A comparison of two biosensing recognition elements using SPR for the detection of drug-resistant genes

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Abstract. The burden of tuberculosis (TB) infections is disproportionately high in low-income and resource-limited settings. This disparity exacerbates the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Mycobacterium tuberculosis* (Mtb), the bacterium that causes TB. Early detection and treatment of TB remain key strategies to reduce the spread and disease progression, particularly for the detection of drug-resistant forms. Therefore, optical-based diagnostic devices could solve this problem. Surface plasmon resonance (SPR) biosensors offer various advantages, including rapid analysis, high specificity, and sensitivity, as well as requiring small amounts of samples for analysis. For this study, two multidrug-resistant genes, namely, *catalase-peroxidase* (*KatG*) and *enoyl reductase* (*InhA*), were detected using a custom-built surface plasmon resonance (SPR) setup. Biotinylated and thiolated deoxyribonucleic acid (DNA) probes, specific to the two genes (*KatG* and *InhA*), were used as biorecognition elements to capture *KatG* and *InhA* target DNA. The SPR setup was used for the analysis of the binding interactions occurring on the gold-coated slides. The SPR biosensor setup indicated binding interactions through the changes in reflected intensities. The reflected intensities indicated the differences in the resonance angle between each experimental test. This is the initial step to identifying the best characterization of DNA as biorecognition elements for detecting drug-resistant mutations using an SPR-based setup.

1 Introduction

Tuberculosis (TB) is one of the most widespread diseases caused by a single bacterial infection [1]. The causal agent of TB is *Mycobacterium tuberculosis*. Globally, 10 million infections and 1.2 million deaths are attributed to TB infections [1]. This is because the bacteria, if not detected early and the correct treatment administered, can gain resistance to first-line drug regimens and develop into drug-resistant TB [2]. Other factors that contribute to drug resistance include poor adherence to the medication, poor drug quality, and late disease detection [3].

The most common drug resistance is to first-line drugs, Isoniazid and Rifampicin [4], [5]. The drug targets for these two drugs are *RpoB* (for rifampicin), *enoyl-reductase* (*InhA*) (for isoniazid), and *catalase-peroxidase* (*KatG*) (for isoniazid). The pill/antibiotic/medication burden of TB treatment often leads to disadvantages of poor enzyme level elevation, adverse drug side effects, and poor bioavailability. As such, this can lead to the conversion of TB to drug-resistant strains such as multi-drug-resistant TB (MDR-TB) and extremely drug-resistant TB (XDR-TB) [1].

One of the main strategies to mitigate the conversion and spread of MDR-TB is early disease detection. Currently, this is done using molecular-based assays such as the line-probe assay, drug susceptibility testing (Gold standard), and Polymerase chain reaction (PCR)-based assays [6]. Some of these diagnostic assays/tools/methods, such as the GeneXpert and the UltraMDRplus, require facilities and equipment that are associated with expensive regular maintenance costs [7], [8]. Thus, other methods, such as optical-based methods, have attracted attention as novel, effective, and rapid alternatives. One specific optical-based technique that has been studied is Surface Plasmon Resonance (SPR).

SPR is characterized by its label-free, sensitive, and rapid detection capabilities, with the ability to detect small changes in complex analytes [9]. SPR is based on the principle of monitoring changes in optical parameters such as resonance angle, refractive index, and reflected intensity, which are interpreted by a signal transducer [10], [11]. In the form of a biosensing technique, SPR can offer real-time detection of samples/molecules in complex analytes [12]. Detecting these molecules in complex analytes can be assisted by biorecognition elements, which aid in capturing the target molecules. The biorecognition elements can be in the form of antibodies, deoxyribonucleic acids (DNA) in the form of ssDNA probes, and/or enzymes [9], [13].

Therefore, given that various biorecognition elements can be used in biosensing devices, this study aimed to determine which biorecognition element, between biotinylated probes and thiolated probes, would produce better detection results in the detection of MDR-TB, using a SPR-based platform.

2 Experimental

2.1 Custom-built SPR setup

A custom SPR setup was used in this study. The SPR setup consisted of a Helium-Neon (HeNe) (632.8 nm wavelength) laser with a 10-mW maximum power output. The laser beam was passed through a polarizing beam splitter to select the p-polarization. As shown in Figure 1, a sensor chip consisting of a glass slide coated with gold and mounted on a BK7 prism was immobilized on a rotating stage. The reflected p-polarization laser beam from the prism was directed to a photodiode detector connected to a computer for signal transduction.

