Molecular-scale Investigations on Antibody-antigen Interactions in Liquids by FM-AFM

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Visualization of molecular recognition process in antibody-antigen interactions is essentially important for the understanding of the immune system at the single molecule level. We recently succeeded in FM-AFM imaging sub-molecular structures of a monoclonal immunoglobulin G (IgG) antibody molecule such as protein domain structures (Ig domains). We also revealed that the IgG antibodies spontaneously assembled into hexamers and successively formed into a two-dimensional (2D) crystal in an electrolyte solution (e.g. 50 mM $MgCl_2$ solution) [1]. In this study, we visualized the change in the 2D antibody crystal after the addition of the antigenic molecules for the molecular-scale understanding of antibody-antigen interactions in liquids.

A low-thermal-drift FM-AFM, developed based on a commercial AFM apparatus (Shimadzu SPM-9600), was used. Mouse anti-serum albumin monoclonal antibody (IgG) in a 10 mM phosphate buffer solution (PBS, pH7.5) containing 50 mM $MgCl_2$ was deposited onto a mica substrate. The IgG antibody molecules immediately turn into the 2D crystal in this solution condition. We first imaged the 2D antibody crystal structures, as shown in Fig. 1(a), and then added PBS holding antigenic molecules (serum albumin) into the solution. Five minutes after the addition we rinsed it with a PBS containing 50 mM $MgCl_2$ for removing non-specific adsorbents. Figure. 1(b) shows an FM-AFM image of the 2D antibody crystals interacted with antigenic serum albumin molecules (50 mM $MgCl_2$). Although the image shows the surface covered with many irregular structures, we can barely see regular crystal pattern in some areas below the irregular adsorbents, which means that these are the antigenic molecules adsorbed onto the antibody crystal. We also confirmed that non-antigenic serum albumin originating from another mammal did not bind the 2D antibody crystals by non-specific interactions. The results indicate the biological function of the antibody-antigen interaction is still active in this 2D crystal structure.

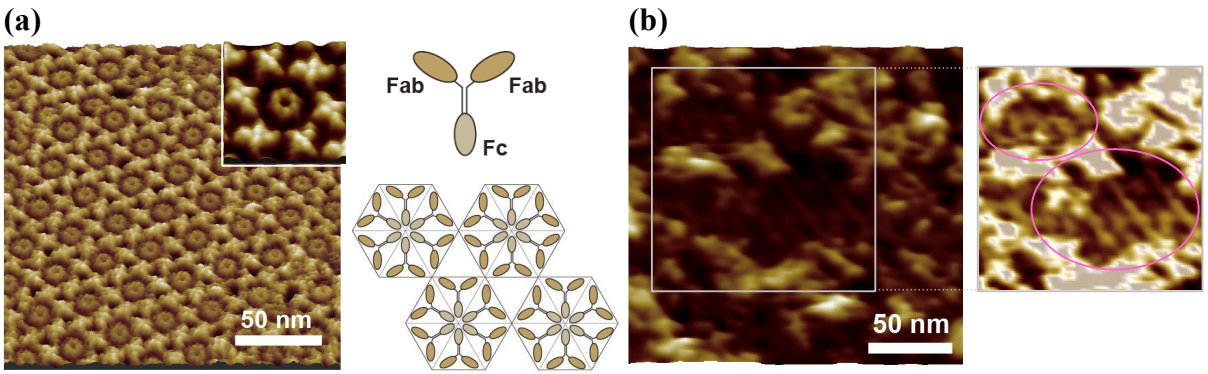

Figure. 1 FM-AFM images of a 2D crystal of IgG monoclonal antibody (in 50 mM $MgCl_2$) before **(a)** and after **(b** *left***)** the additon of the antigenic selum albumin solution (50 mM $MgCl_2$). A crystal regular pattern (surrounded by pink circles) can be seen in the contrast enhanced image **(b** *right***)**.

References

[1] S. Ido et al., 14th International Conference on Non-contact Atomic Force Microscopy, Symp-03, Lindau, Germany (2011).