

# *SPR Enzyme Biosensors: A Comprehensive Overview*

**Zexuan Ye**

*HD Ningbo School, Ningbo, China*

*2301446@stu.hdschools.org*

**Abstract.** This paper offers an in - depth exploration of surface plasmon resonance (SPR) enzyme biosensors. Biosensors, especially those based on enzymes, are of great significance in modern analysis due to their ability to precisely detect analytes. SPR technology, which is highly sensitive to refractive index changes at the metal - dielectric interface, enables real - time and label - free detection when integrated with enzyme - based biosensors. The working principle of SPR enzyme biosensors relies on the SPR phenomenon and refractive index variations caused by enzyme - substrate reactions. Their structural components, including the sensing chip, optical system, and flow system, are meticulously designed. These biosensors exhibit excellent performance characteristics such as high sensitivity for biomedical detection, which helps in early disease diagnosis; high selectivity in food safety monitoring, allowing for accurate detection of harmful residues; stability in environmental monitoring, withstanding complex environmental conditions; and a short response time in emergency detection. However, challenges like signal interference, enzyme immobilization problems, and complex sample analysis exist. Corresponding solutions, such as anti - fouling coatings, new immobilization technologies, and sample pretreatment, have been proposed. Looking ahead, technological innovations like nanotechnology - enabled sensors and multimodal sensing are expected to further enhance their performance and expand their applications, making SPR enzyme biosensors more crucial in improving human health, environmental protection, and food safety.

**Keywords:** SPR enzyme biosensors, Working principle, Structural components, Performance characteristics, Applications

## **1. Introduction**

In the contemporary landscape of analytical techniques, biosensors have emerged as indispensable tools, revolutionizing the way we detect and analyze various substances. These devices, which ingeniously integrate physical or chemical transducers with biological identification components, offer unparalleled precision and specificity in analyte detection. Among the diverse array of biosensors, those based on enzymes have garnered acclaim due to the unique catalytic properties of enzymes. Enzymes possess an inherent ability to recognize and bind to specific substrates with remarkable selectivity, making enzyme - based biosensors highly effective in accurately identifying and quantifying analytes. This characteristic has led to their widespread application across multiple

critical domains, including food safety assessment, environmental monitoring, and medical diagnostics, thereby significantly contributing to the progress and well - being of these fields.

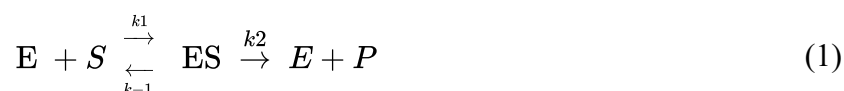
Surface plasmon resonance (SPR) technology has emerged as a powerful and widely - used approach within the realm of biosensors. SPR is an optical phenomenon that occurs at the interface between metals, typically noble metals like gold and silver, and dielectrics. When light impinges on this interface at a specific angle, it excites the free electrons in the metal, giving rise to surface plasmons, which are collective oscillations of these free electrons. The resulting SPR signal is exquisitely sensitive to even the slightest alterations in the refractive index of the adjacent dielectric medium. This sensitivity makes SPR an ideal candidate for biosensor development, as it enables real - time and label - free detection of biological and chemical interactions.

The combination of enzymes and SPR technology has given birth to SPR enzyme biosensors, which have opened new frontiers in biosensing capabilities. By harnessing the catalytic specificity of enzymes and the high - sensitivity refractive index detection of SPR, these biosensors can monitor enzymatic reactions in real - time with exceptional accuracy. This review aims to provide a comprehensive and in - depth exploration of SPR enzyme biosensors. It will delve into their underlying working principles, meticulously examine their structural components, evaluate their performance characteristics, explore their diverse application sectors, identify the challenges impeding their development, and offer insights into their prospects. Through an extensive elaboration, accompanied by references to relevant research and practical applications, readers will be able to gain a systematic and thorough understanding of SPR enzyme biosensors, thus highlighting their significance and potential in modern analytical chemistry and related disciplines.

