Photonic-biosensing towards drug-resistant Tuberculosis diagnosis

Sipho Chauke[1,2], Mabotse Tjale[1], Charles Maphanga[1], Felix Dube[2], Saturnin Ombinda-Lemboumba[1,3], and Patience Mthunzi-Kufa[4]

[1]Manufacturing, Council for Scientific and Industrial Research (CSIR), Pretoria, South Africa
[2]Department of Molecular and Cell Biology, University of Cape Town, Cape Town, South Africa
[3]Départemente de Physique, Université des Sciences et Techniques de Masuku, Franceville, Gabon
[4]Engineering and Technology, University of South Africa (UNISA), Johannesburg, South Africa

E-mail: schauke@csir.co.za

Abstract. Early detection and treatment of tuberculosis (TB) remain key strategies to reduce transmission and disease progression. However, this is hampered by time-consuming, insensitive diagnostic methods, particularly for the detection of drug-resistant forms and in patients with human immunodeficiency virus infection (HIV). Several genes, such as *the RpoB* and *InhA* genes, contain mutations that are responsible for drug resistance. This study aimed to use an SPR-based biosensor platform to detect *RpoB* and *InhA* genes. DNA probes, specific to *RpoB* and *InhA*, were used as biorecognition elements to capture the corresponding target DNA sequences. The *RpoB* and *InhA* gene-specific thiolated DNA probes were immobilized on a gold-coated glass substrate before the target DNA was introduced for detection. As a negative control, a non-specific target to both genes was used to confirm the binding of the specific target. The shifts in the resonance angles indicated the binding properties associated with DNA hybridization between the specific target and the capture probe. The results obtained from this study demonstrated the use of a simple SPR setup and its potential for identifying genes associated with drug-resistant TB.

1 Introduction

Tuberculosis (TB) is a curable disease treated with first-line drugs such as rifampicin, isoniazid, pyrazinamide, and ethambutol [1]. This treatment regimen can last between six and nine months [1]. Due to the prolonged treatment periods, infected individuals will often stop adhering to the treatment regimen, thus increasing their chances of developing drug-resistant TB [1,2].

Multi-drug-resistant TB, on the other hand, is resistant to the two primary first-line TB drugs, rifampicin and isoniazid [2]. These first-line drugs target the *RNA polymerase β subunit* (*RpoB*) (rifampicin gene target) and *enoyl reductase* (*InhA*) (isoniazid gene target) within the TB bacteria [3]. Current diagnostic tests and assays that target the detection of these drug-resistant genes include the XpertUltra test and line probe assays [4,5]. Although effective, these diagnostic tests often require well-equipped facilities and can be quite laborious [4,6]. Some solutions include effectively diagnosing and monitoring the treatment using inexpensive and simple-to-use

diagnostic tests. Hence, easy-to-use, accurate, and sensitive methods can be developed as alternative diagnostic and/or detection tools [7].

Optical-based techniques can be used as alternatives since they have been applied to detect a wide range of pathogens, including *Mycobacterium tuberculosis* [7–9]. One such optical technique is surface plasmon resonance (SPR). SPR is an optical phenomenon that occurs when light interacts with the collective oscillations of free electrons, known as surface plasmons, at the interface between a metal and a dielectric material. When the frequency of the incident light matches the natural oscillation frequency of these electrons, resonance is achieved. In thin metal films, these surface plasmons can propagate along the metal-dielectric interface [10–12]. According to the solutions derived from Maxwell's equations, the oscillation of these surface plasmons can be described by the surface plasmon propagation wave constant, which can be mathematically derived from the dispersion relation [11,13]. The surface plasmon propagation wave constant (k_{spp}) is a key element for describing the continuous wave of the surface plasmons in the metal-dielectric material interface. Furthermore, the k_{spp} depends on several parameters, including the frequency of the incident light (ω), the speed of light (c), and the permittivity of both the metal ($\mathcal{E}_m$) and the dielectric ($\mathcal{E}_d$) [11,13]. Equation 1 below describes how light interacts with the metal surface by exciting the free electrons in the metal-dielectric interface.

$$k_{spp} = \frac{\omega}{c}\sqrt{\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}} \qquad (1)$$

These interactions of the surface plasmons on the thin metal film are sensitive to changes in the refractive index on the metal surface [10,11,13]. This sensitivity characteristic is an advantage for various biosensing applications. Additional advantages of SPR include its label-free nature and requirement of small sample sizes for effective detection [14–16]. In addition, SPR-based biosensors have been used to detect the affinity binding of molecules in real-time due to their sensitivity [7]. The present study evaluates the use of SPR to detect two drug-resistant TB genes using single-stranded deoxyribonucleic acid (ssDNA) probes as biorecognition elements and the effects of changes in target concentration on the SPR angle.

