

# Quaternary Ammonium Salt-Functionalized Tetraphenylethene Derivative Boosts Electrochemiluminescence for Highly Sensitive Aqueous-Phase Biosensing

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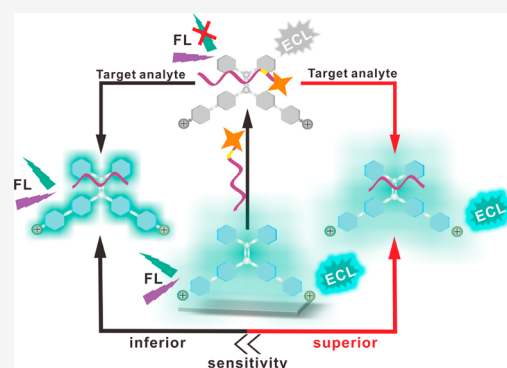


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Supporting Information

**ABSTRACT:** Aggregation induced emission active compounds (AIEgens) have appeared as a new kind of electrochemiluminescence (ECL) emitters due to their bright emission in the aggregated state but lack functional groups. Herein, we report a quaternary ammonium salt groups-functionalized AIEgen (QAU-1) and discover that coating QAU-1 on the indium tin oxide (ITO) surface (QAU/ITO) enabled QAU-1 to display significant cathodic ECL emission compared with that of QAU-1 in the dissolved state. Inspired by this, we applied QAU-1 as emitters to develop a novel ECL biosensor (Fc-DNA/QAU/ITO) through electrostatic attraction between QAU/ITO and a ferrocene-labeled ssDNA (Fc-DNA), and the developed biosensor was employed to detect bleomycin (BLM) with high sensitivity based on the target-initiated specific cleavage and subsequent removal of Fc molecules from the electrode. We envision this work will open up a new avenue to development of high-performance ECL biosensors, which will display a significant potential application in the field of analysis.



Electrochemiluminescence (ECL) couples the advantages of electrochemistry and chemiluminescence and profits from the complete separation in energy forms of excitation voltage and output light,<sup>1–4</sup> and thus it has been considered as a powerful and promising tool in sensitive and reliable determination of various analytes, such as heavy metal ions,<sup>5,6</sup> tumor biomarkers,<sup>7,8</sup> pesticides,<sup>9</sup> and antibiotics.<sup>10</sup> It is generally accepted that ECL emitters play a crucial part in ECL-based analysis. In this context, emitters including ruthenium complex,<sup>11</sup> luminol,<sup>12</sup> metal clusters,<sup>13,14</sup> and quantum dots<sup>15</sup> have been explored, and have been proven to be positive for sensing performance to a certain degree. Whereas, most of reported emitters are detrimental to modification and are enslaved to high price, weak stability, and high toxicity.<sup>16</sup> A strategy to address these issues is to explore organic-based emitters for developing ECL biosensors. Organic-based emitters possess high activity, do not contain metal elements, and are easily modified to adjust their luminescent performance.<sup>17–19</sup> However, to our best knowledge, most of organic-based emitters in the ECL system are applied in the organic phase. For example, Maran et al.<sup>20</sup> reported a triphenylamine-based ECL system in *N,N*-dimethylformamide (DMF). Shao et al.<sup>21</sup> reported the ruthenium(II) and iridium(III) complexes-based ECL systems in acetonitrile. These organic solvents are highly toxic and cause a significant effect on the detection of target analytes in the aqueous phase.<sup>22</sup> To avoid the use of organic solvents, directly coating emitters at the electrode surface appeared and

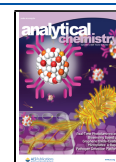
became an effective method.<sup>23,24</sup> Nevertheless, research of organic-based emitters in the ECL system through the coating process are surprisingly scarce. The fundamental cause is that most of organic-based emitters are nonemissive or weakly emissive in the aggregated state due to the severe aggregation caused quenching effect, and thus they are particularly unsuitable for ECL biosensor development.<sup>25</sup> Hence exploring highly efficient organic-based emitters that can emit bright light in the aggregated state is momentous to address this issue.

