

补充材料

基于无监督学习方法的细胞膜内单分子扩散运动分析：胆固醇对模型膜和活细胞膜流动性的不同影响*

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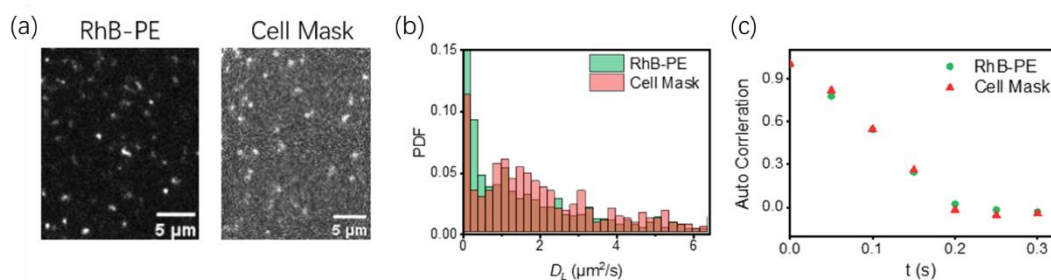


图 S1 荧光磷脂 (RhB-DOPE) 和 CellMaskTM 分子在支撑平板膜内扩散运动行为比较 (a) 单分子追踪视频截图; (b) 单分子扩散系数 (D_L) 的频数分布 (PDF). 绿色为 RhB-DOPE, 红色为 CellMaskTM 分子, 橙色为两者重合的区域; (c) 速度自相关函数分布 ($\delta = 4$). 视频拍摄速率为 50 ms/frame, 用于分析的轨迹长度为 50 帧, 两个体系的轨迹数目分别为 4800 和 4200. 数据来自 3 个平行样本.

Fig. S1. Comparison of the diffusion behavior between fluorescent phospholipid (RhB-DOPE) and CellMaskTM molecules in supported bilayer membranes. (a) Snapshot of single-molecule tracking video; (b) PDF distribution of the diffusion coefficient (D_L) values of individual molecules. RhB-DOPE is represented by green, CellMaskTM molecules are red, and their overlap is orange. (c) Distribution of velocity autocorrelation function ($\delta = 4$). The video was captured at 50 ms/frame, and the trajectory length used for analysis was 50 frames. The number of trajectories for the two systems was 4800 and 4200 respectively, with data obtained from three parallel samples.

样品制备情况说明: 制备平板膜 (成分为 DOPC, 以 0.0001% 比例 (摩尔比) 掺以 RhB-DOPE), 以 CellMaskTM 溶液 (5×10^{-5} mg/mL 浓度溶解于 PBS) 孵育 30 min 后以 PBS 冲洗, 之后立刻置于 TIRFM 下观察.

