Synthesis and Application of CeO₂/SnS₂ Heterostructures as a Highly Efficient Coreaction Accelerator in the Luminol−Dissolved O₂ System for Ultrasensitive Biomarkers Immunoassay

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ABSTRACT: Electrochemiluminescence (ECL) immunoassay amplified by coreaction accelerators has experienced major breakthroughs in ultrasensitive detection of biomarkers. Herein, CeO₂/SnS₂ heterostructures were synthesized and applied as a novel coreaction accelerator to enhance the ECL efficiency of the luminol−dissolved O₂ system for the first time. Benefiting from the well-matched lattice spacing, ultrafine CeO₂ nanoparticles (NPs) were grown in situ on layered SnS₂ nanosheets (NSs) with improved dispersion. CeO₂/SnS₂ as an electroactive substrate can remarkably accelerate the generation of abundant superoxide anion radicals (O₂⁻) to react with luminol anion radical (L⁻), achieving about 2-fold stronger ECL intensity than that of pure CeO₂ NPs. To avoid harsh chemical synthesis of conventional ECL labels and simplify the antibody conjugation process, ferritin (Ft) was served as a natural nanocarrier to immobilize luminol molecules (Lum@Ft) via a one-step linkage, whose protein nanocage can easily connect with the detection antibody. Moreover, a robust site-oriented immobilization strategy using HWRGWVC heptapeptide as specific capturer was further adopted to maintain the bioactivity of the capture antibody on the amine-functionalized CeO₂/SnS₂ surface, which promoted the incubation efficiency markedly. On account of this advanced sensing strategy, a brand new biosensor was constructed for the accurate detection of heart failure biomarkers, which performed with favorable linearity in the range of 0.0001−50 ng/mL and achieved the detection limit of 36 fg/mL.

Electrochemiluminescence (ECL) immunoassay has attracted tremendous attention in modern biosensing fields for protein or peptide determinations.¹,² An advanced ECL sensing strategy rests mainly on the outstanding ECL property of applied luminous reagents. With merits of nontoxic, water-soluble, and ecofriendly properties, luminol has been regarded as an extensively discussed ECL reagent in the past decades.³,⁴ With the use of hydrogen peroxide (H₂O₂) as the coreactant, luminol can generate extremely strong ECL emission in alkaline solutions. However, the self-decomposition of H₂O₂ in electrolytes usually causes the unstable ECL signals of luminol during the detection, which is adverse to the reliability and accuracy of the biosensors.⁵,⁶ As a secondary coreactant, the dissolved O₂ is usually treated as a negligible factor since the reaction efficiency of the luminol−dissolved O₂ system is much weaker than that of the luminol−H₂O₂ system.⁷ Notably, the nontoxic and stable characteristics of dissolved O₂ make it a potential candidate that could eliminate the negative effects of H₂O₂ for stable luminol emission. Nevertheless, great efforts should be devoted to boost the ECL efficiency of the luminol−dissolved O₂ system to fulfill widespread applications in future bioanalysis.

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Coreaction accelerators are known as some specific substances that can significantly promote the generation of reactive intermediate radicals from coreactants to enhance the ECL efficiency of luminophores. Recently, CuS nanoparticles, silver ions, and Pt nanoparticles have all been employed as coreaction accelerators to construct ECL sensing systems.\(^7\)\textsuperscript{−}\(^9\)

However, most of the coreaction accelerators were applied in S\(_2\)O\(_8\)\(^2\textsuperscript{−}\)-based ECL systems, with only several reports on the luminol–dissolved O\(_2\) system. Hence, the designs and applications of novel coreaction accelerators in the luminol–dissolved O\(_2\) system are urgently needed. Ceria (CeO\(_2\)) has drawn extensive attention due to its outstanding electrocatalytic property and oxygen storage ability in electrocatalysis, fuel cells, and sensors.\(^10\)\textsuperscript{−}\(^14\)

We found that CeO\(_2\) nanoparticles (NPs) performed good signal-amplification effect on the luminol–dissolved O\(_2\) system. It should be noted that the feasible reiterative conversion between Ce\(^{3+}\) and Ce\(^{4+}\) in CeO\(_2\) NPs endowed themselves with high catalytic and electrical activities,\(^15\) which could promote the generation of superoxide anion radicals (O\(_2\)\(^{−}\)). This effect can obtain higher production of excited-state species 3-aminophthalate (AP\(^2\textsuperscript{−}\)) for amplifying ECL signals. Even so, the inevitable aggregations of nanosized CeO\(_2\) NPs adversely affected the catalytic and electrochemical properties in practical applications.

