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Research Paper

Exploring an ultra-sensitive electrochemiluminescence monitoring strategy for SARS-CoV-2 using hairpin-assisted cycling and dumbbell hybridization chain amplification

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HIGHLIGHTS
- Hairpin DNA and exonuclease III were employed for target cycling.
- Ti₃C₂@Ag-Au was used to construct the biosensor interface.
- Dumbbell hybridization chain amplification was conducted for signal amplification.
- The strategy was applied for SARS-CoV-2 RdRp detection in environmental samples.
- Ru(phen)₃²⁺ was embedded in a stacked dsDNA groove for signal output.

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ABSTRACT

Rapid and accurate discrimination of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an available approach to implement a rapid diagnosis of the coronavirus disease 2019 (COVID-19). Here we fully exploited the cleavage properties of exonuclease III (Exo III) and hairpin DNA-assisted target cycling technology to generate bulk single-stranded DNA (ssDNA) that was employed to facilitate the constitution of a three-way junction structure on polymeric particle (Ag-Au NPs) and Ti₃C₂ (Ti₃C₂@Ag-Au) complexes. Ag-Au NPs presented favorable stability without adding extra stabilizers, demonstrating the potential value of Ag-Au NPs as an alternative to Au NPs in the field of bioanalysis. Upon the three-way junction structure, the dumbbell hybridization chain amplification (DHCA) was occurred which generated DNA nanostructure with tight conformation. Target cycling and DHCA reactions improved the electrochemiluminescence (ECL) signal, which
1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which provokes coronavirus disease 2019 (COVID-19), has overwhelmed modern healthcare systems due to its exceedingly high pathogenicity and infectivity, imposing health, lifestyle and economic implications on humans worldwide (Zhang et al., 2020a). And the prevalent clinical manifestations of SARS-CoV-2 such as fever, dry cough, fatigue, sore throat and diarrhea, are not unique to it (Da Silva et al., 2020). However, SARS-CoV-2 virus-infected patients are at high risk of transmission in the early stage of symptomatology, therefore, quarantine and isolation of symptomatic patients is an invaluable tool of modern medical epidemic prevention (Moitra et al., 2020). To diagnose SARS-CoV-2 virus-infected patients prematurely, provide tailored regimens, and screen their close contacts, it is imperative to establish expeditious, accessible, and reliable molecular diagnostic methods (Cazares et al., 2020).

Quantitative reverse-transcription real-time PCR (qRT-PCR) is the gold standard for screening SARS-CoV-2 patients in the public health system, being an invaluable screening tool to suppress the COVID-19 pandemic (Wang et al., 2020). Its turnaround time can be within 6 h, but requires medical professionals, specialized reagents and pieces of facilities (Li et al., 2021). Hence, it becomes imperative to explore promising alternative analytical methods to accommodate the demands for speed, accuracy, expediency, and magnitude (Zeng et al., 2022a; Zeng et al., 2022b; Li et al., 2022). Electrochemiluminescence (ECL) has advanced to a formidable and versatile analytical technique by integrating chemiluminescence (EL) and electrochemistry (EC) techniques (Sun et al., 2009). ECL can not only yield high sensitivity for precise quantification, but also has the characteristics of simplified apparatus and convenient operation, which serves an invaluable role in tackling biomedical, clinical diagnosis, food hygiene and environmental monitoring (Fan et al., 2019, 2021a). Meanwhile, ECL-based immuno- and nucleic acid hybridization assays have also gained new vitality and promoted the development of bioanalysis (Fan et al., 2020).

Nucleic acid hybridization is programmed to assemble into multiple conformations, upon which the hybridization chain reaction (HCR) is a reaction initiated by the promoter (target) to produce long nicked double-stranded DNA (dsDNA) (Choi et al., 2014; Zhang et al., 2020b; Zeng et al., 2019, 2012). In addition, HCR is an enzyme-free reaction that allows the triggering of a cascade of numerous oligonucleotides from a single target under relatively mild conditions (Shang et al., 2021). Thus, HCR is capable of achieving high sensitivity and selectivity comparable to PCR reactions, demonstrating the tremendous potential for signal amplification applications in bioanalysis (Chen et al., 2021). The orientation of the polymerization extension of oligonucleotides in the conventional HCR is linear, perpendicular to the EC or ECL biosensing interfaces (Chen et al., 2012; Huang et al., 2020). In particular, the signal amplification performance is correlated with the DNA nanostructures, so the form and performance of HCR can be improved by tailoring the DNA nanostructures (Chu et al., 2019). Subsequently, the solid dumbbell-shaped DNA has been applied to substitute the nonlinear polymeric extension of hairpin DNA, which will generate tighter DNA nanostructures that lead to stronger signal gain effects (Miao and Tang, 2020).