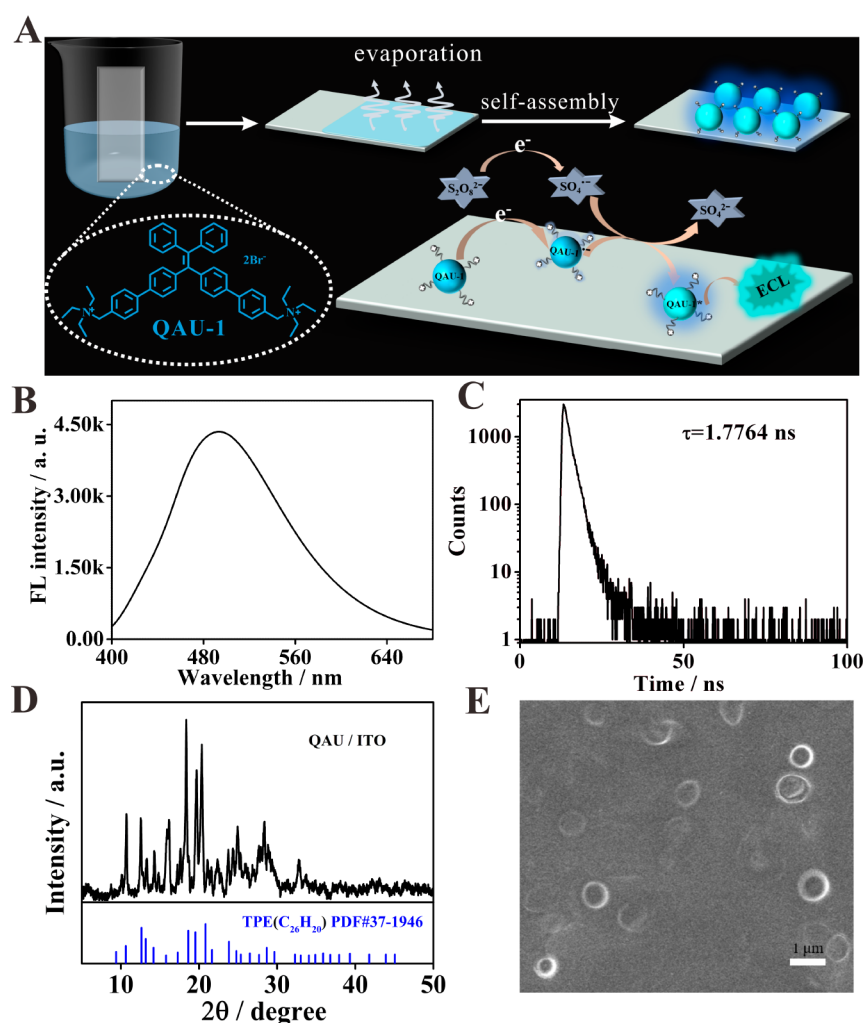
Aggregation induced emission active compounds (AIEgens) are a subclass of organic-based emitters and have attracted substantial interest since the pioneering work reported by Tang in 2001 due to their strong emission in the solid state and thus are regarded as a potential kind of emitter in the development of ECL biosensors.<sup>26,27</sup> Very recently, Yuan et al.<sup>28</sup> found that AIE aggregates on the surface of ITO electrode emit strong ECL signal with the excitation of voltage. Lu et al.<sup>29</sup> employed tetraphenylethene (TPE) derivative as emitters to achieve I<sup>–</sup> ECL biosensing. Ju et al.<sup>30</sup> reported the anodic ECL property of TPE-based conjugated polymer dots. These works undoubtedly justified that AIEgens indeed enhanced ECL

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**Figure 1.** (A) Diagram illustration of QAU-1-functionalized ITO electrode based on an evaporation-induced self-assembly method. Fluorescence spectrum (B), fluorescence lifetime curve (C), XRD pattern (D), and SEM image (E) of QAU/ITO.

sensing performance and displayed a great potential as emitters for high-performance ECL biosensors development. Still, studies of AIEgens on ECL are scarcely reported. As far as we are concerned, there are only nine emitters reported for ECL biosensors.<sup>1,28–35</sup> More notably, most of the ones (six) belong to anodic ECL emitters, which are unfavorable to probe target analytes in complex environments due to the oxidation reactions. At the same time, these reported AIE emitters lack functional groups ( $-\text{CHO}$ ,  $-\text{COOH}$ ,  $-\text{OH}$ , and  $-\text{NH}_2$ ) and thus are detrimental to modification with functional receptors, subsequently prohibiting their wide and deep application in the analytical field.

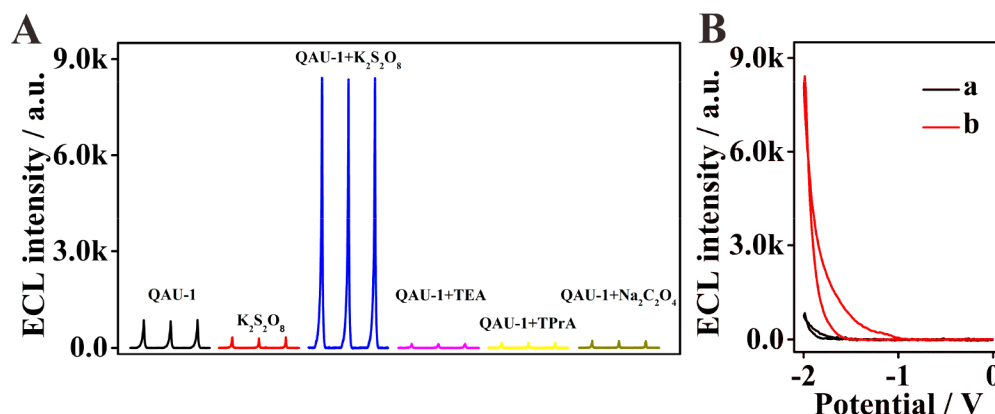
Herein, for addressing the obstacles mentioned above, we introduced a positively charged TPE derivate (QAU-1) containing quaternary ammonium salt groups and subsequently coated QAU-1 on the surface of the indium tin oxide (ITO) electrode to fabricate a QAU-1-modified ITO electrode (QAU/ITO) through an evaporation-induced self-assembling strategy, in which highly stable and significant cathodic ECL emission was observed in the aqueous phase by using  $\text{K}_2\text{S}_2\text{O}_8$  as a coreactant. This fabricated QAU/ITO reacted with a ferrocene-labeled ssDNA (Fc-DNA, Figure S1 and Table S1) to generate Fc-DNA/QAU/ITO through electrostatic attraction, and further Fc-DNA/QAU/ITO was applied in the highly sensitive analysis of bleomycin (BLM, Figure S1) based

