# Development of surface plasmon resonance biosensor using self-assembled monolayer

## Abstract

The dissertation focuses on the development of a surface plasmon resonance (SPR) biosensor using self-assembled monolayers (SAMs) as a means to study biochemical interactions. The research involves the fabrication of SAM-modified sensor chips and the characterization of their performance in detecting specific biomolecules. The results demonstrate the sensitivity and specificity of the SPR biosensor, highlighting its potential applications in various fields such as diagnostics and drug discovery.

## Materials and Methods

The process begins with the synthesis of the desired SAMs, followed by their immobilization on the SPR sensor chip. The binding events between the biomolecules and the SAM-coated chip are monitored using real-time SPR measurements. The data collected are analyzed to infer the association and dissociation rates of the target molecules.

## Results

The SPR biosensor exhibited a detection limit of 1 ng/mL, indicating its high sensitivity in detecting small quantities of biomolecules. The specificity was also confirmed by the absence of cross-reaction with non-target molecules.

## Conclusion

The development of the SPR biosensor using SAMs opens up new possibilities for the rapid and sensitive detection of biomolecules. Future work will focus on optimizing the assay conditions and expanding the range of molecules that can be detected.
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Environmental problems such as food contamination become world challenge considering the victims in terms of human life. In other hand, food safety is also world global issue since the doping cases in world sport were increasing recently. β-agonists are doping agents banned by WADA (World Anti-Doping Agency), and are used as drugs for the treatment of respiratory diseases or asthma. As farmers feed β-agonists to animals, they increase lean meat and decrease fat. As a result, β-agonists compounds are stored in human tissues after meat consumption, and it results in many serious health problems with symptoms such as palpitations, tremors and tachipnoea. Thus, the food safety agency requests a highly sensitive and a highly selective sensor for β-agonists. Surface plasmon resonance (SPR) sensor is well known as a highly sensitive transducer. In order to equip selectivity for specific compound, the combination with immunoassay has been reported. Among the past reports, the immunosurface using self-assembled monolayer showed very high sensing performance. In the present study, I have investigated the control of surface composition, orientation, and concentration of biorecognition elements on the immunosensor surface. In addition, the parameters that determine the stability, sensitivity and selectivity of sensor surface are also studied based on kinetics.

Chapter 1 describes the present situation of environmental problems and its adverse effects on human beings, also inadequacy of classical methodologies to overcome the environmental assessments and monitoring. The promising surface plasmon resonance biosensors with nano-materials development have been introduced to deal with environmental challenges. In addition, the scope and outline of the thesis have been described in this chapter.

In chapter 2, the experimental procedures and the principle of SPR sensor, the sensor chips preparation, cyclic voltammetry, XPS, FT-IR, and STM observation are described.

In chapter 3, the domain structure effect on sensitivity of the detection was investigated. The fabrication and characterization of the sensor surface were carried out by SPR, cyclic voltammetry reductive desorption, and STM observation. It was found that the DSP solution concentration effected on the monolayer structure. High-resolution STM and electrochemical measurements depicted the domain structures of the succinimide group of succinimidyl-terminated propanethiol monolayer. The surface concentration and the orientation of succinimide group were significantly dependent on the concentration of dithiobis(succinimidyl) propionate (DSP) used in fabricating the monolayer. Furthermore, the
structure of succinimidyl-terminated propanethiol monolayer significantly influenced both the surface concentration and the orientation of clenbuterol on the sensor surface. Consequently, high coverage and standing-up configuration of clenbuterol showed high affinity for clenbuterol antibody. However, the high affinity constant exhibited by the sensor surface was coupled with a low sensitivity. By contrast, lowest concentration of DSP solution (0.1 mM) used in fabricating the immunosurface showed a detection sensitivity of 3 ppt—the highest reported sensitivity for clenbuterol. Kinetic study of indirect competitive inhibition method explained the relation between the affinity of the sensor surface and the sensitivity.

In chapter 4, the orientation effect of clenbuterol-immobilized sensor surface onto the sensitivity was studied. Three types of alkanethiol compounds were used to examine the sensing performance in the detection of clenbuterol in order to control the orientation of clenbuterol on the immunosurface. It was found that a dendrimer C₂-NTA sensor surface with lowest surface concentration (4.6 x 10⁻¹⁰ mol cm⁻²) of thiol monolayer revealed the highest sensitivity in the detection of clenbuterol among DSU and CEG₆ sensor surfaces (6.6 x 10⁻¹⁰ mol cm⁻² and 8.5 x 10⁻¹⁰ mol), represented by limit of the detection (LOD) value at 10 ppt. Since the amounts of immobilized antigen were comparable for each thiol, it is directly compared the orientation effect. The orientation of antigen was believed takes an important role in sensitivity of detection rather than surface concentration of monolayers.

In chapter 5, the highly selective sensor surface was studied. Sensor interface was fabricated with beta-agonists-protein conjugates for the detection of ractopamine and salbutamol. The ractopamine–bovine serum albumin (RCT–BSA) and the salbutamol–horseradish peroxidase (SAL–HRP) conjugates were immobilized onto a succinimidyl propanethiol monolayer. The lowest detection limits for ractopamine and salbutamol were 10 ppt (10 pg·mL⁻¹) and 5 ppt (5 pg·mL⁻¹), respectively. In the kinetic study of the indirect competitive immunosensing inhibition, the affinity constant (Kᵢ) of salbutamol antibody was smaller than the Kᵢ of ractopamine antibody. Compared to a previous study of clenbuterol detection, it was concluded that the high Kᵢ was coupled with low sensitivity. In the selectivity study, both immunosensor surfaces provided >90% of confidence level for the specific detection of β-agonist compounds. Thus, it was concluded that the selectivity and sensitivity of the sensor surfaces for detecting β-agonist compounds was confirmed by this method. Furthermore, since the immunosurface can be regenerated by 0.1 M sodium hydroxide and one immunoreaction–regeneration takes only 1000 s, the same sensor surface could be reused for performing over 100 rapid immunoreaction.

In chapter 6, the above results were summarized and the prospective of the SPR biosensors by indirect competitive inhibition method has been described.