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Anal. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.analchem.0c01238 • Publication Date (Web): 22 May 2020

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Oxygen Vacancy-Enhanced Electrochemiluminescence Sensing Strategy Using Luminol Thermally Encapsulated in Apoferritin as a Transducer for Biomarker Immunoassay

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ABSTRACT: Oxygen vacancies (OVs) enhanced electrochemiluminescence (ECL) biosensing strategy using luminol thermally encapsulated in apoferritin (Lum@apoFt) as an efficient transducer was investigated for ultrasensitive biomarker detection. By applying the oxygen-defect engineering (ODE) strategy, the OVs enriched cobalt-iron oxide (r-CoFe₂O₄) was fabricated as the sensing substrate to boost the electron mobility and catalyze the generation of superoxide anion radical (O₂^{•-}) for signal amplification. It should be noted that r-CoFe₂O₄ with higher OVs density dramatically accelerated the ECL reaction between O₂^{•-} and luminol anionic radicals, achieving 6.5-fold stronger ECL output than CoFe₂O₄ with no or low OVs density. Moreover, facile encapsulation of approximate 412 luminol molecules in a single apoFt cavity was firstly realized by an efficient thermal-induction method. The obtained Lum@apoFt complexes exhibited well-maintained ECL efficiency and excellent biocompatibility for biological modifications. On this basis, a biosensor was developed for early diagnostics of squamous cell carcinomas by detecting its representative biomarker named cytokeratin 19 fragment 21-1 19 (CYFRA 21-1), from which excellent linearity was achieved in 0.5 pg/mL - 50 ng/mL with a detection limit of 0.14 pg/mL. This work not only put forward a novel idea of creating OVs enriched sensing interface with excellent signal-amplification function but also proposes a facile and robust methodology to design apoFt-based transducers for developing more practical nanoscale biosensors in early diagnostics of diseases.

INTRODUCTION

Biosensors, known as sensing devices that can convert specific biorecognition events into detectable signals, has gained substantial attention in analyzing targets like pathogens, toxins, antigens and nucleic acids.¹⁻⁵ In biosensor constructions, the successful application of a powerful sensing technique is of paramount importance in achieving excellent performances.⁶ Considering the merits of high sensitivity, wide dynamic range, spatial controllability, and facility, electrochemiluminescence (ECL) technique based biosensors have been extensively established for biomarker detection in real samples.⁷ Strikingly, surface modification of advanced nanomaterials on glassy carbon electrode (GCE) can incredibly enhance the electrical conductivity, catalytic activity, and biocompatibility to achieve the excellent signal-amplifying capability of biosensors.^{1, 2} Moreover, widespread participation of excellent ECL luminophores like ruthenium(III) complexes, iridium(III) complexes, luminol, and its derivatives has made it more facile and efficient to develop more innovative signal transducers for biosensor fabrications.⁷⁻¹¹ Despite the pioneering developments in biosensing techniques, effective and innovative strategies are still highly demanded to accelerate the groundbreaking progress of biosensors with higher reliability and wider clinical applications.

Transition-metal oxides with appealing catalytic activity, high abundance, physicochemical stability, and low toxicity have recently attracted significant attention in electro-catalysis, energy materials and batteries.¹²⁻¹⁶ Nevertheless, their biosensing applications are limited due to the inferior electrochemical properties.^{12, 17} To date, the oxygen-defect engineering (ODE) strategy has been advocated in creating oxygen vacancies (OVs) on

the surfaces of transition-metal oxides, which remarkably improved the electrical and catalytic properties.^{12, 14} Inspired by this, we firstly introduced ODE strategy to build a highly-efficient OVs enhanced sensing interface for ECL amplification. Through a controllable solid-state reaction using NaBH₄ as an oxygen scavenger, abundant OVs were created on the nanosurface of cobalt-iron oxide (r-CoFe₂O₄).¹² The crucial role of OVs in enhancing the ECL emission was revealed by employing a traditional luminol-based ECL system using the dissolved O₂ as a nontoxic coreactant. Compared with pristine CoFe₂O₄ and CoFe₂O₄ with low OVs (p-CoFe₂O₄), the r-CoFe₂O₄ was demonstrated that can dramatically accelerate the ECL reaction between O₂^{•-} and luminol anionic radicals to achieve 6.5-fold stronger ECL output, revealing the advances of r-CoFe₂O₄ modified GCE as the sensing element in the biosensor construction.