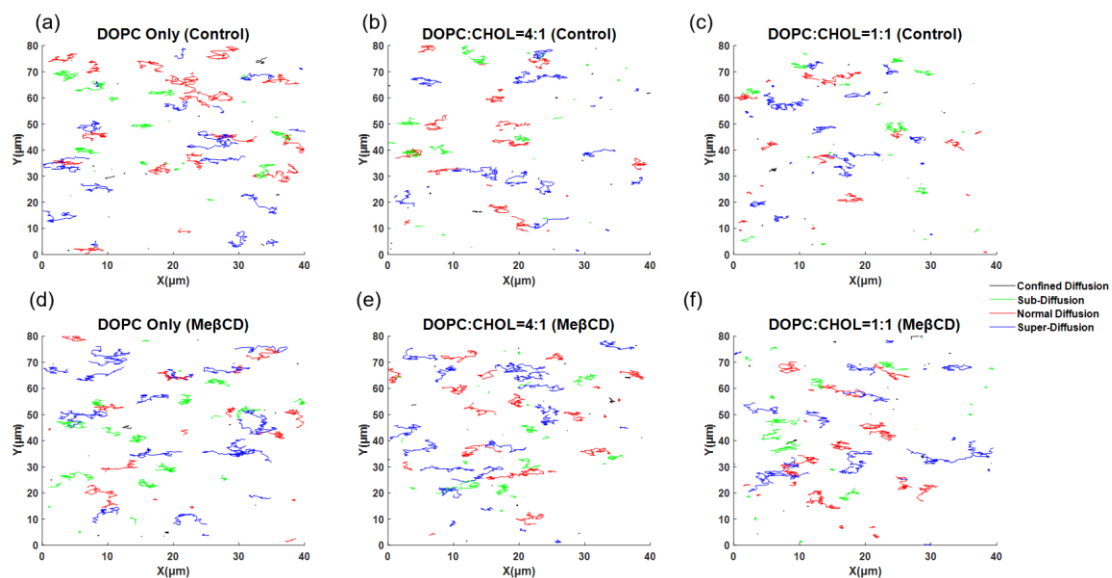


图 S2 不同成分模型膜体系内单脂质分子轨迹的异质性, 成分分别为纯 DOPC, DOPC : Chol = 4 : 1, DOPC : Chol = 1 : 1, 及其经 Me β CD 去除胆固醇后的膜. 4 种颜色分别对应受限扩散 (Confined diffusion)、亚扩散 (Sub-diffusion)、正常扩散 (Normal diffusion)、超扩散 (Super-diffusion) 4 种类型, 每个体系内显示 80 条典型轨迹 (每类 20 条), 轨迹长度皆为 60 帧. 轨迹经平移后显示在一张图内

Fig. S2. Heterogeneity of individual lipid trajectories within model bilayer membranes containing different components, namely pure DOPC, DOPC : Chol = 4 : 1, DOPC : Chol = 1 : 1, and their counterparts after cholesterol removal using Me β CD. The four colors represent distinct diffusion modes: confined diffusion, sub-diffusion, normal diffusion, and super-diffusion. Each panel displays 80 representative trajectories (20 for each diffusion mode), spanning a trajectory length of 60 frames. The trajectories are horizontally shifted to be presented in a single image.

