# Binding Efficiency Improvement of Antibody to Antigen on Mixed Self-Assembly Monolayers

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## ABSTRACT

Protein chip is a potential tool on advanced disease diagnosis and drug screening, due to the capabilities of parallel process and trace-amount sample detection. The detection of proteins mainly depends on immune recognition, very sensitive to the orientation of immobilized proteins on solid surface. However, due to close packing of immobilized proteins on SAMs, the binding efficiency is usually lower than 10 %. This paper proposed a novel way by employing mixed SAMs to generate nano patterns for effective separation of immobilized proteins to allow antibody conjugation from more directions. Testing results by Surface Plasma Resonance (SPR) and Atomic Force Microscope (AFM) investigation demonstrated a almost 3 fold increment on binding efficiency of protein immobilization on mixed SAMs.

***Keywords***: *mixed self assembly monolayer, binding efficiency, protein orientation, antibody, biochip*

## 1 INTRODUCTION

In proteomic era, protein chip has been becoming a powerful tool to resolve relationships between proteins and diseases, thanks that protein chip can perform parallel detection and use only trace amount of sample on a 2-dimension substrate. In protein recognition, antibodies bind to antigens at specific 3-dimension structures. However, owing to steric barrier from close packing of immobilized proteins on Self assembly monolayers (SAMs), the binding efficiency is usually very low. SAMs are generally used to modify reaction surfaces for biomaterial conjugation or changing hydrophilic degree.[1], and have been widely applied to biotechnology, nanotechnology, nanopattern formation [2], material science and Microelectronmechanical System (MEMS)[3] by the optional tailor of SAMs head and tail group , chain length , chain species and substrate fixing methods. Single SAMs usually forms very dense molecular structures, thus carries out a dense protein molecule directly immobilization on the top of the SAMs without any specific orientation. The conventional method to resolve the orientation problem is by modifying protein through molecular biotechnology, ie biotin/avidin [4] or His-tagged[5] structure modification in the specific sections of protein to ensure the orientation of protein immobilization. In this paper we propose a alternative way without the application of molecular biotechnology. In stead, by properly design the nano structures of mixed SAMs system, the immobilized antibodies (in Fig. 1) or antigens (in Fig. 2) can be effectively separated to one another to allow the corresponding antigens or antibodies to access the binding site from not only the top, but also the side ways, thus can greatly enhance the degree of binding freedom and efficiency of the immune recognition process in limited area.

## 2 MATERIALS AND METHODS

### 2.1 Sample preparation

Glass slide preparation: Commercially available glass slides (Kimble 1x3" USA) were subsequently cleaned and oxidized in a freshly prepared 4:1 mixture of 96% sulfuric acid (J.T. Baker, 9684-05) and 30% hydrogen peroxide(J.T. Baker, 2190-14)(piranha solution) at 100°C for 10min. After the substrates cooled to room temperature, the samples were rinsed with DI water and dried in $N_2$ gas.

For the preparation of glass substrate for the control experiment of surface plasma resonance (SPR), glass substrates were subsequently evaporated Cr 50Å, Au 500Å and SiO2 100Å, all at a evaporation rate of 0.5 Å per second. Finally, the substrates were cleaned with ethanol (Riedel, absolute, 32221, $C_2H_5OH$) and DI water several times.

SPR (surface plasma resonance): The employed SPR chips are SIA kit Au (BIACOR) with evaporated 100Å SiO2 for alkylsilane immobilization. The evaporation process is similar to the above.

SAMs preparation: oxidized silicon wafers were immersed into a freshly prepared $5x10^{-3}$ (v/v) solution of APTS (3-aminoproplytrimethoxysilane, Fluka 09326) and C2(ethyltrimethoxysilane, Aldrich 435643 ) in dried ethanol at room temperature (25°C), immediately after drying.

