Surface plasmon resonance aptasensor based on niobium carbide MXene quantum dots for nucleocapsid of SARS-CoV-2 detection

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Abstract
A novel label-free surface plasmon resonance (SPR) aptasensor has been constructed for the detection of N-gene of SARS-CoV-2 by using thiol-modified niobium carbide MXene quantum dots (Nb₂C-SH QDs) as the bioplatform for anchoring N-gene-targeted aptamer. In the presence of SARS-CoV-2 N-gene, the immobilized aptamer strands changed their conformation to specifically bind with N-gene. It thus increased the contact area or enlarged the distance between aptamer and the SPR chip, resulting in a change of the SPR signal irradiated by the laser (He-Ne) with the wavelength (λ) of 633 nm. Nb₂C QDs were derived from Nb₂C MXene nanosheets via a solvothermal method, followed by functionalization with octadecanethiol through a self-assembling method. Subsequently, the gold chip for SPR measurements was modified with Nb₂C-SH QDs via covalent binding of the Au-S bond also by self-assembling interaction. Nb₂C-SH QDs not only resulted in high bioaffinity toward aptamer but also enhanced the SPR response. Thus, the Nb₂C-SH QD-based SPR aptasensor had low limit of detection (LOD) of 4.9 pg mL⁻¹ toward N-gene within the concentration range 0.05 to 100 ng mL⁻¹. The sensor also showed excellent selectivity in the presence of various respiratory viruses and proteins in human serum and high stability. Moreover, the Nb₂C-SH QD-based SPR aptasensor displayed a vast practical application for the qualitative analysis of N-gene from different samples, including seawater, seafood, and human serum. Thus, this work can provide a deep insight into the construction of the aptasensor for detecting SARS-CoV-2 in complex environments.

Keywords Nb₂C MXene quantum dot · Surface plasmon resonance biosensor · Aptasensor · Detection of N-gene · SARS-CoV-2

Introduction
Since the end of 2019, the world has been suffering from the COVID-19 pandemic caused by novel SARS-CoV-2 coronavirus. This disease has spread across 205 countries and territories, infected more than one hundred million individuals, and resulted in 2 million mortalities [1]. The early clinical manifestations of COVID-19 are similar to the symptoms of the severe acute respiratory syndrome and Middle East respiratory syndrome coronavirus disease, namely, fever, headache, myalgia, arthralgia, and lymph node enlargement. SARS-CoV-2 is characterized with moderate mortality rate, high infection rate, and longer incubation period, leading to long-term infection [2]. Thus, COVID-19 threatens the safety of people and causes serious financial crises. SARS-CoV-2 has been identified in diverse environments, such as water systems, frozen foods, or food packaging [3, 4]. Thereby, the rapid diagnosis of the COVID-19 virus is highly essential to control efficiently the spread of the virus and treat patients. Similar to other coronaviruses, SARS-CoV-2 is composed mainly of four structural proteins, namely, the spike (S), membrane (M), envelop (E), and nucleocapsid (N) proteins [5]. The proteins (e.g., S protein) [6] and viral RNA [7] can be used as targets for the qualitative and quantitative analyses of COVID-19. Alternatively, antibodies, such as IgM and IgG, from patient samples can also be detected to probe the infection history [8]. Diverse techniques have been developed to analyze SARS-CoV2, such as real-time polymerase chain reaction (PCR) [9], colorimetric assay [10], surface plasmon...
resonance (SPR) and localized SPR [11], electrochemical approaches [9, 12–15], optics methods [16, 17], optical/chemiluminescence immunosensors [18], fluorescent technique [19], wearable sensor [20], and isothermal rolling circle amplification (RCA) [21]. Although many studies have focused on the sensitive determination methods of SARS-CoV-2 [22–25], COVID-19 has resulted in a heavy burden for health systems and human society. Consequently, advanced and sensitive determination methods should be developed to assess SARS-CoV-2 in different environments and populations.