Ferritin (Ft), which is composed of 24 protein subunits and one ferric nanocore, has been universally studied in nanomaterial science due to its superiorities of uniform size, enzyme-mimic activity, biocompatibility, pH tolerance, and thermal stability.^{18, 19} Recently, modifications of luminol and biomolecules on the outer surface of Ft have been achieved for biosensing.^{20, 21} However, the potential problem was found existed during the modification process. It should be noticed that the binding competition between these two species is subsistent, which can decrease the binding ratio of each other and mutually interfered with their normal functions during the sensing process. To better perform their functions in separate positions, an effective strategy which combines inner encapsulation of luminophores and outer modification of antibodies is firstly proposed by using apoferritin (apoFt), Ft that devoid of ferric nanocore, as the nanocarrier.²² It is worth noticing



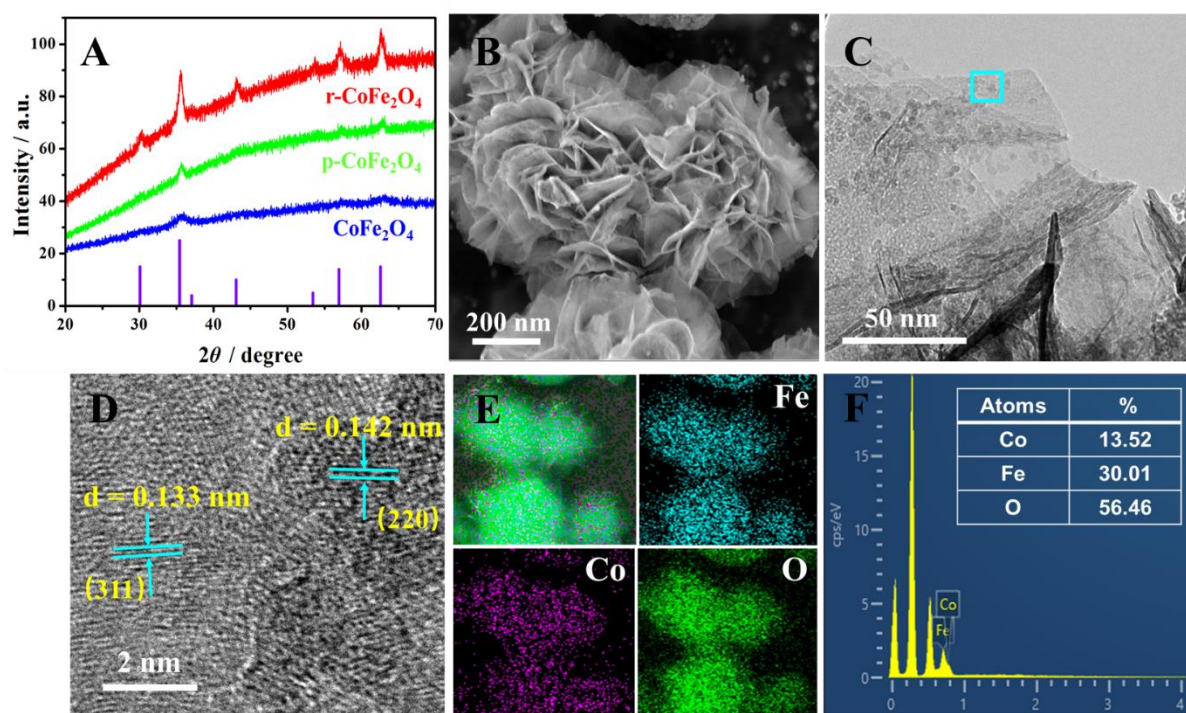


Figure 1. (A) XRD patterns of CoFe_2O_4 , $\text{p-CoFe}_2\text{O}_4$ and $\text{r-CoFe}_2\text{O}_4$. The SEM image (B), TEM image (C), HRTEM image (D), SEM elemental mapping (E) and EDS (F) of $\text{r-CoFe}_2\text{O}_4$.

propyl)-3-ethyl carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) solution at 25 °C for 30 min. After centrifugation, the obtained $\text{r-CoFe}_2\text{O}_4$ -HWR solution stored at 4 °C for electrode modifications.

Preparation of Lum@apoFt-Ab₂. As shown in Scheme 1B, 1 mL of luminol solution (3 mmol/L) was mixed with 5 nmol of apoFt for 15 min of stirring at RT to obtain a uniform mixture. Then, the temperature was slowly adjusted up to 60 °C. After continuous stirring for another 2h, the solution temperature slowly came down to RT to end the preparation process. The apoFt channels will expand along with the uprising of temperature, thus allowing more luminol molecules to enter into the apoFt cavity by the thermal diffusion. As the temperature came down, the channels will contract and the luminol molecules will be encapsulated in apoFt.