on the target-initiated specific cleavage and subsequent removal of Fc molecules. This strategy opens up an important avenue to construct high-performance ECL platforms.

## EXPERIMENTAL SECTION

**Fabrication of QAU/ITO.** QAU-1 was prepared according to the method described in our previously reported literature and then was dissolved in  $\text{CH}_3\text{CN}$  to obtain a 10 mM stock solution.<sup>36</sup> Prior to application, the ITO electrode was first treated with 1.0 M NaOH solution for 4.5 h, and subsequently, ultrapure water and ethanol were sequentially employed to wash the treated ITO electrode three times, respectively. After drying with  $\text{N}_2$  flow, the ITO electrode with a working area of  $0.25\pi \text{ cm}^2$  was immersed in a 100  $\mu\text{M}$  QAU-1 solution for 1 min and then was taken out and dried at room temperature to obtain QAU/ITO.

**Fabrication of Fc-DNA/QAU/ITO.** A volume of 50  $\mu\text{L}$  of Fc-DNA solution (1.0  $\mu\text{M}$ ) was dropped onto the surface of the QAU/ITO electrode and subsequently reacted for 20 min at room temperature to make QAU/ITO adsorb Fc-DNA as many as possible. After that, the electrode was rinsed with 10 mM phosphate buffer (PB, pH 7.4) to remove unreacted Fc-DNA and placed in a 4  $^\circ\text{C}$  environment for further application.



**Figure 2.** (A) ECL intensities vs different systems. The concentration of  $\text{K}_2\text{S}_2\text{O}_8$  was 10 mM, and all concentrations of TEA, TPRA, and  $\text{Na}_2\text{C}_2\text{O}_4$  were 50 mM. QAU-1 was coated on the surface of ITO. (B) ECL-potential profiles of ITO in the presence of 0.1 M tetrabutylammonium bromide (TBAB), 10 mM  $\text{K}_2\text{S}_2\text{O}_8$ , and 100  $\mu\text{M}$  QAU-1 in the mixed solvent containing 75%  $\text{CH}_3\text{CN}$  and 25%  $\text{H}_2\text{O}$  (line a), and QAU/ITO in the presence of 10 mM  $\text{K}_2\text{S}_2\text{O}_8$  in PB (line b).

**Fc-DNA/QAU/ITO-Based ECL Biosensor for BLM Assay.** The detection sample for BLM was prepared by mixing BLM and  $\text{Fe}^{2+}$  with equal amounts for 30 min. A volume of 50  $\mu\text{L}$  of BLM- $\text{Fe}^{2+}$  sample with different concentrations was dropped onto the surface of Fc-DNA/QAU/ITO and reacted for 30 min to complete the detection reaction. The electrode was washed thoroughly using PB. Finally, the reacted electrode was placed in a 1000  $\mu\text{L}$  of PB containing 10 mM  $\text{K}_2\text{S}_2\text{O}_8$  to conduct the ECL measurement with a scan speed of 100 mV/s and a scan potential in the range of 0 to  $-2.0$  V.

