

# Heterojunction-Mediated Dual-Site Synergistic Catalysis for Enhanced Electrochemiluminescence Detection of $\alpha$ -Synuclein

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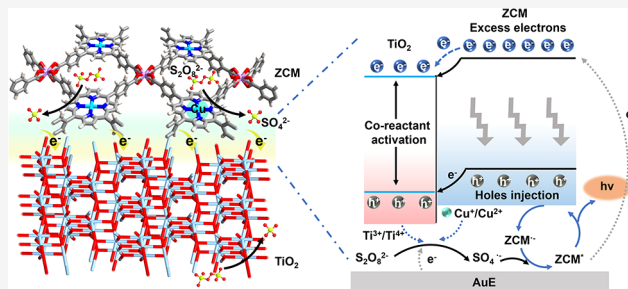


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**ABSTRACT:** Precise quantification of  $\alpha$ -synuclein ( $\alpha$ -Syn), an important species associated with Parkinson's disease and related neurodegenerative disorders, is of paramount importance for early diagnosis and intervention. While electrochemiluminescence (ECL) has emerged as a powerful analytical technique owing to its low background interference and high sensitivity, the performance of MOF-based ECL systems is often limited by sluggish interfacial charge transfer and insufficient co-reactant activation at a single type of catalytic site. To overcome these bottlenecks, a two-dimensional (2D) ZnCu metal-organic framework/ $\text{TiO}_2$  (ZCMT) heterojunction sensing system was constructed by coupling interface engineering with a dual-site synergistic catalysis strategy. Specifically, the heterojunction facilitates directional charge transport by promoting electron delocalization and interfacial charge redistribution. Concurrently, the Cu- and Ti-based redox-active centers synergistically facilitate  $\text{K}_2\text{S}_2\text{O}_8$  activation and accelerate its reduction. This synergistic mechanism substantially enhances its ECL performance. By integrating the ZCMT platform with DNAzyme-assisted amplification strategy, a high-performance ECL biosensor was constructed for the ultrasensitive quantification of  $\alpha$ -Syn. Overall, this study provides an effective strategy for overcoming the charge-transfer and catalytic limitations of MOF-based ECL systems and establishes a promising platform for the ultrasensitive analysis of  $\alpha$ -Syn, with potential applications in the early screening of Parkinson's disease.



## INTRODUCTION

Precise quantitative analysis of  $\alpha$ -synuclein ( $\alpha$ -Syn), a key specie associated with Parkinson's disease and related neurodegenerative disorders, is paramount for early clinical diagnosis.<sup>1</sup> Although conventional analytical techniques, such as enzyme-linked immunosorbent assays (ELISA),<sup>2</sup> fluorescence assays,<sup>3</sup> and nuclear magnetic resonance (NMR),<sup>4</sup> have been used for  $\alpha$ -Syn analysis, they may be limited by insufficient sensitivity, laborious procedures, or high costs.<sup>5</sup> Consequently, these methods may not fully meet the requirements for rapid and routine clinical screening.<sup>6</sup> Against this backdrop, electrochemiluminescence (ECL) has emerged as a compelling alternative owing to its low background interference, rapid response and high sensitivity.<sup>7</sup> Accordingly, ECL provides a promising analytical platform for the ultrasensitive and specific detection of  $\alpha$ -Syn within complex biological matrices.

Despite the tremendous potential of ECL technology in biosensing, its analytical performance remains largely constrained by the charge-transfer efficiency and interfacial catalytic activity of the luminescent platforms.<sup>8</sup> In recent years, metal–organic frameworks (MOFs) have been widely explored as ideal ECL luminophores or signaling platforms owing to their highly tunable porous crystalline structures, exceptionally large surface areas, and abundant metal active sites.<sup>9</sup> However, monometallic MOFs contain only one type of

metal center and often exhibit high charge-transfer resistance, which can limit electron transport and co-reactant activation in complex ECL systems.<sup>10</sup> To circumvent this intrinsic bottleneck, researchers have proposed incorporating secondary metallic nodes into the MOF, thereby developing bimetallic MOFs endowed with tunable electronic structures and abundant synergistic catalytic sites.<sup>11</sup> For instance, Cui and coworkers engineered a noble metal nanoparticle dual-functionalized two-dimensional (2D) MOF (ABEI/AuNPs/Cu-TCPP) via an in situ growth strategy, which exhibited intense and stable ECL emission coupled with remarkable peroxidase-mimicking activity.<sup>12</sup> Furthermore, Hong et al. synthesized functionalized 2D NiZn-MOF nanosheets through controlled thermal treatment to promote  $\text{H}_2\text{O}_2$  generation, thereby achieving highly efficient luminol-based ECL.<sup>13</sup> Overall, the synergistic effect between multiple metal sites (e.g., Cu) not only maintains the structural stability of the porous framework but also endows it with abundant

