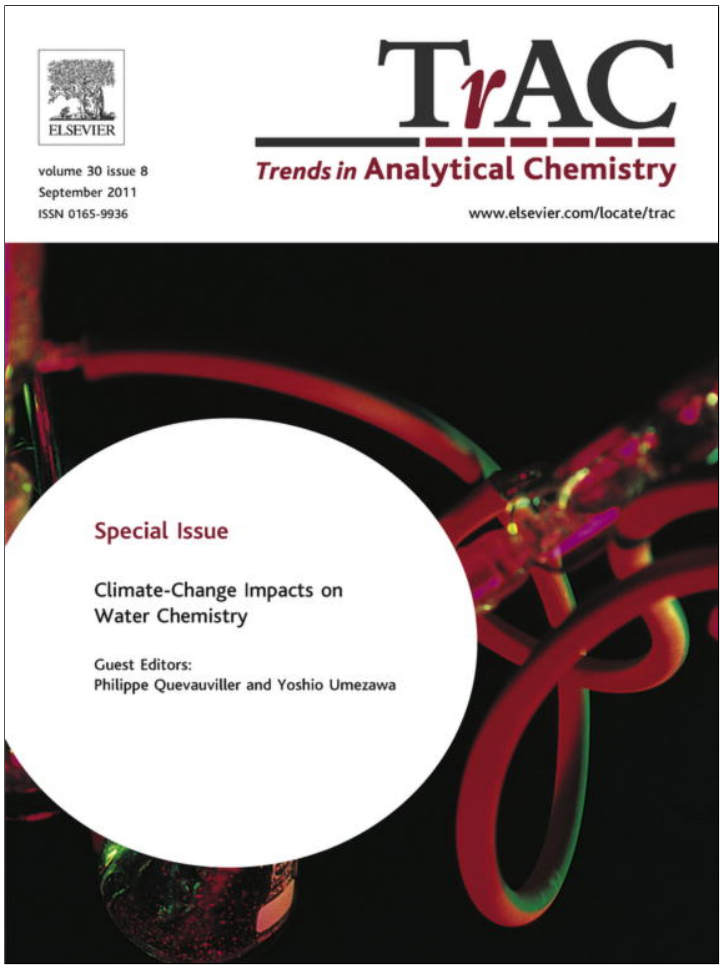

Fundamentals and bioanalytical applications of functional quantum dots as electrogenerated emitters of chemiluminescence

Jianping Lei, Huangxian Ju

Since the electrochemiluminescence (ECL) of quantum dots (QDs) of silicon was reported by *Science* in 2002, lots of QDs (e.g., II-VI, III-V and IV-VI) with different sizes and shapes have been used as ECL emitters for bioanalysis. Especially, QDs functionalized with multitudinous biomolecules offer excellent ECL signal-transduction platforms for designing a new generation of biosensing devices.

In this article, we focus on recent advances in the ECL principles of functional QDs, and their bioanalytical applications in DNA analysis, immunoassay, cytosensing and detection of other biological molecules.

Keywords: Bioanalysis; Biosensing; Cytosensing; DNA analysis; ECL emitter; Electrochemiluminescence; Electrochemistry; Functionalization; Immunoassay; Quantum dot

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1. Introduction

Electrochemiluminescence (ECL) is an energy-relaxation process by optical emission of an excited molecule produced by an applied potential at an electrode surface [1–7]. Compared with photoluminescence (PL), ECL, as an analytical technique, possesses several advantages. First, ECL does not involve a light source. Thus, the attendant problems of scattered light and luminescent impurities are absent, leading to high sensitivity of ECL analysis. Second, ECL possesses better selectivity than PL, because the generation of excited states in ECL can be easily controlled by varying the electrode potentials. Third, ECL is usually non-destructive, and most ECL emitters can be regenerated after ECL emission. ECL has therefore become a very powerful analytical technique and is widely used in the determination of biomolecules in clinical, environmental, and industrial applications.

Since the ECL phenomenon of quantum dots (QDs) was first observed during the study of silicon (Si) semiconductors in 2002 [8], many QD-based ECL emitters (e.g., II-VI, III-V and IV-VI nanocrystals, carbon nanoparticles (NPs), and even aromatic hydrocarbon-NPs) with different sizes, shapes and compositions have been used for bioanalysis [9–11]. Compared with conventional organic emitters, QDs as ECL emitters show excellent advantages (e.g., high quantum yields of fluorescence, size or surface trap-control luminescence, and good stability against photobleaching).