Most techniques have been formulated based on the specific recognition between antibodies and diverse proteins and RNAs in the SARS-CoV-2 virus. Compared with antibodies, as a powerful class of probes, DNA aptamers have some advantages, such as high specificity, strong affinity, rapid and reliable synthesis, ease of conjugation, and high feasibility to bind with other DNA-based reactions for efficient signal amplification. Thus, aptamers can be used in different applications for in vitro and in vivo diagnoses. Diverse techniques, such as PCR [26], electrochemical methods [27], and fluorescence approach [28], have been combined with aptasensors for analyzing biomarkers of SARS-CoV-2. However, the aforementioned techniques have some disadvantages. For example, PCR is more complex with need to achieve appropriate test conditions, often leading to low accuracy [29]; detection kits often exhibit low sensitivity for the analysis of SARS-CoV-2 [30]. As for SPR sensing strategy, it is often applied for the construction of diverse immunoassays, for multiple detection of biomolecules, and in situ determination interactions of multiplexed chemical and biological analytes, such as interactions between antibodies and antigens, RNAs, DNAs, or proteins, and a wide variety of ligands or targets [31]. Although different SPR immunosensors have been constructed for detecting antibodies against SARS-CoV-2 [32–34], no SPR aptasensor for analyzing the RNA of N-gene has been reported yet.

Various nanomaterials, such as functional polymers [35], nanoparticles [36], graphene [37, 38], MXene nanosheets [39, 40], and MoS2 [41], have been utilized as sensing platforms for the fabrication of SPR aptasensors. MXenes, which are known as two-dimensional materials, have attracted extensive attention due to their similar structure and analogous performances to graphene. The versatile chemical structure, compositions, and tunable surface functionalization of MXenes facilitate the diverse applications of MXene, such as in solar cells [42], electronic devices [43], catalysts [44, 45], gas sensors or biosensors [46, 47], and cancer therapy [48]. MXenes have nanosheet-like structure, unique surface chemistry, high conductive properties, and excellent biocompatibility. These characteristics allow MXenes to efficiently act as platforms for the development of diverse biosensors [49, 50]. MXene nanosheets have been explored as a sensitive layer for immobilizing probes and developing SPR biosensors [40, 51]. However, the ultrathin MXene nanosheets are prone to aggregate to bulk size due to the π–π* stacking interaction. Optimal MXene nanosheets for the development of the SPR biosensor are difficult to obtain because of the limitation in the thickness of SPR biosensors, in which the sensitive layer is ~200 nm [52]. By contrast, zero-dimensional Ti3C2 quantum dots (QDs) from 2D bulk MXene demonstrate novel properties relative to their 2D counterparts because of a combination of quantum confinement, edge effect, and surface functionality [53]. Thus, MXene QDs can be utilized as sensitive nanomaterials to construct SPR aptasensors for detecting N-gene of SARS-CoV-2.

In this work, we have designed and constructed a novel SPR biosensor by using niobium carbide MXene QDs functionalized with the thiol group (denoted as Nb2C-SH QDs) as the bioplatform for adsorbing N-gene-targeted N58 aptamer. The biosensor was explored for the sensitive detection of N-gene from diverse complex environments, such as human serum, seawater, and seafood (Fig. 1). Nb2C QDs were derived from Nb2C MXene nanosheets by a solvothermal method, followed by the modification of Nb2C QDs with the thiol group (Fig. 1a). Given the self-assembling effect between the Nb2C-SH QDs and the Au chip of SPR, Nb2C-SH QDs were homogeneously coated over the chip surface, and the N58 aptamer was stably immobilized through π–π* stacking, electrostatic adsorption, and hydrogen bond [54]. In the presence of SARS-CoV-2, the G-quadruplex between the N58 aptamer and N-gene of SARS-CoV-2 was formed [55]. Thus, the aptamer strands changed its conformation [56] when binding with N-gene, leading to the enlargement of the contact area or distance between the probe molecule and the chip. These modifications were then converted to change in the SPR signal to detect N-gene of SARS-CoV-2 (Fig. 1b). Compared with previously reported SPR biosensors for the same detection [11], the SPR aptasensor based on the Nb2C-SH QDs exhibited superior sensing performances, such as high sensitivity, fast response, renewability, and availability in complex environments. These advantages are mainly ascribed to several factors. The multifunctional properties of Nb2C-SH QDs not only can ensure homogeneously deposition onto the SPR chip but also can tightly adsorb a large amount of aptamers, further resulting in high sensitivity. The highly specific recognition between N58 aptamer and N-gene endows the SPR aptasensor with a fast response toward N-gene. In addition, the Nb2C-SH QD-based SPR biosensor can be widely applied for detecting N-gene from diverse environments, such as human serum, seawater, and seafood, because of its easy construction, high stability, and good renewability. Thereby, the present work provides a new insight into the development of the SPR aptasensor for the fast and sensitive analysis of N-gene of SARS-CoV-2 from different real samples.
Experimental section