2 Experimental

2.1 The optical setup

The optical setup used in this study has been described in our previous publication [17]. Briefly, the light source consisted of a Helium-Neon (HeNe) laser with a wavelength of 632.8 nm and an output power of 10 mW, and a beam size of 1 mm. The laser light was directed to a polarizing beam splitter, which split the laser light into the *p*- and *s*-polarized light. The *p*-polarized light beam was attenuated to 2 mW. The attenuated laser beam was directed to the sample through a BK7 prism coupled to a gold-coated sensor chip in the Kretschmann configuration. The reflected beam was subsequently directed to a photodiode detector/amplifier system for data acquisition.

2.2 Sensor chip fabrication and functionalization

Glass slides (5 x 5 cm) were cleaned with 100% ethanol and distilled water by sonicating for 10 minutes each. The glass slides were dried under N_2 gas. The slides were coated with titanium (5 nm) and then gold (50 nm) using a physical vapor deposition system (PVD) (Korvus Technology, United Kingdom). The coated slides (sensor chip) were then rinsed with 100% ethanol and distilled water and dried under N_2 gas. Once dry, silicone wells (9 x 9 mm) were placed on the sensor chip to create wells on the surface of the sensor chip. The first well on each sensor chip was used as the reference well (Au only). On each sensor chip, 30 µL of thiolated probes (10 µm) (*RpoB* probe: **5' HS-***CGTACCGTCGTTTACCGGGC* **3'**; *InhA* probe: **5' HS-***GATATAGCTCCCGTCCTCGG* **3'**) were immobilized in all the wells except for well 1. The sensor chip with the immobilized probes was incubated overnight in the 4ºC fridge. The unattached probes were washed off with 1X PBS, and the sensor chips were allowed to dry. Thereafter, specific targets (*RpoB* target: **5'** *GCCCGGTAAACGACGGTACG* **3'**; *InhA* target: **5'** *CCGAGGACGGGAGCTATATC* **3'**) were added in wells 3 of each sensor chip, while the non-specific target (**5'** *CATGGCAGCAAATGGCCCGC* **3')** was added in wells 4 of each sensor chip. The sensor chips were then incubated for 30 minutes in the 4ºC fridge. The sensor chips were washed with 1X PBS, allowed to dry, and then analyzed using the SPR optical setup.

2.3 Reflectivity analysis of the sensor chip surface and data processing

All the data were recorded at a constant temperature (~20°C). The signal of the changes in the reflected intensities was transduced by a photodiode amplifier/detector, from which the data were then processed and analyzed using OriginPro 8. The repeatability of the SPR angle shift experiments was calculated using equation 2:

$$Repeatability = \frac{\text{Resonance angle shift mean}}{\text{Resonance angle shift mean + Standard Error}} \times 100 \quad (2)$$

3 Results

3.1 Surface plasmon resonance

According to Zheng et al. [18], the sensing responses observed when using SPR are often influenced by the refractive index detected on the surface of the sensor chip. In this study, an SPR optical setup was used to detect two drug-resistant genes (*InhA* and *RpoB*). Single-stranded DNA (ssDNA) probes were used to capture biorecognition elements for target-specific DNA. Furthermore, the experimental data were obtained by monitoring changes in the shifts of the resonance angle of incident light and the reflected intensities of the sensor chips before and after the immobilization of the probes and target DNA (Figures 1 and 2).