The ECL mechanism of QDs involves general annihilation and coreactant ECL reaction pathways [12]. Although annihilation ECL does not need additional reagent for emission, radical QDs must be chemically stable and maintain their charged states long enough to transfer charge upon colliding with oppositely-charged QDs. Thus, modern ECL applications of QDs are almost exclusively based on coreactant ECL. At an applied potential, both luminophore and coreactant species can first be oxidized or reduced at the electrode to form radicals, which then react with the oxidized or reduced

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luminophore to produce the excited states. The latter can emit to produce light.

Further, to achieve ECL bioanalysis, the functionalization of various QDs with multitudinous biomolecules through physical adsorption, electrostatic interaction, covalent binding and specific affinity interaction, has attracted considerable interest in finding new emitters with higher ECL efficiency and offering excellent ECL-signal-transduction platforms for biosensing [13–17].

In this review, we highlights recent advances in the ECL principles of functional QDs, and their biosensing applications in the selective detection of small molecules, DNA, antigen-antibody, cell and other biologically important targets.

2. Mechanism for ECL emission of QDs

2.1. Annihilation pathway

In the annihilation pathway, both oxidized and reduced QD species are produced on the electrode surface by potential sweeping or pulsing. These species then interact to produce a ground state and an electronically excited state, which then emits light. The emission mechanism can be expressed in Scheme 1.

$$QDs - e^- \rightarrow QDs^{\bullet +} \quad (1)$$

$$QDs + e^- \rightarrow QDs^{\bullet -} \quad (2)$$

$$QDs^{\bullet -} + QDs^{\bullet +} \rightarrow QDs^{*} + QDs \quad (3)$$

$$QDs^{*} \rightarrow QDs + h\nu \quad (4)$$

Scheme 1.

A typical annihilation pathway is the ECL system of Si-QDs [8]. When Si-NPs are oxidized or reduced by injecting holes or electrons under electrochemistry conditions, electron-transfer annihilation of electrogenerated anion and cation radicals results in the production of excited states for ECL emission. Recently, water-soluble carbon nanocrystals (CNCs) have been prepared and released into aqueous solution from a graphite rod by applying a scanning potential (Fig. 1). It was suggested that the ECL mechanism of the CNCs involves the formation of excited-state CNCs via electron-transfer annihilation of negatively charged and positively charged CNCs [3]. For the efficient generation of ion-annihilation ECL, three conditions should be met as follows:

(1) stable radical ions of the precursor molecules should survive until colliding with oppositely charged species in an annihilation reaction;
(2) good PL efficiency of a product of the electron transfer reaction; and,
(3) sufficient energy in the electron-transfer reaction to produce the excited state for ECL emission.

2.2. Coreactant pathway

In the coreactant pathway, ECL is usually generated by one-directional potential scanning on the electrode in the presence of both the luminophore and corectant. The coreactant is a species that, upon oxidation or reduction, produces an intermediate to react with an ECL luminophore to produce excited states. Different from the ion-annihilation routine, in which the electrolytic generation of both oxidized and reduced QDs is required, the coreactant pathway involves only electron transfer between electrochemically-generated nanocrystal species and coreactant. Thus, the coreactant routine can be achieved conveniently and is of wider biosensing