To overcome inevitable aggregation and improve electrochemical activity of CeO\(_2\) NPs, tin disulfide nanosheets (SnS\(_2\) NSs) as typical metal sulfides with layered structure and large specific surface area were chosen as the ideal substrate to improve CeO\(_2\) NPs dispersion.\(^16\)\textsuperscript{−}\(^20\) Crystalline analysis of hexagonal SnS\(_2\) and cubic CeO\(_2\) revealed the well-matched lattice spacing between the (100) lattice of SnS\(_2\) and (111) lattice of CeO\(_2\), which might facilitate the fabrication of CeO\(_2\)/SnS\(_2\) heterostructures.\(^21\) Inspired by this, we used a one-step hydrothermal reaction to prepare CeO\(_2\)/SnS\(_2\) heterostructures, in which the CeO\(_2\) NPs were grown in situ on SnS\(_2\) NSs with excellent dispersion. By taking advantage of the lattice matching, the obtained CeO\(_2\)/SnS\(_2\) heterostructures exhibited more efficient property for accelerating the generation of O\(_2\)\(^{−}\) to achieve excellent ECL signal amplification, thus holding great potential in building the luminol–dissolved O\(_2\) system for proteins and nucleic acids determination.

In view of the above, we first proposed a brand new ECL strategy using CeO\(_2\)/SnS\(_2\) heterostructures as a coreaction accelerator to enhance the ECL efficiency of the luminol–dissolved O\(_2\) system. First, amine-functionalized CeO\(_2\)/SnS\(_2\) was prepared as the substrate for incubating capture antibody (Ab\(_1\)) to establish the sensing platform. To maintain the bioactivity of incubated Ab\(_1\), HWRGWVC (HWR), a heptapeptide that can specifically capture the Fc fragment of the antibody in a site-oriented manner, was introduced onto the CeO\(_2\)/SnS\(_2\) surface.\(^22\) This site-oriented strategy has been demonstrated that it can improve the incubation efficiency with better maintained bioactivity than traditional methods in our previous works.\(^23\)\textsuperscript{−}\(^24\) Considering the superiorities of thermal stability, pH tolerance, and enzyme mimic property, ferritin (Ft) as a biocompatible nanocarrier was utilized to immobilize luminol (Lum@Ft) via a one-step cross-linking method for the first time.\(^24\)\textsuperscript{−}\(^25\) Due to the abundant amino groups on the Ft outer nanocage, the detection antibody (Ab\(_2\)) could easily be captured via an amide bond. Using natural protein as a signal label is advanced since conventional harsh chemical synthesis of nanomaterials and complex antibody conjugations can be avoided.\(^26\) More importantly, the luminol molecules can still exhibit high ECL efficiency but with a better stability. On the basis of the above, a sandwich-type ECL biosensor using CeO\(_2\)/SnS\(_2\)−HWR−Ab\(_1\) as the sensing platform and Lum@Ft−Ab\(_2\) as the ECL indicator was successfully developed. Aiming at providing a practical tool...
for early clinical diagnosis of heart failure disease, a typical biomarker named N-terminal-prohormone B-type natriuretic peptide (NT-proBNP) was chosen as the analyte. The biosensor performed with favorable linearity in the range of 0.0001–50 ng/mL and achieved the detection limit of 36 fg/mL.