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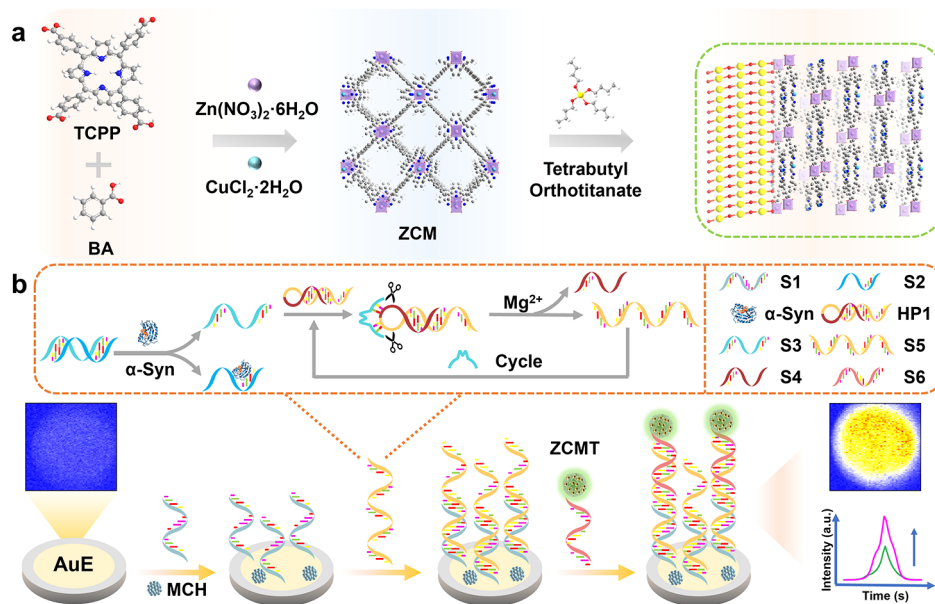
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**Scheme 1.** (a) Synthetic Procedure for the ZCMT Nanocomposite; (b) Fabrication of the Proposed ECL Biosensor and the Corresponding DNAzyme-Assisted Signal Amplification Strategy



catalytically active sites. These sites can promote co-reactant activation and radical generation, thereby enhancing the ECL performance of the system.

However, despite these distinct catalytic and structural advantages, the practical applications of pristine MOFs are severely hindered by their poor intrinsic conductivity.<sup>14</sup> Moreover, conventional physical blending strategies offer negligible improvements to electron transfer pathways, making it imperative to develop novel strategies to overcome this bottleneck. For instance, Li et al. constructed a 2D Fe-MoS<sub>2</sub>/CoFe-MOF heterojunction, which not only introduced novel active sites but also generated a built-in electric field at the interface driven by electronegativity differences, thereby significantly promoting the reaction kinetics.<sup>15</sup> Similarly, Xu et al. coupled MXene with bimetallic MOF nanosheets to fabricate a 2D sandwich-like heterostructure featuring dual Cu/CuCo-MOF and MXene-MOF heterojunctions, which drastically enhanced the catalytic activity of the nonprecious metals.<sup>16</sup> These studies convincingly demonstrate that constructing a heterojunction via interfacial semiconductor engineering provides a well-matched energy band structure. Such a structure not only drives directional charge migration through the built-in electric field but also suppresses nonradiative recombination, ultimately leading to significant ECL signal amplification.<sup>17</sup> Guided by heterojunction engineering and band alignment principles, semiconducting TiO<sub>2</sub> was rationally selected to optimize the luminescence of the bimetallic MOF owing to its exceptional chemical stability and robust redox activity.<sup>18</sup> In the 2D ZnCu-MOF/TiO<sub>2</sub> (ZCMT) heterojunction system, the well-matched band structure promotes the directional transfer of excess electrons to the TiO<sub>2</sub> conduction band. The formation of the heterojunction facilitates interfacial charge separation, which is consistent with the reduced nonradiative decay and prolonged excited-state lifetime observed for ZCMT. In addition, Ti sites in TiO<sub>2</sub> and Cu sites in the ZnCu-MOF component form dual redox-active centers that synergistically promote co-reactant activation. Owing to the combined effects of interfacial charge transfer

and dual-site catalysis, the ZCMT exhibits strong and stable ECL performance and serves as the core luminophore platform (Scheme 1a). Through the strategic integration of this platform with a Mg<sup>2+</sup>-dependent DNAzyme-assisted target recycling amplification strategy, an ECL biosensor was successfully constructed for the ultrasensitive and quantitative detection of α-Syn (Scheme 1b). The development of this sensing platform not only provides novel insights into the design of high-performance luminescent systems based on MOF heterojunctions but also offers a sensitive, reliable, and powerful analytical tool for the early screening and clinical diagnosis of neurodegenerative disorders such as Parkinson's disease.

## EXPERIMENTAL SECTION

### Preparation of ZnCu-MOF (ZCM)

The preparation procedure of ZCM was further refined compared to previous studies.<sup>17</sup> In a typical synthesis, 40 mg of meso-tetra(4-carboxyphenyl)porphine (TCPP) and 25 mg of BA were dissolved in 100 mL of dimethylformamide (DMF), heated at 150 °C, and designated as mixture A. Subsequently, 100 mg of CuCl<sub>2</sub>·2H<sub>2</sub>O was dissolved in 5 mL of ethanol and added dropwise to mixture A in batches. Thereafter, 100 mg of Zn(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O was dissolved in 5 mL of ethanol, and the solution was added dropwise to mixture A in batches over 5 min. The resulting mixture was refluxed at 150 °C for 1 h. After the termination of the reaction, the product (ZCM) was thoroughly washed with ethanol several times and dried in vacuum at 60 °C.

