Direct Imaging of Water/Lipid Interface by Frequency Modulation Atomic Force Microscopy at Sub-Angstrom Resolution

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Frequency modulation atomic force microscopy (FM-AFM) has been used in ultra high vacuum (UHV) environments for investigating subnanometer-scale structures and functions of various surfaces. Until recently, however, its operating environment had been limited only to UHV, which has prevented its practical applications in air and liquids. Recently, Fukuma *et al.* presented a way to overcome this limitation using an ultra low noise cantilever deflection detection system and thereby operating FM-AFM with extremely small cantilever oscillation amplitude [1]. This has made it possible to obtain true molecular [2] and atomic [3] resolution with FM-AFM in liquid. One of the most interesting applications of this new technique is high-resolution imaging of biological systems under physiological conditions. However, true subnanometer resolution with FM-AFM in liquid has not been demonstrated on biological systems.

Biological membranes are amongst the most fundamental elements in biological systems. They form the walls of cells and boundaries between the organelles therein with a selectively permeable structure. The structure and function of the membranes are determined by the chemical interactions between the constituent molecules mediated through water and ions in physiological solution. Thus, understanding of the interactions between lipid molecules (main constituents of biological membranes) and water or ions are of great importance. To date, various spectroscopic methods have been utilized for investigating water/lipid interface. However, these methods provide only global information averaged over micrometer-scale area and hence molecular-scale details of water-lipid and ion-lipid interactions have mostly remained unknown.

Here we investigate a dipalmitoylphosphatidylcholine (DPPC) lipid bilayer in phosphate buffer solution as a model biological membrane under physiological conditions by FM-AFM [4]. The force vs. distance curves measured between the bilayer and the AFM tip show oscillatory force profiles with a peak spacing of 0.28 nm, indicative of the existence of up to two hydration layers next to the membrane surface. FM-AFM imaging at the water/lipid interface visualizes individual hydration layers in three-dimensions with molecular-scale corrugations corresponding to the lipid headgroups. Furthermore, we visualize extensive lipid-ion interaction networks and their transient formation between headgroups in the bilayer. The spatial distribution of ion occupancy, visualized in real-space with the unprecedented lateral resolution of 90 pm, reveals the existence of two equivalent binding sites associated with the phosphate groups and the network formation between them.

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[3] T. Fukuma, K. Kobayashi, K. Matsushige, H. Yamada, *Appl. Phys. Lett.* **87**, 034101 (2005).
[4] T. Fukuma, S. P. Jarvis, *Rev. Sci. Instrum.* **77**, 043701 (2006).