**EXPERIMENTAL SECTION**

**Preparation of CeO₂/SnS₂ Heterostructures.** The materials, regents, and apparatus are shown in the Supporting Information. The synthesis of CeO₂/SnS₂ heterostructures was conducted in terms of a modified method. After dissolving Ce(NO₃)₃·6H₂O (0.434 g), SnCl₄·5H₂O (7.012 g), CH₃CSNH₂ (4.508 g), and 0.5 g of cetyltrimethylammonium bromide (CTAB) orderly into 40 mL of a mixture containing ultrapure water and alcohol, the resulting solution was quickly transferred into an autoclave for 36 h of reaction at 180 °C. After centrifugation and rinsing, the solid product was finally dried in vacuum at 60 °C. After sintering at 500 °C for 2 h in argon, the pristine CeO₂/SnS₂ was obtained. To realize amino functionalization, 2 mL of 3-aminopropyltriethoxysilane (APTES, 3%) was added in 10 mL of CeO₂/SnS₂ solution (2 mg/mL), and the mixture was stirred for 36 h at 60 °C. After centrifugation and washing, CeO₂/SnS₂ heterostructures covered with abundant amino groups were obtained. The above preparation is shown schematically in Scheme 1A. SnS₂ NSs were synthesized without the addition of Ce(NO₃)₃·6H₂O.

**Preparation of the CeO₂/SnS₂−HWR Bioconjugate.** First, 2 mL of HWR (50 μg/mL) was mixed with 20 mmol of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC) and 20 mmol of sulfo-N-hydroxysulfo succinimide (NHS) for 15 min at 25 °C with the purpose of activating the carboxyl groups of HWR. Then, 2 mL of amine-functionalized CeO₂/SnS₂ solution (2 mg/mL) was added with the HWR solution under continuous stirring for 6 h at 4 °C. In this process, HWR could combine with CeO₂/SnS₂ via the cross-linking of EDC/NHS. After the centrifugation at 10,000 rpm, the CeO₂/SnS₂−HWR was dispersed into 1 mL of phosphate-buffered saline (PBS) (pH 7.4).

**Preparation of Lum@Ft−Ab₂.** First, 8 mL of Ft (50 μg/mL) solution, 200 μL of luminol (10 mmol/L), and 50 μL of glutaraldehyde (GA) (50%) were mixed in a 50 mL beaker by 2 h of stirring to finish the cross-linking (Scheme 1C). The obtained Lum@Ft was purified by dialyzing for 6 h in NaOAc solution (0.1 mol/L, pH 5.5). Then, EDC (40 mmol), NHS (10 mmol), and 100 μL of Ab₂ solution (10 μg/mL) were added orderly into the mixture for 2 h of incubation. By centrifugation at 10,000 rpm, the resulting Lum@Ft−Ab₂ was then scattered into PBS (pH 7.4) and stored at 4 °C.

**Fabrication Process of the Biosensor.** The fabrication diagram of the biosensor is illustrated in Scheme 1B. In the beginning, a clean glassy carbon electrode (GCE) was polished to a smooth mirror surface by 0.3 and 0.05 μm Al₂O₃ slurries. After rinsing with ultrapure water and drying in N₂, the GCE was cleaned and ready for the stepwise modification. In the beginning, 10 μL of CeO₂/SnS₂−HWR (2 mg/mL) was first coated on the clean GCE surface as the substrate. After each step below, the unabsorbed biomolecules were removed by mildly rinsing with PBS (10 mmol/L, pH 7.4). After centrifugation and washing, CeO₂/SnS₂ heterostructures covered with abundant amino groups were obtained. The above preparation is shown schematically in Scheme 1A. SnS₂ NSs were synthesized without the addition of Ce(NO₃)₃·6H₂O.

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analyzer was carried out by immersing the sensor into 10 mL of PBS (0.1 mol/L, pH 8.0). The ECL signals were obtained by scanning the electrode from 0 to 0.6 V with scan rate of 100 mV/s. The photomultiplier tube voltage was set at 600 V during the ECL detection.