Fabrication Process of Biosensor. As shown in Scheme 1C, bare GCE was carefully polished with different sizes of slurry polishing powder. After ultrasonic cleaning with ethanol and ultrapure water for three times, the resultant GCE was dried under N_2 atmosphere. First, 10 μL of $\text{r-CoFe}_2\text{O}_4$ -HWR solution (2 mg/mL) was modified onto the bare GCE surface. Then, 3 μL of BSA (2%) was coated on its surface to block the nonspecific binding sites to ensure the site-oriented capture of Ab₁ by $\text{r-CoFe}_2\text{O}_4$ -HWR, after which 10 μL of Ab₁ solution (100 $\mu\text{g/mL}$) was incubated for 1 h at 4 °C. After incubating 10 μL of CYFRA 21-1 antigen solution, 10 μL of Lum@apoFt-Ab₂ was modified to fabricate the sandwich-like immunocomplex. Notably, the electrode surface will be mildly rinsed with PBS (pH 7.4) solution to get rid of the unabsorbed biomolecules after each modifying step. In this way, the biosensor was completely established for the ECL measurements that conducted in an MPI-F flow-injection ECL detector accompanied by an electrochemical workstation.

RESULTS AND DISCUSSION

Characterizations of $\text{r-CoFe}_2\text{O}_4$ and Lum@apoFt Bioconjugate. Powerful techniques were utilized to characterize the structural, morphological, and elemental composition

information of $\text{r-CoFe}_2\text{O}_4$ composite. In Figure 1A, the X-ray diffraction (XRD) pattern is displayed to characterize the crystal structure of CoFe_2O_4 , $\text{p-CoFe}_2\text{O}_4$, and $\text{r-CoFe}_2\text{O}_4$, from which all the diffraction peaks are found well-matched with CoFe_2O_4 (JCPDS Card No. 79-1744), indicating that OV's have no influence on the crystal structure of CoFe_2O_4 . In Figure 1B, the scanning electron microscope (SEM) image of $\text{r-CoFe}_2\text{O}_4$ shows a better defined flower-like structure than that of CoFe_2O_4 and $\text{p-CoFe}_2\text{O}_4$ (Figure S1), which is further confirmed by the transmission electron microscope (TEM) image in Figure 1C. The high-resolution TEM (HRTEM) image in Figure 1D shows two spacing distances of 0.133 and 0.142 nm, which are corresponded to (311) and (220) crystal planes of CoFe_2O_4 , respectively. Besides, the elemental mapping images in Figure 1E exhibits the elemental composition and even distribution of $\text{r-CoFe}_2\text{O}_4$. In Figure 1F, the atom ratio of Co, Fe, O elements from the energy-dispersive X-ray spectroscopy (EDS) is determined to be 1:2.21:4.17, further demonstrating the formation of CoFe_2O_4 phase. Moreover, the Fourier transform infrared (FT-IR) spectroscopy (Figure S2) are provided to prove the successful amine functionalization of $\text{r-CoFe}_2\text{O}_4$.

X-ray photoelectron spectra (XPS) pattern is provided to confirm the valence states of $\text{r-CoFe}_2\text{O}_4$ and the existence of oxygen vacancies by comparing it with pristine CoFe_2O_4 . In Figure 2A, the XPS data of CoFe_2O_4 and $\text{r-CoFe}_2\text{O}_4$ are provided. As for the core level spectra of Co 2p in Figure 2B, the peaks that situated at 794.1 eV and 778.9 eV are assigned to the Co 2p_{1/2} and Co 2p_{3/2}, respectively. Because there are two satellite peaks that appeared at 803.1 eV and 785.2 eV, the valence of Co is designated as +2 in the composite. Looking at the core level spectra of Fe 2p in Figure 2C, two characteristic peaks can be clearly seen at 722.7 eV and 709.3 eV, which indicates that the valence of Fe is +3. Moreover, the O 1s core-level spectra in Figure 2D displays two typical peaks at 530.2 eV and 529.6 eV, which are attributed to oxygen in hydroxyl groups and metal-oxygen bonds, respectively.¹² As can be further seen in Figure 2D, the shoulder peak located at 531.3 eV is attributed to the defect sites with low oxygen coordination,