## RESULTS AND DISCUSSION

**Characterization of QAU/ITO.** QAU-1 (Figure 1A), as the archetype of AIEgens, was characterized to be weakly emissive in the molecule-dissolved state and brightly emissive in the aggregated state (Figure S2). Further, an ITO electrode was immersed in a 100  $\mu\text{M}$  QAU-1 solution ( $\text{CH}_3\text{CN}$ ) and subsequently dried to obtain QAU/ITO through an evaporation-induced self-assembling strategy (Figure 1A). CV and EIS characterizations revealed that QAU-1 was successfully coated on the surface of the ITO electrode (Figure S3). Thus, obvious blue fluorescence at 490 nm was observed under 365 nm excitation (Figure 1B), and the lifetime and fluorescence quantum yield were determined to be 1.7764 ns and 0.25, respectively (Figure 1C and Table S2). Sharp peaks of the XRD pattern in Figure 1D indicated QAU-1 was highly crystalline and in good line with the standard database of TPE (JCPDS No. 37-1946). SEM characterization implied that QAU-1 formed compactly aggregated films on the surface of ITO electrode, in which 800 nm spheres were observed (Figure 1E). This can be ascribed to the fact that quaternary ammonium salt groups in QAU-1 possess strong hydrogen-bond interaction with  $-\text{OH}$  on the surface of the ITO and that QAU-1 is a typical amphiphile molecule, which can self-assemble into regular aggregates with the solvent volatilizing. It is greatly different from previously reported AIE-based emitters, which were fabricated by a direct precipitation method in water solution and subsequently were coated on the surface of electrodes, leading to weak stability and low repeatability. Meanwhile, the emerged C, N, and Br elements in the EDS spectrum of QAU/ITO compared with that of ITO further justified the fabrication of QAU/ITO (Figure S4).

**ECL Property of QAU/ITO.** Further, we evaluated the ECL behavior of QAU/ITO under the given conditions (0.01 M PB, pH = 7.4, 100 mV/s scanning rate, a potential range of 0 to  $-2.0$  V for  $\text{K}_2\text{S}_2\text{O}_8$ , and another potential range of 0 to 2.0 V for TEA, TPRA, and  $\text{Na}_2\text{C}_2\text{O}_4$ ). Very weak ECL signal was determined for QAU/ITO alone. To generate high ECL intensity,  $\text{K}_2\text{S}_2\text{O}_8$ ,  $\text{Na}_2\text{C}_2\text{O}_4$ , TPRA, and TEA were applied as coreactants to react with QAU-1, respectively, and only  $\text{K}_2\text{S}_2\text{O}_8$  could significantly heighten the cathodic ECL emission of QAU-1 (Figure 2A). It can be clearly found that the ECL intensity of the QAU/ITO and  $\text{K}_2\text{S}_2\text{O}_8$  system was 9 or 26-fold higher than that of QAU/ITO or  $\text{K}_2\text{S}_2\text{O}_8$  alone, which might be understood in terms of the principle that  $\text{K}_2\text{S}_2\text{O}_8$  produced intermediates to stimulate QAU-1 from the ground state to the excited state, subsequently producing higher ECL emission. Conversely, QAU-1 in  $\text{CH}_3\text{CN}$  as a monomolecular state displayed a relatively weak ECL emission (Figure 2B), and the intensity decreased to approximately one-tenth (from 8419 au to 836 au) of that of QAU/ITO. This phenomenon might be ascribed to the AIE effect of QAU-1 that in the dissolved state free intramolecular motions increase the nonradiative energy decay, further rendering QAU-1 ECL weakly emissive, and whereas, in the aggregated state, the motions were prohibited by physical constraint, subsequently switching up the radiative decay and enhancing ECL emission. Besides, it was noted that the ECL and fluorescence spectra highly overlapped and the amplitude between ECL and the fluorescence peak was calculated to be 20 nm. These information indicated that the ECL emission in the QAU/ITO- $\text{K}_2\text{S}_2\text{O}_8$  system was attributed to the emission of QAU-1 and underwent a similar generation principle like fluorescence from the excited state to the ground state (Figure S5).

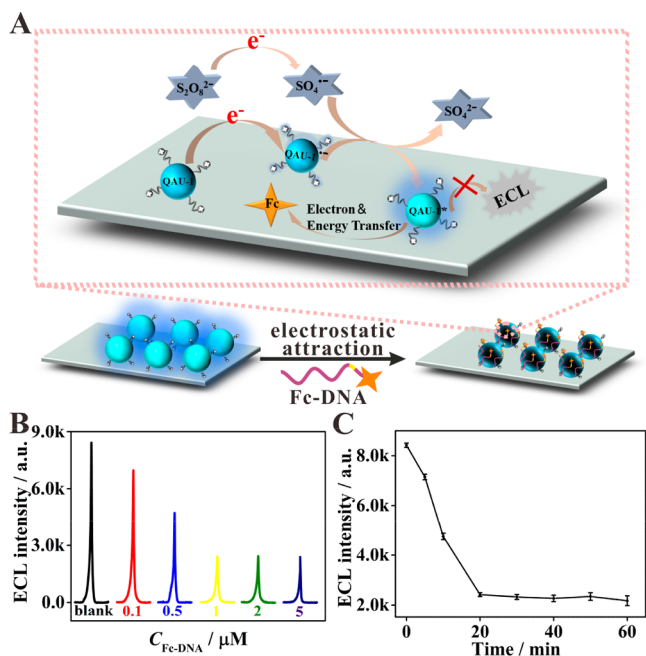
**Conditions Optimization.** ECL properties influenced its sensing performance as a sensor, and thus, the effects of pH,  $\text{K}_2\text{S}_2\text{O}_8$  concentration, and scan speed on ECL emission of QAU/ITO were studied. As shown in Figure S6, when pH value was smaller than 7.0 or larger than 8.4, ECL intensity gradually reduced with the pH value changing. As a comparison, when the pH value was in the range of 7.0–8.4, ECL signal was almost kept constant. This might be due to the reason that low pH value increases the protonation of  $\text{K}_2\text{S}_2\text{O}_8$  and weakens its stability, and that high pH value consumed active species generated in the voltage excitation process. Subsequently, we found that the ECL signal gradually