## 2. Working principles of SPR enzyme biosensors

In biosensor development, achieving high - precision detection is crucial. Surface plasmon resonance (SPR) technology has emerged as a powerful tool [1]. SPR occurs at the interface between metals (such as noble metals like gold and silver) and dielectrics. When light is incident at a specific angle, the free electrons in the metal are excited to form surface plasmons, which are collective oscillations of free electrons. When the wave vector of the incident light matches that of the surface plasmons, the resonance condition is met. The SPR signal is highly sensitive to changes in the refractive index of the adjacent dielectric [2]. Even a slight change in the refractive index, caused by molecular binding or a chemical reaction, will result in a measurable shift in the SPR signal, enabling high - sensitivity detection of biosensors [3].

Enzymes, functioning as highly specific catalysts, play a pivotal role in the operation of SPR enzyme sensors [4]. The fundamental enzyme - catalyzed reaction can be generally represented as equation 1:



where E is the enzyme, S is the substrate, ES is the enzyme - substrate complex, and P is the product.  $k_1$ ,  $k_{-1}$ , and  $k_2$  are the rate constants for the formation of the enzyme - substrate complex, its dissociation, and the formation of the product, respectively.

In an SPR enzyme sensor, as the enzymatic reaction progresses, the consumption of substrates (S) and generation of products (P) lead to alterations in the local chemical environment. According to the equation 2:

$$\frac{n^2-1}{n^2+2} = \frac{N\alpha}{3\epsilon\epsilon_0} \quad (2)$$

Where the refractive index ( $n$ ) of a medium is related to its molecular polarizability ( $\alpha$ ) and number density ( $N$ ). When the enzyme catalyzes the hydrolysis of a substrate, the chemical composition near the sensor surface changes, leading to a change in the number density ( $N$ ) and molecular polarizability ( $\alpha$ ) of the molecules in the vicinity, which in turn causes a change in the refractive index ( $n$ )[5].

SPR technology, with its high sensitivity to refractive index changes, can accurately monitor these alterations. The shift in the SPR signal ( $\Delta\theta$  or  $\Delta\lambda$ , where  $\theta$  is the resonance angle and  $\lambda$  is the resonance wavelength) is related to the change in refractive index ( $\Delta n$ ) through the optical properties of the SPR sensor system. Mathematically, this relationship can be expressed in a simplified form as: “ $\Delta\theta \propto \Delta n$  or  $\Delta\lambda \propto \Delta n$ ”.

By precisely measuring this shift in the SPR signal, one can quantitatively determine the enzyme activity or substrate concentration. For example, the rate of change of substrate concentration ( $dS/dt$ ) can be related to the observed SPR signal shift over time. If the SPR signal shift is  $\Delta\theta(t)$ , and through calibration curves and kinetic models, the relationship between  $\Delta\theta(t)$  and  $dS/dt$  can be established [6].

This working mechanism enables SPR enzyme sensors to monitor the enzymatic reaction process in real - time and accurately, providing an efficient and reliable means for biological analysis, which is crucial for applications such as disease diagnosis [7].

### 3. Structural components of SPR enzyme biosensors

#### 3.1. Sensing chip

The sensing chip is one of the core components of SPR enzyme biosensors [8]. Common substrate materials include glass and quartz, which have good optical transparency and chemical stability, providing a stable foundation for subsequent metal film deposition and enzyme immobilization [9]. A thin gold film is deposited on the chip surface through techniques such as physical vapor deposition. The gold film provides the surface for plasmon resonance. Enzyme molecules are immobilized on the surface of the gold film through various methods. Physical adsorption non - covalently attaches enzymes to the gold surface relying on van der Waals forces; chemical covalent bonding uses cross - linkers to form strong chemical bonds between enzymes and the gold surface; self - assembled monolayers (SAMs) involve the self - assembly of thiol - terminated molecules on the gold surface, and then enzymes are connected to the SAMs in a more orderly manner[10]. Different immobilization methods have their own advantages and disadvantages, and specific needs should be considered in practical applications for selection.

