A Physisorbed Interface Design of Biomolecules for Selective and Sensitive Protein Detection

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We present a unique immobilization technique via physical adsorption/exchange of biomolecules onto a sensing surface of surface plasmon resonance (SPR). The adsorption/exchange is based on competitive bindings of biomolecules to a surface, which does not require a chemical modification of the biomolecules. SPR sensorgrams show that three human serum proteins, albumin, immunoglobulin G (IgG), and fibrinogen, have different adsorption strengths to the surface and the competitive adsorption of the individuals controls the exchange sequence. A target protein displaces a preadsorbed weak-affinity protein; however, a preadsorbed strong-affinity protein is not displaced by the target protein. In a microfluidic device, we engineer two gold surfaces covered by two known proteins. The sensor allows selective protein detection by being displaced by a target protein on only one of the surfaces. We demonstrate that IgG is selectively detected between albumin- and fibrinogen-adsorbed surfaces. Moreover, the physical adsorption without using an additional surface modification can lead to highly sensitive detection in SPR because of the exponential decay of surface plasmon resonance wave (SPW) from the sensing surface. Based on the competitive adsorption and exchange reaction, we may have a complementary detection system to existing complex and labor-intensive biomolecules detector by bypassing relying on bioreceptors and their attachment to the surface. The physisorbed interface may be useful for automated diagnostic systems. (JALA 2010;15:172–8)

INTRODUCTION

Immunosensor has been extensively studied as a common technique in diagnostics, therapeutics, and proteomic research. Fabrication of highly active and robust sensing surfaces is a fundamental key in immunoassays because the quality of the interfacial biomolecules plays a major role in the device performance. A variety of immobilization techniques such as covalent coupling, Langmuir-Blodgett film, polymer thin film, sol-gel, and physical adsorption have been introduced. However, many challenges remain unsolved, including cross-reactivity, nonspecific adsorption, low stability, and short lifetime. Besides these limitations, integrating them on to a surface of a transducer is a time-consuming and labor-intensive process and often becomes the bottleneck of high-throughput sensing systems. Some of those limitations may be solved by direct physical adsorption of the probing molecules to the sensing surface. Yet, it has been generally restricted to a limited number of applications because of the random orientation and inactivation of probing molecules, for example, antibodies, upon direct contact with the surface. Those challenges limit the sensor response to a greater extent, especially in the immunoassay technique. In this article, we present an alternative protein sensing technique using a novel hydrophobic
physisorption of biomolecules. The sensor relies on the competitive nature of protein adsorption onto a surface, namely the Vroman effect. A target protein displaces a preadsorbed weak-affinity protein; however, a preadsorbed strong-affinity protein is not displaced by the target protein. In a microfluidic device (Fig. 1), we engineer two gold surfaces covered by two known proteins. The sensor allows selective protein detection by being displaced by a target protein on only one of the surfaces. The adsorption/exchange of proteins provides the selectivity in detecting a target molecule. The adsorption/exchange processes are monitored by surface plasmon resonance (SPR) and the differential signal of the two surfaces provides quantitative information of a target molecule. Random orientation and inactivation of biomolecules no longer degrade the sensing performance because the entire process is led by thermodynamics; proteins that have different dimensions and morphologies adsorb differently to a surface based upon their thermodynamic energy preferences and behave to minimize the overall system energy. The Vroman effect-based sensor obviates the need to rely on the bioreceptors and their attachment to the sensing surface because it uses nature’s smart system; competitive adsorption/exchange of proteins to a surface. Figure 2 shows that a conventional immunoassay requires a complicated and time-consuming immobilization process, whereas the Vroman effect-based sensor has a much simpler procedure. Self-assembly monolayer (SAM) formation is widely used as an immobilization technique, which provides a convenient and flexible way to generate thin and ordered monolayer of biological molecules on various substrates. However, the

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**Figure 1.** Operating principle of the Vroman effect-based sensor and a schematic of the surface plasmon resonance (SPR) profiles (A) The sensing surfaces are covered by albumin and fibrinogen, respectively. (B) Immunoglobulin (IgG) is injected into both channels. IgG displaces albumin in channel 1, whereas no displacement occurs in channel 2. (C) The sensor has a permanent angle change ($\Delta \theta$).
SAM (COOH(CH₂)nSH, n = 11) formation requires long incubation time (24 h), and probe molecules (e.g., antibody) go through an additional procedure to be bonded on the layer, such as activation of the terminal group of the SAM (Fig. 2A). In addition, immobilization of antibody itself needs pH modification during the process to obtain optimal immobilization condition. In contrast, the Vroman effect-based sensor does not involve immobilization of probe molecules, which eliminates potential chemical modification caused by the immobilization.