### Preparation of 2D ZnCu-MOF@TiO<sub>2</sub> (ZCMT)

8 mg of ZCM was dispersed in 12 mL of H<sub>2</sub>O/ethanol mixture solvent (2:1), which was referred to as mixture B. In addition, 100 μL of tetrabutyl orthotitanate was dissolved in 20 mL of ethanol, and the resulting system was refluxed at 80 °C for 1 h (mixture C). Mixture C was then slowly added dropwise to mixture B, and the reaction was continued under reflux at 80 °C for 4 h. After the reaction was completed, the solid product was collected by centrifugation, washed with ethanol, and redispersed in a mixed solvent consisting of 8 mL of water and 250 μL of DMF. The resulting dispersion was subjected to a solvothermal reaction at 120 °C for 12 h. The product was collected

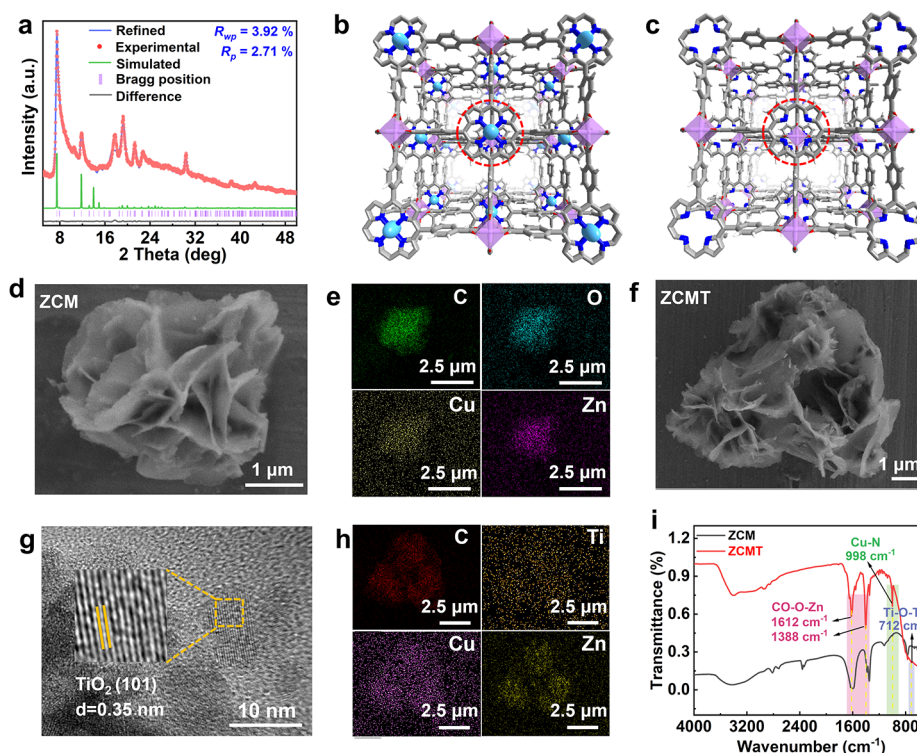


**Figure 1.** (a) PXRD patterns of ZCM: experimental data (red dots) and simulated results (blue line). Structural diagrams of (b) ZCM and (c) Zn-porphyrin MOF (ZM). (d) SEM and (e) the corresponding C, O, Cu, and Zn mappings of ZCM. (f) SEM and (g) HRTEM images of ZCMT. (h) The corresponding C, Ti, Cu, and Zn mappings of ZCMT. (i) FTIR spectra of ZCM and ZCMT.

by centrifugation again, thoroughly washed with ethanol, and then dried in a vacuum drying oven at 60 °C.

## RESULTS AND DISCUSSION

### Characterization of Materials

The crystal structure of ZCM was investigated by combining powder X-ray diffraction (PXRD) with structural modeling and Pawley refinement using Materials Studio. On the basis of previously reported structural models, Pawley refinement yielded the following unit-cell parameters for ZCM:  $a = 16.7891$  Å, Pawley refinement yielded the following unit-cell parameters for ZCM:  $a = 16.7891$  Å,  $b = 15.0023$  Å,  $c = 16.7891$  Å and  $\alpha = \beta = \gamma = 90^\circ$ . The crystal was assigned to the orthorhombic system with a space group of ( $I m m m$ ) (No. 71). The low residual factors low residual factors ( $R_{wp} = 3.92\%$  and  $R_p = 2.71\%$ ) indicated good agreement between the experimental and refined PXRD patterns, supporting the proposed unit cell and space-group assignment of ZCM (Figure 1a–c). As shown in Figure S1a, the characteristic reflections of the ZCM component, including those indexed to the (320), (004), (400), and (440) planes, were retained after the introduction of  $\text{TiO}_2$ .<sup>19</sup> In addition, the emergence of diffraction peaks assignable to anatase  $\text{TiO}_2$ , particularly the prominent (101) reflection, confirmed the presence of crystalline  $\text{TiO}_2$  in the ZCMT composites.

The microstructure and morphology of the samples were characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). As shown in Figure 1d, ZCM exhibited a three-dimensional nanoflower structure with a smooth surface. Energy-dispersive X-ray spectroscopy (EDX) analysis (Figure 1e) demonstrated that Zn, Cu, O, and

C elements were uniformly distributed throughout the nanostructure. After treatment with tetrabutyl orthotitanate, the surface of ZCMT became markedly rougher (Figure 1f). High-resolution transmission electron microscopy (HRTEM) image revealed lattice fringes with an interplanar spacing of 0.350 nm, which can be indexed to the (101) plane of anatase  $\text{TiO}_2$  (Figure 1g).<sup>20</sup> EDX elemental mapping results (Figure 1h) confirmed the homogeneous distribution of C, Ti, Cu, and Zn throughout the ZCMT architecture, supporting the successful incorporation of  $\text{TiO}_2$  into the composite.

To further confirm the interactions among the components, the Fourier-transform infrared (FTIR) spectra are presented in Figure 1i. The characteristic bands at  $1612\text{ cm}^{-1}$  and  $1388\text{ cm}^{-1}$  were assigned to the asymmetric and symmetric stretching vibrations of carboxylate groups coordinated with  $\text{Zn}^{2+}$  (CO–O–Zn coordination).<sup>21</sup> Meanwhile, the characteristic peak at  $998\text{ cm}^{-1}$  corresponded to the N–Cu coordination vibration (the magnified local spectrum was presented in Figure S1b), demonstrating that Cu had successfully coordinated with the pyrrolic N atoms in the TCPP molecules.<sup>17,22</sup> Additionally, the characteristic infrared absorption peak was observed at  $712\text{ cm}^{-1}$  and assigned to the stretching vibration of Ti–O–Ti bonds (the magnified local spectrum was presented in Figure S1c), which directly confirmed the successful introduction of  $\text{TiO}_2$  into the ZCMT. Collectively, the significant changes in the intensity of these characteristic peaks indicated that ZCMT was successfully prepared.