Glass slides and SPR chips were immersed inside mixed SAMs of APTS and C2 for one hour at room temperature, and the mixing ratios are 1/0, 1/3, 1/10, 1/30, 0/1, respectively. The substrates were then cleaned subsequently with ethanol solution and DI water several times, and dried in $N_2$ gas. To obtain homogeneous SAMs film, the substrates were annealed for 2hours at 80°C within

$N_2$ gas environment and cooled to room temperature afterward.

Activation: To conjugate protein, the function groups of SAMs were activated by BS3 (5mg/1cc) for 30min. After activation, the substrates were cleaned with DI water for several times before experiment.

## 2.2 Measurement

SPR: In SPR measurement, PBS buffer was flowed until the stabilization of base line. In the measurement of antibody binding efficiency, 50 μl antibody (0.1mg/ml) with 10 μl/min flow rate, horse serum (0.5%w/w), and antigen (0.1mg/ml) were injected subsequently. Horse serum was used to block area without antibody for the reduction of interference by non-specific bindings.

Fluorescence detection: For fluorescence detection, goat anti-rabbit IgG cy5 (as antibody, Chemicon AP1325) and rabbit anti-mouse IgG cy3 (as antigen, Chemicon AP160c) were employed. The fluorescence was detected by Genepix scanner 4000B. The scanning conditions of all chips were set to 600-volts PMT and 33% laser power. In fluorescence detection, binding efficiency and recognizing efficiency of antibody were calculated by cy3/cy5 ratio and cy5/cy3 ratio, respectively.

AFM: AFM measurement were performed by using JPK NanoWizard AFM (Germany). The AFM operated in tapping mode, and the cantilever was silicon cantilevers (NCH nanoworld). Scan size was 1μm x 1μm, x-y resolution is 512x512 pixels. All height mode and lock-in phase mode were captured at same time.

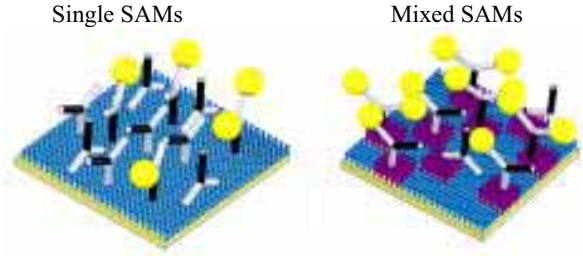


Fig.1 The schematic diagram of the principle for the improvement of recognition efficiency of antibody to antigen by mixed SAMs surface.

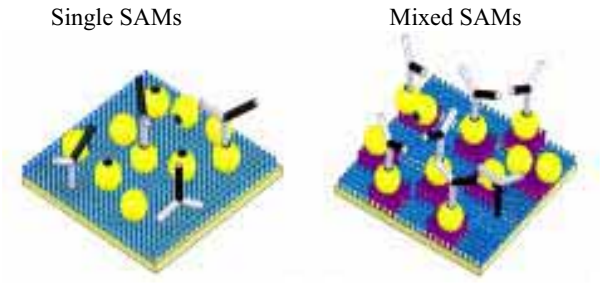


Fig.2 The schematic diagram of the principle for the improvement of binding efficiency of antibody to antigen by mixed SAMs surface

## 3 RESULT AND DISCUSSION

From the SPR (Fig.3,4) and fluorescence (Fig.4,5) measurements, the results obviously indicate that the binding efficiency (antigens immobilized on SAMs) and recognizing efficiency (Antibody immobilized on SAMs) of antibody to antigen were improved on mixed type SAMs, especially in a mixed ratio for APTS to $C_2$ of 1/10. Although the improvements are 1-3 folds in the shown figures, 6-10 fold improvements are possible by the optimization of process conditions and mixing ratio. The mixed SAMs method provides a high-throughput and efficient way on sample preparation for increasing protein binding efficiency. The reasons for the improvement of binding efficiency, as shown in Fig. 1-2, are the open-up of the surrounding area of immobilized proteins on mixed SAMs surfaces, effectively lowering down the steric barrier for protein recognition.