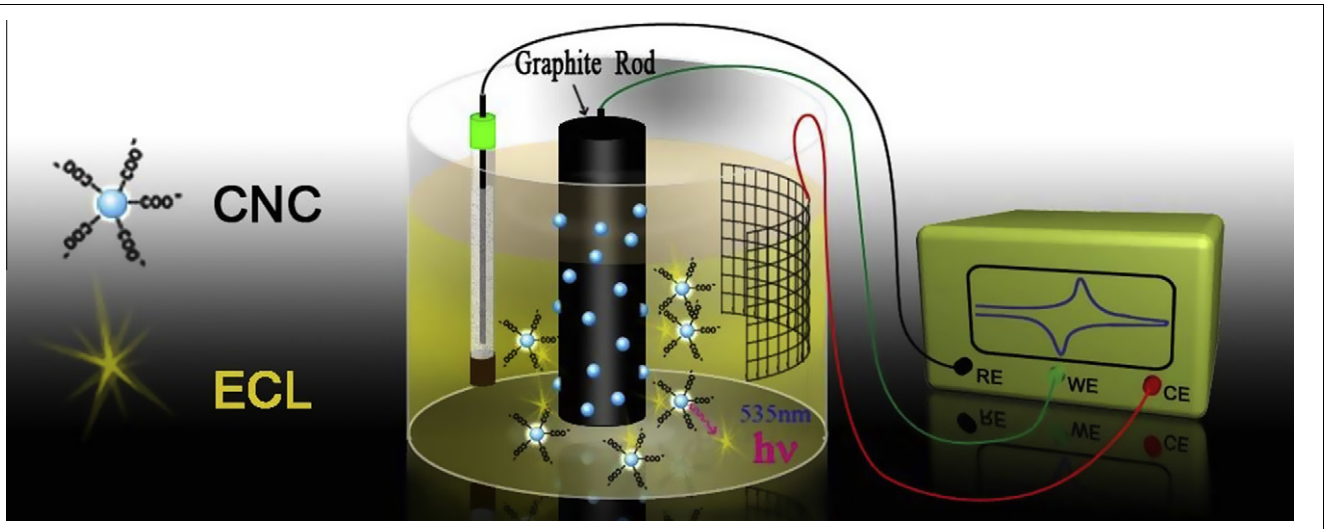

Figure 1. Preparation and electrochemiluminescence (ECL) of water-soluble carbon nanocrystals released into aqueous solution from a graphite rod by applying a scanning potential. (Reprinted from [3] with permission from ACS Publications).

$$QDs - e^- \rightarrow QDs^{\bullet +} \quad (5)$$

$$C_2O_4^{2-} - e^- \rightarrow [C_2O_4^{\bullet -}] \rightarrow CO_2^{\bullet -} + CO_2 \quad (6)$$

$$QDs^{\bullet +} + CO_2^{\bullet -} \rightarrow QDs^* + CO_2 \quad (7)$$

$$QDs^* \rightarrow QDs + h\nu \quad (8)$$

Scheme 2.

application. Especially, when the annihilation reaction between oxidized and reduced species is inefficient, the use of a coreactant may produce more intense ECL emission. A good coreactant of QD ECL should possess good solubility, stability, electrochemical properties, rapid kinetics, and low ECL background. We discuss typical coreactant QD ECL systems and their mechanisms in the following sub-sections.

2.2.1. Oxalate ($C_2O_4^{2-}$) system. As the first coreactant of ECL system, oxalate has been widely used in analysis as an ''oxidative'' or ''oxidative-reductive'' coreactant due to its ability to form a strong reducing agent ($CO_2^{\bullet -}$) upon electrochemical oxidation. By adding excess $C_2O_4^{2-}$ to the QD solution, Bard's group demonstrated strong light emission from an Si-QD solution, in which $CO_2^{\bullet -}$ can inject an electron into the LUMO of an oxidized Si-QD to produce an excited state that then emits light [8]. The corresponding mechanism for oxalate system can be detailed in Scheme 2.

2.2.2. Tri-n-propylamine (TPrA) system. TPrA is another important popular ''oxidative-reductive'' coreactant for ECL systems. The whole procedure for the TPrA involved QDs ECL is deduced as Scheme 3. Upon oxidation, the short-lived TPrA radical cation ($TPrA^{\bullet +}$) is believed to lose a proton from an α-carbon to form the strongly reducing intermediate $TPrA^{\bullet}$. Finally, the reaction between electrogenerated oxidized QD ($QD^{\bullet +}$) and $TPrA^{\bullet}$ produces ECL emission [18]. Further, different kinds of amines have been used for ECL emission of QDs. For example, an ECL method based on QDs has been developed for the detection of 2-(dibutylamino)ethanol with a linear range of 1.7×10^{-8}–1.5×10^{-4} M [19]. Recently, the anodic ECL emissions of 3-mercaptopropionic acid (MPA)-capped CdTe/CdS-QDs [20] and gold (Au) nanoclusters [21] with amines as the coreactant were obtained in aqueous solution.

2.2.3. Peroxydisulfate ($S_2O_8^{2-}$) system. Different from those of oxalate and TPrA systems, the reduction of peroxydisulfate produces the strong oxidant, $SO_4^{\bullet -}$, which can undergo an electron-transfer reaction with an ECL luminophore to generate light. Bard and co-workers first demonstrated that peroxydisulfate can be used as coreactant for the ECL emission of Si-QDs [22]. In the potential region for QD reduction, $SO_4^{\bullet -}$ is generated by electrochemically reducing peroxydisulfate, and then reacts with the negatively-charged QDs by injecting a hole into the highest occupied moleculoar orbital to produce an excited state of the QDs. The general mechanism for peroxydisulfate system can be drawn as Scheme 4. With peroxydisulfate as coreactant, the hollow spherical CdSe-QD nanoassemblies show strong ECL emission in an aqueous solution of pH $\leqslant$ 7.95 [23]. After treated by heating in the presence of ammonia, CdS nanocrystals show greatly enhanced ECL ~310 times greater than that of an untreated CdS/glassy carbon electrode (GCE) in phosphate buffer solution (pH 7.0) containing 0.1 M $K_2S_2O_8$, which facilitates the reduction process of CdS-QDs, and consequently improves the quantity of the excited states (CdS^*), leading to enormous enhancement in ECL signal [24]. An in situ induction-precipitation approach via electrochemical reduction has been successfully used to prepare CdS-polyamidoamine (CdS–PAMAM) nano-composite membranes on electrode surfaces. The resulting film shows 55-fold enhanced ECL compared with that of CdS nanofilm without PAMAM, and the stability of the ECL can be modulated by this electrochemical method. It would open the way to preparation of stable, strong ECL nanocomposite films for analytical application [25].

