Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Information

Size-Selective Adhesion of Calcium Oxalate Monohydrate Crystals to Lipid Membranes

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The fluidity of 16:0-14:0 PC Supported Lipid Bilayers

We utilized fluorescence recovery after photobleaching to investigate the fluidity of 16:0-14:0 PC-supported lipid bilayers. Figure S1 displays the temporal progression of fluorescence recovery after laser bleaching at 20℃ and 37℃. At 20℃, the laser-bleached dark region did not disappear after 60 seconds. At 37℃, however, the dark region became blurred and the intensity significantly recovered within 60 seconds. This rapid recovery of a micron-sized spot in 60 seconds suggests that the 16:0-14:0 PC-supported lipid bilayer at 37℃ is at the fluid state, with the lipid probe having a high diffusivity in the lipid membrane. Conversely, the slow recovery at 20℃ indicates that the membrane had low fluidity.

Interestingly, the size of the bleached spots differed between the two temperatures, 20℃ and 37℃, immediately after bleaching, despite both samples receiving the same laser exposure time of 1 second. This discrepancy could be attributed to the difference in fluidity. At 37℃, the increased fluidity of the membrane enabled the bleached fluorescent lipid probe to spread and mix with the unbleached probe during the 1-second bleaching process, resulting in a larger dark region than the laser beam size. On the other hand, the limited movement of the bleached probes at 20℃ kept the size of the dark region close to the laser beam size.

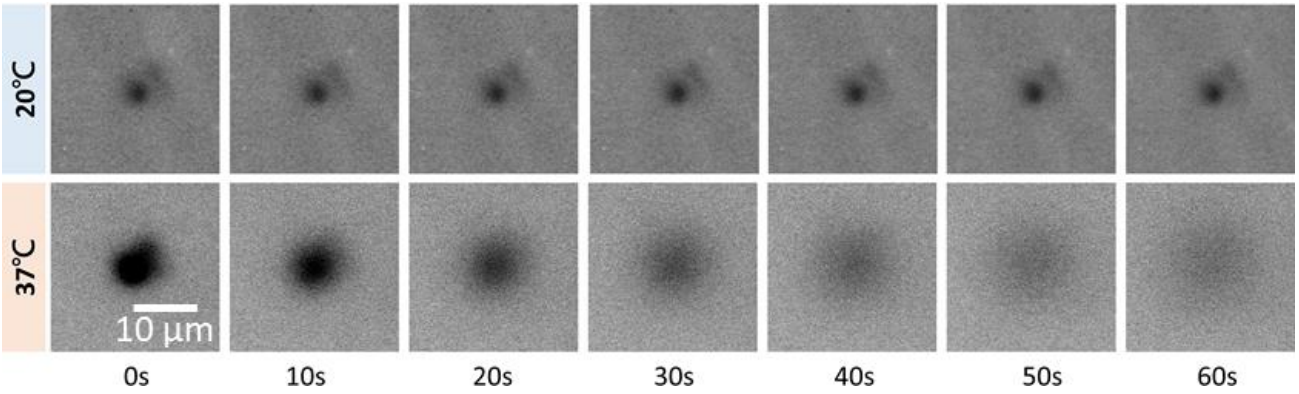

Figure S1. The temporal progression of fluorescence recovery after photobleaching of the 16:0-14:0 PC supported lipid bilayers (with 1 mol% β-BODIPY® FL C5-HPC) at 20℃ and 37℃.

Using VESTA Software to Find Potential Hydrogen Bonding Match

Our hypothesis is that the crystal water molecules at the (100) and (010) faces of COM can form hydrogen bonds with phospholipid head groups to stabilize the interface. To test this hypothesis, we located the crystal waters of COM and attempted to find the best position for the phospholipid head group to form the most hydrogen bonds with the crystal water molecules. Previous studies have demonstrated that the phospholipid head group can form hydrogen bonds with water at the phosphate group and the ester groups.[1-2] However, due to the numerous single bonds in the phospholipid head group, it can rotate and adopt numerous structural orientations. Therefore, we initially focused on determining the position of the phosphate group, which has two electron acceptors (P−O−H) and electron donors (P=O) for the hydrogen bonds. The phosphate group is located further away from the hydrophobic acyl chains, making it more accessible to water molecules and offering more chances to form hydrogen bonds. Next, we examined different potential positions for the remaining atoms in the phospholipid head group to allow the ester bonds to form the largest number of hydrogen bonds with the crystal water.

Figure S2 illustrates the possible hydrogen bonds formed between the phospholipid head group and the crystal water networks in the COM crystal. Figure S2(a) and (b) show how the phospholipid head group can fit in the crystal water networks at the (100) face and (010) face, respectively. The number of phospholipid molecules per unit cell is 2 for the (100) face and 1 for the (010) face, which reflects the ratio of the phospholipid molecule's area to the COM crystal unit cell's area. Our findings suggest that there may be up to 9 hydrogen bonds per phospholipid molecule in the (100) face and 10 hydrogen bonds in the (010) face, as illustrated in Figures S2(c) and (d). The corresponding hydrogen bond lengths are listed in Table S1. The bond lengths all range from 1.44 Å to 2.64 Å, which is consistent with the anticipated range for hydrogen bonds.[3]

