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The Omicron variant has been reported to have undergone 50 mutations [4], giving rise to multiple variations to the spike protein. Presently, as variants of concern (VOC), such as Delta and Omicron [3]. However, since spike proteins produce varying degrees of conformational change, a single catcher may not be able to precisely deal with new variants, such as Omicron with a “very unusual constellation of mutations” [3].

Photoelectrochemical (PEC) sensing, based on the photoelectric conversion effect induced by the photoactive materials, has attracted increasing attention in bioanalysis recently [10,11]. By coupling photoexcitation process of the photosensitive materials and electrochemical detection via photoelectric conversion, PEC sensing can exhibit lower background noise and higher sensitivity than conventional electrochemical/chemiluminescent methods, owing to the completely separated energy forms of material excitation process and signal output [12]. Besides, the electrical signal pattern endows PEC sensing with many merits (e.g., simpler instrumentation, lower cost, miniaturization,  

1. Introduction

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a severe public health and economic crisis worldwide [1]. Although over 12 billion vaccine doses have been administered, the confirmed cases are continuously rising [2]. Worse still, SARS-CoV-2 has gradually evolved into different lineages, and multiple mutants, has caused widespread panic and concern worldwide. The rapid antigen detection method via a single ligand recognition, although currently implemented in many countries, remains challenging for mutated antigens. Herein, we present a novel strategy using a dual recognition by two types of targeted ligands, based on photoelectrochemical (PEC) sensing for detection of SARS-CoV-2 spike protein. To demonstrate this strategy, the specific antibodies are modified onto the photoactive material with a supported nanostructure, created by loading the Pt nanoparticles onto MoS₂ nanosheets (Pt/MoS₂) to boost photon-to-electricity conversion efficiency. By subsequent binding of the targeted aptamers to the Au polyhedra, which act as a signal amplifier to suppress PEC photocurrent by competing with the Pt/MoS₂ for the absorption of excitation light energy, the dual recognition is successfully achieved. The constructed biosensor not only shows satisfactory stability, high sensitivity, and selectivity, but is effective for test of the pseudovirus of SARS-CoV-2. The work provides useful advance for the development of PEC biosensors for sensitive detection of SARS-CoV-2.
portability and easy integration), distinct from spectroscopy detection technologies that usually involve complex and expensive equipment [13]. Specifically, to enhance analytic performance of PEC systems for practical applications, one of the key steps is to develop optoelectronic materials with good light absorption and broad spectral response [14]. In this respect, two-dimensional (2D) nanomaterials have attracted huge interest for PEC sensing, owing to their unconventional physiochemical attributes [11,15-17]. With single/few atoms thickness, these ultrathin nanostructures (typically nanosheets) possess high specific surface areas and extraordinary surface properties (e.g., light/ultrasonic/magnetic responses) because of exposure of abundant atoms on the surface of nanosheets (NSs), thus offering opportunities to develop advanced PEC sensors. As a typical example, few-layered molybdenum disulfide (MoS$_2$) NSs have shown potential in photoelectrochemistry, due to unique optical and electrical properties [18,19]. However, rational design and engineering of 2D nanomaterials to regulate their PEC properties and sensing performance remain a challenge.

Among the various synthetic approaches to access the MoS$_2$ NSs, hydrothermal synthesis as one classical bottom-up method shows merits of high yields and convenience of large-scale production [20]. However, the 2D nanostructure is easily to be self-stacked or aggregated, which could limit its practical applications. Besides, the resulted products often consist of two crystal phases with semiconducting 2H and metallic 1T. Although 1T-phase MoS$_2$ had better electrical conductivity than the natural 2H counterpart, it is thermodynamically metastable due to the distorted crystal structure [20,21]. To improve stability of the MoS$_2$ NSs, one of effective strategies is functionalization with metal nanostructures (e.g., Pt, Au, and Ag nanoparticles), by chemical reduction or photoreduction deposition [22-24]. Moreover, by introducing these plasmonic metal nanostructures, the photogenerated electrons could be more easily to be captured, thereby promoting photoelectric conversion efficiency for sensitive detection of a variety of biological analytes, such as small molecules (e.g., glucose [25]), peptide [26], and RNA [27]. However, the potential of the MoS$_2$-metal heterostructures as PEC biosensors has less explored for the detection of SARS-CoV-2.