As an exemplary two-dimensional nanosheet for augmenting electron conduction and enhancing EC and ECL signal strength, Ti3C2 has exceptionally high electrical conductivity and serves as an optimal sensing interface material for a wide range of applications in electronic energy storage, biosensing, and cancer theranostics fields (Guo et al., 2021; Fan et al., 2022; Tang et al., 2019; Zeng et al., 2021; Cai et al., 2019; Lu et al., 2021). Ti3C2 has excellent metallic conductivity up to 6500 S cm⁻¹ and a large specific surface area, which can significantly improve the electron transfer capability and analytical performances in ECL biosensors, and thus has enormous application potential in ECL biosensing systems (Yao et al., 2021; Qin et al., 2018; Zhang et al., 2019; Jiang et al., 2022). Meanwhile, electroactive monometallic NPs (e.g. Au, Ag, Pt, Zn, Cu NPs) with promising electrochemical catalytic activity, high electrical conductivity, and favorable biocompatibility have also gained substantial interest in EC or ECL biosensors (Molenbroek et al., 2001; Xu et al., 2010). And it has been demonstrated that polynmetallic particles can significantly enhance the stability and electrical activity of the particles (Zhao et al., 2019). In view of this, polynmetallic particles present dramatic possibilities in the design of electrode interfaces as an alternative to monometallic particles.

Herein we exploited a biosensing regime employing Ag-Au nanoparticles (Ag-Au NPs) encapsulated in Ti3C2 as an electrochemical interfacial material coated onto the GCE surface, which enabled a remarkable improvement in electron transfer efficiency, accentuating the unique characteristics of high stability and electrocatalytic activity of Ag-Au NPs. The role of Au is to immobilize the ST1 strand by forming Au-S bonds. Ag can also improve the charge transfer on the electrode due to its outstanding electrical conductivity. Moreover, the introduction of Ag can enhance the stability of monometallic Au nanoparticles and improve their aggregation-prone properties. Utilizing the stem-loop structure of HP DNA and the cleavage property of exonuclease III (Exo III), the target input is converted into the essential ssDNA output to establish the three-way junction structure for stacked dumbbell hybridization chain amplification (DHCA). After the amplification, a large amount of Ru(phen)3Cl2⁺ was captured by insertion into the dsDNA grooves between the and finally the signal is output by the ECL photoelectric system (Chen et al., 2012). Most importantly, our strategy was successfully validated in the monitoring of SARS-CoV-2.

2. Materials and methods

2.1. Materials and chemical reagents

Gold chloride tetrahydrate (HAuCl4), tris(2-carboxyethyl) phosphonate hydrochloride (TCEP), dichlorotris(1, 10-phenanthroline) ruthenium hydrate (Ru(phen)3Cl2⋅H2O), triethylamine (TEA) with other analytical grade chemicals were obtained by Aladdin Biochemical Technology Co. Ltd. (Shanghai, China). Silver nitrate (AgNO3) and Ti3C2 were purchased from Boer Chemical Reagent Co. Ltd. (Shanghai, China) and XFNANO (Nanjing, China), respectively. L-ascorbic acid (AA) was from Sigma-Aldrich Co. Ltd. (Shanghai, China). T4 DNA ligase and buffer reagents consisting of tris-borate-EDTA (TBE) and phosphate buffer saline (PBS) were obtained from Sangon Biotechnology Co. Ltd. (Nanjing, China). The related sequences used in the experiment are depicted in Table S1.

