

Label-free SERS-Based Aptasensor for SARS-CoV-2 S-Proteins Detection

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Abstract

This study presents a label-free surface-enhanced Raman spectroscopy (SERS) aptasensor for the sensitive and selective detection of SARS-CoV-2 spike (S-) proteins of Wuhan and Omicron variants. Gold nanoparticles (Au NPs) were deposited onto hydroxylated silicon (Si) substrates and functionalized with variant-specific aptamers. The SERS aptasensor demonstrated a strong, concentration-dependent Raman response from 1 nM to 20 μ M, with a sensitive and prominent peak at 1590 cm^{-1} attributed to Au-thiolate bonding. The Omicron variant produced significantly higher SERS intensity than the Wuhan variant, suggesting enhanced surface interactions and Raman responses. Beside high sensitivity and capability for variant discrimination, a strong linear correlation between SERS intensity and protein concentration supports the potential of label-free SERS aptasensor for quantitative diagnostics of COVID-19.

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1. Introduction

The global spread of SARS-CoV-2 and the frequent virus mutations especially within the S-protein have emphasized the need for a sensitive and variant-inclusive diagnostic technologies. The Omicron variant exhibit higher transmissibility and can evade immune responses, making an accurate identification as crucial for early intervention [1]. Conventional detection methods like reverse transcription polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) are reliable, however suffer from limitations [2]. Therefore, there is a growing demand for an alternative detection method that can effectively discriminate the variant of SARS-CoV-2 at protein level [3]. The SERS technique is promising for label-free and sensitive detection of viral proteins. This study focuses on the fabrication of SERS substrate using functionalized Si chips with aptamer immobilized-Au NPs to differentiate the S-proteins from both Wuhan and Omicron variants.

2. Materials and Methodology

Au NPs were synthesized using the Turkevich-Frens method with gold(III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), trisodium citrate, and sodium borohydride (NaBH_4). Silicon chips were cleaned and functionalized via a self-assembled monolayer (SAM) process, including hydroxylation using piranha solution and thiolation with (3-mercaptopropyl) trimethoxysilane (MPTMS). Au NPs were then immobilized on the thiolated surface through Au-S bonding to form the SERS-active substrate.

Aptamers were covalently attached by modifying the Au NPs surface with NHS ester and glutaraldehyde, enabling linkage to amine-terminated aptamers. Both aptamer (SP1KRZ-46, aptO7) and SARS-CoV-2 spike protein solutions were prepared in Tris-EDTA (TE) buffer (pH 8.0) and stored at -20°C to -80°C . Aptamer solution (10 μ M) was drop-casted onto the substrate and air-dried at room temperature. Subsequently, 2 μ L of SARS-CoV-2 S-protein (1 nM to 20 μ M) was applied to the surface for target binding. Surface morphology was examined by field emission scanning electron microscopy (FESEM). As illustrated in Figure 1(a), aptamer immobilization enabled specific binding to SARS-CoV-2 S-proteins, resulting in distinct Raman spectral changes based on variant-specific vibrational modes.

Raman spectra were collected using a LabRAM HR Evo spectrometer with a 523 nm green laser, scanning from 400–3000 cm^{-1} using a $\times 10$ objective lens and 25 μ m pinhole. Each final spectrum was averaged from

three scans taken at different points, with 1-second exposure per scan, 3 seconds total exposure, and 20% laser power. Five replicate measurements were collected per concentration to ensure reproducibility.

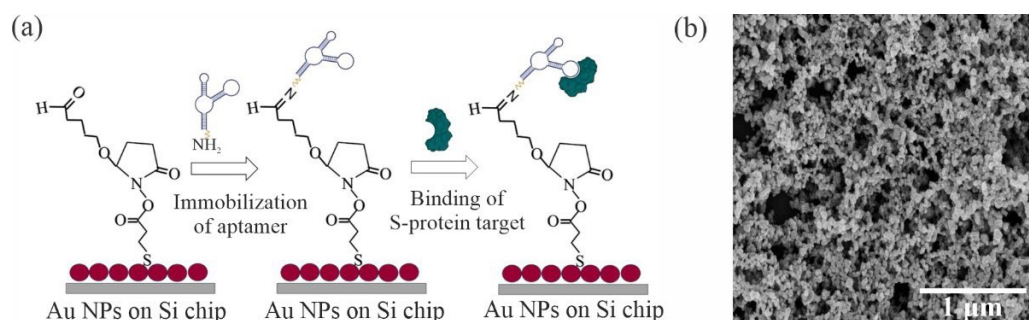


Figure 1. (a) Molecular interactions during aptamer immobilization and subsequent binding with protein target. (b) FESEM image of the random arrangement of Au NPs on Si substrate.

3. Results and Discussions

The successful synthesis and deposition of Au NPs onto the Si substrate were confirmed through visual and spectroscopic evaluations. As shown in Figure 1(b), the Au NPs formed a random nanostructures arrangement on the surface for an intense SERS-active hotspots. These hotspots enhance the localized electromagnetic (EM) field, amplifying the Raman signal of bound protein molecules presence near or in the hotspots.

Figure 2 shows concentration-dependent SERS spectra (1 nM - 20 μM) with rising Raman intensity confirming target-aptamer interaction. A consistent 1590 cm^{-1} peak attributed to Au-thiolate bonding was observed in both variants. Notably, the S-Omicron reaches a double in the intensity at 20 μM , compared to the S-Wuhan. This implies stronger surface interactions and enhanced SERS-amplified Raman responses due to structural flexibility and mutations in S-Omicron. These mutations commonly occur in S1, which is basically the tip of the S-glycoprotein that exposed to the external environment, making it the part that is more exposed to the hotspots [4]. The Omicron variant exhibit subtle shifts or enhanced intensity compared to the Wuhan variant, reflecting structural adaptations such as changes in glycosylation, secondary structure, or binding interactions.