The specific surface area and pore structure distribution of the samples were characterized by nitrogen adsorption–desorption isotherms.<sup>23</sup> As shown in Figure S1d, the isothermal curves of both ZCM and ZCMT exhibited a typical type IV feature. Notably, the pore size distribution curve of

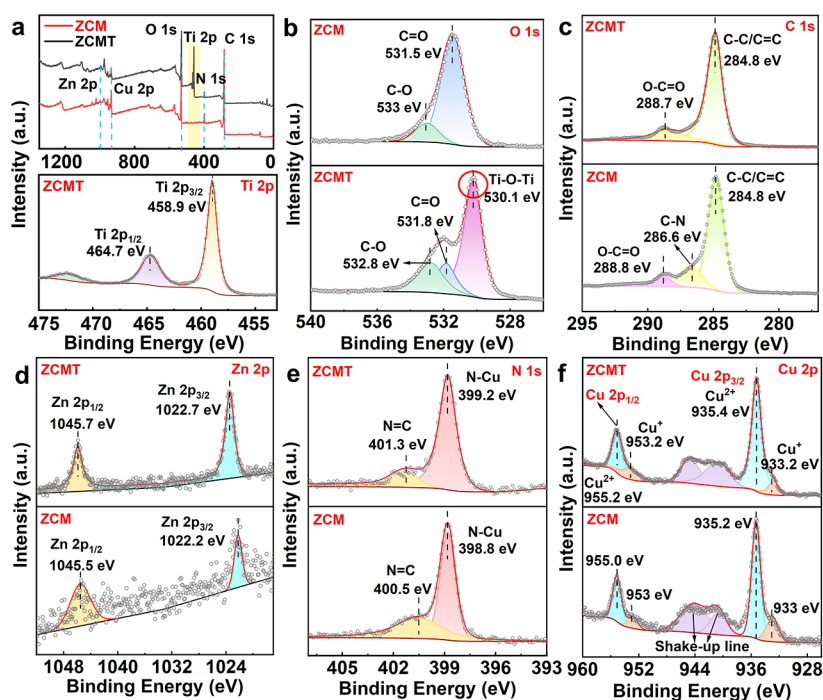


**Figure 2.** (a) XPS survey spectra and high-resolution XPS spectra of Ti 2p; (b) O 1s, (c) C 1s, (d) Zn 2p, (e) N 1s, and (f) Cu 2p spectra of ZCM and ZCMT.

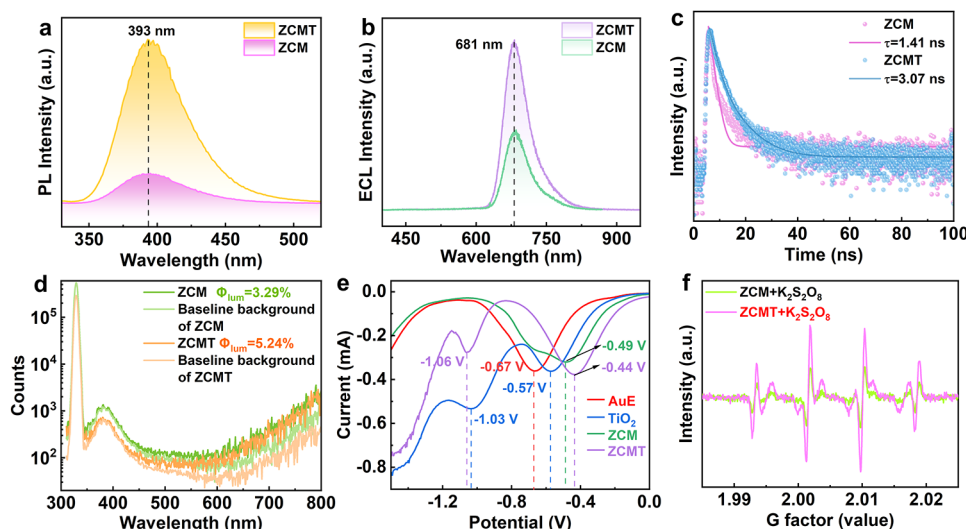


**Figure 3.** (a) PL emission spectra of ZCM and ZCMT. (b) ECL spectra of ZCM and ZCMT in PBS containing 80 mM  $K_2S_2O_8$ . (c) Fluorescence lifetime of ZCM and ZCMT. (d) PL quantum yield of ZCM and ZCMT. Solid lines: samples; lighter lines: corresponding baseline backgrounds. (e) DPV curves (vs Ag/AgCl) of different electrodes in PBS solution containing 80 mM  $K_2S_2O_8$ : AuE (red curve),  $TiO_2$  (blue curve), ZCM (green curve), and ZCMT (purple curve). Instrumental parameters: pulse amplitude of 50 mV, pulse width of 60 ms, pulse period of 500 ms, and sample width of 20 ms. (f) EPR of ZCM and ZCMT in PBS containing  $K_2S_2O_8$ . The *S,S*-dimethyl-1-pyrroline *n*-oxide (DMPO) was used as a trapping agent to test at -1.5 V.