enhanced with the concentration of  $K_2S_2O_8$  and scan speed increasing in the initial stage (2–10 mM for  $K_2S_2O_8$  and 0.03–0.10  $V\ s^{-1}$  for scan speed). Whereas, when the  $K_2S_2O_8$  amount was greater than 10 mM and the scan speed was higher than 0.10  $V\ s^{-1}$ , ECL intensity gradually reduced (Figure S7). These changes might be attributable to the reasons that sufficient coreactant can completely react with QAU-1 to ensure high ECL emission, but excessive coreactant would hamper the generation of excited state of QAU-1 and that fast scan speed accelerates the diffusion of  $K_2S_2O_8$  to the electrode surface, but too fast a rate easily prohibits the reaction of  $K_2S_2O_8$  and QAU-1. As demonstrated from the above experiment information, pH 7.4, 10 mM  $K_2S_2O_8$ , and 100 mV/s are the optimum conditions for ECL measurements. Additionally, ECL stability was investigated through cyclic measurements for 18 consecutive times under working conditions, and the relative standard deviation (RSD) was calculated to be only 1.10%, suggesting the good stability (Figure S8). Thus, QAU-1 can serve as an effective emitter to develop a high-performance ECL biosensor.

#### Development of BLM-Responsive ECL Biosensor.

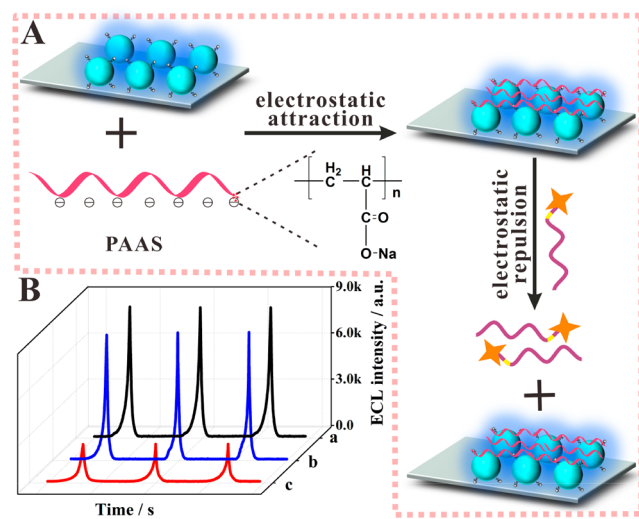
BLM, a typical antitumor drug, has been widely applied in cancer treatment for considerably prolonging life due to its advantages of low myelosuppression and low immunosuppression.<sup>36</sup> Although a large number of biosensors have been reported for BLM sensing,<sup>37–39</sup> they all are limited by some shortcomings such as high background, tedious procedures, long analysis time, and expensive bioenzymes. So, it is of crucial importance to develop simple, rapid, and reliable methods for sensitive detection of BLM in the pharmaceutical and clinical fields. In the present work, we attempt to apply QAU-1 as emitters to develop a novel BLM-responsive ECL biosensor with high sensitivity, and the fabrication diagram was illustrated in Figure 3A. QAU/ITO emitted a strong ECL



**Figure 3.** (A) Schematic diagram of fabrication of a BLM-responsive ECL biosensor based on QAU/ITO and Fc-DNA. (B) ECL intensity of the fabricated Fc-DNA/QAU/ITO in the presence of Fc-DNA with different concentrations. (C) ECL intensity of Fc-DNA/QAU/ITO versus reaction time between QAU/ITO and 1.0  $\mu M$  Fc-DNA.