2.2. Instrument parameters and test conditions

Morphological characterizations were measured by transmission electron microscopy (TEM) using JEOL JEM-2100 F and scanning electron microscopy (SEM) using FEI Quanta 250 FEG. ECL measurements were conducted by an ECL-6B apparatus equipped by the State Key Laboratory of Analytical Chemistry for Life Sciences, Nanjing.
University. ECL experiments were conducted with a three-electrode system that consisted of a 3 mm diameter glassy carbon electrode (GCE), an Ag/AgCl reference electrode and a Pt counter electrode in PBS (0.1 M, pH 7.4) containing 20 mM TEA with a voltage range from 0 to 1.3 V and a scanning rate of 0.1 V/s. X-ray photoelectron spectroscopy (XPS) characterizations were obtained from Thermo Fisher K-Alpha. UV–vis absorption spectra were available on a Spectra Max M5e (Molecular Devices Co. Ltd, USA). The validations of the electrode modifications were accomplished by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), which were experimentally studied with a scanning rate of 100 mV/s and frequency range of 100 kHz to 0.1 Hz in PBS buffer (0.1 M, pH = 7.4) containing (Fe(CN)₆)₃⁻/₄⁻ (5 mM), respectively.

2.3. Synthesis of Ti₃C₂@Ag-Au

Ag-Au nanoparticles were prepared according to the following method. Add 100 μL of 1 % HAuCl₄ aqueous solution to 10 mL of ultrapure water. Then 250 μL of 8 mM AgNO₃ was quickly injected under stirring, and the color of the solution changed from light yellow to beige. After being mixed homogeneously, 1 mL of 100 mM AA was added and the color changed to gray-black. Next, Ti₃C₂@Ag-Au complexes were prepared by the following procedures. 2 mL of Ti₃C₂ (2 mg mL⁻¹) was mixed with 5 mL of Ag-Au nanoparticles, and then the reaction was carried out at room temperature with stirring for 3 h to make the Ag-Au nanoparticles fully adsorbed onto Ti₃C₂.

2.4. Fabrication of biosensor for SARS-CoV-2 monitoring

The following procedures were followed for the construction of the sensor for the determination of the SARS-CoV-2. Firstly, the GCE electrode was polished iteratively with 0.3 and 0.05 μm alumina powder. Subsequently, the electrode was immersed in an ethanol and water solution for ultrasonic washing. Afterwards, the surface-treated and cleaned GCE was plunged into 0.5 M sulfuric acid under a cyclic voltage of –0.2–1.2 V for CV activation until steady CV profiles were observed. 10 μL of the synthesized Ti₃C₂@Ag-Au nanocomplex solution was dripped on the surface of the activated electrode and then dried under nitrogen protection (GCE/Ti₃C₂@Ag-Au). The modified electrode was subsequently soaked in 50 μL ST1 solution (1 μM) with prior activation treatment by adding 0.05 mM TCEP overnight to eventually link the ST1 probe on the modified GCE by forming Au-S bonds (GCE/Ti₃C₂@Ag-Au/ST1). Then, the electrode was dipped in the MCH solution (1.0 mM) for 1 h to passivate the electrode surface, forming (GCE/Ti₃C₂@Ag-Au/ST1/MCH). Finally, the sensor for SARS-CoV-2 monitoring was successfully constructed after the above procedures.

2.5. SARS-CoV-2 determination procedures

First, the HP probe solution was heated to 95 °C and maintained for 5 min, followed by reacting for 3 h in a 4 °C refrigerator to constitute a stabilized hairpin-structured conformation. The HP (1 μM) probe was blended with various target concentrations for 2 h and reacted with exonuclease III (0.5 U mL⁻¹) for 50 min at 37 °C. Then, the blended solution was heated to 65 °C to deprive the activity of Exo III. Therefore, the cycling amplification reaction (CAR) product was obtained. DH1 and DH2 were heated to 95 °C and kept at this temperature for 5 min, respectively, then cooled quickly to 4 °C, followed by reacting for 2 h to form the double-HP-structured conformations. The constructions of cyclic DH1 and DH2 followed the procedures described below. Specifically, DH1 or DH2 (with 5' phosphorylated end and a final concentration of 4 μM), T4 DNA ligase buffer, and T4 DNA ligase (with a final concentration of 20 U μL⁻¹) were blended and reacted at 16 °C for 5 h. Afterward, the reacting enzyme was heated to 65 °C for 10 min to render it inactive. After that, the modified electrodes (GCE/Ti₃C₂@Ag-Au/ST1/MCH) were submerged into the mixture of cyclic DH1, cyclic DH2 and CAR product to react for 2 h. Afterward, the modified electrode was immersed in Ru(phen)₃³⁺ (2 mM) for 7 h. Most importantly, the non-specific substance was washed with PBS (0.1 M, pH = 7.4) after each reaction. Finally, the above-modified electrode was subjected to relevant ECL tests and electrochemical validations were conducted.