On the other hand, to better satisfy practical requirements, it is crucial to effectively integrate the photoactive materials with electrochemical analysis systems and recognition events. Since the Au nanoparticles (NPs) could exhibit strong absorption of visible light to allow energy transfer [28,29], previous studies have shown they could serve as a signal amplifier to compete with the photoactive matrices for the absorption of visible-light, inhibiting the electron transfer and then weakening the photocurrent in PEC sensing [30,31]. Such an amplification strategy could improve sensitivity and selectivity of the sensors, potentially reducing the false positive results in bioanalysis. Meanwhile, the morphology of Au nanostructures is known to largely affect their surface plasmon. The Au polyhedra have displayed superior light absorption to the spherical NPs [32,33]; thus, it could be expected to exhibit enhanced signal amplification for PEC sensing.

Herein, we demonstrate a new dual-targeting strategy based on PEC biosensing for the detection of SARS-CoV-2 spike protein (S1), by using the MoS$_2$ NSs loaded Pt NPs (Pt/MoS$_2$) as a photoactive material and the Au polyhedra serve as a signal amplifier (Fig. 1). This strategy integrates specific antibodies modified Pt/MoS$_2$ by the photochemical immobilization technology (PIT) and the aptamer-functionalized Au polyhedra. Such two types of specific ligands with dual targeting functions can capture multiple binding sites of the spike protein, while the Au polyhedra can suppress photocurrent by steric hindrance and photoelectric conversion dissipation. The constructed dual-ligand recognition platform is effective for the detection of both SARS-CoV-2 S1 protein and SARS-CoV-2 pseudovirus.

2. Materials and methods

The materials and characterization techniques were presented in Text S1 and S2 (see details in Electronic Supplementary material).

2.1. Synthesis of Pt/MoS$_2$ nanocomposites

The synthesis of MoS$_2$ NSs was presented in Text S3. To prepare Pt/MoS$_2$, 1.5 mL of the as-obtained MoS$_2$ dispersion was mixed with cysteine (3 mL, 1 mM) and K$_2$PtCl$_4$ (1.5 mL, 20 mM). Then the resulting mixture was stirred for 24 h at 40 °C in dark. Finally, the products were collected by centrifugation and redispersed in water.

2.2. Synthesis of Au polyhedra

The Au polyhedra were fabricated by the modified secondary seeded growth method. The Au nanocubes were used as seeds to synthesize the Au polyhedra (Text S4). Typically, a growth solution was prepared composed of cetyltrimethylammonium bromide (CTAB, 4.75 mL, 16.84 mM), HAuCl$_4$·3H$_2$O (0.2 mL, 10 mM) and ascorbic acid (0.475 mL, 100 mM). Then, the cysteine solution (5 μL, 100 μM) and Au nanocube solution were added into the growth solution in sequence.

![Fig. 1. Illustration of the dual-ligand recognition PEC biosensor for detection of SARS-CoV-2 by integrating the Pt/MoS$_2$ heterostructure and Au polyhedra.](image-url)
After incubation for 2 h at 30 °C, the mixture was centrifuged (8000 rpm, 5 min) and washed. Next, specific aptamers were modified on the surface of the Au polyhedra for subsequent targeting procedure (Text S5).

2.3. Photoelectrochemical (PEC) measurements

The dual recognition sensor was constructed through a step-by-step modification approach (Test S6). PEC measurement was performed in 0.01 M PBS (pH = 7.4, containing 10 mM ascorbic acid) under a typical three-electrode system and an auxiliary external light source by 300 W Xe lamp. The light source (0.1 W/cm²) was turned on and off every 20 s, and a constant potential of 0.1 V was applied. The electrode was rinsed with 10 mM PBS after each operation.

3. Results and discussions

3.1. Synthesis and characterization of the MoS₂ NSs and Pt/MoS₂ nanocomposites

The as-synthesized MoS₂ NSs had a sheet-like morphology (Fig. S1a and b), with (100) and (110) crystal planes evidenced by the selected area electron diffraction (SAED) pattern (Fig. S1b inset), in line with the previous finding [34]. They were also well characterized by elemental maps (Fig. S2) and X-ray photoelectron spectroscopy (XPS, Fig. S3).