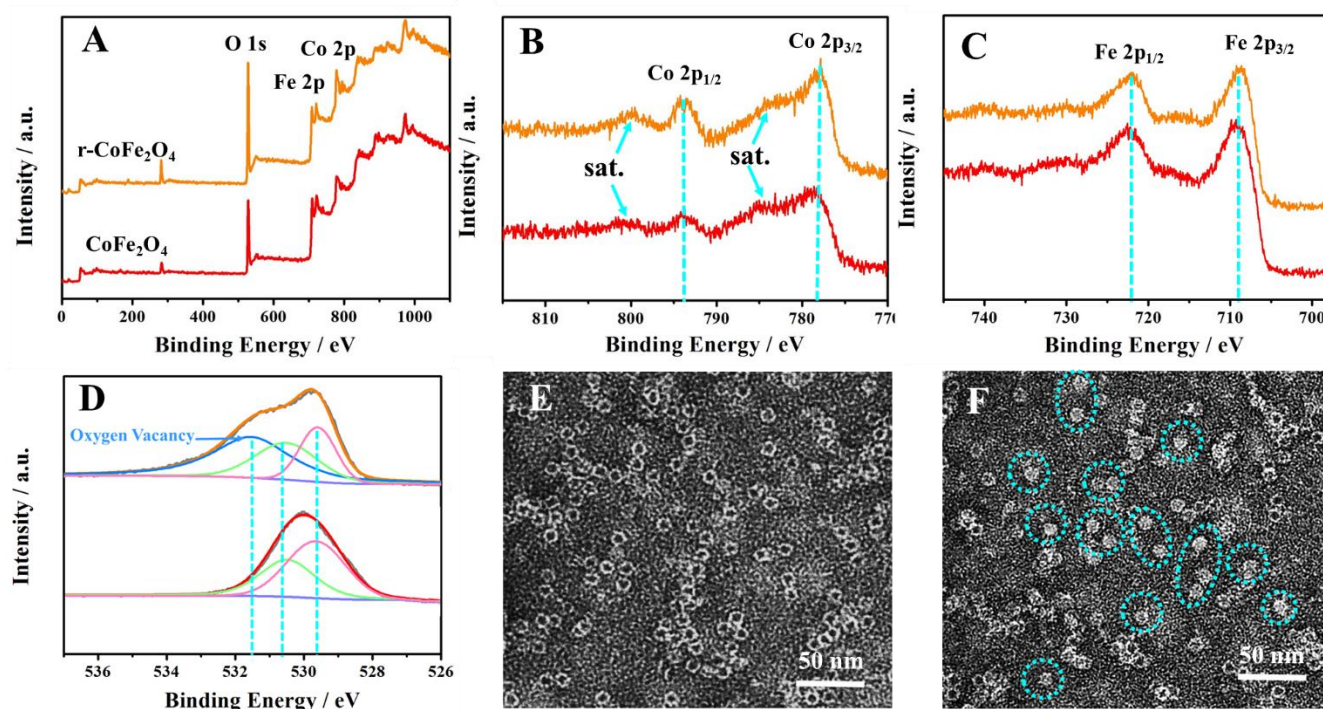


Figure 2. (A) XPS spectrum of CoFe₂O₄ and r-CoFe₂O₄ (A), and the core level spectra of Co 2p (B), Fe 2p (C) and O 1s (D). TEM image of apoFt and the prepared Lum@apoFt.

revealing that the oxygen vacancies have been created on the CoFe₂O₄ surface.^{13, 16}

Considering the inner surface of apoFt is rich in acidic residues like Glu and Asp, the generated high negative charge density makes it more convenient to immobilize the positively charged luminol molecules inside of the cavity.²⁵ After employing the UV-vis spectra demonstration in Figure S3 to prove the successful preparation of Lum@apoFt, TEM was further utilized to characterize the microcosmic morphologies of apoFt and Lum@apoFt using amine molybdate solution (3%, pH 7.4) as the negative staining reagent. Comparing with the TEM image of apoFt (Figure 2E), it can be clearly observed that the Lum@apoFt (Figure 2F) exhibits a similar spherical morphology, indicating the thermally induced encapsulation had negligible effects on the nanocage morphology. Because the amine molybdate molecules penetrated in apoFt through the channels, “black” molybdate cores appeared inside of the apoFt.²⁴ However, most of the apoFt exhibits full “white” cores after the encapsulation of luminol molecules because the amine molybdate molecules could not get into the apoFt. Due to the occupation of luminol molecules, the successful preparation of Lum@apoFt using the proposed thermal strategy.