3. Results and discussion

3.1. Principle of the proposed sensing strategy

Our HP DNA-assisted target cycling and dumbbell hybridization signal amplification for prompt determination of the SARS-CoV-2 are illustrated in Scheme 1. The first segment is a target cycling using the functional stem-loop structure of HP probe and the dsDNA cleavage property of Exo III that specifically cleaves flat or concave end from 3' to 5' direction. The stem and loop of the HP probe are engineered with complementary sequences to the target, so that the target can easily lock the HP DNA and form a duplex with it. In the newly formed DNA duplex, the 3' end of the HP strand lies at the flat end and thus can be considered as a cleavage initiation site for Exo III. However, the target strand has over 4 bases that are not complementarily paired, obviating the possibility of being digested by Exo III. Accompanied by Exo III digestion of the double-stranded binding sequence of the HP DNA, ST2 is produced and the target is released. Thus, the released target subsequently cycles the above process of unlocking HP and Exo III continuously executes the digestion of HP, generating a large amount of ST2. The second segment is the construction procedure of the electrode sensor. In the preliminary process, Ti₃C₂@Ag-Au complex with high electron transfer efficiency was coated on the GCE surface. The ST1 probe was then trimmed on the Ti₃C₂@Ag-Au surface through Au-S covalent bonds. With the incorporation of ST2 generated in the front segment and cyclic hairpins (DH1 and DH2), ST1 and ST2 initially constitute a three-way junction, followed by its single-stranded portion opening the stem of the cyclic hairpins. Ultimately, DH1 and DH2 progressively form a cascade of nanostructures with a mass of tightly packed dsDNA on the modified GCE surface. Then dsDNA grooves supplied many insertion sites for (Ru(phen)₃³⁺), contributing to a formidable ECL signal output.

3.2. Morphological, elemental, and structural characterizations of the Ti₃C₂@Ag-Au

We characterize the synthesized Ti₃C₂@Ag-Au complex as depicted in Fig. 1A) as well as Ag-Au nanoparticles (as depicted in Fig. 1B) by the SEM method. SEM image of Ti₃C₂@Ag-Au indicates that there are some particles adsorbed on Ti₃C₂. Similarly, the Ag-Au nanoparticles in correlation with the size (seen from Fig. 1B inset) and morphology of the
particles on the Ti$_3$C$_2$ surface, which confirms that Ag-Au nanoparticles are indeed adsorbed on Ti$_3$C$_2$. To confirm that the particles adsorbed on the surface of Ti$_3$C$_2$ are indeed Ag-Au bimetallic nanoparticles, we investigated the elemental distribution and element ratio of the particles by enlarging the EDS mapping images, as shown in Fig. S1 and Table S3. The distribution of Au and Ag on the surface of the particles confirms that they are indeed Ag-Au bimetallic particles. The TEM image of Ag-Au NPs was showed in Fig. S2. XRD was also used to analyze the
structure of the Ag-Au particles (as depicted in Fig. S3A). We observed characteristic peaks at $2\theta = 37.9^\circ$, 44.3$^\circ$, 64.6$^\circ$ and 77.4$^\circ$, corresponding to Au and Ag atomic planes (111), (200), (220) and (311), respectively, which were close to the Joint Committee on Powder Diffraction Standards file no. 65-8424.31. We similarly investigated the XPS spectra of Ag-Au NPs (as depicted in Fig. S3B). We could see characteristic peaks of Au 4f (83.8 and 88.3 eV) and Ag 3d (373.4 and 367.5 eV), which further indicated the synthesis of Ag-Au NPs. In order to further validate the synthesis of the complex, we characterize the elemental distribution of the complex. As demonstrated in Fig. 1C, Au and Ag elements were spread on the surface of Ti$_3$C$_2$ with C and Ti elements as the dominant elements, which further verifies the successful synthesis of the complex.