Next, the as-obtained NSs were employed as a support to immobilize the Pt NPs, via in situ reduction of K₂PtCl₄ by cysteine at 40 °C. The NSs in products remained similar morphology before reaction, with several hundred nanometers in size (Fig. 2a). Meanwhile, from a transmission electron microscopy (TEM) image (Fig. 2b), many NPs were immobilized on the NSs. Lots of lattice fringes could be discerned from these NPs by high-resolution TEM (HRTEM, Fig. 2c), with a distance 0.226 nm corresponding to (111) plane of Pt. The results indicate formation of the Pt/MoS₂ heterostructure, which could be further proved by the high-angle annular dark-field scanning TEM (HAADF-STEM), including STEM image (Figs. 2d-2e) and elemental maps (Figs. 2f-2h), with even distribution of elements in the sample. The findings were also obtained from elemental maps by scanning electron microscopy (SEM, Fig. S4) and EDS analysis (Fig. 2i).

Interestingly, the introduction of the Pt NPs (2.3 ± 0.3 nm in size, Fig. 2j) into the material not only afforded stronger UV–vis absorption (Fig. S5), but also caused a larger interlayer spacing for (002) plane of MoS₂ (0.95 nm, Fig. S6) than that of the pure MoS₂ NSs (0.63 nm, Fig. S1c), implying formation of trigonal 1T-phase MoS₂ (1T-MoS₂) [35]. Thus, the 2H-1T phase conversion was induced by the intercalation of Pt atoms, with formation of more 1 T-MoS₂ within the heterostructure. It was further confirmed by the phase composition analysis using Raman spectra. From Fig. 2k, the pure MoS₂ NSs exhibited two characteristic peaks at 381 cm⁻¹ and 407 cm⁻¹, corresponding to the in-plane Mo-S phonon mode (E₂₃) and out of plane Mo-S mode (A₁₃) in 2H-MoS₂, respectively. Meanwhile, the four weak peaks at 153 cm⁻¹, 227 cm⁻¹, 286 cm⁻¹ and 338 cm⁻¹, were related to J1, J2, E₁₃, and J₃ photon modes of 1T-MoS₂, respectively. For comparison, the Pt/MoS₂ gave an obvious enhancement in the signal of 1T phase but a remarkable decline in that of 2H phase, indicating composition increment of the 1T-MoS₂. This intriguing finding was again attested by XPS analysis (Fig. S7).

3.2. Synthesis, characterization, and functionalization of the Au polyhedra

The Au polyhedra underwent a morphological evolution of seeds-nanocubes-polyhedra (Fig. S8), affording a final size of about 150 nm (Figs. 3a-3b). In the Fourier transform infrared spectrum (FTIR, Fig. 3c), the pure Au polyhedra exhibited peaks at 2920 cm⁻¹, 2852 cm⁻¹ and 1580 cm⁻¹, vesting to C-H and N-H of the residual CTAB molecules on the particle surface. When functionalized with aptamers, new peaks at 3365 cm⁻¹, 1408 cm⁻¹ and 1064 cm⁻¹ appeared, corresponding to O-H/N-H, C-N and C-O-C of aptamer, respectively. Such a modification also gave a negative shift in Zeta potential from 12.2 mV to −32.2 mV (Fig. 3d), demonstrating successful functionalization of aptamers on the surface of Au polyhedra.

3.3. Evaluation of electrochemical performance

To obtain a maximum sensitivity, different electric pulses were first applied onto the Pt/MoS₂ cast electrode. From Fig. 4a, the photocurrent gradually enhanced as the electric pulse increased and the highest response was obtained at 0.1 V. However, an excessively high electric pulse will cause an overflow of photocurrent under the same conditions. Then the cyclic voltammetry (CV) curve and photocurrent response of the nanocomposites in 0.01 M PBS with/without ascorbic acid (AA, 10 mM) were tested to investigated the influence of AA (Fig. 4b and S9). The results showed that the introduction of AA greatly enhanced the CV and photocurrent signals, where AA could serve as a sacrificial electron donor in the supply chain.