Increased Electroactive Surface Area of r-CoFe₂O₄. To demonstrate that CoFe₂O₄ with higher OV_s density can dramatically enhance the electrochemical behavior of the sensing interface, the electro-active surface area of CoFe₂O₄, p-CoFe₂O₄ and r-CoFe₂O₄ modified electrodes were scanned by cyclic voltammetry (CV) using [Fe(CN)₆]^{4-/3-} as the redox probe. In terms of the commonly used Randles-Sevcik equation:^{11, 31}

$$I_p = 2.69 \times 10^5 A D^{1/2} n^{3/2} \nu^{1/2} c$$

where the I_p stands for the peak reduction current appears during the scanning process, A is the electroactive surface area (cm²) that will be determined, D is the diffusion coefficient of [Fe(CN)₆]^{4-/3-} whose value can be determined to be $6.70 \pm 0.02 \times 10^{-6}$ cm² s⁻¹ at 25 °C, ν is the CV scanning rate (V/s), and c is the concentration of

the K₃Fe(CN)₆ (5 mmol/L), n is the number of electrons that transported during oxidation-reduction reaction ($n=1$). After scanning these electrodes at different rates, regression equations of pure CoFe₂O₄ (Figure S4A), p-CoFe₂O₄ (Figure S4B) and r-CoFe₂O₄ (Figure S4C) modified electrodes are presented as $I = 522.35 \times \nu^{1/2} + 127.11$ ($R^2=0.982$), $I = 626.27 \times \nu^{1/2} + 155.29$ ($R^2=0.991$) and $I = 987.44 \times \nu^{1/2} + 132.99$ ($R^2=0.989$), respectively. Correspondingly, the electro-active surface areas were calculated to be 15.00 mm², 21.99 mm², and 28.36 mm², respectively, indicating the increased the electro-active surface area by the OV_s generated by the ODE strategy.

Electrochemical Impedance Spectroscopy (EIS) Studies. Though the narrower bandgap of r-CoFe₂O₄ endowed itself with faster electron-transfer ability than the p-CoFe₂O₄ (Figure S5), the enhanced interfacial electron transfer by OV_s was further explored by EIS. The EIS data of electrodes modified with 2 mg/mL of CoFe₂O₄, p-CoFe₂O₄ and r-CoFe₂O₄ were tested in 5 mmol/L of [Fe(CN)₆]^{3-/4-} solution, whose charge transfer resistance (R_{ct}) data were presented in Figure 3A. The R_{ct} value of r-CoFe₂O₄ (curve b) modified electrode is much smaller than that of p-CoFe₂O₄ (curve c) and CoFe₂O₄ (curve d) modified electrodes, which indicates that the higher OV_s density can efficiently accelerate the electron transfer on the sensing interface. Using r-CoFe₂O₄ as the ECL sensing substrate is quite beneficial to boosting the interfacial electron transfer for better performance of biosensor. Besides, EIS was also employed to characterize the step-wise fabrication of the biosensor in Figure S6.

Superiorities of apoFt in luminol immobilization. To prove the improved loading capacity of luminol molecules, high-performance liquid chromatography (HPLC) test was conducted on an Agilent 1260 HPLC system consisting of a UV detector and a Zorbax C18 column (4.6 × 250 mm, 5 μm) using a mobile phase of acetonitrile/water (60:40, v/v). The Lum@Ft solution was obtained by using 5 nmol of Ft to crosslink with 1 mL of luminol (3 mmol/L) via glutaraldehyde under room temperature, then the Lum@apoFt solution was obtained by mixing apoFt with 1 mL of luminol (3 mmol/L) for stirring at 60 °C. After ultrafiltration, filtrates (1 mL)

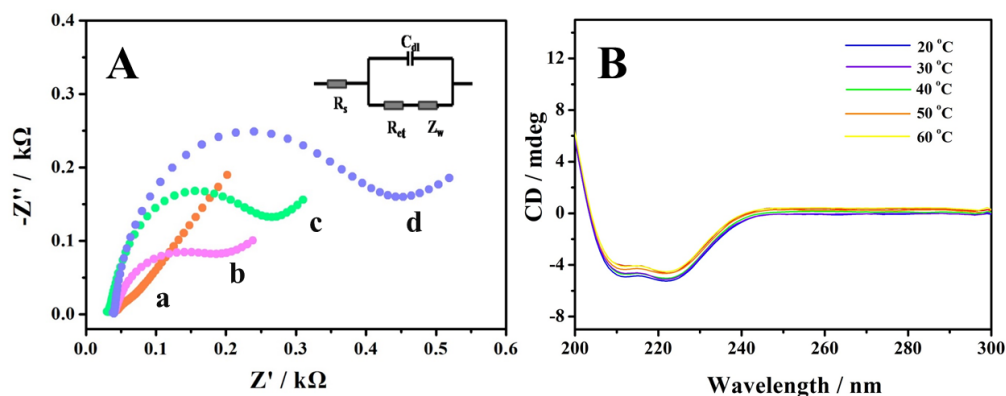


Figure 3. (A) the EIS spectra of bare GCE (curve a), r-CoFe₂O₄ (curve b), p-CoFe₂O₄ (curve c) and CoFe₂O₄ (curve d). (B) the CD spectra of apoFt solution at different temperatures (20 °C to 60 °C).