Moreover, we demonstrate the synthesis of the Ti$_3$C$_2$@Ag-Au complex by UV–vis spectra (as depicted in Fig. 2A). We analyze the characteristic variations during the synthesis of the complex by analyzing the absorption spectra of Ti$_3$C$_2$, Ag-Au NPs. The Ti$_3$C$_2$@Ag-Au, Ti$_3$C$_2$ (black curve) exhibits no significant absorption peaks, while Ag-Au NPs (red curve) represents the characteristic peaks of Ag and Au at approximately 442 nm and 525 nm, respectively, which are also observed in the Ti$_3$C$_2$@Ag-Au complex (blue curve). Moreover, as depicted in Fig. 2B, when Ti$_3$C$_2$ is incorporated, the electronegativity is enhanced compared with Ag-Au nanoparticles, resulting in more cations being adsorbed to the electrode and a more efficient electron transfer. The XPS profile of Ti$_3$C$_2$@Ag-Au complex as depicted in Fig. 2C exhibits two significant peaks at 284 and 460 eV, corresponding to C 1s and Ti 2p, respectively, indicating the existence of Ag-Au NPs. Additionally, Fig. 2D showed the EDS spectra of Ti$_3$C$_2$@Ag-Au nanocomposite and Ti$_3$C$_2$, indicating that the Au and Ag elements are present in the Ti$_3$C$_2$ with C and Ti as the main elements.

### 3.3. Characterizations of sensor fabrications

CV and EIS elaborated the fabrication processes of the sensing system, and the results are presented in Fig. 3. Fig. 3A shows the schematic of the sensor modifications. The Fe(CN)$_6^{3-/4-}$ redox peaks are very sensitive to the electrode interface and thus can be employed to validate the electrochemical behavior during the electrode modifications. The CV curves (as depicted in Fig. 3B) indicate that the bare GCE (curve a) shows a couple of standard reversible redox peaks. However, when the Ti$_3$C$_2$@Ag-Au complex is assembled on the electrode (curve b), an enhanced current is observed due to the superior charge conductivity characteristics of Ti$_3$C$_2$, Ag, and Au nanoparticles. Afterwards, with the assembly of the ST1 probe (curve c), the current decreases due to the fact that the DNA nucleic acid backbone behaves electronegatively and therefore excludes the Fe(CN)$_6^{3-/4-}$. Similarly, the modification of MCH (curve d) decreases the current due to that the MCH blocks the electron transfer of Fe(CN)$_6^{3-/4-}$ with GCE. As DHCA process is completed (curve e), the modified GCE surface is equipped with more electro-negative nucleic acids, thus further lowering the current. The EIS spectra (as depicted in Fig. 3C) also account for the electrochemical behavior during the sensor constructions. The semicircular arc of the EIS spectra reveals that the bare GCE (curve a) exhibits a significant decrease in resistance after being modified on the Ti$_3$C$_2$@Ag-Au complex (curve b) that can dramatically promote the electron transfer efficiency. However, due to the modifications of ST1 (curve c), MCH (curve d) and the completion of DHCA (curve e), the EIS resistance value becomes larger due to the blocked electron transfer between the GCE and the Fe(CN)$_6^{3-/4-}$.

Similarly, we investigate the superiority of GCE/Ti$_3$C$_2$@Ag-Au-based sensors and the ECL luminescence mechanism as depicted in
Fig. S2. From the comparisons between (a), (b), (c), it can be seen that the signal amplification effect is related to the type of modified nanomaterials and that GCE/Ti$_3$C$_2$@Ag-Au is better than Au nanoparticles. The comparison between (c) and (d) shows that the co-reactant TEA is involved in the ECL luminescence process. Based on the above phenomenon and combined with the previous work (Fan et al., 2021b), we summarized the Ru(phen)$_3^{3+}$ luminescence mechanism as illustrated in the following Eqs. 1–4. At first, Ru(phen)$_3^{3+}$ oxidizes with TEA to produce the cationic radicals TEA•$^+$ and Ru(phen)$_3^{3+}$ (Eq. 1). Then TEA•$^+$ rapidly deletes a proton to produce TEA• (Eq. 2). At this point, the Ru(phen)$_3^{3+}$ with strong oxidability and TEA• with strong reducibility undergo an oxidation reaction to produce the excited state Ru(phen)$_3^{2+*}$ and TEA (Eq. 3). Finally, Ru(phen)$_3^{2+*}$ decays to the ground state Ru(phen)$_3^{2+}$ and releases energy (Eq. 4).

