595a Development and Characterization of Antibody Molecules on Peg Tethered Silicon-Based Biosensors by Atomic Force Microscopy

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Of primary concern in biosensor design is the immobilization of biomolecules such as antibodies to the biosensor surface. The process of immobilization is critical and determines the primary properties of the biosensor. The using of spacers, which can increase the flexibility of immobilized molecules, is one promising protocol to improve the bioactivity or capture efficiency of immobilized antibodies. To develop a highly sensitive biosensor, the biospecific recognition of substrates must be optimized.

To this end, we have created PEG interfaces with various grafting densities on silicon to immobilize *E.Coli* K99 pilus antibody by a series of reaction steps. Silicon wafers were modified by forming a self-assembled monolayer (SAM) of (3-glycidyloxypropyl)trimethoxysilane (GPTMS), followed by immobilization of PEG spacer. Antibody molecules were finally bonded through glutaraldhyde. To obtain different grafting densities, initial PEG concentrations were varied by changing the ratio of m-PEG and NH_2-PEG-NH_2. Ellipsometry, X-ray photoelectron spectroscopy (XPS) and Atomic Force Microscopy (AFM) were used to characterize the surface morphology and chemical composition at each reaction step. The bioactivity and sensitivity of the antibody-immobilized surfaces were then evaluated by AFM. The interaction between *E.Coli* K99 and its antibody were obtained by coating different strains of *E.Coli* to a standard AFM tip. Both approach and retraction force curves were obtained and utilized in elucidating the features of interaction between *E.Coli* modified tip and the surface-attached antibody. Scanning Electronic Microscopy (SEM) was also performed to investigate the capture efficiency of antibody. Quantitative results from AFM and SEM indicated that PEG tethered surfaces showed an improved bioactivity and sensitivity. This work provides a novel approach for tailoring a highly sensitive biorecognition system and for developing the application of AFM as a way to evaluate bioactivity and sensitivity.

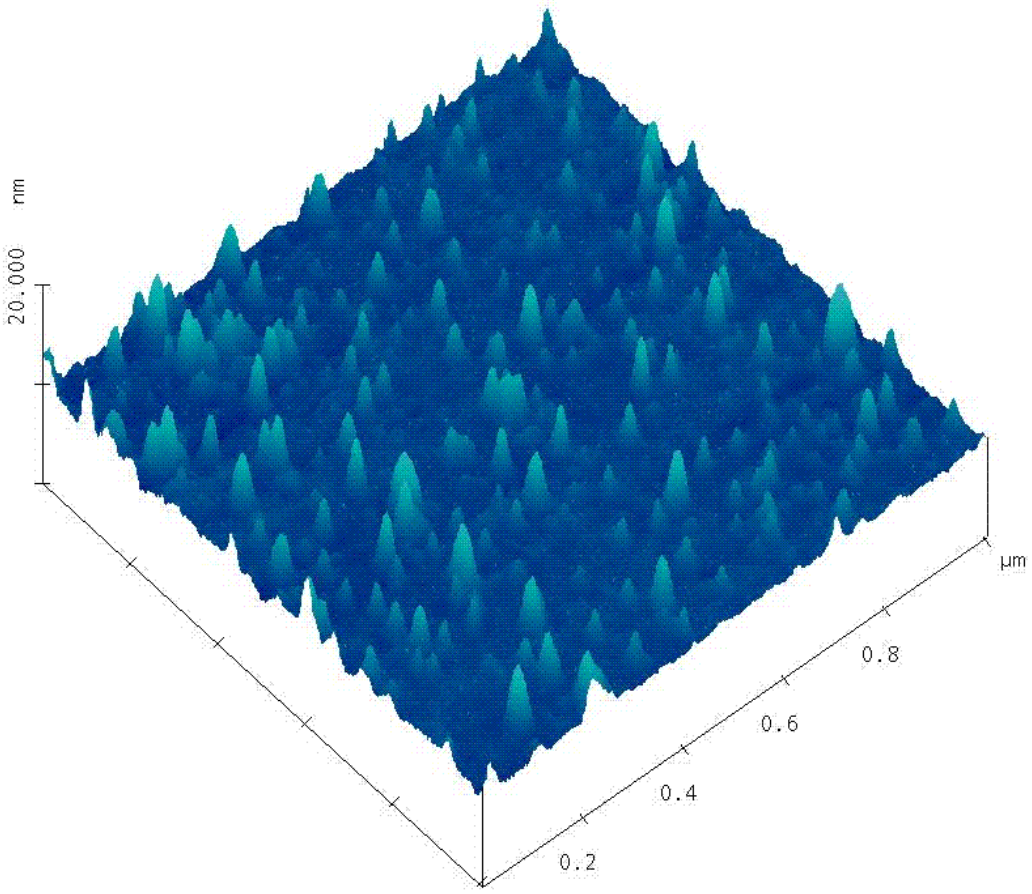

Figure 1. Tapping mode AFM image of E.Coli K99 pilus antibody immobilized on silicon wafer surfaces via PEG spacer taken in ambient conditions. The individual antibody molecular can be identified from the image.