

Site-directed antibody immobilization on gold substrate for quartz crystal microbalance and surface plasmon resonance detection

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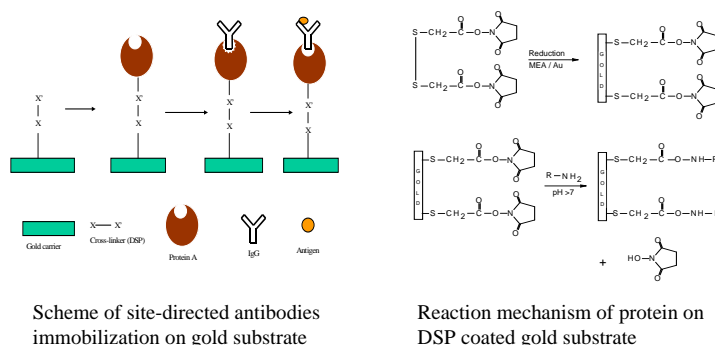
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Introduction

In an immunosensor using quartz crystal microbalance (QCM) or surface plasmon resonance (SPR) based detection approach, the sensing surface is the critical part of the detection system. This surface is usually made of gold [1]. On this surface biomolecular interaction takes place and the binding event is transduced into a detectable signal. The sensor surface is generally derivatized with suitable linkers for binding biomolecules to get reproducible and consistent results. To obtain highly sensitive sensing surfaces, it is necessary to present the receptor molecule in a manner, where the corresponding ligand will be able to bind the receptor without any steric restrictions. Specifically with antibodies, once immobilized to a solid support, they lose a part of their specific binding competence. This is mainly due to their random orientation on the surface. [2, 3]. In the present study, we propose a singular sensor surface using Protein A as a linker on DSP modified gold substrate to achieve uniform, stable, and sterically accessible antibody coating for QCM and SPR based immunosensors.

Experimental:

The gold substrates were cleaned thoroughly, first with Milli-Q deionized water and then with a detergent solution (2 % Hellmanex), before a final wash in deionized water. Freshly prepared gold surface was first treated by dipping into a boiling solution of H₂O₂ (35%), NH₃ (25%) and Milli-Q water in a 1:1:5 ratio for 10 min and then rinsing thoroughly with Milli-Q water. Cleaned gold-coated glass slides were immediately used for thiol linkage by dipping in 0.002 M dithiobissuccinimide propionate (DSP) in dimethylsulfoxide for 2 h at RT followed by rinsing with DMSO and then with phosphate buffered saline (PBS), pH 7.4. The protein layer was covalently attached to thiol linked gold slides by soaking the slides overnight at 4°C in a Protein A solution (1 mg/ml) in phosphate buffer. These are then followed by treatment with ethanolamine hydrochloride (1 M), pH 8.6, for 1 h to block the residual reacting sites. Slides were then washed thoroughly with distilled water, air dried, and stored at 4°C before use in the SPR or QCM set up.



Results

Atomic Force Microscope (AFM) was used to estimate the uniformity of the gold layer on the glass slide and the protein A layer on the gold-coated glass slide. Surface roughness and flatness was analyzed using AFM in the contact-mode. Figure 1 shows these surfaces.

The stability of Protein A binding on modified gold surface was analysed by using fluorescence isothiocyanate (FITC) labeled antibody. The labelled antibody was used for binding with protein A modified gold substrate at different time interval (up to 8-weeks time). The Protein A coating on modified gold surface did not show significant loss of activity up to 8-weeks time, primarily due to the strong covalent linkage of Protein A with DSP chemisorbed on the gold.

Table 1 and Fig. 2 show the SPR and QCM response curves for (a) thiolated glass slides using octanethiol in ethanol and (b) Protein A modified glass slides. The SPR angle scans were observed for equal amounts of immunoglobulin (IgG) (10 µg/ml) added onto Protein A on DSP modified gold and thiolated gold surface. The signal response observed in the case of both (SPR and QCM) for the Protein A modified surfaces was higher than on thiolated gold surface.

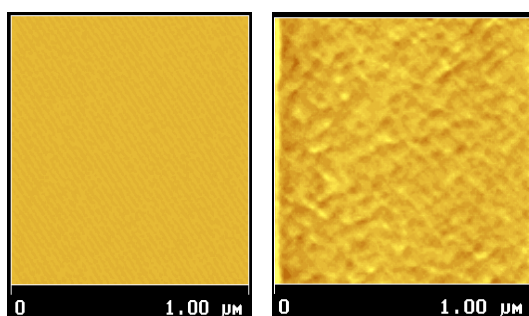


Fig. 1: AFM image of the protein A self-assembled monolayer on gold substrates (a) plain gold and (b) protein coated gold substrates.

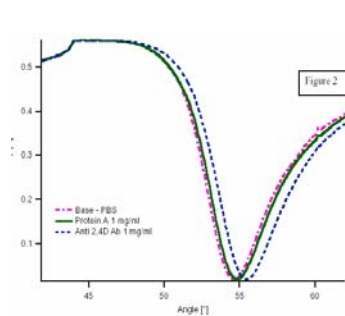


Fig. 2: SPR response for different conc. of protein

Table 1: QCM response for different conc. of IgG concentrations on (a) Protein A modified crystal and (b) Thiolated crystal.

IgG Conc. mg/ml	Frequency shift (Hz) (Prt A modified surface)	Frequency shift (Hz) (thiolated surface)
10 ⁻¹	251	121
10 ⁻²	187	109
10 ⁻³	83	53
10 ⁻⁴	37	13
10 ⁻⁵	05	--

Conclusion

This work demonstrates an efficient site-directed antibody immobilization on a gold-coated glass slide for SPR and QCM applications. The thiol (DSP) based linker arm on gold surface provides the covalent linkage of protein molecules to bind on the surface. Protein A coated slides can be used several times repeatedly by removing the bound antibody with low pH (2.6) buffer. The suggested scheme could be employed for the modification of solid support coated with gold for other immunobiosensor applications i.e., electrochemical or other optical transducers.

Acknowledgement

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