#### 3.2. Optical system

The optical system plays a crucial role in SPR enzyme biosensors [11]. Lasers are often used as light sources, such as a helium - neon laser with a wavelength of 632.8 nm, because they can provide high - intensity and monochromatic light, which is conducive to achieving accurate SPR measurements [12]. The optical path is carefully designed to precisely control the incident light angle to meet the resonance condition. Reflective optical elements are used to direct the incident light to the sensing chip and collect the reflected light. Detectors (such as photodiodes and charge - coupled devices CCD) operate based on the principle of converting light energy into electrical signals, measure the

intensity of the reflected light, and analyze the electrical signals to obtain the SPR signal [13]. The stability and accuracy of the optical system directly affect the performance of the sensor, so various optical parameters need to be strictly controlled in the design and use process.

### 3.3. Flow system

The flow system is designed to ensure the smooth and accurate flow of sample solutions and buffer solutions on the surface of the sensing chip [14]. The flow path is generally made of materials resistant to chemical corrosion, such as polytetrafluoroethylene (PTFE). Peristaltic pumps or syringe pumps are used to control the flow rate, ensuring the stable and accurate flow of the solution. Appropriate sealing techniques are adopted to prevent solution leakage and cross - contamination. The flow system also has valves to control the flow direction of the solution and to switch between different solutions, such as sample loading, buffer rinsing, and calibration solutions. A reasonable flow system design can ensure the efficient and accurate detection of different samples by the sensor.

## 4. Performance characteristics of SPR enzyme biosensors

### 4.1. Sensitivity - the key to biomedical detection

In the diagnosis of cancer, traditional diagnostic methods have many limitations, such as invasiveness, high cost, and low sensitivity in early - stage detection (as mentioned in the literature, early and accurate diagnosis of cancer is important, but traditional methods have limitations). The SPR enzyme biosensor, with its high sensitivity, shows unique advantages in cancer diagnosis.

Cancer cells over - express certain enzymes, and these enzymes are present in body fluids such as blood and urine. Just as the literature mentions that electrochemical biosensing can detect cancer biomarkers, the SPR enzyme biosensor, similar to it, can detect extremely low - concentration substances. It can acutely capture the extremely subtle changes in the concentration of these enzymes in body fluids, even changes at the nanomolar or picomolar level, with a detection limit of up to  $10^{-9}M$ . This high sensitivity helps to detect abnormalities in the early stage of cancer when the changes in the concentration of relevant enzymes in the body are still very weak, achieving early diagnosis and thus improving the cure rate and survival rate of patients. It represents a significant improvement in early - stage detection sensitivity compared to traditional diagnostic methods.

In the diagnosis of metabolic diseases such as diabetes, the literature points out that research on tumor diagnosis has the potential for personalized medicine, and the same is true in the field of metabolic diseases. The SPR enzyme biosensor can accurately measure the changes in the activity of enzymes related to glucose metabolism, providing accurate evidence for disease monitoring and the evaluation of treatment effects. For example, during the treatment of diabetic patients, doctors can adjust the treatment plan in a timely manner based on the changes in the activity of relevant enzymes detected by the sensor, achieving personalized medicine. This reflects its important value in practical medical applications and corresponds to the significance of diagnostic techniques for disease treatment and intervention mentioned in the literature [15].

### 4.2. Selectivity -a pillar for precise food safety detection

In the realm of food safety monitoring, where the rapid and accurate detection of contaminants is of utmost importance, SPR enzyme biosensors have emerged as a powerful tool. Selectivity, a crucial

performance characteristic of these biosensors, plays a pivotal role in ensuring reliable and specific detection.