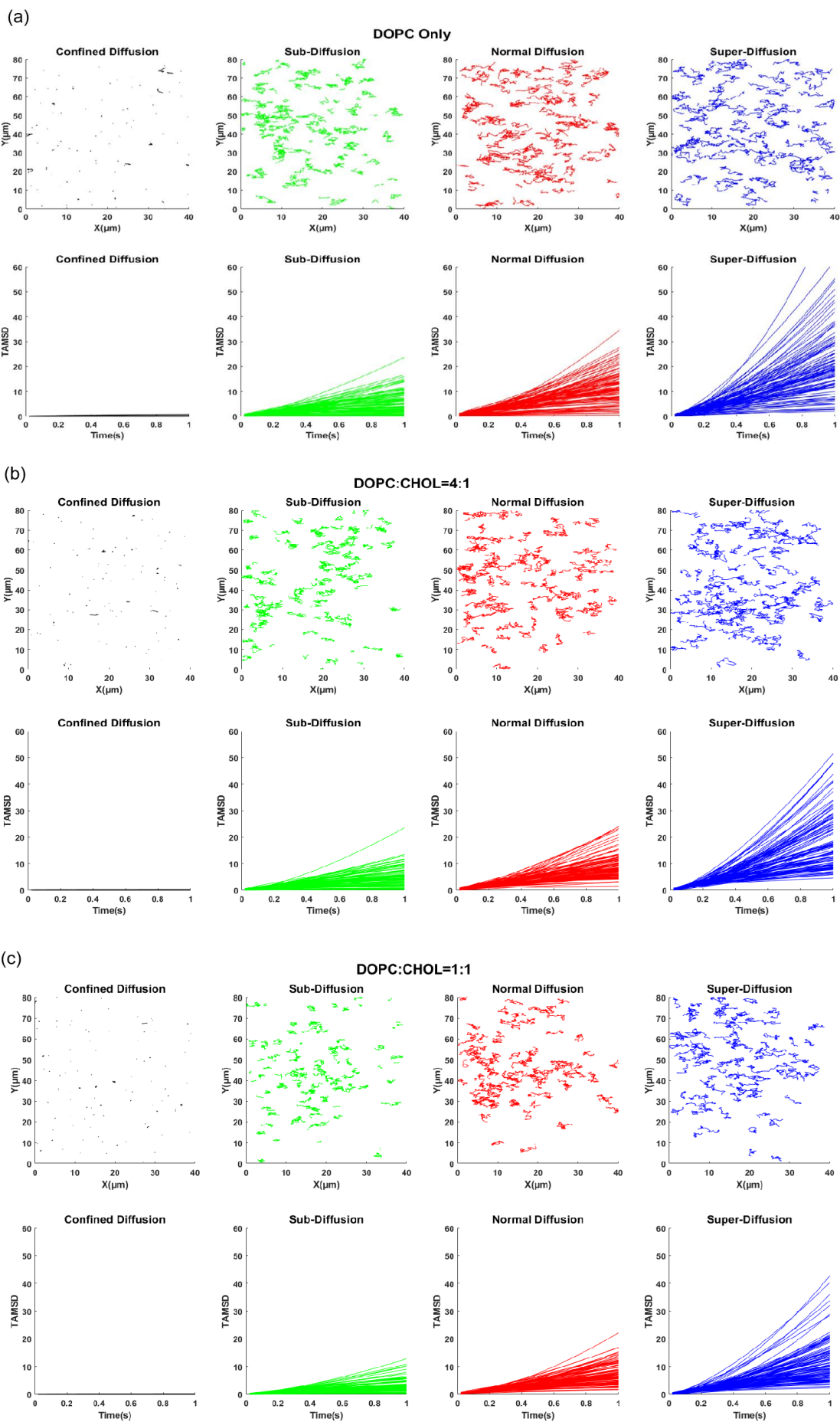


图 S3 利用“两步归类法”对各体系内单脂质分子扩散轨迹进行识别和分类后, 每种扩散模式下的典型轨迹及其对应的时间平均 MSD 曲线, 其中每组随机选择 100 条轨迹 (a) — (c) 分别对应 3 种成分模型膜体系, 即纯 DOPC, DOPC : Chol = 4 : 1, DOPC : Chol = 1 : 1 (未经 Me β CD 处理)

Fig. S3. Identification and classification of individual lipid molecule diffusion trajectories within each system using the "two-step classification method" and the typical trajectories and corresponding time-averaged MSD curves for each diffusion mode, with 100 randomly selected trajectories for each subgroup. (a) - (c) respectively represent the typical trajectories and corresponding time-averaged MSD curves for three types of model membrane systems, namely pure DOPC, DOPC : Chol = 4 : 1, and DOPC : Chol = 1 : 1 (untreated by Me β CD).

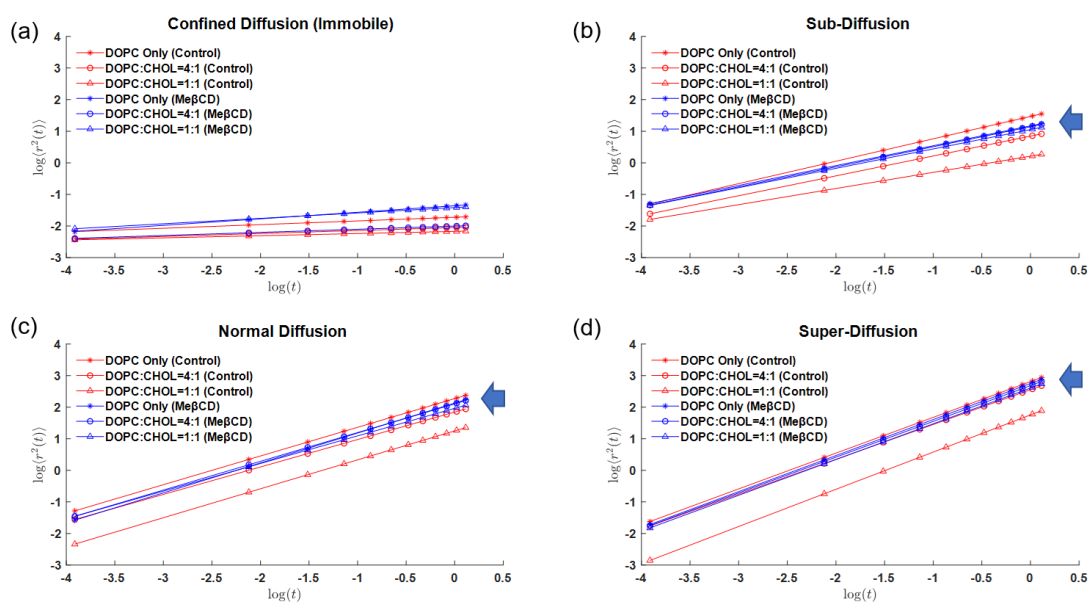


图 S4 利用“两步归类法”对各体系内单脂质分子扩散轨迹进行识别和分类后, 各子集内分子轨迹的系综平均 MSD 曲线, 其中轨迹数目见正文表 1

Fig. S4. Ensemble-averaged MSD profiles of molecular diffusion trajectories within each sub-group. The trajectories have been identified and classified using the "two-step classification method" for each system, with the corresponding number of trajectories listed in Table 1 of the main text.