Chemicals and materials

Bulk Nb$_2$AlC powder, hydrofluoric acid (HF), dimethyl sulfoxide (DMSO), $N,N$-dimethylformamide (DMF), and $n$-octadecyl mercaptan were purchased from Aladdin Reagent Co. Ltd. Biologicals including influenza A virus (Flu A), influenza B virus (Flu B), M. pneumonia (P1), chlamydia pneumoniae (CPN), immunoglobulin (IgG), prostate-specific antigen (PSA), bovine albumin (BSA), and SARS-CoV-2 and SARS-CoV-2-N58 aptamer sequence: 5$'$-GCT GGA TGT CAC CGG ATT GTC GGA CAT CGG ATT GTC TGA GTC ATA TGA CAC ATC CAG C-3$'$ were ordered from Solarbio Life Sciences Co. Ltd. Additionally, human serum was obtained from the First Affiliated Hospital of Zhengzhou University after informed consent of the patient. This research protocol was in accordance with the ethical standards of the 1964 Declaration of Helsinki and its subsequent revisions and was approved by the ethics committee of the First Affiliated Hospital of Zhengzhou University.

Preparation of Nb$_2$C QDs and Nb$_2$C-SH QDs

The preparation of the Nb$_2$C QDs was realized through a solvothermal method according to the reported literature [57]. The detailed preparation methods of the Nb$_2$C QDs and Nb$_2$C-SH QDs were described in the Electronic Supplementary Material.

SPR measurements

All SPR measurements were performed by a Biacore™ X100 device (GE-Healthcare Bio-Sciences AB, Sweden, the wavelength of the laser (He-Ne) of 633 nm) at 25 °C with flowing 0.01 M phosphate buffer saline (PBS) (pH = 7.4) containing 2.7 mM KCl, 137 mM NaCl, and 0.5% DMSO. In prior to use, the work chip, a standard 10 × 12 × 0.3 mm glass chip coated with gold film (~50 nm), was cleaned with H$_2$SO$_4$/H$_2$O$_2$ (70/30 v/v) for 1 min, following by washing with Milli-Q water and drying in N$_2$ stream. Afterwards, 10 μL of Nb$_2$C-SH QDs suspension (0.1 mg mL$^{-1}$) was dropped on the gold chip surface, forming the homogeneous Nb$_2$C QDs layer via the covalent bond of Au-S between thiol group bearing on Nb$_2$C-SH QDs and gold atom by the self-assembling interaction [58]. Equilibration of the baseline was completed by a continuous flow (5 μL min$^{-1}$) of running buffer through the chip for 0.5 h. Afterward, the Nb$_2$C-SH QDs-modified gold chip was incubated with the aptamer immobilization (100 nM) at a flow rate of 5 μL min$^{-1}$ for 0.5 h. After obtaining a stable baseline, the SPR flow cell was washed with running buffer to remove the unbounded aptamer. Each binding experiment for the detection of N-gene was dissolved in PBS at predetermined concentrations.
(0.05, 0.1, 1, 5, 10, 50, and 100 ng mL\(^{-1}\)) and introduced into the flow cell at a flow rate of 5 μL min\(^{-1}\). The specificity analysis of the SPR aptasensor was performed by injecting several kinds of interferents, which may be coexisted with N-gene of SARS-CoV-2, including Flu A, Flu B, P1, CPN, IgG, PSA, and BSA, respectively, at a flow rate of 5 μL min\(^{-1}\). The loading of the SPR chip was calculated considering one reflectivity unit (RU) corresponding to a change in refractive of 10\(^{-6}\) and approximately 1 pg mm\(^{-2}\) of bound protein.

### Real samples

For the analysis of real samples, human serum without pretreatment was firstly spiked with different amounts of SARS-CoV-2 N-gene. Afterward, human serum was diluted 10 times with PBS. Sea water was obtained from the Yellow Sea. Different amounts of N-gene of SARS-CoV-2 were added to sea water. In addition, seafood was purchased from seafood market in Zhengzhou and treated according to a previous report [59]. After being spiked with different amounts N-gene of SARS-CoV-2, 2 g of shrimp was added to 4 mL of 3% trichloroacetic acid (TA) under stirring for 10 min. Next, the resulting extract was centrifuged at 12,000 rpm for 10 min. Moreover, the pH value of supernatant was then adjusted to 7 with 1.0 M NaOH, following by diluted 100-fold with deionized water.