The selectivity of SPR enzyme biosensors is inherently rooted in the unique properties of enzymes. Enzymes possess a high degree of specificity towards their substrates, a characteristic that forms the foundation of the biosensor's selectivity. For instance, an enzyme engineered to catalyze the hydrolysis of a particular substrate will exhibit a preferential reaction solely with that specific substrate. This inherent specificity allows for the targeted detection of analytes, minimizing the likelihood of false positives.

Moreover, the selectivity of SPR enzyme biosensors can be further enhanced through surface modification techniques. By immobilizing specific receptor molecules onto the chip surface, non-specific binding of unwanted substances can be significantly reduced. This targeted approach not only improves the sensor's ability to discriminate between analytes but also enhances the overall accuracy of the detection process.

In the context of food safety, the selectivity of SPR enzyme biosensors is harnessed to detect a wide range of contaminants. Pesticides and veterinary drugs, which pose significant risks to human health, can be effectively monitored using these biosensors. These contaminants often interact with specific enzymes in food, either inhibiting or activating their activity. SPR enzyme biosensors are capable of precisely detecting these changes in enzyme activity, enabling the accurate determination of the presence and concentration of pesticide and veterinary drug residues in food products.

Furthermore, in the detection of microbial contamination, the selectivity of SPR enzyme biosensors proves invaluable. Microorganisms produce specific metabolic enzymes during their growth and metabolism. By targeting these enzymes, the biosensor can detect the activity of enzymes involved in the decomposition of nutrients by bacteria. This allows for the early detection of food spoilage and the identification of harmful bacteria, safeguarding public health.

In conclusion, the selectivity of SPR enzyme biosensors is a key factor in ensuring the accuracy and reliability of food safety monitoring. By capitalizing on the inherent specificity of enzymes and leveraging surface modification techniques, these biosensors offer a highly effective solution for the detection of various contaminants in the food industry. As the demand for rapid and sensitive food safety detection methods continues to grow, the further development and optimization of SPR enzyme biosensors with enhanced selectivity will be crucial in meeting these challenges [16].

### 4.3. Stability - the foundation for environmental monitoring

In environmental monitoring, the stability of surface plasmon resonance (SPR) enzyme biosensors hold utmost importance, as demonstrated in the following aspects:

**Temperature:** Temperature exerts an influence on both enzyme activity and the refractive index of the solution. Typically, it is necessary to maintain the temperature within a restricted range of  $25 \pm 1$  °C to guarantee the stable functioning of the sensor.

**pH Level:** Each enzyme has its own optimal pH range. The pH level can interfere with enzyme activity, consequently affecting the stability of the sensor.

**Enzyme Immobilization Technique:** In contrast to physical adsorption, the covalent bonding approach can render enzyme immobilization more stable, playing a crucial role in determining the sensor's stability.

**Enzyme Category and Storage Circumstances:** The lifespan of the sensor is associated with the type of enzyme and storage conditions. Storing it appropriately in a buffer solution at low temperatures can prolong its service life.

The Significance of Stability in Environmental Monitoring: Robust SPR enzyme biosensors are capable of withstanding complex environmental conditions. In water quality assessment, even when the water temperature and pH level fluctuate constantly, the sensor can precisely measure the enzyme activity in aquatic organisms, utilizing this as a gauge of water pollution. In soil monitoring, when confronted with soil environments featuring diverse acid - base levels and temperatures, it can efficiently detect the effects of organic pollutants and heavy metal ions on soil enzyme activity, enabling a comprehensive assessment of soil pollution levels [17].