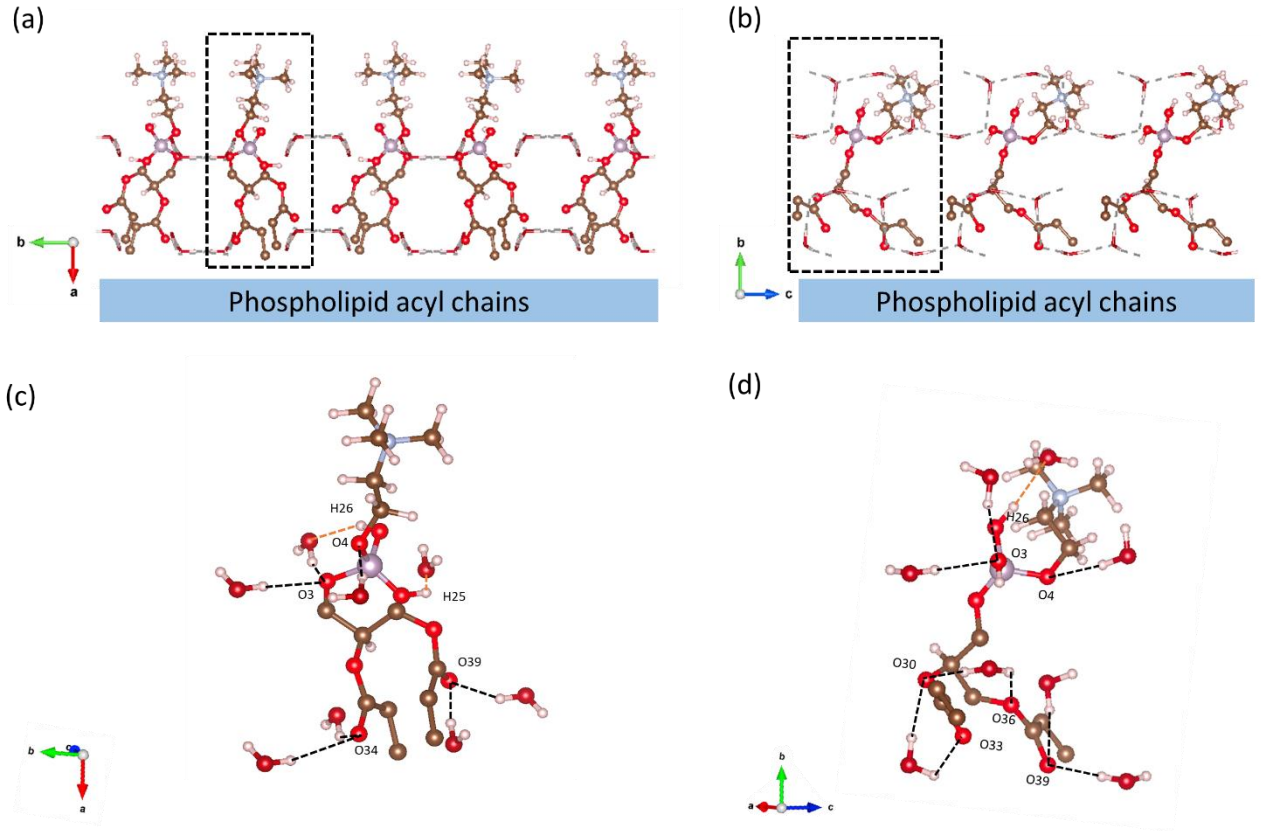

Figure S2. The hypothetical hydrogen bonds that could form between the phospholipid head group and the crystal water networks in the COM crystal. (a)(b) The potential positions of the phospholipid head group in the crystal water networks at the (100) face and (010) face, respectively. The dashed rectangles indicate the area occupied by a single phospholipid head group. (c)(d) The detailed depiction of the hydrogen bond interactions between the phospholipid head group and the crystal water molecules

at the (100) face and (010) face, respectively.

Table S1. The anticipated lengths of the hydrogen bonds that could potentially form between the phospholipid head group and the crystal water molecules at the (100) face and (010) face in the COM crystal.

(100)		(010)	
HB length		HB length	
PC atom	bond length (Å)	PC atom	bond length (Å)
H25	1.59	H26	2.10
H26	2.32	O3	2.02, 2.24
O3	2.19, 2.23	O4	1.98
O4	2.31	O30	1.44, 2.14
O34	1.53, 2.64	O33	1.71
O39	1.83, 2.22	O36	1.85
		O39	1.92, 1.94

Reference

1. Zhang, R.; Cross, T. A.; Peng, X.; Fu, R., Surprising Rigidity of Functionally Important Water Molecules Buried in the Lipid Headgroup Region. *Journal of the American Chemical Society* **2022,** *144* (17), 7881-7888.

2. Yamada, T.; Takahashi, N.; Tominaga, T.; Takata, S.-i.; Seto, H., Dynamical Behavior of Hydration Water Molecules between Phospholipid Membranes. *The Journal of Physical Chemistry B* **2017,** *121* (35), 8322-8329.

3. Grabowski, S. J., *Understanding Hydrogen Bonds: Theoretical and Experimental Views*. Royal Society of Chemistry: 2020.