In this article, molecular behaviors are monitored by SPR. SPR is a surface-sensitive analytical tool responding to slight changes in refractive index occurring adjacent to a metal film. The presence of target molecules on the surface of the metal film can be accurately detected in real time. The SPR system consists of several components, including a light source, a prism, a sensing surface, biomolecules, a flow regulator, and a photodetector. Figure 3 shows a simple schematic of the SPR operating principle. A glass slide covered by a thin gold film is optically coupled to a prism via a refractive index matching liquid. Plane polarized light is directed through a prism to the gold over a wide range of incident angles and the intensity of the reflected light is detected by a photodetector. At certain incident light angles, a minimum in the intensity is observed, which is denoted as an SPR angle. The angle is very sensitive to the dielectric properties of the medium adjacent to the sensing surface. The SPR sensorgrams show that three human serum proteins, albumin, immunoglobulin G (IgG), and fibrinogen, have different adsorption strengths to the surface and the competitive adsorption of individuals controls the exchange sequence. Based on the exchange reaction, we demonstrate that the sensor has a high selectivity for IgG. Moreover, physical adsorption without using an additional chemical modification can lead to high sensitive detection in the SPR experiment because surface plasmon resonance wave (SPW) is exponentially decaying from the sensing surface.

**EXPERIMENTAL METHODS**

**Chemicals**

We used three human serum proteins to characterize the sensor: albumin (66 kDa), IgG (150 kDa), and fibrinogen (340 kDa) (Sigma-Aldrich, St. Louis, MO, and CalBIOCHEM, San Diego, CA). All the chemicals were received as lyophilized powders and used without further purification. The proteins were made up to 0.05% (w/v) concentrations in phosphate buffered saline (PBS) 1× (1.15 g/L—Na₂HPO₄, H₂PO₄, NaCl).

**Figure 2.** A schematic procedure for protein detections. (A) Conventional immunosensor using covalent coupling. (B) Vroman effect-based sensor. Conventional immunosensor is time consuming and labor intensive, whereas Vroman effect-based sensor is quick and simple.
Microfluidic Device Fabrication

We used the soft-lithography technique to fabricate the microfluidic device. For a top layer, photore sist, AZ4330, was spin-coated on a silicon wafer and patterned for channels. Then, the pattern was transferred to silicon by a deep reactive ion etching, \( \text{width} = 100 \text{ mm} \). The width of the channel is 2.1 mm and two channels are separated by 1.3 mm. Polydimethylsiloxane (PDMS) was poured and cured on the silicon wafer and was peeled off as molded. The thickness of the layer is approximately 1 cm. Intel/outlet tubes (Upchurch Scientific, Oak Harbor, WA) (inner diameter: 25 \( \text{mm} \); outer diameter: 360 \( \text{mm} \)) were inserted through the PDMS layer using a syringe needle and glued by an adhesive. A bottom substrate has two patterned Cr/Au (2 nm/48 nm) pads on the glass substrate for the detection of the protein adsorption/exchange. Glass substrates (BK7, \( n = 1.517, 150 \text{ mm} \)) were first cleaned in piranha solution (a 3:1 ratio of \( \text{H}_2\text{SO}_4 \) and \( \text{H}_2\text{O}_2 \)) for 10 min. The substrates were then rinsed with water and ethanol sequentially and were dried under \( \text{N}_2 \) stream. Using a thermal evaporator, Cr layer was coated first on the glass substrates to a thickness of 2 nm followed by Au to a thickness of 48 nm. Then, the substrates were cleaned by oxygen plasma (Harrick Plasma Inc., Ithaca, NY) at 18 W for 10 min. The size of the sensing surfaces is 1.8 mm wide and 8.0 mm long. The width of the channel is slightly wider than that of the gold surface to completely bond two substrates using oxygen plasma.

RESULTS AND DISCUSSION

Physisorption Strength

In its first implementation, we demonstrate that three human serum proteins, albumin, IgG, and fibrinogen, have different adsorption strengths onto a hydrophobic gold surface (contact angle measurement: 83.2° ± 0.75°). The different strengths induce an exchange reaction among them. First, a protein is preadsorbed on the surface. Then, another protein subsequently reaches to the surface to interact with the preadsorbed protein. The adsorption/displacement behaviors of proteins on the surface are monitored by SPR in real time. When the protein exchange occurs, a permanent angle change of SPR profiles is generated. Figure 5 shows all the cases of the displacement among three different proteins. The surface was saturated using 0.05% (w/v) proteins to form a fully packed monolayer. When albumin was preadsorbed on the surface, IgG and fibrinogen displaced the albumin producing 111 and 276 mDeg angle changes, respectively (Fig. 5A). IgG was displaced by fibrinogen with 141 mDeg of angle change (Fig. 5A). In contrast, the SPR angle change was almost negligible when those proteins were injected in the reverse sequence (Fig. 5B). IgG was displaced by fibrinogen with 141 mDeg of angle change (Fig. 5A). In contrast, the SPR angle change was almost negligible when those proteins were injected in the reverse sequence (Fig. 5B), demonstrating that the angle changes are not because of the multilayer formation but because of the protein displacement. As we used neutral PBS (phosphate buffered solution, pH 7.4) and all the proteins used for experiments have the pI (isoelectric point) less than pH 7.0, multilayer formation as the result of protein–protein