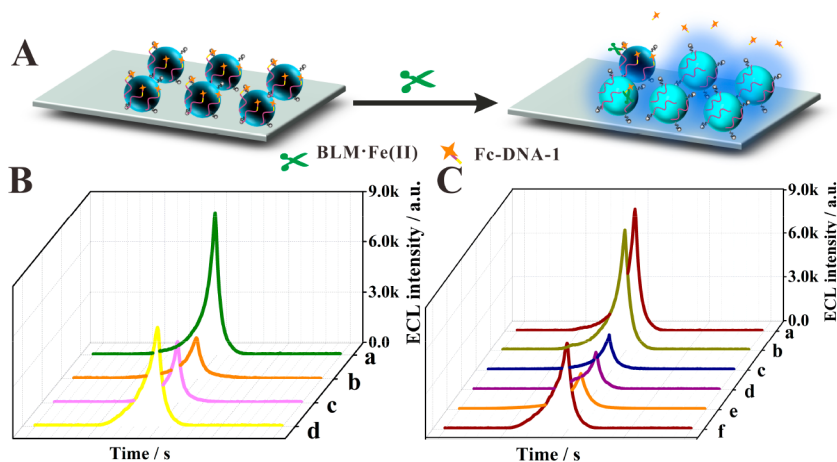
signal and possessed a high-charge density cationic surface. Fc-DNA contained 5'-GCT-3' sequences that could be recognized by BLM and was labeled by Fc molecules that could quench the ECL emission of QAU-1 due to resonance energy transfer and electron transport effects.<sup>40–42</sup> QAU/ITO reacted with Fc-DNA to produce an ECL biosensor (denoted as Fc-DNA/QAU/ITO), and Fc-DNA/QAU/ITO displayed very weak ECL emission. To validate the development of Fc-DNA/QAU/ITO, ECL measurements of QAU/ITO subjected to different concentrations of Fc-DNA were conducted. As manifested in Figure 3B, when the amount was  $\leq 1\ \mu M$ , ECL intensity gradually reduced with the amount elevating, which might be understood in terms of the capture of Fc-DNA and the quenching effect of Fc on ECL emission of QAU-1. Whereas, when the amount was  $> 1\ \mu M$ , ECL signal displayed negligible variation due to the saturated interaction of Fc-DNA and QAU/ITO, indicating 1  $\mu M$  was the optimum amount of Fc-DNA. Moreover, it only requires 20 min to complete this reaction (Figure 3C). Further, we explored the stability of Fc-DNA/QAU/ITO in PB comprising metal ions and small molecules and in 20-fold diluted serum sample (Figure S9). It is noted that complex environments are difficult to interfere with the ECL emission, and thus, the stability of Fc-DNA/QAU/ITO is good, and the resistance to interferences of Fc-DNA/QAU/ITO is high, providing a potential tool for assessment of BLM in biological fluids.

To gain more insight into the interaction of QAU/ITO and Fc-DNA, electrostatic attraction was deeply studied through prereaction of QAU/ITO with anionic polymer poly(acrylic acid) sodium (PAAS) and subsequent addition of Fc-DNA (Figure 4A). Obviously, PAAS masked the positive charges on

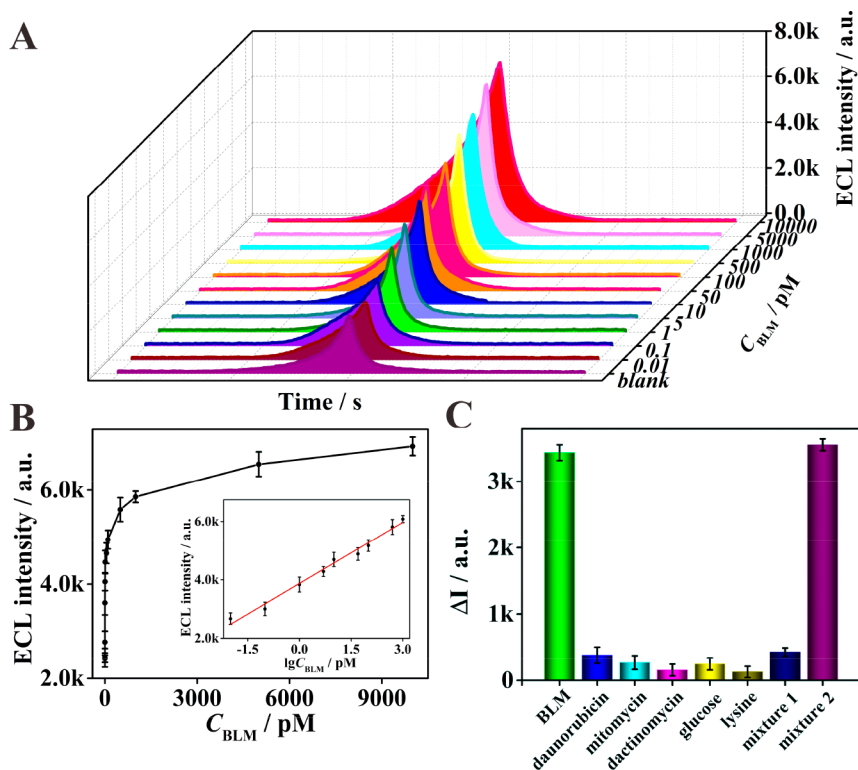


**Figure 4.** (A) Schematic illustration of the influence of PAAS on the interaction of QAU/ITO and Fc-DNA. (B) ECL intensity of QAU/ITO (a), prereacted QAU/ITO with PAAS + Fc-DNA (b), and Fc-DNA/QAU/ITO (c).

the surface of QAU/ITO and thus prevented Fc-DNA from being adsorbed, leading to no resonance energy transfer and electron transport from QAU-1 to Fc molecules. Consequently, the ECL emission of QAU/ITO was not quenched. As illustrated in Figure 4B, prereaction of QAU/ITO and PAAS resulted in an ECL emission similar to that of QAU/ITO alone and much higher than that PAAS did not exist.