This idea can further be verified by AFM investigations. Fig. 7 shows the 3-D topographies of AFM height mode scanning results on antibody immobilized on single SAMs of APTS and mixed SAMs of APTS to $C_2$ in a ratio of 1/10, respectively, revealing a larger roughness, higher peak value, and wider protein-protein interspaces in the mixed SAMs case. On the other hand, in the lock-in phase mode measurement shown in Fig. 8, stronger interaction forces between cantilever and antibody/mixed SAMs surface demonstrating a higher capability for antigen binding to antibody in mixed SAMs case than that in singe SAMs case. Besides, from the cross section views, the gap among each protein cluster is larger in the mixed SAMs case, indicating a looser paving of proteins on mixed SAMs surface, which allows antigens more room to bind on antibodies. As a result, AFM investigations successfully provide evidence and mechanism on the binding efficiency improvement for antibody-antigen recognition on mixed SAMs surfaces.

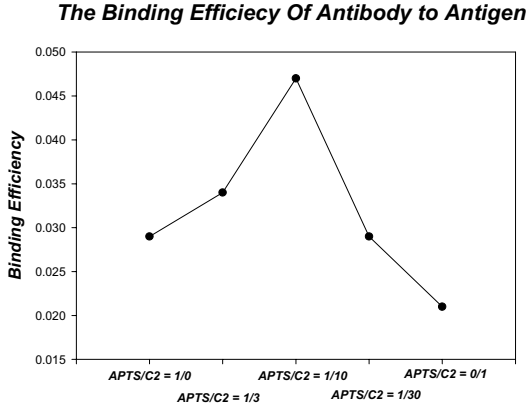


Fig.3 The SPR measured binding efficiencies of antibody to antigen for different ratios of APTS/$C_2$ mixed SAMs, ranging from 1/0 to 0/1. the binding efficiency becomes 1.62 fold for the APTS/$C_2$ mixing ratio of 1/10 than that of 1/0.

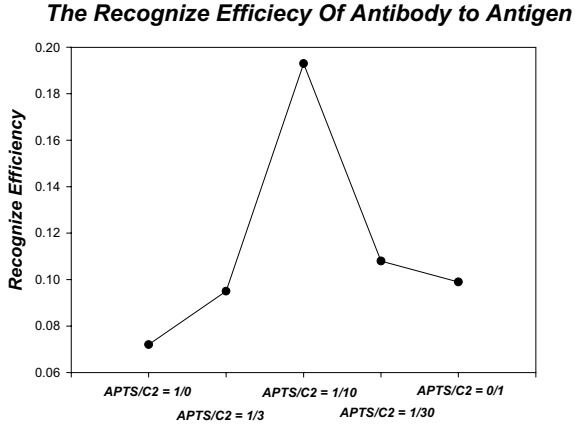


Fig.4 The SPR measured recognition efficiencies of antibody to antigen for different ratios of APTS/$C_2$ mixed SAMs, ranging from 1/0 to 0/1. the binding efficiency becomes 2.68 fold for the APTS/$C_2$ mixing ratio of 1/10 than that of 1/0.

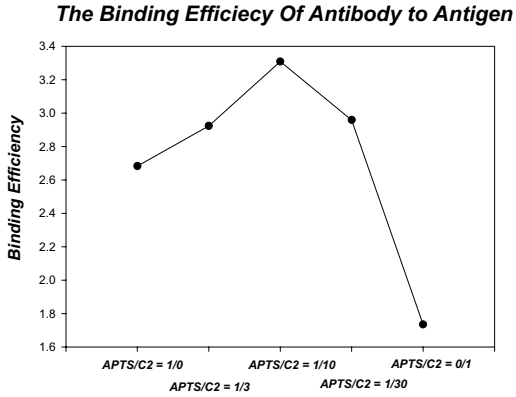


Fig.5 The measured binding efficiencies by fluorescent information of antibody to antigen for different ratios of APTS/$C_2$ mixed SAMs, ranging from 1/0 to 0/1. the binding efficiency becomes 1.233 fold for the APTS/$C_2$ mixing ratio of 1/10 than that of 1/0.

