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 targets of these two primary drugs are specific proteins in the TB bacteria: *RpoB* for rifampicin, and two targets for isoniazid - *enoyl-reductase* (*InhA*) and *catalase-peroxidase* (*KatG*). 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.

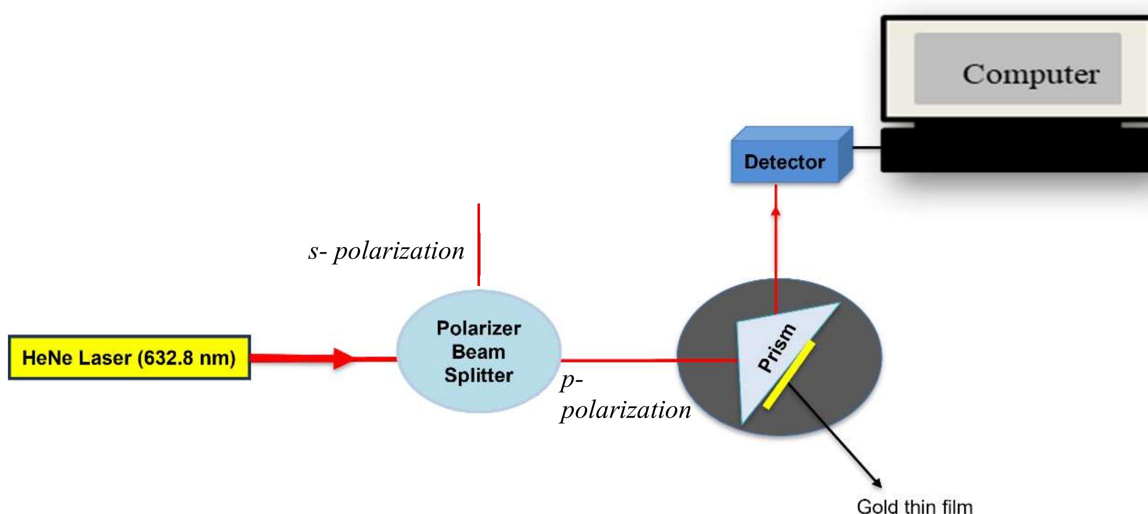

Figure 1: A schematic diagram of the custom-built SPR setup used in this study [14].

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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 [14]. 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 by pipetting 30 µL of the thiolated probe solution. The sensor chip with the probes was then 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.

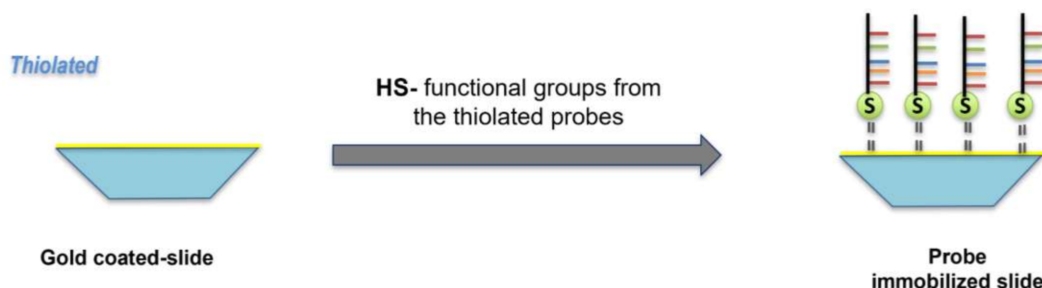

Figure 2: A simplified diagram of the immobilized thiolated DNA probes on the sensor chip surface.

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 [14]. 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 NH functional groups using 30 µL of 1mM solutions of N-ethyl-N-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 1mM N-hydroxysuccinimide (NHS) (Sigma-Aldrich, South Africa) dissolved in distilled water. The sensor chips were incubated 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 [14]. 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.

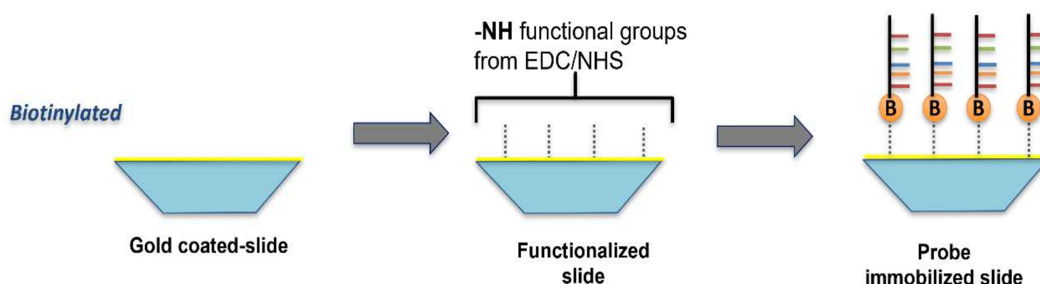

Figure 3: A simplified diagram of the immobilized biotinylated DNA probes on the sensor chip surface.

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).