■ RESULTS AND DISCUSSION

Characterizations of CeO2/SnS2 Heterostructures and Lum@Ft Bioconjugates. Compositional and morphological data of the as-synthesized CeO2/SnS2 heterostructures and Lum@Ft bioconjugates were characterized. The powder X-ray diffraction (XRD) patterns (Figure 1A) showed two sets of diffraction peaks that indexed to cubic CeO2 (in red, JCPDS no. 43-1002) and hexagonal SnS2 (in green, JCPDS no. 23-0677). By comparing with the scanning electron microscope (SEM) images in Figure 1B and Figure S1, spherical CeO2 NPs were proved that had grown on the SnS2 surface uniformly with fine dispersion. From the transmission electron microscopy (TEM) image of CeO2/SnS2, CeO2 NPs with average diameters of about 20 nm (Figure 1C) could be seen clearly. High-resolution transmission electron microscopy (HRTEM) was further applied to obtain microstructural information on the heterostructures. As seen in Figure 1D, several lattice fringes were corresponding to the (111) and (220) lattice planes of cubic CeO2 and the (100) planes of hexagonal SnS2, respectively. The measured lattice spacing of the (100) plane of SnS2 (3.16 Å) and (111) plane of CeO2 (3.12 Å) were in good agreement with the lattice matching, which promoted the formation of CeO2/SnS2 heterostructures.

X-ray photoelectron spectroscopy (XPS) analysis of CeO2/SnS2 is shown in Figure S2, which corresponded to the Ce, S, Sn, and O elements in the heterostructures. The amino functionalization of CeO2/SnS2 was successfully characterized by Fourier transform infrared (FT-IR) spectroscopy that is shown in Figure S3.

In addition, TEM and HRTEM images of pure Ft (Figure 1E) proved its good dispersibility with no aggregations, and the outer and inner diameters of a single Ft were about 12 and 8 nm, respectively. The UV−vis spectrum (Figure 1F) was to prove the successful cross-linking of luminol with Ft via GA. When compared with the UV−vis spectra of Lum@Ft (green curve), the characteristic peak of luminol (red curve) at 214 nm was slightly red-shifted to 236 nm, which could be explained by the conjugation enhancement between the ferritin nanocage and luminol molecules. Moreover, the characteristic peak of luminol at 347 nm faded away, which may be attributed to the consumption of the −NH2 of luminol during the GA cross-linking with ferritin. Lastly, the characteristic peak of ferritin (blue curve) around 302 nm became wider, which was resulted from the successful cross-linking with luminol molecules.

Advances of HWR in the Antibody Incubation. To conduct the investigation, GCEs modified with pure CeO2/SnS2−HWR and CeO2/SnS2 were both incubated with 20 μg
of Ab1 solution at 4 °C. The unhatched Ab1 were collected after different incubation times for the UV–vis absorption test. It could be observed that the HWR curve leveled off at 180 min, proving that the antibody incubation had finished (Figure 2A). The amount of antibody incubated on the CeO2/SnS2–HWR surface was calculated to be 12.10 μA by using the equation $A = 0.00405 + 0.00909c$ (Ab). Similarly, the immobilized antibody on the CeO2/SnS2 surface was calculated to be 7.70 μg, but this incubation process did not finish until 540 min later. From the comparison it can be concluded that HWR dramatically facilitated incubation efficiency, which was an efficient strategy that can incubate large amounts of antibody in a short time.

Circular dichroism (CD) spectra have been regarded as a simple and reliable tool to characterize the maintenance of antibody bioactivity from different immobilization methods.27 Because the specific absorption peaks of the α-helix in secondary protein structure are shown at wavelengths of 210 and 222 nm,23,24 the antibody bioactivity from different immobilization methods can be distinguished. As shown in Figure 2B, CD spectra of CeO2/SnS2–HWR (curve a), CeO2/SnS2–Ab1 (curve b), CeO2/SnS2–HWR–Ab1 (curve c), and pure Ab1 (curve d) are presented. The CD value of CeO2/SnS2–HWR–Ab1 was similar to that of pure Ab1, but much higher than that of CeO2/SnS2–Ab1, indicating the better maintained bioactivity of Ab1 captured on the CeO2/SnS2 surface by HWR. In short, the specific interaction between HWR and antibody Fc fragment is more efficient than random immobilization via amide reaction in the incubation process, underlining the advance of HWR in improving the antibody incubation efficiency with a better maintained bioactivity.

