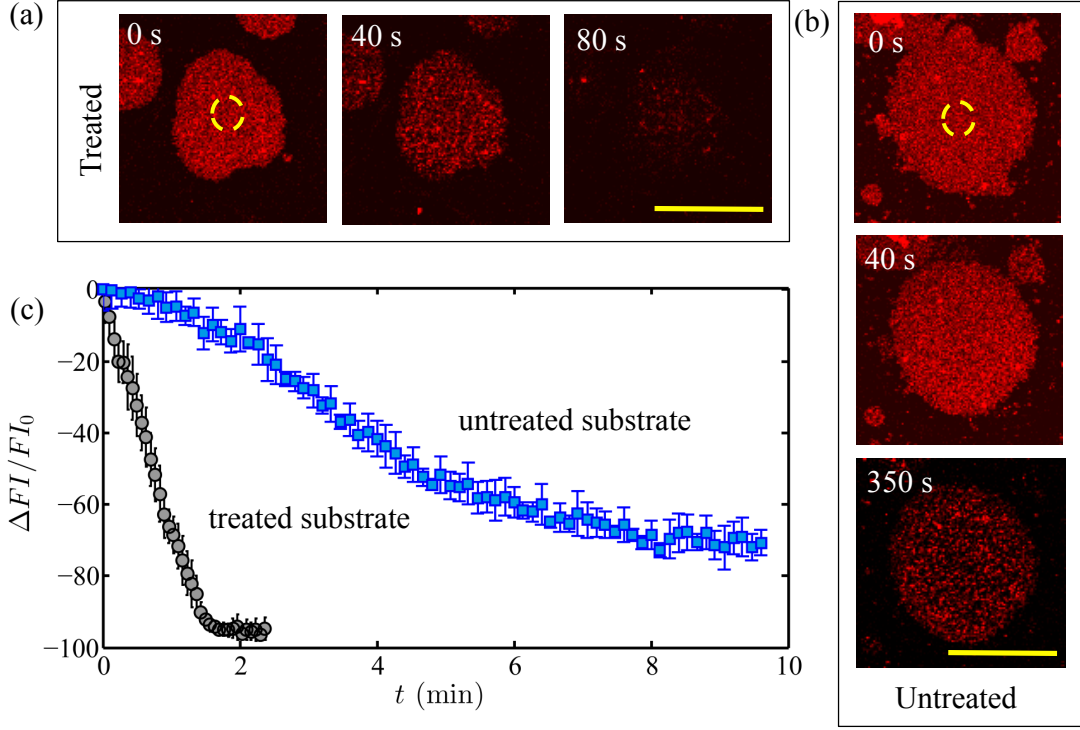


Figure 3: Disintegration of SLB patches on $M\beta CD$ -treated (a) and untreated (b) substrates, following exposure to 50mg/ml $M\beta CD$ solution. The yellow circle marks the membrane area selection for fluorescent intensity measurements. The time after exposure to 50mg/ml $M\beta CD$ solution is noted on the upper left corner of each image. Scale bars 30 μm . (c) Normalized fluorescent intensity change of the SLB patches as a function of time, following exposure to 50mg/mL $M\beta CD$.

Following equations 3 and 4, we can obtain A_{chol} as a function of ϕ , such as $\dot{A}_{chol} = \dot{\phi} d(A_{chol})/d(\phi)$, where $\dot{\phi} = 1/\tau (\bar{\phi} - \phi(x, t))$ (see the main text for details). If we assume that the lipid removal occurs only in the newly generated membrane area, A_{new} , and that there is no limit for the lipid removal, then $\dot{A}_{removal} = 1/\bar{\tau} A_{new}$, where $\bar{\tau}$ is the lipid removal time scale. We rewrite the area change dynamics equation as $\dot{A}_{new} = \dot{\phi} d(A_{chol})/d(\phi) - 1/\bar{\tau} A_{new}$, and solve it for $\bar{\tau}$, for the adsorption rates and amplitudes reported in Fig.2 (main text). For 50mg/ml $M\beta CD$ -Chol, we obtain $\bar{\tau} = 750$ s. The numerical results are presented in Fig S4.

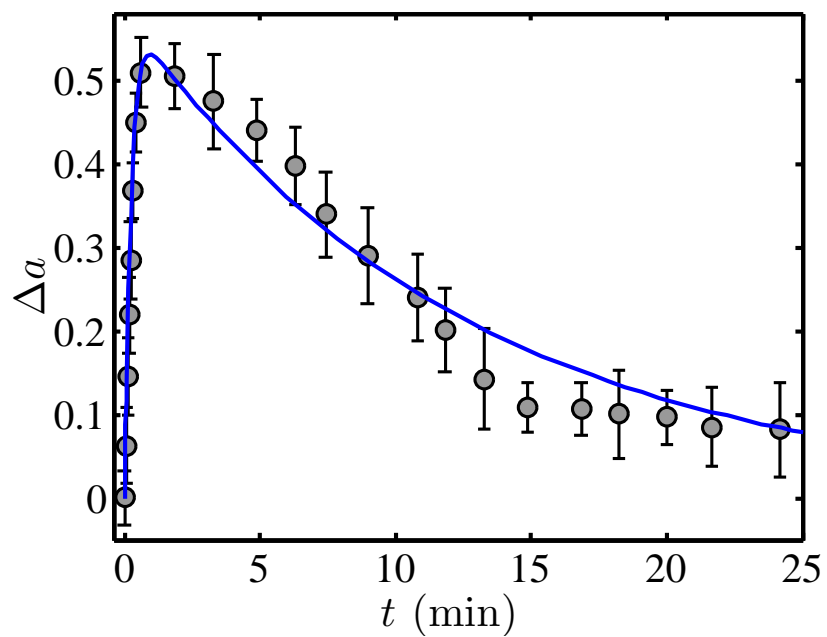


Figure 4: Normalized area deviation of SLB patches $\Delta a = (A - A_0)/A_0$ exposed to 50mg/mL M β CD-Chol as a function of time. Circles are experimental results and solid line is the numerical solution for the cholesterol adsorption, lipid removal model.

References

- [1] W.C. Hung, M.T. Lee, F.Y. Chen, and H.W. Huang. The condensing effect of cholesterol in lipid bilayers. *Biophys. J.*, 92(11):3960–3967, 2007.
- [2] Jonathan P Litz, Niket Thakkar, Thomas Portet, and Sarah L Keller. Depletion with cyclodextrin reveals two populations of cholesterol in model lipid membranes. *Biophysical journal*, 110(3):635–645, 2016.