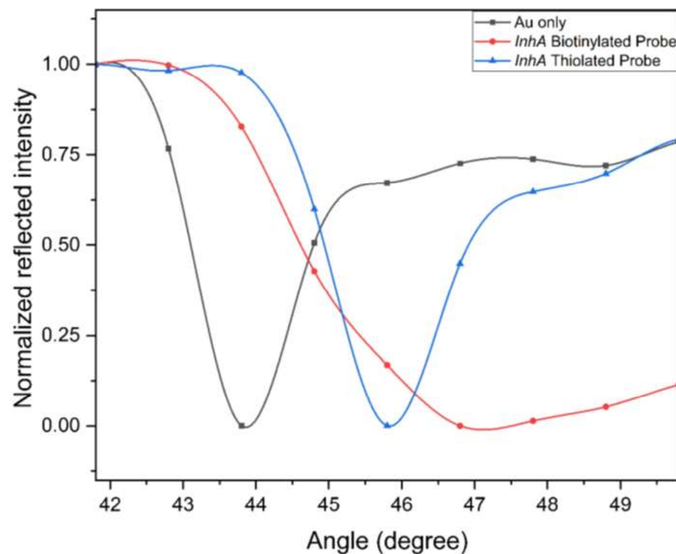

Figure 4: The normalized reflected intensity spectra as a function of angle variation showing the resonance angle shifts obtained for the biotinylated and thiolated *InhA* probe, relative to the sensor ship surface alone.

The data obtained for the *InhA* gene (Figure 4) showed notable changes in the resonance angle compared to the unmodified gold-coated sensor chip and those functionalized with biotinylated and thiolated *InhA* probes. The resonance angle of the bare gold-coated sensor chip was 43.8 degrees, whereas the functionalized chips with biotinylated and thiolated InhA probes exhibited a right shift to 46.8 and 45.8 degrees, respectively. Interestingly, the biotinylated *InhA* probe displayed a 1-degree greater resonance angle shift than the thiolated probe. The immobilization of biotinylated probes on the gold sensor chip surface introduced varying diluents and chemical properties, influencing DNA probe adsorption. According to Peterson et al. [16], thiolated probes enable covalent bonding between the gold and thiol group, facilitating self-assembly monolayer formation and probe attachment kinetics. In contrast, Avidin molecules (streptavidin and neutravidin) have been shown to enhance the signal and adsorption properties of DNA on gold sensor surfaces [16–18]. This may explain the greater resonance angle shift observed for the biotinylated *InhA* probe compared to the thiolated probe.

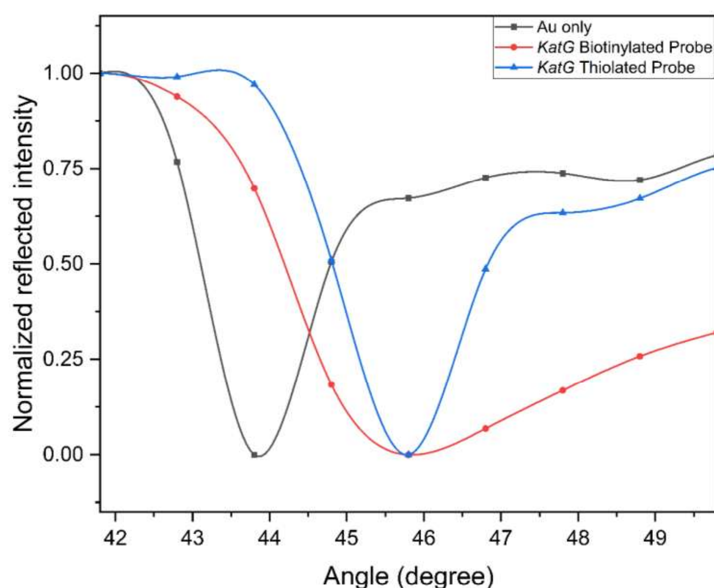

Figure 5: The normalized reflected intensity spectra as a function of angle variation showing the resonance angle shifts obtained for the biotinylated and thiolated *KatG* probe, relative to the sensor ship surface alone.

The *KatG* gene data, obtained using biotinylated and thiolated probes (Figure5), revealed changes in the resonance angle compared to the bare gold-coated sensor. Specifically, the resonance angle shifted from43.8 degrees for the gold-coated chip to 45.8 degrees for both the biotinylated and thiolated *KatG* probes. The observed red shift in resonance angles indicates changes in the molecular composition on the sensor chip surface, suggesting successful immobilization of the probes. This shift can be attributed to the formation of covalent and non-covalent bonds between the probes and the sensor surface. According to Soares [19] a red shift in the resonance angle is primarily due to the increased mass of newly bound molecules on the sensor surface [19].

The immobilization methods for biotinylated and thiolated probes differ significantly in terms of underlying chemistry and surface attachment mechanisms. Biotinylated probes require a multi-step process, where the sensor surface is first functionalized with amide groups using EDC/NHS, enhancing the binding affinity between neutravidin, NHS groups, and biotin on the probes. In contrast, thiolated probes can be immobilized directly onto the sensor chip surface without additional surface modifications. Notably, the multiple adhesion layers required for biotinylated probe immobilization can impact the SPR incident angle dips, influencing the observed signal. This highlights a key difference between the two immobilization approaches, with implications for probe performance and signal quality [20].

Depending on the implementation and optimization of the SPR-based methods, the specificity and sensitivity of an SPR-based method can vary. Furthermore, SPR-based platforms offer a complementary approach to the current diagnostic tools by providing real-time kinetic information and label-free detection [5,8,11]. While some of the current diagnostic tools for MDR-TB may excel in sensitivity and specificity, SPR-based platforms offer advantages in multiplexing and kinetic analysis [11].

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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