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Single Molecule Sensing via Kelvin Probe Force Microscopy

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Kelvin probe Force microscopy (KPFM) is a non-invasive tool to analyze with nanometric spatial resolution, changes in work function for metal or surface potential for nonmetals by measuring contact potential difference between the sample surface and a conducting cantilever tip. [1] It allows the simultaneous high-resolution topographic imaging of the sample surface, as well as electrical and electronic characterization. Since KPFM does not measure a current flow, but rather it records the electrostatic-force interaction between two objects, i.e., the tip and the sample, not requiring a direct contact, it is well suited for the study of fragile and soft samples, such as proteins. In addition, it does not require particular sample treatments, such as exposure to high electric fields or electron or photon beams [2]. With this principle, KPFM may be suitably adopted to investigate molecular interactions that lack topological differences but have distinguishable differences in surface potential. Furthermore, the capability for discriminating the difference in surface potential before and after bioassay allows the use of KPFM for developing a diagnostic platform for testing biomolecular interactions. Human-IgM (IgM) is the affinity ligand that selectively binds to the anti-Human-IgM. Kelvin probe microscopy has been employed for the quantitative analysis of anti-IgM/IgM interactions occurring at a gold electrode surface with a patterned physisorbed anti-IgM layer, after the incubation with IgMs in PBS standard solutions, down to the single molecule level. The analysis has been performed always at the same scanning location, to detect changes in charging properties of the bio-modified gold surface after the highly specific IgM binding, with micrometer-scale resolution without labeling (Fig. 1). The electric fields produced by the electric dipole of the a-helix and by ionic charges in stabilizing the immunoglobulins have been extensively studied [3]. These electrostatic interactions are involved, at the active sites, in binding the homologous antibody, affecting the protein structure. As a result of their long range, extending over the whole protein molecule, we can assume that the physisorbed bio-receptors on the gold surface consist of a dense and compact layer, divided into domains with a characteristic electric charge distribution. The binding with very low concentration of IgM molecule (100 zM), in minute timescale, affects the charging properties of the bioreceptor and also of the entire corresponding domain. On the basis of a propagation effect, an overall shift of the investigated area surface potential occurs, as already demonstrated by Torsi and coworkers in the SiMoT platform, where the formation of at least one antigen/antibody complex on the gate biofunctionalized electrode surface gives rise to a shift in the device threshold voltage [4].

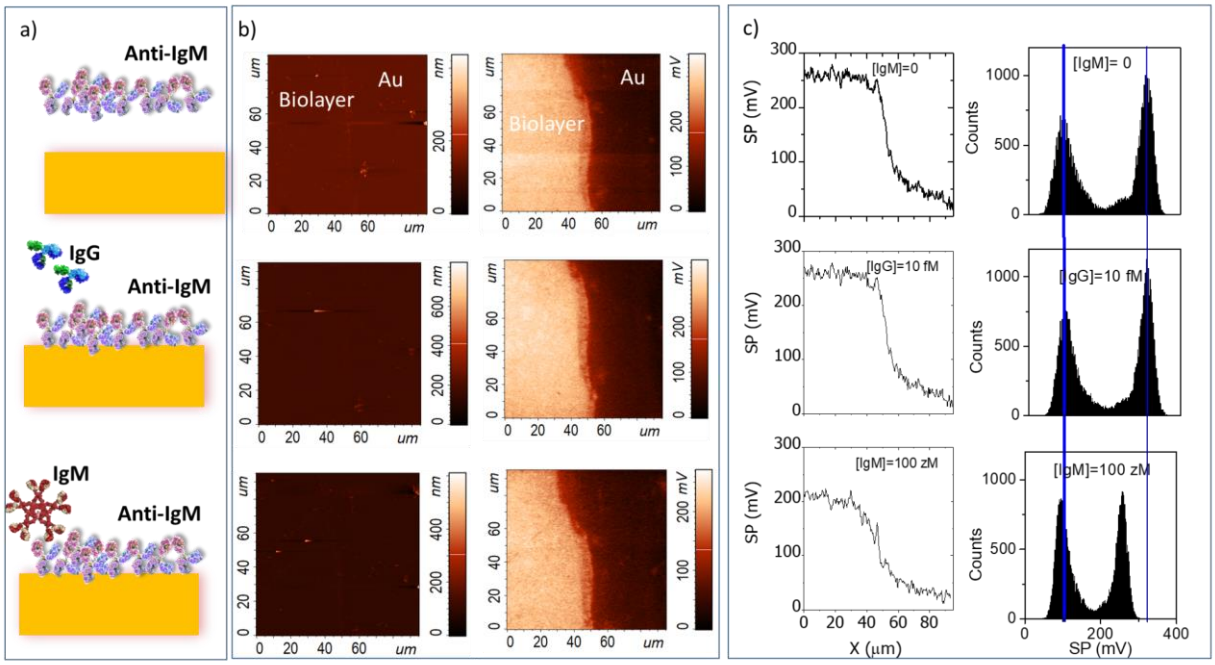

Figure 1. *The immunoglobulin IgM recognition assay has been performed by measuring the surface potential landscapes after the incubation of a diluted standard solution of ligand (([IgM]= 100 zM) for 10 min, in 100 µl in PBS. A standard-solution of IgG 10 fM in PBS has been used for negative control experiments (panel a). After the ligand exposure, the substrate has been electrically biased by applying a sweeping potential between the sample and a reference electrode in the range [0.1 V, -0.5 V]. The area overlapping gold and anti-IgM patterned regions has been scanned via KPFM two pass mode. The tip has been held at 250 nm above the surface (panel b). Quantitative analysis of the surface potential changes induced by the formation of anti-IgM/IgM biocomplex and the corresponding histograms of the SP distributions (panel c).*

References

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