$$\text{Ru(phen)}^{3+} + \text{TEA} \rightarrow \text{TEA}^{+} + \text{Ru(phen)}^{3+}$$ \hspace{1cm} (1)

$$\text{Ru(phen)}^{3+} + \text{TEA•} \rightarrow \text{Ru(phen)}^{3+} + \text{TEA•} + \text{H}^{+}$$ \hspace{1cm} (2)

$$\text{Ru(phen)}^{3+} + \text{TEA} \rightarrow \text{Ru(phen)}^{2+*} + \text{TEA}$$ \hspace{1cm} (3)

$$\text{Ru(phen)}^{2+*} \rightarrow \text{Ru(phen)}^{2+} + h\nu$$ \hspace{1cm} (4)

3.4. Reaction validations and superiorities of polymetallic Ag-Au NPs and our DHCA-based strategy

We verified the hairpin-assisted cycling and DHCA by polyacrylamide gel electrophoresis (PAGE). As shown in Fig. S4A, the target, HP DNA and the duplex of target and HP DNA showed bright single bands (lanes 1–3), but with Exo III cutting the duplex portion from 3′, the target and short bands (lane 4) were left. As shown in Fig. S4B, lanes 1–3 show ST1, DH1 and DH2. ST1 does not react directly with DH1 (lane 4). However, ST2 combined with ST1 can react with DH1 (lane 5). In addition, with the introduction of DH2, a slower band was observed (lane 6). All these evidences supported the occurrence of DHCA. Polymetalllic NPs are used as an alternative to monometalllic NPs for bioassays and it is crucial to determine their stability. Fig. 4A showed that UV–vis spectra of Ag-Au NPs did not change significantly within 20 days, indicating that Ag-Au NPs still exhibited excellent stability without the addition of stabilizers, which showed that Ag-Au NPs had potential value in replacing Au NPs in the bioanalysis area. We then compared the ECL responses of the assays performed by HCR and DHCA methods, respectively, as depicted in Fig. 4B. The ECL intensity of DHCA was considerably enhanced compared to that of HCR at the same concentration (1 pM) because the compact conformation of DHCA could enrich more Ru. Therefore, our protocol could obtain a stronger ECL intensity, which significantly enhanced the signal amplification and improved the monitoring sensitivity.

3.5. Analytical performances of the DHCA-based biosensor

We have evaluated the performance of the sensor by analyzing the ECL responses of the sensor at different target concentrations, in the presence of non-specific targets, in the continuous potential scanning condition and during reproducible fabrications of the sensor. Fig. 5A illustrates the ECL responses under different concentrations that ECL
performances are enhanced upon increasing concentrations. From the inset in Fig. 5A, we can see that the ECL intensity and target concentration present an excellent correlation with a linear regression equation of \( y = 4425.2 + 1275.64 \lg C \) \( (R^2 = 0.9989) \), where \( y \) represents the ECL intensity and \( \lg C \) refers to the logarithmic value of the target concentration. The limit of detection (LOD) is 0.59 fM according to 3\( \sigma \) method, which is comparable to the sensitivity reported in previous work (see table S2). We evaluated the specificity of the sensor (as depicted in Fig. 5B) which is essential to distinguish virus analogues and to implement accurate analysis of the target virus. In this work, we discriminated non-specific sequences, including RdRp genes from SARS-CoV, Frankfurt-1 and BM48–31/BGR/2008, at 10-fold target concentration. The results revealed that in the presence of the target, the ECL intensity values of other non-specific sequences were approximate to the blank sample and significantly smaller than the ECL intensity of the target sequence, indicating the superior specificity of our sensing method. In addition, to investigate the stability of the sensor, we explored the ECL performances of the sensor at 10 fM, 1 pM and 0.1 nM under 20 cycles of potential scanning as shown in Fig. 5C. The calculated relative standard deviation (RSD) were 2.9 %, 1.8 % and 2.2 %, respectively, indicating the excellent stability of our sensor. Similarly, the performances of six electrodes at 1 pM (as depicted in Fig. 5D) were also studied with a calculated RSD of 1.84 %, implying the outstanding repeatability of our sensor. We also simulated the assay process of detecting targets in environmental samples such as exposed commodities and food products. 1 pM of the targets were sprayed on bags and pork, respectively, and then stored in a 4 °C refrigerator. After one week, the target solution was collected from the environmental samples and then tested using our method. As shown in Fig. 4C, the larger S/N values (12.8 and 12.3 for the packaging bags and pork, respectively) indicate that our protocol is still effective in detecting the target in environmental samples.