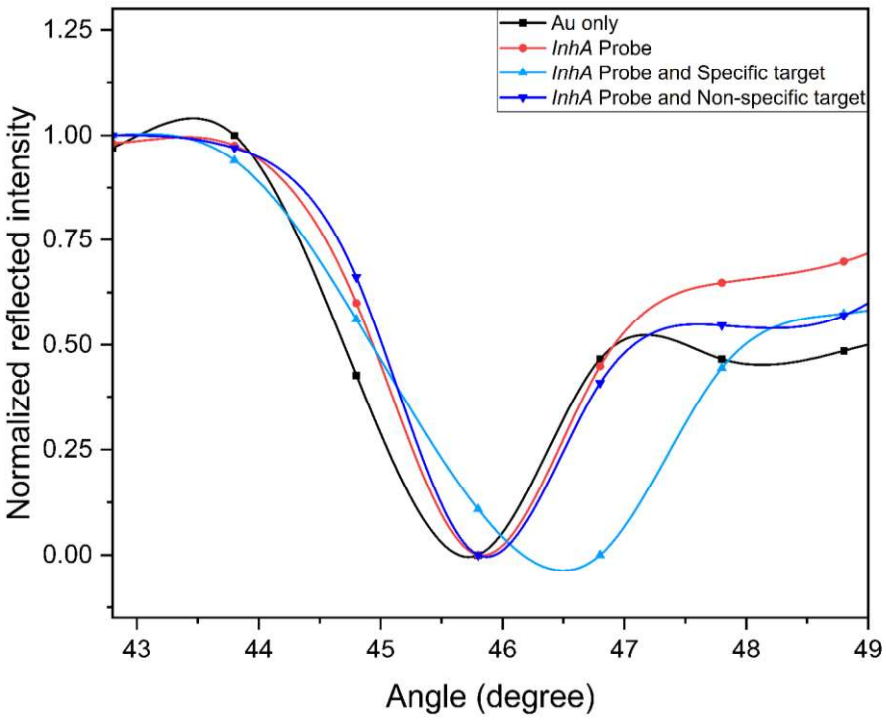

Figure 1: The normalized reflected intensities and resonance angle obtained for the *InhA* gene during the analysis of the hybridization kinetics between the gene's probe, its specific target, and the non-specific target.

In Figure 1, the resonance angle of incidence observed for the reference (Au only) was 45.80 degrees, while the resonance angles for the *InhA* probe, the probe with the specific target, and the probe with the non-specific target were 45.95, 46.74, and 46.03 degrees, respectively. The addition of the probe and the specific and non-specific target resulted in a shift towards the right when compared to the reference sample. According to Zheng et al. [18], the angle shifts that result from molecules binding to each other result in changes in the SPR angle of incidence due to changes in the refractive index and an increase in the mass detected on the sensor surface. The repeatability of three independent experiments evaluating the sensor chip surface and detecting the immobilized *InhA* probe

with the addition of the specific and non-specific targets, at constant concentrations of 10 µM, was calculated to be 99.4, 99.3, 99.5, and 99.0%, respectively.

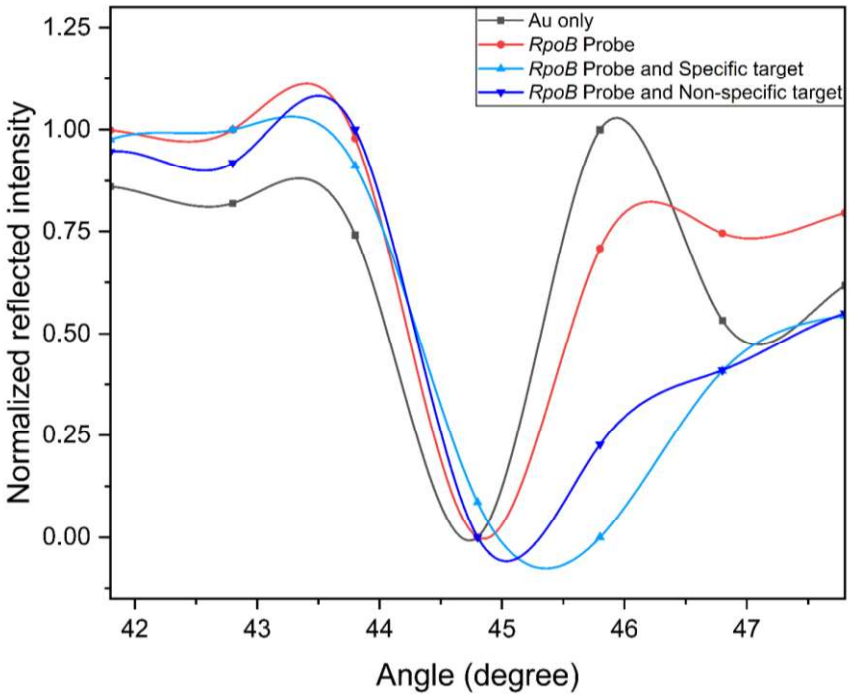

Figure 2: The normalized reflected intensities and resonance angles obtained for the *RpoB* gene during the analysis of the hybridization kinetics between the gene's probe, its specific target, and the non-specific target.

Similarly, Figure 2 shows the resonance angle peak of the incidence beam obtained for the *RpoB* probe with the specific target and the non-specific target. The resonance angle of incidence beam for the reference (Au only) was 44.70 degrees. In contrast, the immobilized *RpoB* probe and the *RpoB* probe with the non-specific target resulted in minor resonance incident angle shifts of 44.76 and 44.78 degrees, respectively, when compared to the reference (Au only). In addition, the *RpoB* probe with a specific target resulted in a resonance incident angle shift of 45.78 degrees. This observation is similar to what was noted in a study by Soares et al. [16], which investigated the hybridization of ssDNA targets and probes on a sensor chip. The repeatability of three independent experiments evaluating the sensor chip surface and detecting the immobilized *RpoB* probe with the addition of the specific and non-specific targets, at constant concentrations of 10 µM, was calculated to be 98.8%, 99.2%, 99.05%, and 98.9%, respectively.