ZCMT exhibited a broader and more uniform pore-size distribution, confirming that the introduction of  $TiO_2$  regulated the pore structure of ZCM and rendered the pore distribution to be more uniform. After the growth of  $TiO_2$ , the specific surface area of ZCMT rose from 3.612  $m^2/g$  to 12.169  $m^2/g$  relative to ZCM, which indicated that the introduction of  $TiO_2$  effectively enhanced the specific surface area and optimized the pore structure of the material.

The surface chemical composition and electronic states of the samples were analyzed by X-ray photoelectron spectroscopy (XPS) (Figure 2a). The Ti 2p spectrum of ZCMT

exhibited Ti 2p<sub>3/2</sub> (458.9 eV) and Ti 2p<sub>1/2</sub> (464.7 eV) peaks, respectively, indicating that Ti predominantly existed in the  $Ti^{4+}$  state. The O 1s spectrum of ZCMT (Figure 2b) exhibited a Ti-O-Ti signal at 531.8 eV, confirming the successful introduction of  $TiO_2$ .<sup>24</sup> The high-resolution C 1s spectra (Figure 2c) exhibited characteristic peaks at 284.8 eV, originating from the carbon framework of the porphyrin units. Specifically, the characteristic peaks of Zn 2p and N 1s in ZCMT both exhibited significant positive shifts compared to

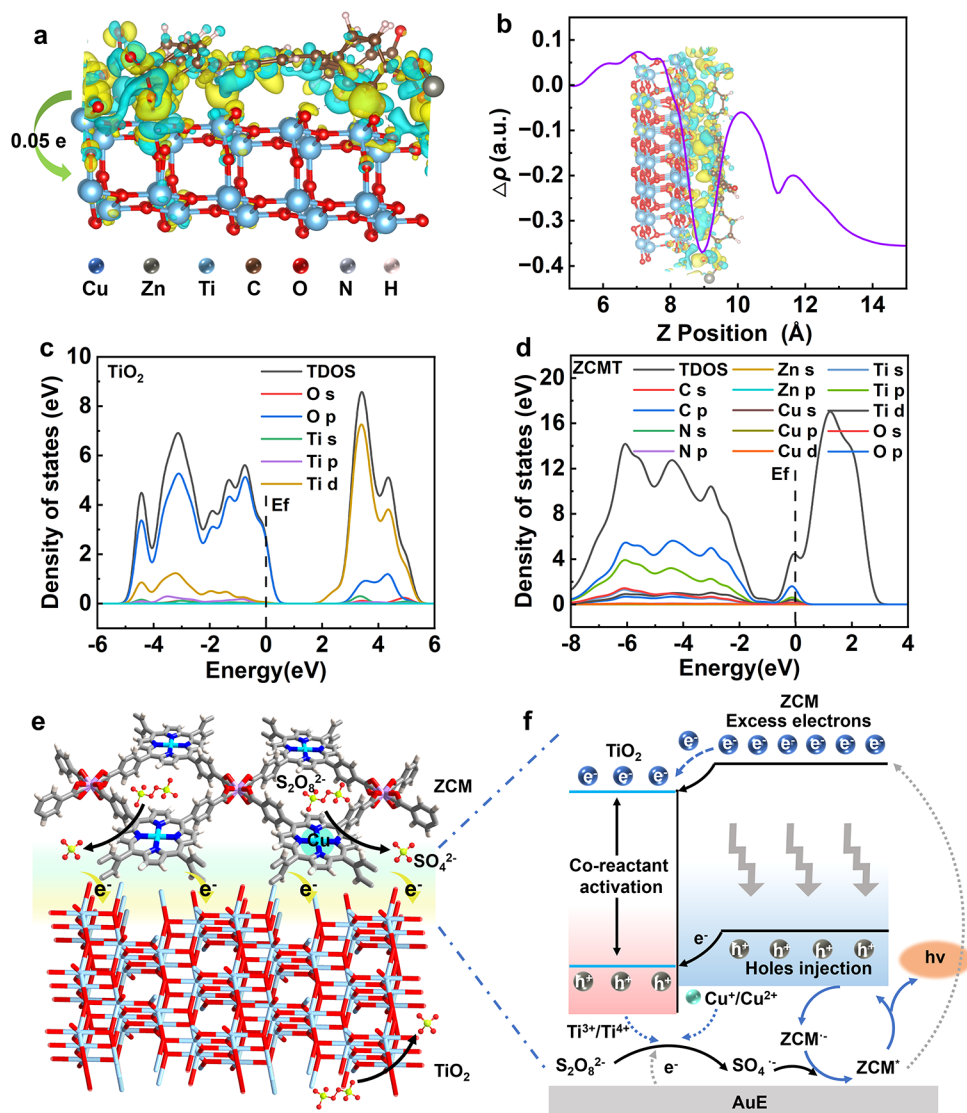


**Figure 4.** (a) Charge density difference of ZCMT with an isosurface. (Charge accumulation is presented in yellow, and charge depletion is presented in cyan). (b) Plane-averaged charge-density difference along the Z-direction. PDOS of (c) TiO<sub>2</sub> and (d) ZCMT. (e, f) Possible ECL emission mechanisms in the heterojunction structure.

those of ZCM (Figure 2d,e), reflecting a diminished electron cloud density around the Zn centers and porphyrin N atoms. Such synergistic increases in binding energy provided compelling evidence for the spontaneous directional electron transfer from the ZCM to TiO<sub>2</sub>. Additionally, the Cu 2p spectra (Figure 2f) confirmed the coexistence of mixed Cu<sup>+</sup>/Cu<sup>2+</sup> valence states, with the Cu 2p peaks shifting toward higher binding energies in ZCMT.<sup>25</sup> These results collectively demonstrate the successful construction of the ZCMT heterojunction with strong interfacial electronic interactions that are conducive to efficient charge separation.