#### 4.4. Response time - the advantage in emergency detection

The response time of SPR enzyme biosensors from sample injection to obtaining a stable detection signal is relatively short, generally ranging from a few minutes to tens of minutes. The solution flow rate and enzyme reaction kinetics are two key factors affecting the response time. A higher flow rate can accelerate the transport of the substrate, but it may also cause turbulence and affect the measurement accuracy; enzymes with fast - acting kinetics can make the response time shorter. In emergency detection scenarios, including the fields of biomedicine, food safety, and environmental monitoring, a short response time has obvious advantages. In the biomedical field, when an infectious disease breaks out, an SPR enzyme biosensor with a short response time can quickly detect the activity of relevant enzymes in the patient's body fluids, assisting in rapid disease diagnosis and winning time for timely treatment; in terms of food safety, in the face of emergencies such as food poisoning, the sensor can quickly detect harmful substances in food, such as microbial toxins that affect enzyme activity; in environmental emergency monitoring, for example, in the event of a sudden water pollution incident, the sensor can quickly detect the impact of pollutants in the water on enzyme activity, providing an immediate basis for taking timely countermeasures.

### 5. Challenges and solutions in SPR enzyme biosensor development

#### 5.1. Sources of interference

Non - specific adsorption is a major culprit in signal interference. When the sensor is exposed to samples, various molecules in the sample, other than the target analyte, can adsorb onto the sensor surface, especially in complex biological or environmental samples. For example, in blood samples for biomedical detection, proteins, lipids, and other biomolecules can non - specifically bind to the sensing chip, altering the local refractive index and creating background noise that masks the true SPR signal related to the enzyme - substrate interaction. Additionally, sample impurities, such as contaminants from the sampling process or inherent in the sample matrix, can also contribute to signal interference. In environmental samples, suspended particles, heavy metals, or organic pollutants can interfere with the SPR signal, making it difficult to accurately detect the enzyme - related changes.

In the development process of surface plasmon resonance (SPR) enzyme biosensors, signal interference is a key challenge, and there are multiple effective solutions to this problem.

Anti - fouling coatings reduce non - specific adsorption: When using SPR technology to detect specific enzymes in blood, numerous biomolecules in the blood other than the target enzyme, such as proteins and lipids, can bind non - specifically to the sensing chip, generating background noise that interferes with the real signal. For example, when detecting thrombin in blood, the non - specific adsorption of other proteins can cause deviations in the test results. If an anti - fouling coating based on polyethylene glycol (PEG) is applied to the surface of the sensing chip, the PEG

chains can form a hydrated layer on the sensor surface, repelling non-specific binders through steric hindrance. Just like in actual detections, when using a sensing chip coated with PEG to detect blood samples, non-specific binding is significantly reduced, and the detection signal of thrombin becomes more accurate [18].

**Buffer solutions optimize the detection environment:** When detecting phosphatase in environmental water samples, impurities in the water sample, such as suspended sediment particles, heavy metal ions, and organic pollutants, can interfere with the SPR signal. At this time, by rationally preparing buffer solutions and adjusting their ionic strength and pH value, the non-specific interactions between the impurities in the water sample and the sensor surface can be reduced. For instance, adjusting the pH value of the buffer solution to the optimal activity pH range of phosphatase and simultaneously optimizing the ionic strength can effectively reduce the interference of impurities in the water sample, making the detection results of phosphatase more reliable.

**Signal processing algorithms improve signal quality:** In the detection of complex biological samples, signal interference is more severe. For example, when detecting enzymes related to tumor markers, the complex signals generated by multiple components in the sample are intertwined. By using signal processing algorithms based on machine learning, in-depth analysis of these complex SPR signals can be carried out, accurately identifying and removing the signals generated by non-specific adsorption and impurities, enhancing the signal-to-noise ratio, and correcting baseline drift. In practical applications, after adopting such algorithms, the detection accuracy of enzymes related to tumor markers has been greatly improved, providing stronger support for the early diagnosis of tumors.

## 5.2. Enzyme immobilization challenges

Enzyme immobilization is of great significance in sensor preparation, but it faces many challenges, and there are corresponding solutions as well:

### 5.2.1. Challenges in enzyme immobilization

**Blocked Active Sites:** Take physical adsorption as an example. Although this method is easy to operate, it causes enzymes to be randomly distributed on the sensor surface. As a result, some active sites are blocked, which affects the catalytic activity of the enzymes and leads to poor detection performance.