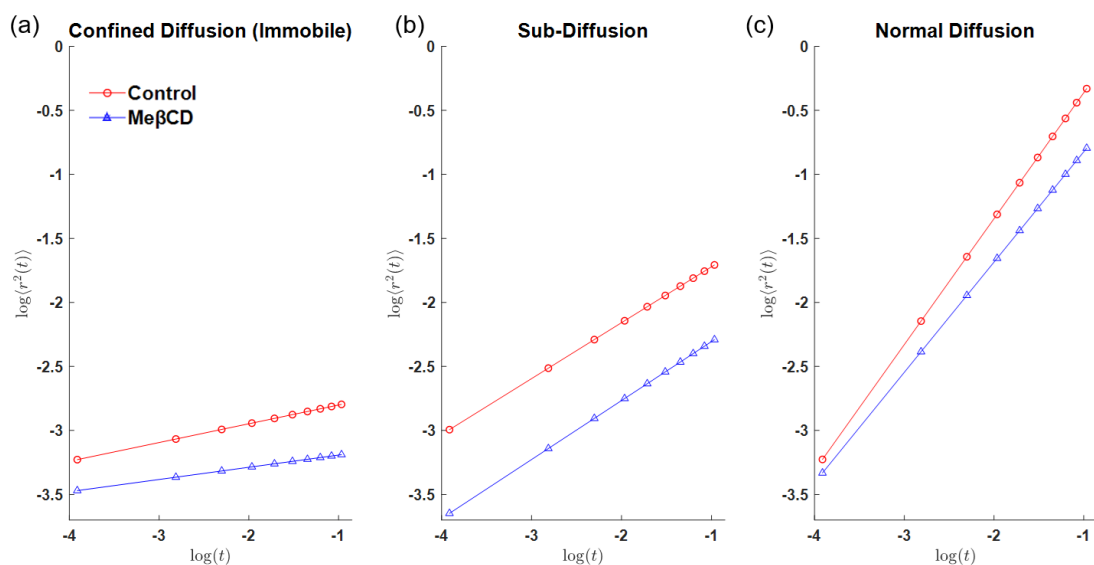


图 S5 B16 活细胞膜在 Me β CD 处理前（红色）后（蓝色）的单分子扩散运动系综平均 MSD 曲线，轨迹利用“两步归类法”分为受限扩散（Confined diffusion）、亚扩散（Sub-diffusion）、正常扩散（Normal diffusion）3 种类型，其中轨迹数目见正文表 2

Fig. S5. Ensemble-averaged MSD profiles of molecular diffusion trajectories in live cell membranes, both before (red) and after (blue) Me β CD treatment. The trajectories have been identified and classified into three subgroups for each system using the "two-step classification method," namely confined diffusion, sub-diffusion, and normal diffusion. The corresponding number of trajectories is listed in Table 2 of the main text.