#### Photophysical and Electrochemical Characterizations

The luminescence enhancement mechanism was investigated in depth through photoluminescence (PL), ECL, fluorescence lifetime and PL quantum yield.<sup>26</sup> As shown in the PL spectra (Figure 3a), both ZCM and ZCMT exhibited a broad PL emission band centered at approximately 393 nm under excitation at 250 nm, with ZCMT showing a markedly higher intensity than ZCM. ECL emission spectra (Figure 3b) revealed well-defined peaks at 681 nm for both samples,

indicating that the ECL signals originated from the radiative transition of excited-state ZCM.<sup>27</sup> Consistent with the transient ECL-time profiles (Figure S2a-b), ZCMT exhibited a distinctly higher ECL intensity than pristine ZCM. To further examine the excited-state decay dynamics, time-resolved fluorescence decay curves were recorded (Figure 3c). An average fluorescence lifetime ( $\tau$ ) of 1.41 ns was obtained for ZCM via a biexponential model fit, whereas that of ZCMT was extended to 3.07 ns. Furthermore, the PL quantum yield ( $\Phi_{\text{lum}}$ ) increased from 3.29% for ZCM to 5.24% for ZCMT (Figure 3d). The above results indicated that the introduction of TiO<sub>2</sub> had improved the optical properties of ZCM.

To investigate the interfacial electrocatalytic behavior, differential pulse voltammetry (DPV) was conducted for systematic characterization (Figure 3e). In PBS buffer containing 80 mM K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, the bare gold electrode (AuE) exhibited a dominant reduction peak at -0.67 V, which was assigned to the reduction of S<sub>2</sub>O<sub>8</sub><sup>2-</sup> (red curve). After the electrode was modified with the ZCM (green curve), the

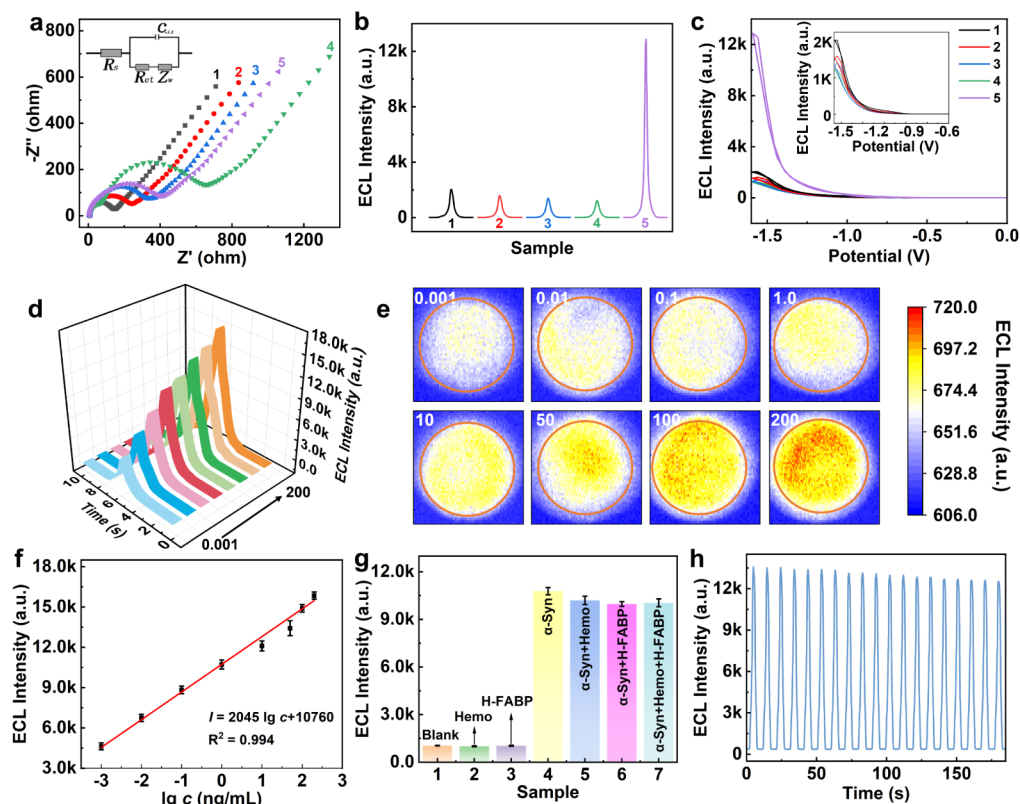


**Figure 5.** (a) EIS Nyquist plots, (b) ECL intensity-time curves, and (c) ECL-potential curves of different electrodes: (1) bare AuE, (2) AuE/S1, (3) AuE/S1/MCH, (4) AuE/S1/MCH/S5, and (5) AuE/S1/MCH/S5/ZCMT-S6. (d) ECL -time profiles of the biosensor for different concentrations of  $\alpha$ -Syn (0.001, 0.01, 0.1, 1.0, 10, 50, 100, and 200 ng/mL) and (e) the corresponding ECL images. (f) Calibration curve of the biosensor. (g) ECL intensities of different samples ( $n = 3$ ,  $\pm$  SD): (1) Blank, (2) Hemo (10 ng/mL), (3) H-FABP (10 ng/mL), (4)  $\alpha$ -Syn (1.0 ng/mL), (5)  $\alpha$ -Syn (1.0 ng/mL) + Hemo (10 ng/mL), (6)  $\alpha$ -Syn (1.0 ng/mL) + H-FABP (10 ng/mL), and (7)  $\alpha$ -Syn (1.0 ng/mL) + Hemo (10 ng/mL) + H-FABP (10 ng/mL). (h) Stability of the ECL sensor in the presence of  $\alpha$ -Syn (50 ng/mL).

catalytic reduction peak of  $\text{S}_2\text{O}_8^{2-}$  shifted remarkably toward a more positive potential of  $-0.49$  V. For the ZCMT-modified electrode (purple curve), the corresponding reduction peak shifted further positively to  $-0.44$  V. The positive shift of the peak potential indicated that after the introduction of ZCMT,  $\text{S}_2\text{O}_8^{2-}$  was more prone to reduction. In addition, the  $\text{TiO}_2$ -modified electrode (blue curve) showed a reduction peak at  $-1.03$  V, while ZCMT displayed a characteristic peak at  $-1.06$  V, which confirmed that Ti active sites had been successfully introduced into ZCMT.