of Lum@apoFt and Lum@Ft were collected for the HPLC test, the peak areas of which were found to be 169.7 and 328.9 mAU*s, respectively. According to the HPLC calibration curve of standard luminol solutions with concentration from 0.1 mmol/L to 5 mmol/L (Figure S7), the corresponding concentration values of the above were calculated to be 0.94 mmol/L and 1.96 mmol/L. Therefore, the loading capacities of single Ft and apoFt were determined averagely to be 412 and 208 luminol molecules, respectively. Moreover, the reproducibility of this proposed thermally induced encapsulation method was proved by the *intra* and *inter*-assays (Figure S8), whose SD values were calculated to be 4.40% and 5.68%, respectively. The results highlighted the advance of apoFt in loading larger amounts of luminol via the internal encapsulation method.

As well known, pH-induced disassembly/reassembly is regarded as the most conventional pathway to encapsulate molecules or ions in apoFt for biosynthesis and applications.²⁴ However, extremely acid/alkaline conditions might destroy the functional groups on the nanocage, thus decreasing its biocompatibility for bioanalysis, especially for the outer biomodifications.²⁵ To conquer this limitation, a thermally induced strategy was first brought up to include luminol molecules inside of apoFt without dramatic pH adjustments. To prove the thermal stability of apoFt under 60 °C, circular dichroism (CD) data of the Lum@apoFt samples prepared under temperatures from 20 to 60 °C for 1 h were recorded by scanning from 200 to 300 nm utilizing a MOS-450 spectrometer consisting of a quartz cuvette of 1 mm optical path length at 25 °C. As shown in Figure 3B, negligible variation was found, proving the favorable thermal stability of apoFt under 60 °C. Thus, the feasibility of this method is well demonstrated.

Discussions of Signal Amplification and Luminous Mechanism. To investigate the signal amplification of the OV's involved ECL emission of luminol-dissolved O₂ system, electrodes modified with 10 μ L of 2 mg/mL CoFe₂O₄, p-CoFe₂O₄ and r-CoFe₂O₄ were detected in air-saturated PBS (pH 8.2) containing 0.5 mmol/L of luminol, respectively. As shown in Figure 4A, the r-CoFe₂O₄ modified electrode (magenta curve) achieved the maximum ECL intensity among the samples. By comparing CoFe₂O₄ (green curve) with p-CoFe₂O₄ (blue curve), it can be concluded that the existence of OV's can enhance the ECL signal of luminol. By comparing p-CoFe₂O₄ (blue curve) with r-CoFe₂O₄ (magenta curve), it can be concluded that higher OV's concentration can further boost the ECL process with stronger intensity. According to the corresponding CV curves in Figure 4B, it can be seen that the r-CoFe₂O₄ (magenta curve) owns a negatively shifted

anodic potential (0.47 V) and a greatly increased anodic current, which means that CoFe₂O₄ with higher OV's density not only decreased the overpotential of the redox reaction between luminol and O₂ but also promoted the current intensity, thus dramatically enhancing the ECL performance of the biosensor during the detection.

To have a better understanding of the luminous mechanism, two possible arguments are proposed: 1) The OV's can serve as the adsorption and reaction sites of dissolved O₂ and then facilitate the electron transport to catalyze the generation of O₂^{•-} for the ECL reaction. 2) The OV's can serve as the adsorption site for H₂O molecules, and then electro-catalyze the generation of more O₂ as coreactant via the oxygen evolution reaction (OER) process. To demonstrate the above, the r-CoFe₂O₄ electrodes were further detected in N₂-saturated PBS. Nevertheless, luminol can hardly generate ECL signals in the N₂-saturated environment (Figure 4C). As shown in Figure 4C, the ECL intensity of r-CoFe₂O₄ electrodes dramatically decreased after the addition of Tiron (1,2-dihydroxy-3,5-benzene disulfonic acid disodium salt), a specific O₂^{•-} scavenger.^{32, 33} This decrement was not caused by the electronic block on the electrode surface since the current only decreased a little in Figure 4D. Apparently, it was the O₂^{•-} elimination that decreased the ECL signal. Therefore, it can be included that the r-CoFe₂O₄ with higher OV's density can absorb dissolved O₂ on the interface and then catalyze the generation of more O₂^{•-} efficiently to react with luminol anionic radicals (L^{•-}) that generated by the electrochemical oxidation of luminol anions (LH⁻). In this way, more excited-state species of luminol (AP^{2-*}) can be produced to obtain greatly amplified signals (eqs 1-5).