To evaluate photoelectric conversion performance, the photocurrent of the material was measured. Compared with the MoS₂ NSs, the Pt/MoS₂ displayed markedly enhanced photoelectrochemical performance (Fig. 4c). This could be ascribed to a wider photoresponse range and stronger light absorption by Pt decoration, as revealed by UV–vis spectrum in Fig. S5. To further investigate its stability, time-dependent photocurrent response was tested under more than 20 times on/off irradiation cycles for 1000 s (Fig. 4d). The photocurrent was reproducible over a long time with a relative standard deviation (RSD) of about 4.80 %, indicating excellent stability of the heterostructure. Besides, 50 cycles of CV test were performed without interruption in 0.01 M PBS containing 5 mM [Fe(CN)₃]⁻³/⁻⁴. The stable CV curves also demonstrated the Pt/MoS₂ had good PEC stability (Fig. S10).

3.4. Characterization of the dual recognition sensor

The stepwise modification process of the sensor was monitored by electrochemical impedance spectroscopy (EIS) and CV. The casting of Pt/MoS₂ on the substrate resulted in a decrease in Rₚ and an increase in CV current. Subsequently, the antibody (Ab) was irradiated by the simple PtI₂ technology and fixed on the surface of the nanocomposites (Fig. S11), which caused an increase of Rₚ about 4.6 KΩ and inhibition of the reversible conversion of [Fe(CN)₃]⁻³/⁻⁴ redox couples, indicating that the process achieved effective fixation of Ab (Fig. 5a). Due to weak electron transfer ability and steric hindrance of BSA and S1 protein, further modification to the interface led to blocking effect of charge transfer between the solution and interface (Rₓ = 5.9 and 6.7 KΩ, respectively). After incubating with Au-Apt, the target-based dual recognition approach was completed. The recorded current responses of the sensor in CV curves distinctly reduced while the Rₓ increased (Fig. S12), owing to steric hindrance by the spatial conformational change and electrostatic barrier of the formed Ab-S1-Apt-Au complex.

The photocurrent response of the constructed sensor was further investigated under the natural light of 0.1 W. Benefitting from excellent conductivity and high specific surface area, the Pt/MoS₂ produced a high photocurrent of 2.36 μA (Fig. 5b). The continuous fixation of Ab and BSA resulted in a reduction of the photocurrent to 1.53 μA and 1.26 μA, respectively, due to the increase of steric hindrance effect and obstruction of electron diffusion. The specific binding of S1 protein and Apt-Au induced a significantly reduced photocurrent response (0.41 μA). Compared with S1 protein alone (1.12 μA), the suppression photocurrent generated by the S1-Apt conjugate was increased by 6.2 times (Fig. S13). This could be attributed to three factors: (1) inhibition of the recombination of photogenerated electrons and holes, by means of Pt decoration, improved sensitivity of the sensor; (2) the dissipation of the photoelectric conversion efficiency by the Au polyhedra at the far end; and (3) weak electron transport between negatively
Fig. 2. Characterization of the Pt/MoS$_2$. (a) SEM image, (b) TEM image, (c) HRTEM image, (d) HAADF-TEM image, (e) magnified image of the area in (d), (f-i) EDS maps, (j) size distribution of the Pt NPs (by counting 200 particles) and (k) Raman spectra.
charged protein and Apt, as well as the charge repulsion between negatively charged AA molecules. To achieve the best analytic performance, the concentrations of Ab (40 μg/mL) and Apt-Au (75 μg/mL) were further obtained by parameter optimization (Fig. S14).

3.5. The photoelectrochemical detection of SARS-CoV-2 spike protein

Fig. 6a illustrated the integrated PEC detection platform, where the photocurrent response to S1 protein was measured. After incubating with different concentrations of spike protein (from 0.1 ng/mL to 1 μg/mL), the photocurrent response gradually decreased as protein concentration increased (Fig. 6b and Fig. S15), resulting from a steric hindrance effect. Notably, the sensor could provide a rapid signal acquisition for the target when performing the light “on/off” procedure and passing through the solution, and returned to a stable state quickly after the light source was turned off. The sensor response was as low as 0.1 ng/mL with a favorable linear relationship (R² = 0.9939, Fig. 6c). Compared with other immunosensors (Table S1) including colorimetric sensors, electrochemical immunosensors, and chemiluminescent biosensors [5,36–39], the proposed sensor showed a comparable linear range (0.1–1000 ng/mL) and limit of detection (0.53 ng/mL), indicating that the strategy is a promising method for detecting spike protein.