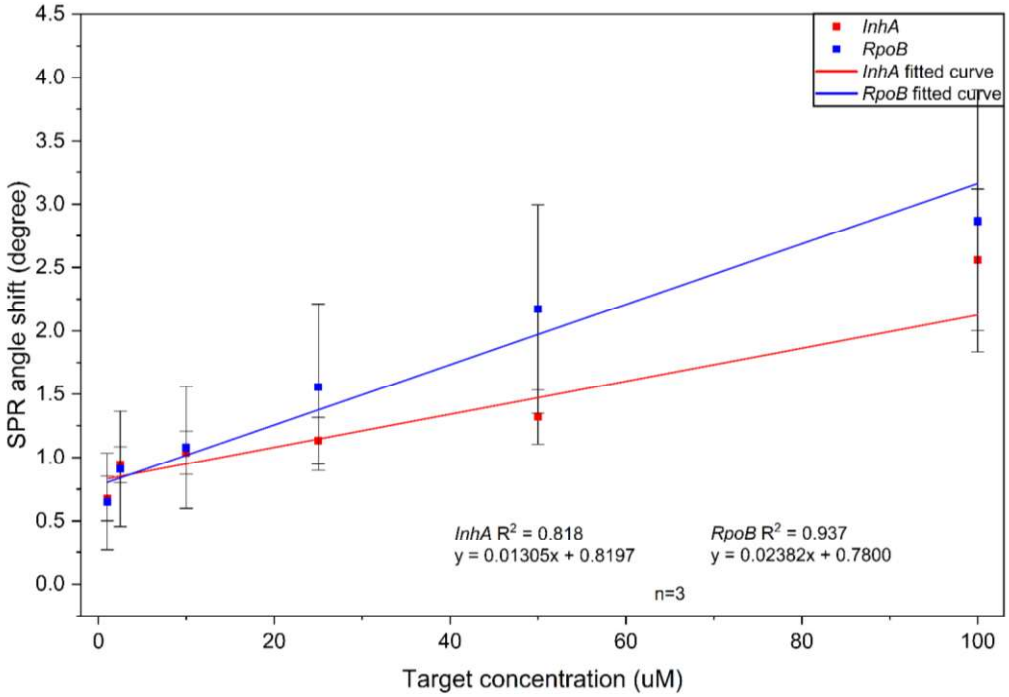

Figure 3: Linear fitted curves of the effects of changing the target concentration (in µM) bound to the probe on the SPR angle of incidence. Each curve represents the SPR angles obtained for each gene, indicating R^2 values of 0.818 and 0.937 for genes *InhA* and *RpoB*, respectively.

Figure 3 illustrates the variation of the SPR resonance angle shifts as a function of changing the target concentration. For *InhA*, target concentrations of 1, 2.5, 5, 10, 25, 50, and 100 µM resulted in resonance angle shifts of 0.677, 0.942, 1.037, 1.133, 1.32, and 2.56 degrees, respectively. Similarly, for *RpoB*, target concentrations of 1, 2.5, 5, 10, 25, 50, and 100 µM resulted in resonance angle shifts of 0.651, 0.911, 1.08, 1.554, 2.174, and 2.867 degrees, respectively. These results indicate that with increasing target concentration, there is an increase in the SPR resonance angle. This observation coincides with what was reported in studies that attribute SPR angle shifts to the increase in the mass detected on the sensor chip surface [18–21].

4 Conclusion

In this study, the sensitivity of the SPR platform was demonstrated by its ability to distinguish between the presence of the probe alone and its interaction with both specific and non-specific targets on the sensor chip surface. The SPR setup successfully detected target concentrations as low as 1 µM, with molecular interactions reflected as shifts in the SPR resonance angle. These findings highlight the effectiveness of single-stranded DNA (ssDNA) probes as biorecognition elements in SPR-based optical biosensing. Future studies may look at the use of different biorecognition elements for the detection of drug-resistant TB on an SPR-based sensor chip and SPR setup.