**Enzyme Denaturation:** The chemical covalent bonding method can make the combination between the enzyme and the sensor surface more stable. However, the reaction conditions are often rather harsh, which is likely to change the structure of the enzyme, causing it to lose activity and unable to function properly.

**Inconsistent Sensor Responses:** During the enzyme immobilization process, if the enzymes are not evenly distributed on the sensor surface, inconsistent responses will occur. For instance, in the case of self-assembled monolayers (SAMs), improper formation or subsequent modification can lead to uneven enzyme attachment, thus affecting the accuracy of sensor detection.

### 5.2.2. Solutions

**Utilizing Nanocomposites:** Nanocomposites such as carbon nanotube - enzyme composites or metal - organic framework - enzyme hybrids have a large specific surface area, which can provide good

support for enzyme immobilization. For example, carbon nanotubes can not only disperse enzymes better but also enhance the electron transfer performance of enzymes, improving their catalytic activity and effectively solving the problems of enzyme activity and dispersibility.

**Adopting Electrodeposition Technology:** By applying an electric field, the electrodeposition technology can precisely control the deposition process of enzymes on the sensor surface, enabling the enzymes to be more evenly distributed on the sensor surface and achieving controllable immobilization. Moreover, this technology can co - deposit enzymes with other functional materials (such as conductive polymers), further improving the performance of the sensor [19].

### 5.2.3. Impact of complex sample matrices

Complex samples, such as those from environmental sources or biological fluids, contain a multitude of components. In environmental samples, the presence of various organic and inorganic substances can compete with the target analyte for enzyme binding sites. In wastewater samples, different types of pollutants may inhibit or activate the enzyme, leading to false - positive or false - negative results. In biological fluids like serum, the complex matrix can interfere with the SPR signal directly. The high protein content in serum can cause significant background scattering, making it challenging to detect the subtle refractive index changes associated with enzyme - substrate reactions.

### 5.3. Sample pretreatment and sensor adaptation

Sample pretreatment methods, such as filtration, centrifugation, and extraction, can remove large - sized impurities and concentrate the target analyte. Filtration can eliminate suspended particles, while extraction techniques can selectively isolate the analyte from the complex matrix. Surface chemistry modification of the sensor is also essential. By functionalizing the sensor surface with specific ligands or receptors, the sensor can be made more selective towards the target analyte. For example, adding antibody - based recognition elements can enhance the sensor's ability to detect a specific biomarker in a complex biological sample.

## 6. Conclusion

In conclusion, surface plasmon resonance (SPR) enzyme biosensors represent a remarkable advancement in the field of biosensor technology. Their working principle, rooted in the SPR phenomenon at the metal - dielectric interface and the refractive index changes during enzyme - substrate reactions, enables real - time and accurate monitoring of enzymatic processes. The structural components, namely the sensing chip, optical system, and flow system, work in harmony to ensure the proper functioning of these sensors.

The performance characteristics of SPR enzyme biosensors, including high sensitivity for biomedical detection, high selectivity in food safety monitoring, stability in environmental monitoring, and short response time in emergency detection, render them versatile and highly effective in diverse applications. They have proven to be invaluable tools in early disease diagnosis, ensuring food safety, and assessing environmental pollution.

Nevertheless, the development of SPR enzyme biosensors is not without challenges. Signal interference, enzyme immobilization issues, and complex sample analysis pose obstacles that require continuous research and innovation. However, solutions such as anti - fouling coatings, new

immobilization technologies, and sample pretreatment methods have been proposed and are being refined.

Looking ahead, the future of SPR enzyme biosensors is promising. Technological innovations, such as nanotechnology - enabled sensors and multimodal sensing, hold great potential for further enhancing their performance and expanding their applications. As research and development in this field continue, SPR enzyme biosensors are likely to play an even more significant role in improving human health, safeguarding the environment, and ensuring food security.

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