Increased Electroactive Surface Area of CeO2/SnS2 Heterostructures. The electrical property of CeO2 NPs could be greatly enhanced by the supporting superiority of SnS2 NSs, which can be verified by the increment of the electroactive surface area. To conduct the investigation, GCEs were modified with pure CeO2 NPs and CeO2/SnS2 heterostructures, respectively. Then, cyclic voltammetry (CV) was measured using the commonly used [Fe(CN)6]4−/3− as redox probe. The regression equations are $I = 703.9n^{1/2} + 33.82$ and $I = 1228.4n^{1/2} = 46.63$, respectively. According to the Randles–Svcek equation28,29

$$I_p = \left(2.69 \times 10^6\right)AD^{1/2}n^{3/2}v^{1/2}c$$

It should be noted that $I_p$ stands for the peak reduction current of K3Fe(CN)6. $A$ stands for the electrochemically active area (cm2) of electrodes modified with different materials, $n$ stands for the number of electrons that are transported during the oxidation–reduction reaction ($n = 1$), $c$ is the concentration of the K3Fe(CN)6 ($c = 5$ mmol/L), $D$ is the diffusion coefficient of [Fe(CN)6]4−/3− [$D = (6.70 \pm 0.02) \times 10^{-6}$ cm2 s−1 at 25 °C], and $v$ is the CV scanning rate (V/s).

According to the Randles–Svcek equation, the A values of the CeO2 NPs modified GCE (Figure 2C) and CeO2/SnS2 modified GCE (Figure 2D) were calculated to be 20.21 and 35.28 mm2, respectively.

The remarkably increased electroactive surface areas demonstrated the excellent electrochemical activity of CeO2/SnS2 heterostructures.

**ECL Enhancement by CeO2/SnS2 Heterostructures.** After the successful electrochemical characterization of the biosensor (Figure S4), the important role of CeO2/SnS2 in the luminol–dissolved O2 system was studied. In Figure 3A, the
synchronous CV and ECL responses that were individually measured in different solutions are illustrated. Low ECL intensity (660 a.u.) was obtained from detecting bare GCE (curve a) in N₂-saturated luminol solution. After the modification of pure CeO₂ (2 mg/mL) and CeO₂/SnS₂ (2 mg/mL), no noticeable signal was found (curves b and c), proving that both CeO₂ and CeO₂/SnS₂ could not directly enhance the ECL emission of luminol. Nevertheless, when detecting bare GCE in the O₂-saturated luminol solution (curve d), the ECL signals were greatly increased to 2544 a.u., which indicated that the ECL emission of luminol occurred when using dissolved O₂ as coreactant. With respect to GCE/CeO₂/SnS₂ (curve f), about 2-fold stronger ECL signal (15531 a.u.) was achieved. This fact revealed that CeO₂/SnS₂ could facilitate the ECL emission of luminol, which is more efficient than pure CeO₂ NPs when using dissolved O₂ as the coreactant.

Furthermore, consistent CV curves of bare GCE, GCE/CeO₂, and GCE/CeO₂/SnS₂ tested in different solutions are displayed in Figure 3B. When detecting bare GCE in the N₂-saturated luminol solution, a distinctive anodic peak at 0.6 V was found from curve a. When GCE/CeO₂ (curve b) and GCE/CeO₂/SnS₂ (curve c) were tested in N₂-saturated PBS solution containing 20 mmol of luminol, the anodic currents were found to be increased, while no shift was found from peak potentials. When testing the bare GCE in O₂-saturated solution, the anodic peak (curve d) displayed a negative shift from 0.6 to 0.44 V, which could be regarded as the result of the reaction between luminol and O₂⁻. Then, GCE/CeO₂ and GCE/CeO₂/SnS₂ were both tested in O₂-saturated luminol solution; the current of GCE/CeO₂/SnS₂ (curve f) was found to be stronger than that of GCE/CeO₂ (curve e), which demonstrated that CeO₂/SnS₂ was more efficient than CeO₂ in enhancing the ECL efficiency of the luminol–dissolved O₂ system. All the above results verified that CeO₂/SnS₂ played a crucial role of being a highly efficient coreaction accelerator to react with O₂ to promote the ECL emission of luminol.