### 3.6. Application of the sensor in biological samples

We estimated the accuracy of the sensor for application in real sample analysis as depicted in Table 1. Samples to be determined were spiked in 10-fold and 20-fold dilutions of real human serum, and then the assay capabilities of the sensor were investigated. All the results indicated that the recoveries of the sensors ranged from 99.12 % to 103.33 %, indicating that our proposed method demonstrates high accuracy in the determination of complicated samples.

### 4. Conclusions

In summary, we successfully implemented the analysis of SARS-CoV-2 using polymetallic particles and mxene complexes (Ti$_3$C$_2$ @Ag-Au)

| Sample          | Target   | Recovery (%) | RSD (% n = 3) |
|-----------------|----------|--------------|---------------|
| Spiked          | Detected |              |               |
| Human serum (10 %) | 10 fM    | 10.44 fM     | 104.4         | 2.9 |
|                 | 0.2 pM   | 0.2056 pM    | 102.8         | 3.2 |
|                 | 1 pM     | 0.9654 pM    | 96.54         | 2.5 |
|                 | 20 pM    | 20.64 pM     | 103.2         | 3.8 |
| Human serum (5 %) | 10 fM    | 9.635 fM     | 96.35         | 3.1 |
|                 | 0.2 pM   | 0.2078 pM    | 103.9         | 3.3 |
|                 | 1 pM     | 1.048 pM     | 104.8         | 2.7 |
|                 | 20 pM    | 20.59 pM     | 103.0         | 2.7 |

Table 1: Determination of target in 10-fold and 20-fold real human serum.
complex), HP-assisted target cycling, and DHCA techniques. Ti$_3$C$_2$ @Ag-Au is an efficient interfacial material that remarkably boosts the nucleic acid capacity and electron transfer efficiency on the electrochemical interface, thereby enhancing the efficiency and intensity of ECL. Impressively, the input SARS-CoV-2 target enabled the enrichment of short-stranded DNA (ST2) through HP structural features and the cleavage property of Exo III with 3′ to 5′ direction, which was essential for the construction of three-way nodal structures to support DHCA. Although the hairpin-assisted cycling and DHCA increases the complexity of the reaction, it enhances the assay capabilities including sensitivity and specificity. In addition, the sensor proved to be feasible for the monitoring of SARS-CoV-2 and was successfully implemented in complex biological and environmental samples.

Environmental implication

Early and rapid detection of SARS-CoV-2 plays an important role in suppressing pandemics and reducing the lethality of the virus. We constructed a three-way junction structure on the surface of polyanionic particles and Ti3C2 complexes to support the dumbbell hybridization chain amplification (DHCA) for SARS-CoV-2 detection both in environmental conditions and real human serum samples. Our proposed SARS-CoV-2 monitoring strategy obtained a limit of detection (LOD) of 0.59 fM with high specificity and reproducibility, which was of guiding significance for currently monitoring methods of mutant SARS-CoV-2 in biological and environmental conditions.

Supplementary data

Supplementary data to this article can be found online at ****.

CRediT authorship contribution statement

Biaoliang Wu: Data curation, Writing – review & editing. Xiaojie Wen: Formal analysis. Jiaying Cui: Data curation. Xiangpeng Qiao: Material synthesis; Zheng Li: Probe synthesis. Yuanyun Gong: Material synthesis. Qianli Tang: Data curation, Writing – review & editing. Kai Zhang: Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2022.129868.
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