High-Resolution Imaging of Proteins by Dynamic Force Microscopy in Liquid

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High-resolution DFM imaging in liquid is severely hindered by the extreme reduction of the Q-factor due to the hydrodynamic interaction between the cantilever and the liquid. Nevertheless, DFM imaging is essential especially for visualizing nanometer-scale structures of biological materials such as protein molecules and DNAs. We have recently succeeded in imaging organic crystal surfaces in liquid with true molecular resolution by FM-DFM. The above-mentioned difficulty was overcome mainly by the use of the small amplitude mode (amplitude < 1 nm) and the noise reduction in cantilever deflection sensor, leading the success in high-resolution DFM imaging in the low Q-factor environment.
A commercially available AFM head (JEOL: JSPM-4200) was modified and used in this experiment with our developed FM detector based on phase-locked loop circuit using a voltage controlled crystal oscillator. In addition, we used homebuilt low-noise optical deflection sensor of which deflection noise density was about 17 fm/√Hz. This remarkable low-noise feature is essential for high-resolution frequency measurements and also for stable cantilever self-oscillation with a small amplitude. A highly-doped n-Si cantilever (Nanosensors: NCH) with a resonance frequency of about 150 kHz in buffer solution and a nominal spring constant of 40 N/m was used as a force sensor. The Q-factor was about 20. The cantilever vibration amplitude was regulated at as small as about 0.6 nm.
The sample in this experiment is purple membrane consisting of protein molecules (bR: *bacteriorhodopsin*). The sample was suspended in phosphate buffer solution. Then it was adsorbed to freshly cleaved mica. After 30-min. adsorption at room temperature, it was gently washed with the imaging buffer to remove weakly attached fragments of the membranes. Imaging was conducted in the phosphate buffer solution. Figure 1(a) shows an FM-DFM image of a membrane deposited on mica. Figure 1(b) is a magnified image of (a). A hexagonally packed structure of bR protein trimers was clearly observed.

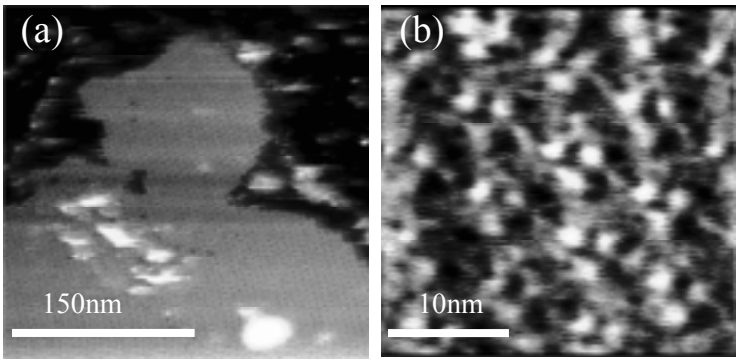

Figure 1 FM-DFM images of purple membrane obtained in 1 mM PBS, pH 7.4, 200 mM KCl.