Possible ECL Mechanism Discussion. The possible ECL mechanism of our proposed ECL biosensor is described in Figure 3C. Due to the lack of coreactants, no efficient ECL emission of luminol could be generated (a). When dissolved O₂ was used as coreactant, O₂⁻ was produced from the GCE surface which led to a fine ECL response (b). Tiron (1,2-dihydroxy-3,5-benzenedisulfonic acid disodium salt) solution, a specific Ce³⁺ scavenger, was utilized to further prove the existence of O₂⁻ (Figure S5). Notably, as CeO₂/SnS₂ participated in the ECL emission process (c), the production of O₂⁻ was remarkably promoted, which achieved excellent signal amplification. On this basis, a possible mechanism of this biosensor could be described as follows (eqs 1–7).

\[
\text{Route 1:} \quad \text{O}_2^- + e^- \rightarrow \text{O}_2^{*-} \quad \text{(2)}
\]

Figure 4. (A) Calibration curve of biosensors constructed with (red curve) and without (blue curve) HWR incubation to detect NT-proBNP in different concentrations: (a) 100 fg/mL, (b) 1 pg/mL, (c) 10 pg/mL, (d) 100 pg/mL, (e) 1 ng/mL, (f) 10 ng/mL, and (g) 50 ng/mL. (B) Corresponding ECL intensity–time curve to the red curve in panel A. (C) Selectivity of the proposed ECL biosensor. (D) Stability of the biosensor for 11 scans. (E) Reproducibility of five different electrodes in the same batch. (F) Reproducibility of the same electrode in five different batches. Error bars, SD, n = 5.
Table 1. Recovery Results Obtained from Human Serum Detection of NT-proBNP Using the Proposed ECL Biosensor

<table>
<thead>
<tr>
<th>detected concn (ng/mL)</th>
<th>spiked concn (ng/mL)</th>
<th>theor concn (ng/mL)</th>
<th>found concn (ng/mL)</th>
<th>recovery/%</th>
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<td>10</td>
<td></td>
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<td>15.28</td>
<td>101</td>
</tr>
</tbody>
</table>

Route 2:

\[
\begin{align*}
O_2 + 2H_2O + 2e^- & \rightarrow H_2O_2 + 2OH^- \\
Ce^{4+} + H_2O_2 & \rightarrow Ce^{4+} + O_2^{--} \\
\text{ECL emission:} \\
I^{--} + O_2^{--} & \rightarrow LO_2^{--} \\
LO_2^{--} & \rightarrow AB^{--*} + N_2 \\
AB^{--*} & \rightarrow AB^{--} + h\nu
\end{align*}
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**ECL Responses for NT-proBNP.** After the optimizations of the involved experimental conditions (Figure S6), the biosensor was incubated with a series of NT-proBNP concentrations. As shown in Figure 4A (red curve), the calibration curve performed with a favorable linearity toward NT-proBNP concentration in the range from 100 fg/mL to 50 ng/mL. From this linearity, \( I_{ECL} = 1964.1 \lg c + 10205.6 \) was expressed as the linear equation whose correlation coefficient was 0.993. The limit of detection (LOD) of this established biosensor was computationally determined as 36 fg/mL. To further highlight the crucial role of HWR in improving the sensitivity of biosensor, the calibration curve of a biosensor constructed without HWR is displayed in Figure 4A (blue curve) with a linear equation of \( I_{ECL} = 1134.5 \lg c + 6890.1 \), \( R^2 = 0.998 \). From this comparison, the advance of HWR in improving the sensitivity could be further proved. Additionally, the analytical performance of the proposal method was compared with that of other reported methods (Table S1). As seen, it can be sure that our developed biosensor owned obvious advances in the ultrasensitive detection of NT-proBNP than other methods.