The Raman spectrum reveals distinct molecular vibrations across three key regions. In the fingerprint region (green shaded, $\sim 400\text{--}1800\text{ cm}^{-1}$), strong peaks such as $\sim 1590\text{ cm}^{-1}$ and amide I ($\sim 1650\text{ cm}^{-1}$) were observed, with S-Omicron showing higher intensity than S-Wuhan, suggesting stronger aptamer interaction. The silent region (blue shaded, $\sim 1800\text{--}2700\text{ cm}^{-1}$) exhibited minimal activity. In the high-wavenumber region (purple shaded $\sim 2700\text{--}3200\text{ cm}^{-1}$), a prominent peak at $\sim 2900\text{ cm}^{-1}$ in the Omicron spectrum, attributed to C-H group vibrations, further indicates variant-specific surface interactions [5]. These spectral differences highlight this sensor ability to distinguish SARS-CoV-2 variants.

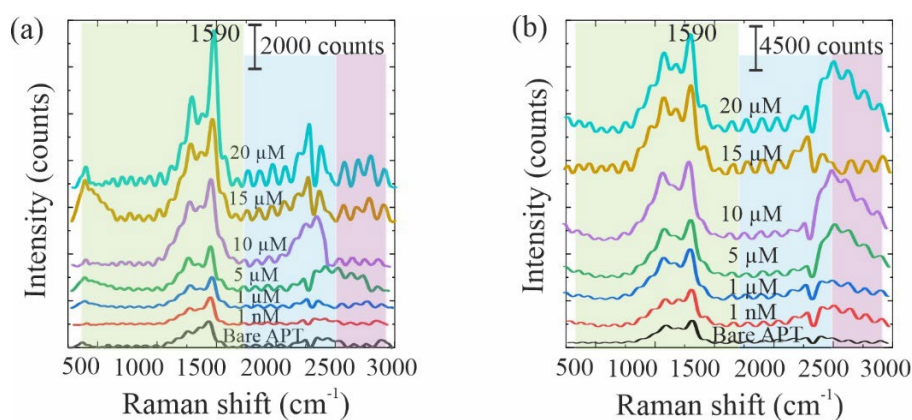


Figure 2. Concentration-dependent Raman spectra from SARS-CoV-2 S-protein (a) Wuhan and (b) Omicron variants with 1.0 nM - 20.0 μM concentrations.

To evaluate the quantitative capability of the SERS aptasensor, the 1590 cm^{-1} peak intensity values were plotted against protein concentrations. As shown in Figure 3, strong linear relationship $R^2 = 0.95$ (S-Wuhan)

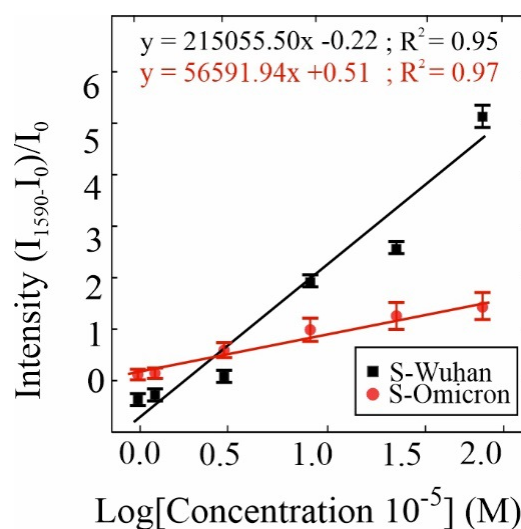


Figure 3. Linearity evaluation of the SERS peak intensities versus corresponding S-protein Wuhan and Omicron variants concentrations.

and $R^2 = 0.97$ (S-Omicron) were observed, indicating excellent linearity and detection reliability. The calculated limits of detection (LOD) were 6.59×10^{-15} M (0.307 ng/mL) for S-Wuhan and 2.51×10^{-14} M (1.17 ng/mL) for S-Omicron, confirming the capability of this sensor for sensitive quantification of SARS-CoV-2 S-proteins. The observed difference in LOD between the S-Wuhan and S-Omicron variants is closely related to both the binding affinities of the aptamers used and the performance of the developed SERS substrate. The SP1KRZ-46 aptamer, designed for the S-Wuhan protein, exhibited a remarkably low dissociation constant ($K_d = 79.45$ fM), reflecting very high binding affinity. In contrast, the aptO7 aptamer targeting S-Omicron protein had a substantially weaker binding affinity ($K_d = 69.7$ nM), resulting in less efficient binding. This strong correlation between K_d and LOD highlights the critical role of aptamer-target interaction strength in determining the sensitivity of the SERS aptasensor.

4. Conclusion

This study demonstrated a label-free SERS aptasensor based on Au NPs substrate capable of detecting and differentiating SARS-CoV-2 S-proteins from Wuhan and Omicron variants. A sensitive and variant-specific Raman responses across 1 nM to 20 μ M proteins concentrations were obtained. The S-Omicron showed dynamic spectral characteristics than S-Wuhan, indicating stronger surface interactions and responses. The LOD results confirm that the combination of high-affinity aptamers and the fabricated SERS substrate provides a platform for femtomolar-level detection of SARS-CoV-2 S-proteins.

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