### Results and discussion

#### Basic characterizations of Nb\(_2\)C-SH QDs

Transmission electron microscopy (TEM) was performed to characterize the microstructure and morphological characteristics of Nb\(_2\)C-SH QDs (Fig. 2a–c). The QDs are clearly observed in the low-magnification TEM image of Nb\(_2\)C-SH QDs (Fig. 2a), which have average lateral sizes from 2.3 to 5.4 nm (Fig. 2b). The inset image in Fig. 2a depicts the corresponding size distribution curve. A clear fringe is obtained in the high-resolution TEM image of the Nb\(_2\)C-SH QDs (Fig. 2c), indicating the single-crystalline feature of the Nb\(_2\)C-SH QDs. The distance between adjacent lattice fringes is 0.217 nm, which well matches with the [001] direction plane of graphene carbon [60].

Fourier transform infrared spectra (FT-IR) of the Nb\(_2\)C QDs and Nb\(_2\)C-SH QDs were shown in Fig. S1 (see the Electronic Supplementary Material), indicating that a sufficient number of oxygen-bearing functional groups are decorated on both QDs. To further evaluate the chemical structure and component of the Nb\(_2\)C QDs and Nb\(_2\)C-SH QDs, X-ray photoelectron spectroscopy (XPS) characterization was performed. The XPS survey spectrum of the Nb\(_2\)C QDs (curve i, Fig. S2) indicates four peaks at the binding energies (BEs) of 286.1, 532.1, 407.1, and 232.1 eV, which are assigned to C 1s, O 1s, N 1s, and Nb 3d, respectively. For Nb\(_2\)C-SH QDs, an additional signal due to S 2p at the BE of 168.1 eV is obtained (curve ii, Fig. S2). To further probe the chemical environments and valence states of each element, the high-resolution C 1s, Nb 3d, and N 1s XPS spectrum of the Nb\(_2\)C QDs and Nb\(_2\)C-SH QDs were analyzed (Fig. 2d–f). The high-resolution C 1s (Fig. 2 (d1)) of Nb\(_2\)C QDs can be deconvoluted into three peaks at BEs of 284.5, 285.5, and 287.4 eV, which are attributed to graphitic C–C, C–N, and O=C=N, respectively. The presence of O=C–N indicates that the Nb\(_2\)C QDs are slightly oxidized during the preparation. The high-resolution Nb 3d XPS spectrum of Nb\(_2\)C QDs (Fig. 2 (e1)) is fitted by two peaks at 207.3 and 210 eV, which are assigned to the BEs of Nb 3d\(_{5/2}\) and Nb 3d\(_{3/2}\) of Nb\(_5^+\), respectively. This finding reveals that the Nb\(_2\)C phase is substantially oxidized [61, 62]. The high-resolution N 1s spectrum of the Nb\(_2\)C QDs (Fig. 2 (f1)) has been commonly simulated with two peaks at the BEs of 399.8 and 402.1 eV, corresponding to pyridinic N and quaternary N [63].

Compared with the C 1s X-ray photoelectron spectrum of Nb\(_2\)C QDs, an additional peak at the BE of 285 eV due to C-S bond appears in the C 1s X-ray photoelectron spectrum of the Nb\(_2\)C QDs-SH (Fig. 2 (d2)). This peak is caused by the modified thiol group. No clear change is observed for the Nb 3d and N 1 s X-ray photoelectron spectra of the Nb\(_2\)C-SH QDs compared with that of Nb\(_2\)C QDs but with lower peak intensity. A discernable observation of the N-species is that the conversion from pyrrolic N to graphitic N occurs upon the additional doping of S into the Nb\(_2\)C QDs [64]. Moreover, the S 2p XPS spectrum of Nb\(_2\)C-SH QDs (Fig. S3) is analyzed in Section S3 of the Electronic Supplementary Material. These results verify the coverage of the self-assembling layer over the Nb\(_2\)C QDs.