**Figure 5.** (A) Schematic illustration of the working principle of Fc-DNA/QAU/ITO-based ECL biosensor for BLM detection. (B) ECL intensity of the developed biosensor under different conditions: (a) QAU/ITO, (b) Fc-DNA/QAU/ITO, (c) Fc-DNA/QAU/ITO + 1.0 pM BLM, and (d) Fc-DNA/QAU/ITO + 1000 pM BLM. (C) ECL intensity of different biosensors in the absence/presence of 1000 pM BLM: (a) Fc-DNA'/QAU/ITO, (b) Fc-DNA'/QAU/ITO + BLM, (c) Fc-DNA'/QAU/ITO, (d) Fc-DNA'/QAU/ITO + BLM, (e) Fc-DNA/QAU/ITO, and (f) Fc-DNA/QAU/ITO + BLM.



**Figure 6.** (A) ECL responses of Fc-DNA/QAU/ITO-based biosensor corresponding to BLM with different concentrations. (B) ECL intensity of Fc-DNA/QAU/ITO-based biosensor versus different BLM concentrations. Inset shows the linear curve of ECL intensity versus the logarithm of the concentration of BLM. (C)  $\Delta I$  ( $\Delta I = I - I_0$ , where  $I$  is the ECL intensity of Fc-DNA/QAU/ITO-based biosensor in the presence of analyte, and  $I_0$  is the ECL intensity of the Fc-DNA/QAU/ITO-based biosensor in the presence of different analytes. Mixture 1 consisted of daunorubicin, mitomycin, dactinomycin, and lysine, and mixture 2 consisted of BLM and mixture 1. The concentrations of all analytes were 1000 pM.

**Analytical Performance of Fc-DNA/QAU/ITO-Based Biosensor.** Based on the properties described above, Fc-DNA/QAU/ITO was exploited to sensitively determine BLM with  $K_2S_2O_8$  as a coreactant by coupling target-initiated specific cleavage and the Fc-mediated quenching effect. Figure 5A gave the working principle. When BLM did not exist, Fc-DNA was not cleaved, the attraction between Fc-DNA and QAU/ITO was not influenced, and thus, Fc-DNA/QAU/ITO

displayed low and unchanged ECL emission. Nevertheless, upon the addition of BLM, Fc-DNA was selectively cleaved into two segments at 5'-GCT-3', one of which was labeled by Fc molecules and only contained three bases (denoted as Fc-DNA-1). The few bases resulted in an obviously decreased negative charge density in Fc-DNA-1 compared to Fc-DNA, thus liberating Fc-DNA-1 from the QAU/ITO electrode into solution due to the weak electrostatic attraction. Given the

removal of Fc molecules, a drastically enhanced ECL emission was received as the “turn-on” state. On the basis of ECL emission enhancement, a Fc-DNA/QAU/ITO-based ECL biosensor for highly sensitive detection of BLM was readily developed.

In order to demonstrate the role of Fc-DNA/QAU/ITO, BLM solutions with amounts of 1 pM and 1000 pM were, respectively, added into the ECL analysis system. As shown in Figure 5B, if no BLM existed, the ECL intensity was 2418 au. However, with 1 pM BLM being added, a significantly enhanced ECL emission was determined with a value of 3605 au. This was no surprise to us that BLM cleaved Fc-DNA into two parts and resulted in the removal of Fc molecules from the QAU/ITO electrode. In another validation experiment, the increase in the concentration of BLM brought about a higher ECL emission than that of 1 pM BLM, demonstrating an evidently positive relationship and corresponding well to the sensing principle. The above data indicated that it is absolutely feasible to probe BLM using the Fc-DNA/QAU/ITO-based ECL biosensor. Additionally, the ratio of BLM and  $\text{Fe}^{2+}$ , reaction time between BLM and Fc-DNA/QAU/ITO were optimized to be 1:1 and 30 min for subsequent experiment, respectively (Figure S10).

In this study, Fc-DNA serves as a dual-functional quencher and recognizer for BLM sensing, and thus, the influences of sequences and labeled molecule on analytical performance were evaluated (Figure 5C). Fc-DNA' was made up of a Fc molecule and 25 bases, had different sequences with that of Fc-DNA, and thus was employed to replace Fc-DNA to assess the effect of sequences on sensing ability. It is noted that the biosensor developed by Fc-DNA' displayed very weak ECL emission (2395 au) in the absence of BLM, which is approximately identical with that of the biosensor developed by Fc-DNA but still showed very weak ECL signal in the presence of target BLM. This causation might be due to the fact that BLM only cleaves the ssDNA at the scission site (5'-GCT-3'), conforming well to previously reported works. Fc-DNA" had the same sequences with that of Fc-DNA but was not labeled by Fc molecules. With Fc-DNA being substituted by Fc-DNA", the established biosensor emitted a strong ECL signal whether BLM is present or absent in the sensing system due to the nonexistence of Fc molecules on the surface of the electrode. So, for BLM sensing, elaborate design of the sequences and labeled molecules counted for a great deal.