To study selectivity of the sensor, 10 substances that may cause interference were detected by the same procedure. The concentrations of spike protein and interferences were 10 ng/mL and 100 ng/mL, respectively. From Fig. 6d, the PEC responses of the interfering substances were close to the blank group or had a slight interference. However, there was a memorably reduced photocurrent when the S1 protein was used. Therefore, the developed sensor exhibited excellent selectivity for S1 protein, considering dual targeting of the high-affinity antibodies and aptamers. Besides, by measuring 0.1 ng/mL S1 protein, the sensor showed good reproducibility (Fig. 6e), with a low RSD of 5.69 % (n = 5).

3.6. The real-time detection of SARS-CoV-2 pseudovirus

The performance of the sensor to commercially available SARS-CoV-2 pseudovirus was investigated in PBS (Fig. 7a). In the assay, different numbers of pseudoviral particles (from 10³ to 10⁶ transducing unit (TU)/mL) were incubated with the PEC equipment under same conditions. Fig. 7b showed a gradually suppressed photocurrent strongly dependent on the number of virus particles, along with a lower current base. There was a good linear correlation in the range of 10³ to 10⁵ TU/mL (Fig. 7c). Higher concentrations revealed a stagnation of PEC current inhibition, likely due to the influence of saturation of the bound virus particles and stacking between particles. Taken together, the photoelectric detection results of pseudovirus of SARS-CoV-2 were exciting with a minimum detectable rate of 1000 TU/mL. This is essential to achieve more sensitive and immediate detection of SARS-CoV-2.

4. Conclusions

In summary, we developed an efficient dual ligand-induced PEC biosensor for S1 protein and pseudovirus detection of SARS-CoV-2,
using the Pt/MoS$_2$ as a photoactive material. The introduction of Pt not only induced formation of more 1 T-MoS$_2$ within the Pt/MoS$_2$ heterostructure to improve conductivity of the material, but improved its stability, light harvesting, and photoelectric conversion capacity, leading to enhanced photoelectrochemical performance. As dual-targeted recognition elements, the anti-SARS-CoV-2 S1 protein antibodies and specific aptamers were successfully modified on the surface of Pt/MoS$_2$ and Au polyhedra, respectively. They could specifically capture S1 protein, causing a decrease of photocurrent intensity due to high steric hindrance and low conductivity. The constructed PEC platform exhibited satisfactory performance for S1 protein detection of SARS-CoV-2 and its pseudovirus.

CRediT authorship contribution statement

Haolin Li: Conceptualization, Methodology, Investigation, Data

Fig. 4. Evaluation of photoelectrochemical properties of the Pt/MoS$_2$ heterostructure. (a) The influence of different voltage on the photocurrent response of Pt/MoS$_2$; (b) photocurrent responses of Pt/MoS$_2$ in the presence/absence of AA (10 mM) in 0.01 M PBS; (c) photocurrent responses of MoS$_2$ and Pt/MoS$_2$; (d) PEC stability of Pt/MoS$_2$ ($V = 0.1$ V).

Fig. 5. Functionalization of dual-ligand-based PEC biosensing electrodes. (a) EIS curves and (b) photocurrent response of the step-by-step modification process.
Fig. 6. Illustration and performance analysis of the PEC biosensor. (a) Illustration of detection for S1 protein by the constructed dual ligand PEC platform; (b) photocurrent response vs. different concentration of S1 protein; (c) photocurrent and its change (inset) curve as a function of S1 protein concentration; (d) selectivity and (e) repeatability evaluation of the PEC biosensor.

Curation, Formal Analysis, Visualization, Writing – Original Draft; Jia- lin Zhao: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Visualization, Writing – Original Draft; Ting Wu: Resources, Validation; Zhao Fu: Formal Analysis, Software; Wei Zhang: Conceptualization, Funding Acquisition, Project Administration, Supervision, Writing – Review & Editing; Zheng Lian: Resources, Formal Analysis; Shuangfei Cai: Conceptualization, Formal Analysis, Validation, Writing – Original Draft, Writing – Review & Editing; Rong Yang: Conceptualization, Funding Acquisition, Project Administration, Validation, Supervision, Writing – Review & Editing.

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.snb.2022.132970.

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