References

[1] World Health Organization 2024 *2024 Global tuberculosis report* (Geneva)
[2] Naidoo K, Perumal R, Ngema S L, Shunmugam L and Somboro A M 2024 Rapid Diagnosis of Drug-Resistant Tuberculosis–Opportunities and Challenges *Pathogens* **13**
[3] Liu Q, Yang D, Qiu B, Martinez L, Ji Y, Song H, Li Z and Wang J 2021 Drug resistance gene mutations and treatment outcomes in mdr-tb: A prospective study in Eastern China *PLoS Negl Trop Dis* **15** 1–15

[4] World Health Organization 2021 *Use of Xpert MTB/RIF and Xpert MTB/RIF Ultra on GeneXpert 10-colour instruments: WHO POLICY STATEMENT*
[5] Rahman A, Sahrin M, Afrin S, Earley K, Ahmed S, Rahman S M M and Banu S 2016 Comparison of Xpert MTB/RIF assay and genotype MTBDRplus DNA probes for detection of mutations associated with rifampicin resistance in mycobacterium tuberculosis *PLoS One* **11**
[6] Ocheretina O, Byrt E, Mabou M M, Royal-Mardi G, Merveille Y M, Rouzier V, Fitzgerald D W and Pape J W 2016 False-positive rifampin resistant results with Xpert MTB/RIF version 4 assay in clinical samples with a low bacterial load *Diagn Microbiol Infect Dis* **85** 53–5
[7] Srivastava S K, Van Rijn C J M and Jongsma M A 2016 Biosensor-based detection of tuberculosis *RSC Adv* **6** 17759–71
[8] Joshi H, Kandari D, Maitra S S and Bhatnagar R 2022 Biosensors for the detection of Mycobacterium tuberculosis: a comprehensive overview *Crit Rev Microbiol* **48** 784–812
[9] Apollonov V 2021 *Laser Therapy for Treating Tuberculosis*
[10] Butt M A 2025 Surface Plasmon Resonance-Based Biodetection Systems: Principles, Progress and Applications—A Comprehensive Review *Biosensors (Basel)* **15**
[11] Mendes J P, dos Santos P S S, Dias B, Núñez-Sánchez S, Pastoriza-Santos I, Pérez-Juste J, Pereira C M, Jorge P A S, de Almeida J M M M and Coelho L C C 2024 Exciting Surface Plasmon Resonances on Gold Thin Film-Coated Optical Fibers Through Nanoparticle Light Scattering *Adv Opt Mater* **12**
[12] Hong Y, Huh Y M, Yoon D S and Yang J 2012 Nanobiosensors based on localized surface plasmon resonance for biomarker detection *J Nanomater* **2012**
[13] Treebupachatsakul T, Boosamalee A, Chaithatwanitch K and Pechprasarn S 2022 Generalized figure of merit for plasmonic dip measurement-based surface plasmon resonance sensors *Biomed Opt Express* **13** 1784
[14] Singh P 2016 SPR Biosensors: Historical Perspectives and Current Challenges *Sens Actuators B Chem* **229** 110–30
[15] Trzaskowski M, Napiórkowska A, Augustynowicz-Kopeć E and Ciach T 2018 Detection of tuberculosis in patients with the use of portable SPR device *Sens Actuators B Chem* **260** 786–92
[16] Soares L, Csáki A, Jatschka J, Fritzsche W, Flores O, Franco R and Pereira E 2014 Localized surface plasmon resonance (LSPR) biosensing using gold nanotriangles: Detection of DNA hybridization events at room temperature *Analyst* **139** 4964–73
[17] Chauke S H, Ombinda-Lemboumba S, Dube F and Mthunzi-Kufa P 2024 Biosensing multidrug-resistant TB genes using SPR (SPIE-Intl Soc Optical Eng) p 39
[18] Zheng F, Chen Z, Li J, Wu R, Zhang B, Nie G, Xie Z and Zhang H 2022 A Highly Sensitive CRISPR-Empowered Surface Plasmon Resonance Sensor for Diagnosis of Inherited Diseases with Femtomolar-Level Real-Time Quantification *Advanced Science* **9**
[19] Beusink J B, Lokate A M C, Besselink G A J, Pruijn G J M and Schasfoort R B M 2008 Angle-scanning SPR imaging for detection of biomolecular interactions on microarrays *Biosens Bioelectron* **23** 839–44
[20] He L, Musick M D, Nicewarner S R, Salinas F G, Benkovic S J, Natan M J and Keating C D 2000 Colloidal Au-enhanced surface plasmon resonance for ultrasensitive detection of DNA hybridization *J Am Chem Soc* **122** 9071–7
[21] Ekgasit S, Tangcharoenbumrungsuk A, Yu F, Baba A and Knoll W 2005 Resonance shifts in SPR curves of nonabsorbing, weakly absorbing, and strongly absorbing dielectrics *Sens Actuators B Chem* **105** 532–41