**Specificity, Stability, and Reproducibility of the Biosensor.** With aim of evaluating the specificity, four representative interfering proteins (10 ng/mL) in human serum including carcinoembryonic antigen (CEA), insulin, procalcitonin (PCT), and prostate-specific antigen (PSA) were detected. In Figure 4C, ECL biosensors incubated with interferences (10 ng/mL) showed negligible influences. Meanwhile, biosensors incubated with mixed samples (1 ng/mL of NT-proBNP and 10 ng/mL of interferences) also showed slight changes, suggesting that the biosensor had favorable specificity. Operating stability was investigated by scanning the electrodes for 11 cycles. The ECL intensities (Figure 4D) were found with no significant changes with an RSD of 2.19%, illustrating that the stability of the biosensor was good. Reproducibility of the biosensor was inspected by inter- and intra-assay precision. Five different ECL biosensors incubated with NT-proBNP (1 ng/mL) in the same batch were all detected (Figure 4E). Then, the same ECL biosensor incubated with PCT (1 ng/mL) was detected in five different batches (Figure 4F). The RSDs of intra- and interassays were 1.31% and 3.08%, respectively, indicating the favorable reproducibility of proposed ECL biosensor.

**Analysis of NT-proBNP in Human Serums.** Heart failure (HF), one of the most common cardiovascular disorders that frequently exerts a detrimental effect on cardiovascular health, has become a major cause of human mortality. \(^{31,32}\) NT-proBNP has been identified as a representative biomarker for HF early diagnosis. \(^{33}\) With aim of verifying the accuracy and reliability of the biosensor, NT-proBNP was chosen as the representative biomarker for HF diagnostics. The ratio between the actual concentration (defined as added) and the detected concentration based on the linear equation (defined as found) was the recovery. \(^{29}\) In Table 1, the recovery values were from 96.0% to 101% with RSD values from 0.86% to 3.24% (n = 5), highlighting its great prospects in practical applications for NT-proBNP detection in various samples.

**CONCLUSIONS**

In summary, CeO\(_2\)/SnS\(_2\) heterostructures, an excellent coreactant accelerator proposed for enhancing the ECL efficiency of the luminol–dissolved O\(_2\) system, were first synthesized and applied in a highly efficient biosensor for HF biomarker detection. Compared to pristine CeO\(_2\) NPs, the lattice-matched SnS\(_2\) NSs as supporter improved the dispersion of CeO\(_2\) NPs with remarkably increased catalytic and electrical properties. Moreover, using Ft as a natural carrier to immobilize luminol and Ab\(_3\) could perfectly avoid harsh chemical synthesis of nanomaterials and complex antibody conjugations in traditional ECL strategies, making the biosensor construction more efficient and reliable. Furthermore, polypeptide HWR greatly promoted the incubation efficiency, ameliorating the practical performance of the biosensor markedly. The biosensor achieved ultrasensitive NT-proBNP detection in the range of 0.0001–50 ng/mL with an LOD of 36 fg/mL. This strategy provides not only new insights of fabricating CeO\(_2\)-based heterostructures for enhancing the ECL efficiency of the luminol–dissolved O\(_2\) system, but also further insights of using heptapeptide as a site-oriented immobilizer to maintain bioactivity in modern bioanalysis.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.9b03796

Materials and regents, apparatus, molar structure of HWR, SEM image of pure SnS\(_2\) NSs and CeO\(_2\) NPs, FT-IR and XPS analysis of CeO\(_2\)/SnS\(_2\), electrochemical characterization of the biosensor, Tiron test, optimization of experimental conditions, and comparison of this method with other reported methods for NT-proBNP detection (PDF)
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Notes
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