To gain deeper insights into the catalytic mechanism, electron paramagnetic resonance (EPR) spectroscopy was employed to systematically characterize the reaction systems.<sup>28</sup> The results revealed that, in the presence of  $\text{K}_2\text{S}_2\text{O}_8$ , the ZCM system exhibited distinct EPR signals compared to the pristine ZM (Figure S2c), confirming that the incorporation of Cu sites effectively endowed the system with the capability to catalytically activate  $\text{K}_2\text{S}_2\text{O}_8$ . Furthermore, the ZCMT system demonstrated a significantly enhanced EPR signal intensity relative to the ZCM system (Figure 3f), indicating the generation of abundant  $\text{SO}_4^{\bullet-}$ .

#### Possible ECL Enhancement Mechanism

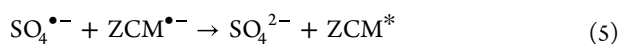
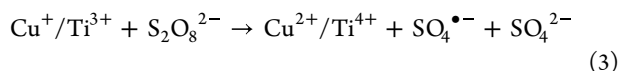
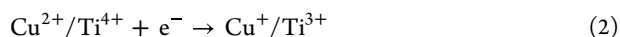
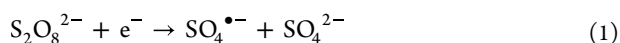
Density functional theory (DFT) calculations elucidated the interfacial charge transfer mechanism in the ZCMT composite. As shown in Figure 4a, electrons migrated from ZCM to  $\text{TiO}_2$ . Formation of the heterointerface induced spatial charge redistribution between the two components. Bader charge analysis indicated a net transfer of approximately 0.05 e from

the ZCM component to  $\text{TiO}_2$  within the constructed interfacial model. The plane-averaged charge density difference ( $\Delta\rho$ ) along the Z-direction (Figure 4b) indicated that electrons transferred from ZCM to  $\text{TiO}_2$ .<sup>17</sup> (Figure 4. The electron states and orbital contributions near the Fermi level were further analyzed using the partial density of states (PDOS) analysis.<sup>29</sup> Compared to pristine  $\text{TiO}_2$ , the ZCMT composite (Figure 4c, d) exhibited more hybridization near the Fermi level, which indicated that interfacial reactions and electron transfer occurred between the heterojunctions.<sup>30</sup>

The band gaps of the materials were investigated via UV-vis diffuse reflectance spectroscopy (DRS) (Figure S3a,b). Pristine  $\text{TiO}_2$  exhibited an intrinsic absorption peak at 282 nm, whereas ZCM displayed only weak absorption at 421 and 541 nm, ascribed to the organic ligands.<sup>31</sup> To gain deeper insights into the ECL mechanism of the ZCMT, Mott-Schottky (M-S) analysis and DRS (Figure S3c-e) were further employed to characterize the flat-band potentials ( $E_{\text{fb}}$ ) and energy band structures.<sup>32</sup> The  $E_{\text{fb}}$  values for  $\text{TiO}_2$  and ZCM were determined to be  $-0.49$  and  $-0.78$  V (vs Ag/AgCl), respectively. To elucidate the mechanism underlying the significantly enhanced ECL signal, the charge transfer and catalytic behavior at the ZCMT heterojunction interface were discussed<sup>33</sup> (Figure 4e, f). The excess electrons on ZCM were injected from the conduction band (CB) of ZCM into that of  $\text{TiO}_2$ , while the holes generated in  $\text{TiO}_2$  captured electrons from the valence band (VB) of ZCM and thereby produced holes at the VB of ZCM. The recombination of electrons and

holes enabled the excited ZCM\* to return to the ground state, accompanied by the generation of ECL emission. In addition, the  $\text{Ti}^{3+}/\text{Ti}^{4+}$  and  $\text{Cu}^+/\text{Cu}^{2+}$  active sites contributed to the catalytic reduction of  $\text{K}_2\text{S}_2\text{O}_8$ .<sup>34</sup>

During the ECL process, the system exhibited multiple reduction pathways for the coreactant  $\text{K}_2\text{S}_2\text{O}_8$ . Initially,  $\text{S}_2\text{O}_8^{2-}$  underwent direct reduction at the electrode surface (eq 1). The  $\text{Cu}^{2+}$  and  $\text{Ti}^{4+}$  centers could separately accept electrons to generate  $\text{Cu}^+$  and  $\text{Ti}^{3+}$  species, which may subsequently mediate  $\text{S}_2\text{O}_8^{2-}$  reduction through parallel redox cycles (eqs 2–3). Concurrently, the cathodic potential reduced the ZCM luminophore to its radical anion  $\text{ZCM}^{\bullet-}$  (eq 4). The combined effects of synergistic catalysis and direct reduction generated an abundance of highly reactive sulfate radicals ( $\text{SO}_4^{\bullet-}$ ). These radicals subsequently engaged in electron transfer with  $\text{ZCM}^{\bullet-}$  to generate the excited-state  $\text{ZCM}^*$  (eq 5), which returned to the ground state via radiative transition, yielding a strong and stable ECL signal (eq 6).