For evaluating the sensitivity, we monitored the ECL emission of the Fc-DNA/QAU/ITO biosensor subjected to BLM samples with different amounts, and the data was illustrated in Figure 6A,B. It was found that ECL intensity varied positively when BLM (from 0.01 to 10000 pM) was added into the Fc-DNA/QAU/ITO and  $\text{K}_2\text{S}_2\text{O}_8$  sensing system, demonstrating a great potential for BLM detection. Meanwhile, as far as we are concerned, this is the first documented example of an ECL biosensor in the detection of BLM. To fully understand the relationship and quantitatively determine the expression level of BLM, a standard working curve was obtained with high correlation ( $R^2 = 0.9870$ ) by plotting ECL intensity ( $I$ ) versus the logarithm of the amount of BLM ( $\lg C_{\text{BLM}}$ ). The equation was  $I = 705.6788 \lg C_{\text{BLM}} + 3647.2927$ , and the detection limit (LOD) was estimated to be 4.64 fM based on the  $3\sigma$  rule, which was comparable or lower than that of previously reported BLM sensors (Table S3). Moreover, the LOD was much lower than that (230 fM) of the fluorescence biosensor developed by QAU/ITO and Q-DNA

(Figure S1) under the same conditions with Fc-DNA/QAU/ITO (Figure S11). This low LOD might be derived from QAU-1's high ECL emission and the complete separation of the excitation source and output signal, further justifying the competence of QAU-1 as an emitter to develop an ECL biosensor.

In order to assess the selectivity of Fc-DNA/QAU/ITO in analysis of BLM, a collection of experiments were carried out to investigate the effect of interferences on the ECL intensity. As shown in Figure 6C, the  $\Delta I$  was calculated to be 3438 au for BLM. While the  $\Delta I$  were 380 au, 269 au, 156 au, 248 au, and 127 au for daunorubicin, mitomycin, dactinomycin, glucose, and lysine, respectively, which were much smaller than that of BLM. In addition, simultaneous addition of daunorubicin, mitomycin, dactinomycin, glucose, and lysine into the sensing system made the ECL intensity increase to 2847 au with  $\Delta I$  of only 429 au. With BLM further being added, ECL intensity enhanced to 5976 au, and the  $\Delta I$  was calculated to be 3558 au. The difference in  $\Delta I$  justified the good selectivity and strong anti-interference ability for BLM sensing. Beyond that, the repeatability of the proposed sensor was investigated through applying five different Fc-DNA/QAU/ITO electrodes to detect 10 pM BLM. As manifested in Figure S12, no evident fluctuation was observed, suggesting the satisfactory repeatability of our proposed biosensor.

**Real Sample Analysis.** Encouraged by the above-described high performance, the Fc-DNA/QAU/ITO-based ECL biosensor was anticipated to determine BLM in biological fluids. To confirm this, a standard spiking experiment was carried out by adding BLM into a diluted serum sample at the amounts of 1 pM, 50 pM, and 200 pM, followed by ECL measurements. The serum was obtained from a healthy person in Qingdao Agriculture University Hospital and was subsequently diluted to 20-fold with PB. As listed in Table S4, the recovery was in the range of 98.5–101.3%, implying a good agreement between added and measured amounts, and all RSDs were smaller than 3.23%, suggesting the excellent repeatability. Thus, Fc-DNA/QAU/ITO-based ECL biosensor displayed a significant potential for BLM diagnosis in biological samples.

## CONCLUSIONS

For the first time, in this work, a positively charged AIE molecule (QAU-1) was applied in the fabrication of AIE aggregates-modified ITO (QAU/ITO) through an evaporation-induced self-assembling strategy, and the QAU/ITO was characterized to emit a strong cathodic ECL signal using  $\text{K}_2\text{S}_2\text{O}_8$  as a coreactant with high stability and quaternary ammonium salt groups on the surface. Due to the strong electrostatic attraction of Fc-DNA and QAU/ITO, Fc-DNA/QAU/ITO-based ECL biosensor was developed and displayed an ultralow ECL signal due to the resonance energy transfer and electron transport. This character enabled it to selectively determine BLM at approximately the femtomolar level based on the target-initiated specific cleavage and subsequent removal of Fc molecules, which is lower than that of QAU/ITO-based fluorescence biosensor. We expect that this study will not only broaden the application area of AIEgens but also open up a new path to the development of high-performance ECL biosensors.



## ■ ASSOCIATED CONTENT

## ■ Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.analchem.0c01796>.

Sequences of the oligonucleotides; real sample analysis; FL, CV, and EIS characterizations; conditions optimization; and stability investigation (PDF)

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## Notes

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