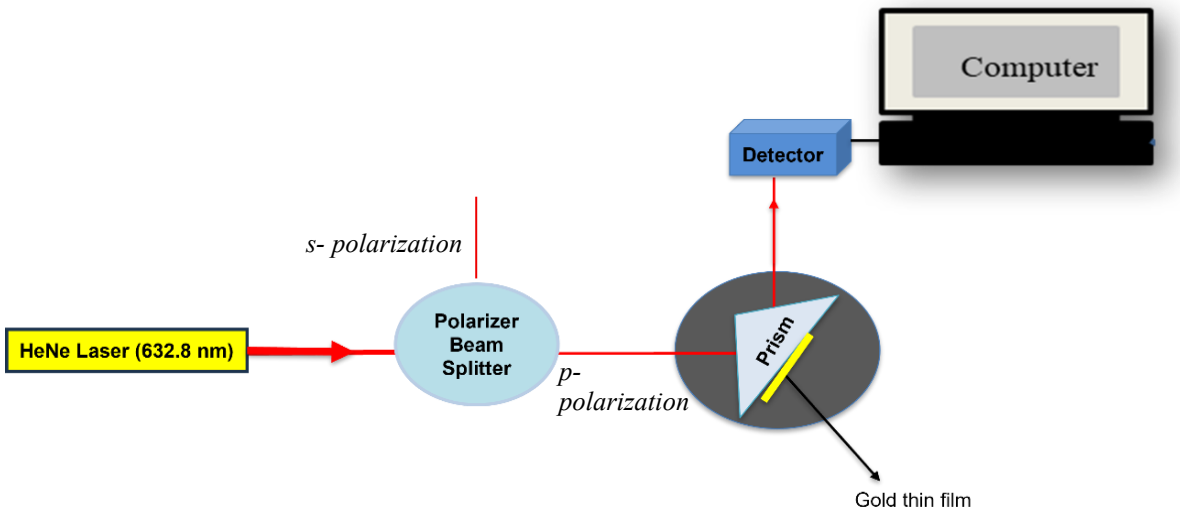

Figure 1: A schematic diagram of the custom-built SPR setup used in this study [14].

2.2 Biosensor chip surface functionalization

The sensor chips (gold-coated slides, 50 nm gold thickness) used in this study were initially cleaned with 100% ethanol and distilled water before use. The sensor chips were dried under Nitrogen gas (N_2).

2.2.1 Thiolated probes immobilization

The thiolated probes (10 µM) (Figure 2) (*KatG* probe: 5' SH-GTCGAACCCGGTGAGGCCCA 3'; *InhA* probe: 5' SH-GATATAGCTCCCGTCCTCGG 3') were immobilized on the sensor chip surface and incubated overnight at 4 ºC (in the fridge). Afterward, the sensor chip surface was rinsed with distilled water and dried in the fume hood. Once dry, the sensor chips were analyzed using the custom-built SPR setup.

Figure 2: A simplified diagram of the immobilized thiolated DNA probes on the sensor chip surface.

2.2.2 Biotinylated probes immobilization

The sensor chip surface for the immobilization of the biotinylated probes (Figure 3) was treated with 1 mM 11-Mercaptoundecanoic acid (MuA) (Sigma-Aldrich, South Africa) at room temperature for 2 hours in the dark. Afterward, the surface of the sensor chip was rinsed with 1X Phosphate-buffered saline (PBS, pH 7.4) to remove any residual silane/chemicals. The sensor chip was dried and then functionalized with 1mM of N-ethyl-N-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) (Sigma-Aldrich, South Africa), for 10 minutes at room temperature and in the dark. The sensor chip surface was rinsed twice with 1X PBS (100 µL) and allowed to dry. Once dry, Neutravidin was added to the sensor chip's surface and incubated for 1 hour at room temperature. Following the incubation period, the sensor chip surface was rinsed with 1X PBS (100 µL) and allowed to dry. Six microliters (6 µL) (10 µM) of the biotinylated probes (KatG probe: 5' Biotin-GTCGAACCCGGTGAGGCCCA 3'; *InhA* probe: 5' Biotin-GATATAGCTCCCGTCCTCGG 3') were immobilized on the sensor chip surface overnight, in the dark at 4 ºC. Afterward, the sensor chip was rinsed twice with 1X PBS and allowed to dry. Once dry, the sensor chips were analyzed using the custom-built SPR setup.