In prior to the SPR measurements, XPS was used to investigate whether the N-58-targeted aptamer can be immobilized over the Nb\(_2\)C QDs-modified Au chip. For the C 1s X-ray photoelectron spectrum of the Apt\(_{58}\)-aptamer/Nb\(_2\)C-SH QDs (Fig. 2 (d3)), the two peaks at the BEs of 285.0 and 285.9 eV, which are due to C-S and C-O, respectively, are deconvoluted. The C-O bond is caused by the immobilized aptamer strands. In addition, no clear Nd 3d XPS signal is present in the Apt\(_{58}\)-aptamer/Nb\(_2\)C-SH QDs (Fig. 2 (e3)), indicating that the full coverage of the aptamer strands over the modified layer. The N 1 s X-ray photoelectron spectrum of the Apt\(_{58}\)-aptamer/Nb\(_2\)C QDs can be fitted to two peaks at the BEs of 399.7 and 401.7 eV, which are attributed to pyrrolic-N and graphitic N, respectively [65]. As an indicator of the aptamer strands, the P 2p XPS signal was obtained in the XPS survey scan spectrum of the Apt\(_{58}\)-aptamer/Nb\(_2\)C-SH QDs. The high-resolution P 2p XPS spectrum (Fig. S4) can be separated into two peaks at the BEs of 132.9 and 133.9 eV, which are due to
P\textsubscript{2p\textsubscript{3/2}} and P\textsubscript{2p\textsubscript{1/2}}, respectively. Apparently, the clear P\textsubscript{2p} XPS signal is from the phosphate backbone of the N\textsubscript{58} aptamer [66].

The optical properties of Nb\textsubscript{2}C-SH QDs were studied by UV-vis spectrum and fluorescence spectroscopy (Fig. S5). The results and analysis are supplied in Section S4 of the Electronic Supplementary Material.

**Sensing performance of the Nb\textsubscript{2}C-SH QD-based aptasensor toward N-gene**

Ahead of investigating the optimization of the determination conditions, the electrochemical surface area (ECSA) of the aptasensor was calculated from corresponding CVs (Fig. S6). The ECSA of the aptasensor is 10.5 cm\textsuperscript{2}, revealing good electrochemical activity. In prior to the analysis of N-gene by using the Nb\textsubscript{2}C-SH QD-based aptasensor, the determination conditions or parameters, including the concentration of aptamer, pH value of PBS, and the concentration of Nb\textsubscript{2}C-SH QDs, were investigated to obtain the optimal sensing performance of the Nb\textsubscript{2}C-SH QD-based SPR aptasensor. The results (Fig. S7) and analysis are supplied in Section S6 of the Electronic Supplementary Material. The optimal conditions for the construction of the Nb\textsubscript{2}C-SH QD-based aptasensor are aptamer solution concentration of 100 nM, 0.01 M PBS with pH 7.4, and the Nb\textsubscript{2}C-SH QDs concentration of 1 mg mL\textsuperscript{−1}.

Under the optimal detection conditions, the sensing performance of the Nb\textsubscript{2}C-SH QD-based aptasensor toward N-gene was probed. The Nb\textsubscript{2}C-SH QD-coated Au chip was incubated with different concentrations of N-gene and measured by the SPR instrument (Fig. 3a). The SPR response gradually increases from 40 to 270 RU with increasing N-gene concentration from 0.05 to 100 ng mL\textsuperscript{−1} within 1000 s (Fig. 3b). As aforementioned, the N58 aptamer strands would bind with N-gene of SARS-CoV-2, specifically forming the G-quadruplex between the aptamer and N-gene. This characteristic further increases the thickness of the Au chip for the SPR biosensor, which can vary the refractive index of the overlayer [67]. After 1000 s, the Nb\textsubscript{2}C-SH QD-coated SPR aptasensor was rinsed with 0.01 M PBS. The resulting decrease in the SPR response is ~15 RU, which is considerably low. This finding indicates the strong binding interaction between N-gene and aptamer strands. When \(\Delta RU\) of the Nb\textsubscript{2}C-SH QD-based SPR aptasensor before and after the detection of N-gene (\(\Delta RU = RU_{N\text{-gene}} - RU_{Apt}\)) is defined as the detected signal, the \(\Delta RU\) value is proportional to the logarithm value of N-gene concentration from 0.05 to 100 ng mL\textsuperscript{−1}. The linear regression
The equation was \[ \Delta RU = 70.26 \log C_{\text{N-gene}} + 121.09 \], with the correlation coefficient \((R^2)\) of 0.9923 (the inset of Fig. 3b). The detection limit (LOD) was estimated to be as low as 4.9 pg mL\(^{-1}\) at a signal-to-noise ratio of 3 \([68]\). The LOD was calculated with the equation \((\text{LOD} = 3.3 S_B/m)\) derived from the criterion of the IUPAC recommendation, where \(S_B\) refers to the standard deviation of the blank \((n = 3)\), and \(m\) refers to the slope of the linear calibration curve \([69]\). Compared with the reported SPR, optical, or electrochemical aptasensors for analyzing N-gene of SARS-CoV-2 (Table S1), the SPR aptasensor based on Nb\(_2\)C-SH QDs demonstrates lower LOD and faster response. The low LOD and fast response toward N-gene by using the Nb\(_2\)C-SH QDs-based aptasensor is mainly due to several aspects. (i) The strong self-assembling interaction between thiol bearing on the Nb\(_2\)C-SH QDs and Au chip surface provides the outstanding stability of the aptasensor in the aqueous solution \([70]\). (ii) The strong bioaffinity between the aptamer and highly conjugated Nb\(_2\)C-SH QDs via \(\pi-\pi^*\) stacking, hydrogen bonds, and Van der Waal force facilitates the full coverage of the aptamer onto the Nb\(_2\)C-SH QD-modified Au chip, leading to the high detection efficiency toward N-gene \([71]\). (iii) The highly specific recognition between aptamer and N-gene can reduce the nonspecific adsorption of other interferents on the Nb\(_2\)C-SH QD-based biosensor \([72]\). No substantial \(\Delta RU\) change is found for the BSA adsorption, hinting the absence of a strong nonspecific binding (Fig. S8).