### Analytical Performance of the Biosensor

To feasibility of the proposed sensing strategy was evaluated by examining both the solution-phase DNA reactions and the stepwise assembly of the sensing interface. Polyacrylamide gel electrophoresis (PAGE) verified the stepwise DNA assembly and reactions (Figure S4a–b),<sup>35</sup> with the corresponding DNA sequences listed in Table S1. Furthermore, electrochemical impedance spectroscopy (EIS) was employed to monitor the interface modification. As shown in Figure 5a, the sequential assembly of S1, 6-mercapto-1-hexanol (MCH), and S5 onto AuE led to a progressive increase in electron transfer resistance ( $R_{\text{et}}$ ), owing to the increasingly blocking DNA and organic layers at the electrode interface. The corresponding fitted  $R_{\text{et}}$  values are summarized in Table S2. The marked decrease in impedance upon ZCMT-S6 introduction confirmed that the target-triggered S5 sequence successfully templated the hybridization of ZCMT-S6 onto the electrode. In addition, the ECL responses (Figure 5b) and ECL potential curves (Figure 5c) of the stepwise modified electrodes revealed that a significantly enhanced ECL signal was obtained when ZCMT was successfully loaded onto the electrode. These results were intuitively confirmed by ECL imaging (Figure S4c), where the ZCMT-S6 modified electrode was filled with luminescent spots. The consistency of these experimental phenomena validated the rationality of the proposed ECL biosensor platform.

Under the optimal experimental conditions (Figure S5), the analytical performance of the developed ECL biosensor was systematically evaluated. As shown in Figure 5d, the ECL intensity of the system exhibited a progressive increase with the ascending concentration of  $\alpha$ -Syn across a broad range from 0.001 to 200 ng/mL. The simultaneously recorded ECL

images (Figure 5e) intuitively corroborated this concentration-dependent behavior. With the increase in target concentration, both the quantity and the brightness of luminescent spots on the electrode surface gradually increased. Quantitative analysis (Figure 5f and S6a) revealed an excellent linear relationship between the ECL intensity ( $I$ ) and the logarithm of the  $\alpha$ -Syn concentration ( $\lg c$ ). The corresponding linear regression equation was formulated as  $I = 10760 + 2045 \times \lg c$  ( $R^2 = 0.994$ ), yielding a remarkably low limit of detection (LOD) of 19.8 fg/mL. Figure S6a. Compared with previously reported detection strategies (Table S3), the proposed biosensor exhibited superior sensitivity and a broader linear range, underscoring its immense potential for trace analysis in complex clinical biological samples. As illustrated in Figure 5g, hemoglobin (Hemo) and heart-type fatty acid-binding protein (H-FABP) were selected as potential interfering proteins to evaluate the specificity of the platform. The results indicated that even when the concentration of the interferents was 10-fold higher than that of the target  $\alpha$ -Syn, the ECL response of the system remained virtually unaffected. This confirmed that the sensor possessed satisfactory specific recognition capability toward  $\alpha$ -Syn. During the continuous scanning stability test, the ECL intensity was basically consistent even after 19 consecutive ECL cycles (Figure 5h). Moreover, after storage at 4 °C for 15 days, the fabricated biosensor retained 86.2% of its initial ECL intensity (Figure S6b), demonstrating satisfactory long-term storage stability. Collectively, these results indicated that the constructed ECL sensing platform integrated high specificity and good storage stability, demonstrating remarkable value for practical applications.

### Artificial Sample Testing

To assess the practical viability of the developed biosensor, quantitative analysis of artificial serum was conducted via recovery tests.  $\alpha$ -Syn at known concentrations was spiked into 100-fold diluted samples to determine the analytical recoveries. The results (Table S4) revealed excellent recovery rates spanning from 102.0% to 106.0%, with low relative standard deviations (RSDs) between 2.90% and 4.95%. This compelling analytical performance underscored the remarkable selectivity and accuracy of the biosensor, demonstrating its great promise as a tool for clinical auxiliary diagnostics.

## CONCLUSION

In summary, a ZCM/ $\text{TiO}_2$  heterojunction was developed as an ECL-emitting platform by integrating interfacial electronic modulation with proposed Cu/Ti dual-site co-reactant activation. The reconstructed electronic states at the ZCM/ $\text{TiO}_2$  interface may further facilitate interfacial electron transport. In addition, Cu sites in the ZCM component and Ti sites in  $\text{TiO}_2$  provided parallel redox-active pathways for  $\text{S}_2\text{O}_8^{2-}$  activation, contributing to the enhanced ECL response. Combined with a  $\text{Mg}^{2+}$ -dependent DNAzyme-assisted amplification strategy, the biosensor achieved a low detection limit for  $\alpha$ -Syn and exhibited satisfactory selectivity and storage stability. Overall, this work provides a strategy for improving interfacial charge transport and co-reactant activation in MOF-based ECL biosensors and establishes a sensitive platform for  $\alpha$ -Syn analysis. These findings provide valuable theoretical support for the design of efficient and reliable biosensing platforms.

## ■ ASSOCIATED CONTENT

## ■ Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.analchem.6c02862>.

Apparatus and material, XRD patterns, N<sub>2</sub> adsorption–desorption isotherms and pore size distribution curves, UV–vis diffuse reflectance spectroscopy (DRS), Kubelka–Munk-transformed reflectance spectra, Mott–Schottky (M–S) plots, band structures, preparation of DNA strands, the construction process of the sensor, polyacrylamide gel electrophoresis (PAGE) analysis, and the calculation of LOD (PDF)

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## Notes

The authors declare no competing financial interest.

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