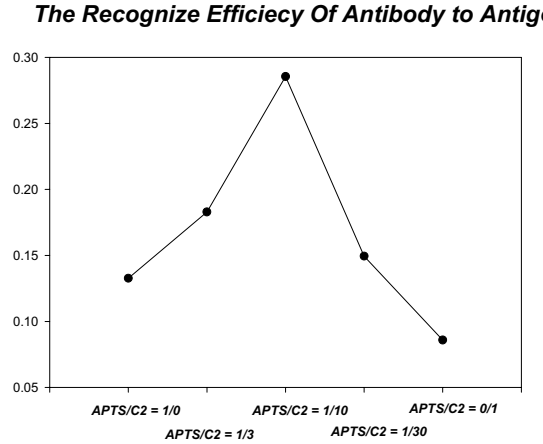


Fig.6 This diagram show recognize efficiency of antibody to antigen that been measured by fluorescence scanner, the binding efficiency obvious increase 2.15 fold form 0.1327 to 0.2855 when the ratio of APTS / C2 from 1/0 to 1/10

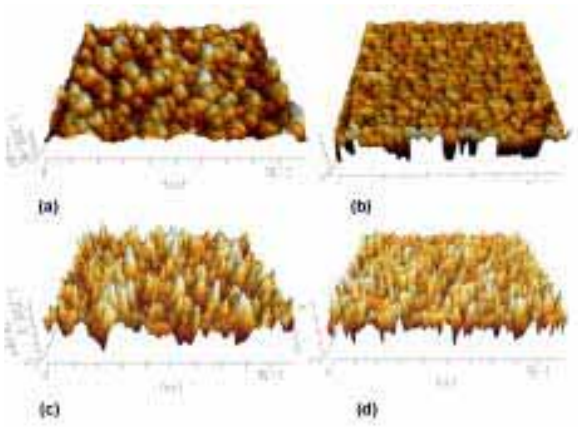


Fig.7 3-D views of AFM measurement results: (a) antibody on APTS/C2 = 1/0 by height mode (b) antibody on APTS/C2 =1/0 by lock-in phase mode (c) antibody on APTS/C2 = 1/10 by height mode (d) antibody on APTS/C2 = 1/10 by lock-in phase mode.

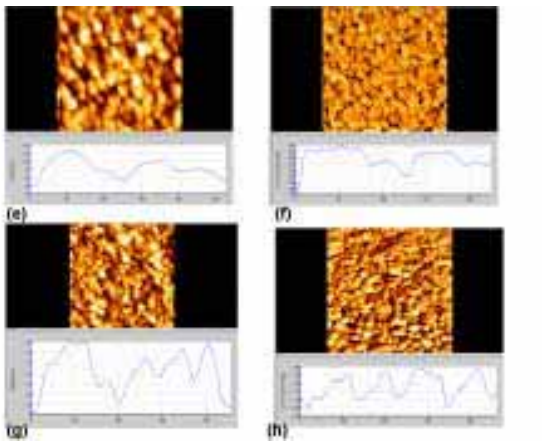

Fig.8 Top views and cross sections of AFM measurement results: (a) antibody on APTS/C2 = 1/0 by height mode (b) antibody on APTS/C2 =1/0 by lock-in phase mode (c) antibody on APTS/C2 = 1/10 by height mode (d) antibody on APTS/C2 = 1/10 by lock-in phase mode

## 4 CONCLUSION

This paper has demonstrated that the binding and recognizing efficiency of proteins on solid surfaces can be improved through the increment of degree of freedom for immobilized proteins on mixed SAMs. This method can eliminate the traditional way for protein binding orientation control by protein modification through bioengineering, a tedious and difficult process. Results show at least a 3 fold increment on antigen-antibody binding efficiency when the mixed SAMs, APTS and $C_2$, at a ratio of 1/10. The protein immobilized surfaces have also been investigated by AFM operated in height mode and lock-in phase mode, and results show more protein-protein inter-spaces in mixed SAMs case than that in single SAMs case, responsible for the increment of protein binding efficiency.

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