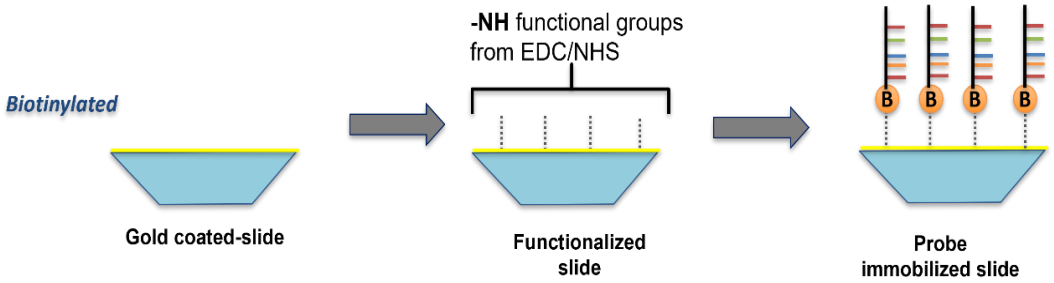

Figure 3: A simplified diagram of the immobilized biotinylated DNA probes on the sensor chip surface.

2.2.3 Sensor chip analysis and data processing (reflectance)

The signals detected by the photodiode amplifier system were recorded as changes in reflected intensities as a function of changes in the incident angle. Measurements were conducted for the sensor chip containing only the gold-coated slide, and the gold-coated slide functionalized with each probe. The reflected intensity data were collected at one-degree increments in the incident angle. The acquired data were subsequently processed and analyzed using OriginPro 8.

3 Results

The ssDNA probes (thiolated and biotinylated) were immobilized on the sensor chip surface due to their strong adsorption properties on the gold-coated surface [15]. Zhang et al. [15] reported an increase in DNA concentration on the gold-sensor chip leads to a broader and more pronounced SPR angle shift and dip. Simplified diagrams (figures 2 and 3) illustrate the immobilization process of DNA probes onto the sensor surface before analysis using the SPR system. The sensing surface of the gold-coated chip was excited by a HeNe laser source at a wavelength of 632 nm. The reflected light was continuously monitored in real-time using an optical analyzer (photodiode amplification system).

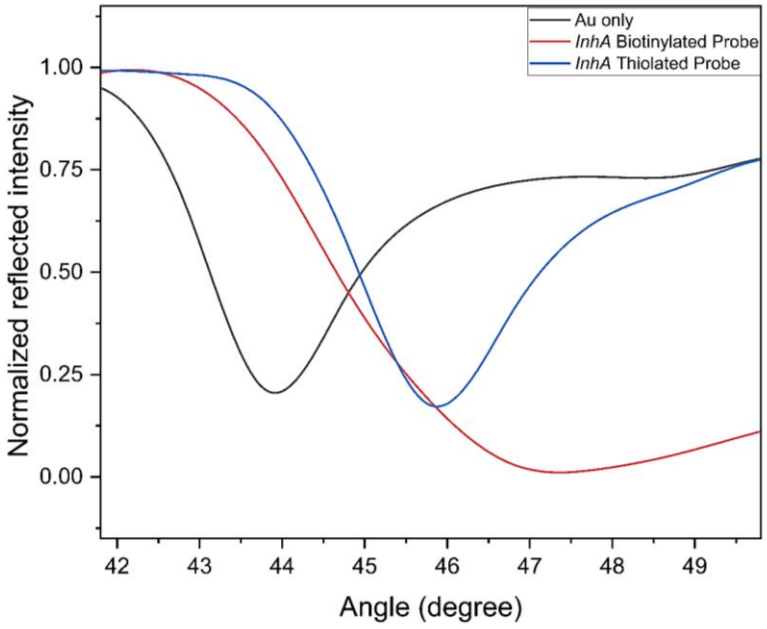

Figure 4: The normalized reflected intensity spectra as a function of angle variation showing the resonance angle shifts obtained for the biotinylated and thiolated *InhA* probe, relative to the sensor ship surface alone.

The obtained data for the *InhA* gene (depicted in Figure 4) indicated distinct changes in the resonance angle when compared to the unmodified gold-coated sensor chip and those functionalized with the biotinylated and thiolated *InhA* probes. The resonance angle of the gold-coated sensor chip was recorded at 43.8 degrees, while the functionalized chip with biotinylated and thiolated *InhA* probes resulted in a right shift to 46.8 and 45.8 degrees, respectively. The resonance angle shift for the biotinylated *InhA* probe was 1 degree more than the angle shift observed for the thiolated probe. In this study, the immobilization of the biotinylated probes onto the gold sensor chip surface introduced different diluents and chemical properties that can influence the adsorption of the DNA probes to the sensor surface. According to Peterson et al. [16], using thiolated probes enables covalent bonding between the gold and the thiol group. This interaction facilitates the formation of self-assembly monolayers required to monitor the kinetics of probe attachment [16]. In contrast, the use of Avidin molecules (streptavidin and neutravidin) has been associated with increasing the signal of the adsorption properties of DNA to gold sensor surfaces [16], [17], [18]. This could be why the biotinylated probe for *InhA* resulted in a greater resonance angle shift than the thiolated probe.