The selectivity of the constructed Nb\(_2\)C-SH QD-based aptasensor was also examined by analyzing different interferents, including other kinds of respiratory viruses (i.e., Flu A, Flu B, P1, IgG, PSA, and BSA) \((100 \text{ ng mL}^{-1})\). The selectivity of the constructed Nb\(_2\)C-SH QD-based SPR aptasensor for N-gene detection \((1 \text{ ng mL}^{-1})\) in the presence of CPN, flu A, flu B, P1, IgG, PSA, and BSA \((100 \text{ ng mL}^{-1})\) is negligible, greatly lower than that of the Nb\(_2\)C-SH QD-based SPR response of N-gene. This finding suggests that the Nb\(_2\)C-SH QD-based aptasensor has the outstanding selectivity for analyzing N-gene.

### Determination of N-gene in different environments and human serum

Given that the Nb\(_2\)C-SH QD-based aptasensor displays high sensitivity and good selectivity, the aptasensor can be employed to detect N-gene in diverse environments, such as in seawater, seafood (frozen shrimp), and human serum, to verify the suitability of the aptasensor. The pretreatment procedure of each sample is provided in the Real samples of the Experimental section. The original concentration of the target analyte (before the sample being spiked) in the real samples was also investigated. The result (Fig. S9) shows that the \(\Delta RU\) of the Nb\(_2\)C-SH QD-based SPR aptasensor before and after the detection of N-gene in seawater, seafood, and human serum is negligible. This finding verifies that no
N-gene exists in real samples. Consequently, the applicability of the developed aptasensor can be assessed by spiking a series of N-gene solutions with different concentrations into real samples. Different concentrations of N-gene were spiked into diverse samples. Then, the determination using the Nb$_2$C-SH QD-based aptasensor was carried out as described in previous sections. According to the calibration curve, the real concentrations of N-gene were deduced. All results are summarized in Tables 1, 2, and 3. As indicated in Table 1, the recoveries for detecting N-gene in seawater range from 97.70 to 111.30%, along with low RSD values of 1.04–2.79%. For N-gene in seafood (Table 2), the found recoveries are from 91.80 to 95.86%, while they are ranging from 98.67 to 104.86% in the human serum (Table 3). The corresponding RSD values are 1.99–4.21% and 1.76–4.12%. These results verify that the Nb$_2$C-SH QD-based aptasensor can be utilized to analyze N-gene from different environments.

### Conclusion

A novel SPR aptasensor was designed and constructed by using Nb$_2$C-SH QDs as the sensitive layer for anchoring N-gene-targeted aptamer strands, which were used for the specific determination of N-gene of SARS-CoV-2. Given the thiol-functionalized, highly conjugated structure, and graphene-like MXene phase, the prepared Nb$_2$C-SH QDs not only displayed high binding interaction with the Au chip via the self-assembling force by forming Au–S bond but also demonstrated strong bioaffinity toward the aptamer strands, and the amplified SPR effect. Apart from low detection limit within a wide linear range of N-gene concentration, high selectivity, excellent repeatability exhibits, and good applicability, the provided SPR aptasensor based on Nb$_2$C-SH QDs apparently demonstrates the advantage of feasible construction, fast response, and in situ analysis. This work paves the way for the fabrication of N-gene aptasensor and provides a new sensing strategy for the early and sensitive analysis of N-
gene. Nonetheless, the Nb₂C QDs-based SPR aptasensor still exhibits some unsatisfied features such as poor repeatability and regenerability when detecting N-gene.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no competing interests.

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