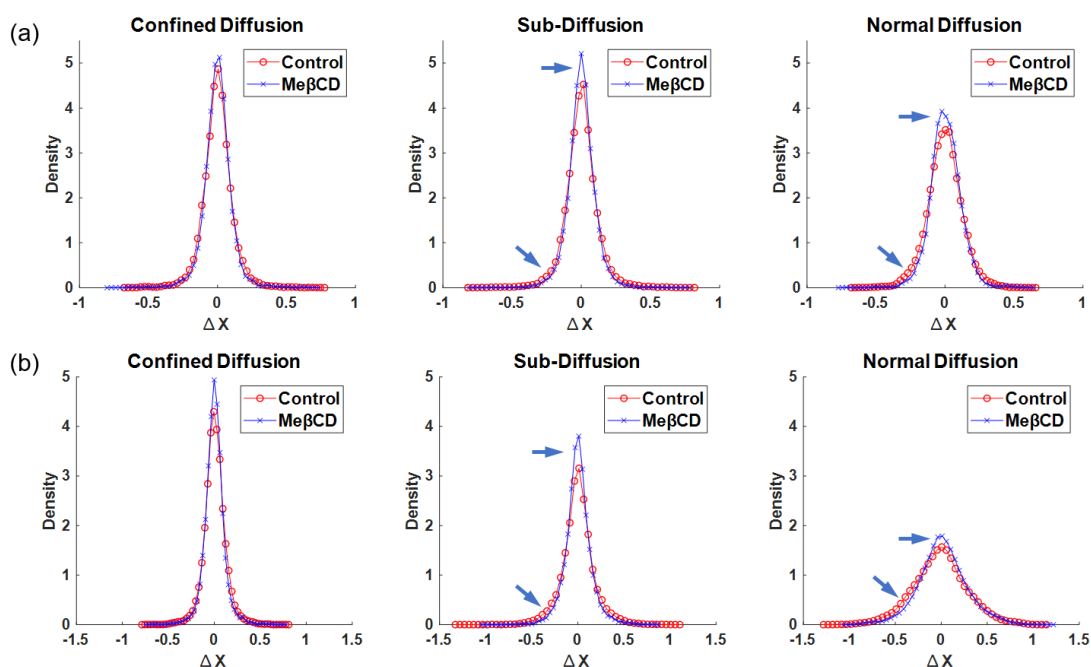


图 S6 活细胞膜在 Me β CD 处理前 (红色) 后 (蓝色) 的单分子扩散运动位移 (以 x 方向为例) 的概率分布, 其中轨迹利用“两步归类法”分为受限扩散 (Confined diffusion)、亚扩散 (Sub-diffusion)、正常扩散 (Normal diffusion) 3 种类型 (a), (b) 中的位移长度分别对应于 1 帧和 5 帧, 轨迹数目见正文表 2. 蓝色箭头强调了 Me β CD 处理导致的分布曲线变化

Fig. S6. PDF distribution of the displacement (in the x direction) of single-molecule diffusion trajectories live cell membranes, both before (red) and after (blue) treatment with Me β CD. The trajectories have been identified and classified into three subgroups for each system using the "two-step classification method", namely confined diffusion, sub-diffusion, and normal diffusion. The corresponding number of trajectories is listed in Table 2 of the main text. In panels (a) and (b), the displacement length corresponds to one frame and five frames respectively. The blue arrows are utilized to accentuate the alteration in the distribution profile induced by Me β CD treatment.

算法 S1 对单轨迹进行分类的计算过程.

输入: 单分子轨迹数据 X_{t_i} , 约束参数 λ

输出: 扩散类别 C , 扩散系数 D , 异常指数 α

- 1) 将数据 X_{t_i} 和约束参数 λ 代入 (8) 式进行优化求解;
- 2) 若 $P_1 > P_2$, 则轨迹为受限扩散, 否则转到 3;
- 3) 根据选择的方法计算 $c = t_{\delta/2}(n-2) \text{SE}(\hat{a})$ 或 $T_N = D_N / \sqrt{(t_N - t_0)\hat{\sigma}_N^2}$ 和分位数 $q_N(\delta/2)$ 的值;
- 4) 比较异常指数 α 与截断参数 c 或统计量 T_N 与分位数 $q_N(\delta/2)$ 的大小关系, 判断轨迹扩散类型 (亚扩散、正常扩散、超扩散);
- 5) 返回扩散类型结果

算法 S2 Monte Carlo 方法模拟 n 个样本的 $(T_N^{(1)}, \dots, T_N^{(n)})$ 在自由扩散下统计量 T_N 的分布.

输入: 单分子轨迹长度 N , 分位值 $\delta/2$, Monte Carlo 模拟数量 n

输出: T_N 的分位数 $q_N(\delta/2)$ 和 $q_N(1 - \delta/2)$

- 1) 模拟产生布朗运动的单分子轨迹, 设 $x_0^{(i)} = (0,0)$, 单位位移时间 $\Delta = 1$, 从正态分布中随机抽取 ϵ , $x_{t+1}^{(i)} = x_t^{(i)} + \epsilon$, $\epsilon \sim N(0, \mathbf{I}_2)$.
- 2) 由 $(x_0^{(i)}, \dots, x_N^{(i)})$ 计算统计量 $T_N^{(i)} = D_N^{(i)} / \sqrt{N \cdot \hat{\sigma}_N^{(i)2}}$.
- 3) 重复步骤 1 和 2, 计算 $(T_N^{(1)}, \dots, T_N^{(n)})$.
- 4) 对 $(T_N^{(1)}, \dots, T_N^{(n)})$ 排序计算 T_N 的分位数 $q_N(\delta/2)$ 和 $q_N(1 - \delta/2)$.