Instrumentation

As shown in Figure 4, the fabricated microfluidic device was mounted to the semicylindrical prism of SPR instrument (Bi SPR 1000, Biosensing Instrument Inc., Tempe, AZ) by using a refractive index matching liquid. We monitored the angle shift in real time as protein solution flowed through the microfluidic channels driven by an external syringe pump. Initially, PBS was circulated for 20 min until the angle shift stabilized. Once the angle shift stabilized, the protein sample flowed through the microfluidic channels at 10 \( \mu \text{L/min} \), which generated an angle shift proportional to molecular interactions on the surface. When protein adsorption completed, we let PBS wash the surface to remove excess weakly bound proteins. The experimental setup is equipped with a computer-controlled data acquisition system. The SPR produces two sensorgrams in real time; one on a reference channel and the other on a sensing channel. Throughout the experiments, room temperature was maintained at 25°C.

Figure 3. Schematic view of the surface plasmon resonance (SPR)-based biosensor. Any adsorption of biomolecules on the gold layer produces the SPR angle changes.
The displacement strength is ranked in the following order: fibrinogen (340 kDa) > IgG (150 kDa) > albumin (66 kDa). In other words, fibrinogen can displace all the other proteins, whereas IgG can only displace albumin, but albumin cannot displace any of them.

**Selectivity**

Based on the results, we can identify a specific target protein without using the conventional immunosensor technique. Figure 1 shows how to detect IgG using a pair of surfaces preadsorbed by two known-size proteins: albumin and fibrinogen. IgG displaces albumin in channel 1, but flows...
through the fibrinogen-covered surface in channel 2 without any exchange reaction. The differential measurement of the SPR angle change from channel 1 and 2 allows the detection of IgG and the angle change provides quantitative information; the number of IgG molecules displacing albumin.

Figure 6 shows that the sensor integrated in a microfluidic device has the SPR angle difference between the channels. First of all, IgG and fibrinogen were simultaneously injected from inlet 1 and inlet 2 and flowed through both microfluidic channels at 10 μL/min. They adsorbed on each surface generating angle changes, 490 mDeg in channel 1 and 729 mDeg in channel 2. When IgG was introduced from both channels and reached the surfaces, channel 1 showed 92 mDeg angle change and the channel 2 had little angle change (<1 mDeg). This competitive adsorption/exchange reaction offers the selectivity of the sensor. Figure 6C shows the final angle changes (%) of a series of experiments on both surfaces.

**Sensitivity**

Immunosensors requiring long linker molecules and antibodies suffer from the sensitivity degradation because the SPW is proportional to the depth of the area within which the refractive index change occurs. Figure 7 shows the sensitivity comparison between a conventional immunosensor and Vroman effect-based sensor. Conventional immunosensors often use ω-carboxylic acid alkanethiols as a linker molecule. When commonly used alkanethiols with $n = 10$ are formed on a surface, the surface forms a monolayer of approximately 2 nm in thickness. On top of the monolayer, antibodies are formed of more than 5 nm. Therefore, sensitivity degrades as a target analyte binding to the antibodies being monitored by the SPR. In addition, the Vroman effect-based sensor can achieve higher sensitivity because the analyte displacement occurs within a much shorter distance from the metal surface than that of the conventional immunosensors. Figure 8 shows a plot of SPR angle difference.
between albumin- and fibrinogen-adsorbed surfaces versus IgG concentration (1 ng/mL to 10 μg/mL). The plot demonstrates that the assay is linear over four orders in concentration. IgG (1 ng/mL) was detected generating 50-mDeg angle changes, which means that the sensor showed the $10^3$-fold enhancement of sensitivity comparing to IgG detection of a conventional immunosensor.8

**CONCLUSION**

Immunosensor technique has been extensively studied as a common technique in diagnostics, therapeutics, and proteomic research. Many attempts to develop new bioelements and their immobilization strategies have been pursued, nonetheless many challenges remain, including cross-reactivity, non-specific adsorption, low stability, short lifetime, and lack of bioelement diversity. To date, few alternative platforms for protein detection have been active in biosensor communities.

Here, we reported a fundamentally different protein detection method that relies on the competitive nature of protein adsorption onto a surface and is implemented in a microfluidic system. The SPR sensorgrams showed that three human serum proteins, albumin, IgG, and fibrinogen, have different adsorption strengths to the surface and the competitive adsorption of individuals controls the exchange sequence. Based on the exchange reaction, IgG was selectively detected. By using this unique technique, we obviate the reliance on antibodies as capturing probes and their attachment to transducers.

**Competing Interests Statement:** The authors certify that they have no relevant financial interests in this manuscript.

**REFERENCES**