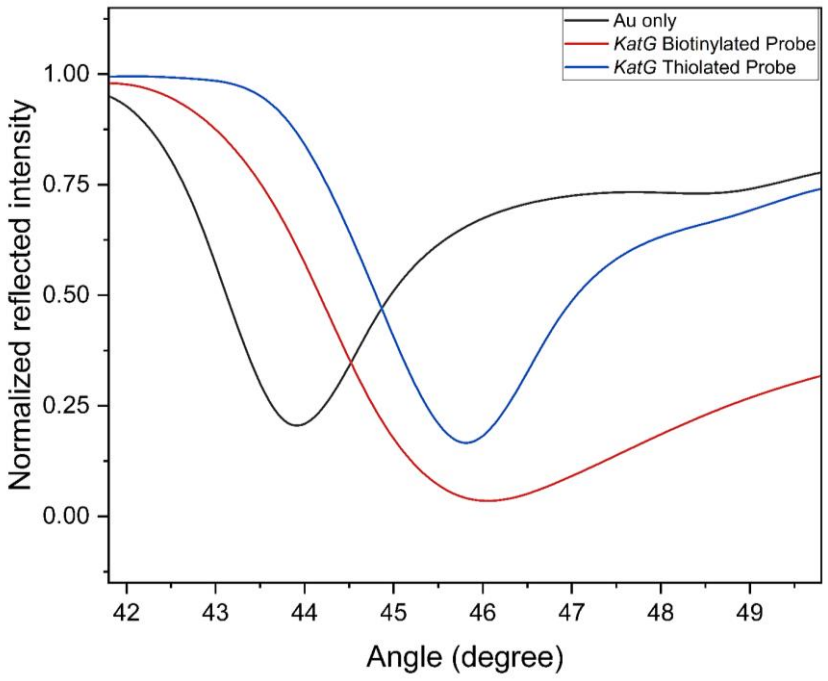

Figure 5: The normalized reflected intensity spectra as a function of angle variation showing the resonance angle shifts obtained for the biotinylated and thiolated *KatG* probe, relative to the sensor ship surface alone.

The data obtained for the *KatG* gene using the biotinylated and thiolated probes (shown in Figure 5) indicated changes in the resonance angle when compared to the data obtained for the gold-coated sensor. The resonance angle for the gold-coated sensor chip alone was noted at 43.8 degrees. In contrast, the resonance angles for the biotinylated and thiolated *KatG* probes shifted to 45.8 degrees.

The observed red shift in the resonance angles for the immobilized probes indicates changes in the molecule's composition on the sensor chip surface. This can also indicate the binding of the probes to the sensor chip surface through covalent and non-covalent bonds for both probes. In addition, a study by Soares [19] reported that a red shift in the resonance angle is mainly attributed to the newly bound molecules on the sensor surface, which increase the detected mass on the chip [19].

The methods of probe immobilization for biotinylated probes and thiolated probes differ in their underlying chemistries and surface attachment mechanisms. For the biotinylated probes, the sensor surface was first functionalized with amide functional groups using EDC/NHS to further increase the binding affinity of the neutravidin to the NHS functional groups and the biotin on the probes. In contrast, immobilizing the thiolated probes did not require any additional constituents to be functionalized on the sensor chip surface. In addition, the presence of many adhesion layers on the sensor chip surface in preparation for the immobilization of biotinylated probes influences the prominence of the SPR incident angle dips observed for the biotinylated probes [20].

4 Conclusion

Both types of probes (biotinylated and thiolated) for each gene displayed a red resonance angle shift. Several factors, such as the binding of the probes to the sensor chip surface, through covalent and non-covalent strategies, can be attributed to some of the findings obtained in this study. Therefore, the use of thiolated probes is a more cost-effective method compared to biotinylated probes, making it a preferred choice for low-cost applications. In addition, the data obtained affirms that an optical-based technique such as SPR can effectively detect changes on the sensor chip's surface, such as the binding of single-stranded DNA probes. This shows that different DNA-based biorecognition elements can be used in optical-based detection.

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