Calibration Curve. Under optimized experimental conditions (Supporting Information, Figure S9), the calibration relationship between ECL intensity and standard CFERRA 21-1 concentration was investigated. The developed ECL biosensor was used to detect standard CFERRA 21-1 samples of different concentrations in 10 mL of PBS (pH 8.2). As shown in Figure S10, it can be seen the

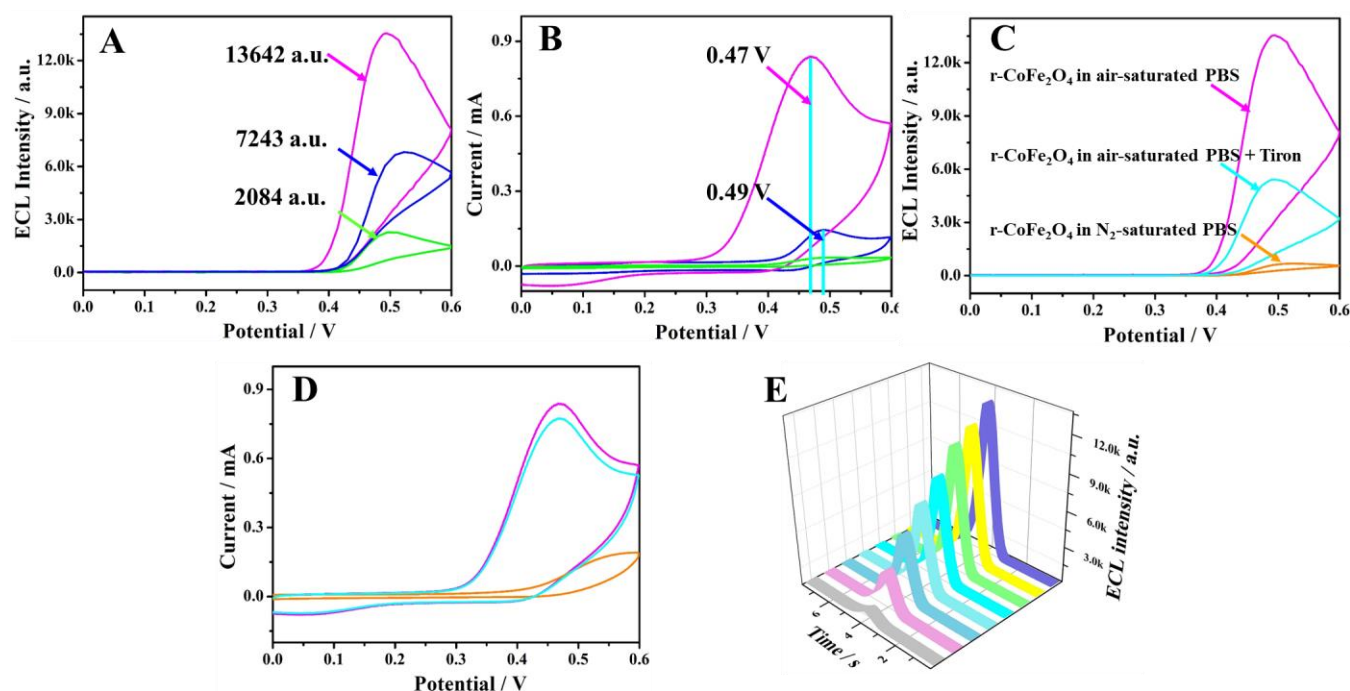


Figure 4. The ECL intensity-potential curves (A) and their corresponding CV curves (B) of CoFe₂O₄, p-CoFe₂O₄ and r-CoFe₂O₄ modified electrodes detected in 0.5 mmol/L of air-saturated luminol solution. The ECL intensity-potential curves (C) and the corresponding CV curves (D) of r-CoFe₂O₄ modified electrode detected in 0.5 mmol/L of luminol solution with different treatments. (E) ECL intensity-time curves for CYFRA 21-1 with concentrations of 0.5 pg/mL, 5 pg/mL, 50 pg/mL, 500 pg/mL, 1 ng/mL, 10 ng/mL, 20 ng/mL and 30 ng/mL.

ECL intensities of the biosensors were in an excellent linear relation with the logarithm of CFRA 21-1 concentration (0.5 pg/mL – 50 ng/mL). The linear regression equation was expressed as $y = 2343.04 \times \log c + 8432.43$, $R^2 = 0.994$, and the detection limit is estimated to be 0.14 pg/mL ($S/N = 3$). Moreover, the corresponding ECL intensity-time curve of this linearity was shown in Figure 4E. Given that most of the methods for CFRA 21-1 were using electrochemical sensors,^{27–30} this first proposed ECL biosensor exhibited sparkling superiorities of linear range and detection limit by the comparison in Table 1.

Table 1. Comparison Between this Proposed Method and Other Reported Sensors for CYFRA 21-1 Detection

electrode materials	linear range (ng/mL)	detection limit (pg/mL)	ref
3D graphene-Au NPs	0.25 — 800	100	27
CeO ₂ nanocube/RGO	6.25×10^{-4} — 0.01	0.625	29
ZrO ₂ -RGO	2 — 22	122	30
OVs enriched CoFe ₂ O ₄	5×10^{-4} — 50	0.14	this work

Real Sample Analysis. Based on the favorable specificity, stability, and reproducibility of the biosensor (Figure S11), the standard addition method was used to evaluate the accuracy and practicality of the constructed ECL biosensor for CYFRA 21-1 analysis. Normal human serum was spiked with standard CYFRA 21-1 samples with different concentrations (0.1 ng/mL, 2 ng/mL, 10 ng/mL and 30 ng/mL). Then, all the samples were detected by the developed biosensors. As shown in Table 2, the recovery data of the above were found from 95.5% to 103.0%. Therefore, the accuracy and practicality of the proposed ECL biosensor were successfully proved, showing great potential in the early diagnostics of squamous cell carcinomas.

Table 2. Recovery Data of Normal Human Serum Spiked with Different Concentration of CYFRA 21-1

Sample number	Spiked concentration (ng/mL)	Found (ng/mL), n=5	Recovery %
1	0.1	0.10, SD=1.98%	103.00
2	2	1.91, SD=2.69%	95.50
3	10	10.23, SD=1.76%	102.30
4	30	29.22, SD= 3.12%	97.40

CONCLUSIONS

Conclusively, the OV enhanced ECL biosensing strategy is successfully applied to fabricate a highly-efficient biosensor for biomarker immunoassay. The r-CoFe₂O₄ with higher OV density was fabricated as the biosensor substrate by the ODE strategy. In-depth characterization of the r-CoFe₂O₄ showed significant improvement in facilitating the interfacial electron-transfer and catalyzing the generation of O₂^{•−} in comparison to CoFe₂O₄ with low or no OV density. With further introducing the site-oriented HWR linkers, the formed r-CoFe₂O₄-HWR substrate leads to high antibody binding specificity. Then, a novel thermally induced channel expansion method was first proposed and applied to design the biocompatible transducer of Lum@apoFt, which perfectly avoided the inevitable denaturation caused by the pH-induced disassembly/reassembly and complicated procedures in conventional apoFt-complex synthesis. Using the developed biosensor to monitor the concentration of CFRA 21-1 in human serum, excellent linearity between 0.5 pg/mL – 50 ng/mL with a low detection limit of 0.14 pg/mL was achieved along with the favorable stability, specificity, and reproducibility. This work not only put forward new ideas of fabricating OV enhanced sensing interface with excellent signal amplifying function, but also

proposes a facile and robust methodology to design apoFt-based transducers in developing practical nanoscale biosensors for early diagnostics of diseases.

ASSOCIATED CONTENT

Supporting Information.

Materials, reagents, apparatus, SEM images of pure CoFe₂O₄ and p-CoFe₂O₄, FT-IR spectra, UV-vis absorbance spectrum, the linear relation between the peak reduction current *I* and $\nu^{1/2}$, UV-vis diffuse reflectance spectra, EIS characterization, HPLC calibration curve, reproducibility of preparing Lum@apoFt, optimization of experimental conditions, the calibration curve of biosensor, specificity, stability, and reproducibility of biosensor.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Key Scientific Instrument and Equipment Development Project of China (No.21627809); National Natural Science Foundation of China (No. 21777056), Special Foundation for Taishan Scholar Professorship of Shandong Province (No. ts201712052) Jinan Scientific Research Leader Workshop Project (2018GXRC024), the Innovation Team Project of Colleges and Universities in Jinan (No.2019GXRC027).

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