2.2.4. Hydrogen-peroxide (H_2O_2) system. Since hydrogen peroxide is the substrate of many enzymes, many bioanalytical applications of ECL have been developed by using hydrogen peroxide as a coreactant [26]. Zou and Ju firstly demonstrated that the electron-transfer reaction between electrochemically-reduced nanocrystal species and H_2O_2 can produce ECL emission from CdSe-QDs [13]. Because the dissolved oxygen can be electrochemically reduced to H_2O_2 and then works as

$$QDs - e^- \rightarrow QDs^{\bullet +} \quad (9)$$

$$TPrA - e^- \rightarrow TPrA^{\bullet +} \rightarrow TPrA^{\bullet} + H^+ \quad (10)$$

$$QDs^{\bullet +} + TPrA^{\bullet} \rightarrow QDs^* + TPrA \quad (11)$$

$$QDs^* \rightarrow QDs + h\nu \quad (12)$$

Scheme 3.

$$QDs + e^- \rightarrow QDs^{\bullet -} \quad (13)$$

$$S_2O_8^{2-} + e^- \rightarrow SO_4^{\bullet -} + SO_4^{2-} \quad (14)$$

$$SO_4^{\bullet -} + QDs^{\bullet -} \rightarrow QDs^* + SO_4^{2-} \quad (15)$$

$$QDs^* \rightarrow QDs + h\nu \quad (16)$$

Scheme 4.

$$QDs + e^- \rightarrow QDs^{\bullet -} \quad (17)$$

$$O_2 + 2H_2O + 2e^- \rightarrow H_2O_2 + 2OH^- \quad (18)$$

$$2QDs^{\bullet -} + H_2O_2 \rightarrow 2OH^- + 2QDs^* \quad (19)$$

$$QDs^* \rightarrow QDs + h\nu \quad (20)$$

Scheme 5.

$$QDs - e^- \rightarrow QDs^{\bullet +} \quad (21)$$

$$SO_3^{2-} - e^- \rightarrow SO_3^{\bullet -} \quad (22)$$

$$2OH^- + SO_3^{\bullet -} + 2O_2 \rightarrow O_2^{\bullet -} + SO_4^{2-} + H_2O \quad (23)$$

$$QDs + O_2^{\bullet -} \rightarrow QDs^{\bullet -} + O_2 \quad (24)$$

$$QDs^{\bullet -} + QDs^{\bullet +} \rightarrow QDs^* + QDs \quad (25)$$

$$QDs^* \rightarrow QDs + h\nu \quad (26)$$

Scheme 6.

coreactant for ECL emission, the general mechanism of ECL for H_2O_2 (or dissolved oxygen) system can be drawn as Scheme 5. By using a controlled solution-precipitation method, hierarchical CdS-nanotube arrays assembled in an anodic aluminum oxide template can display considerably enhanced solid-state ECL in H_2O_2 solution compared with those of random NP aggregates [27].

2.2.5. Sulfite (SO_3^{2-}) system. SO_3^{2-} as a coreactant was developed to enhance the anodic ECL of MPA-capped CdTe-QDs by Ju's group in 2008 [15]. This system combines coreactant and annihilation pathways. In the potential sweep range 0–+1.10 V, $QDs^{\bullet +}$ are directly generated by the electric oxidation of QDs, and $QDs^{\bullet -}$ can be formed by the interaction between QDs and the electrooxidition-produced superoxide anion. The excited QDs^* can be produced from the combination of $QDs^{\bullet +}$ and $QDs^{\bullet -}$, and then return to the ground state accompanied with photon irradiation. The whole ECL process can be expressed as Scheme 6. The sulfite-enhanced anodic ECL emission provides an alternative for traditional ECL-light emitters and a new methodology for extremely sensitive ECL detection of monohydroxybenzenes and dihydroxybenzenes [15], dopamine [28] and nitrite [29] at relatively low anodic potential.

3. Biosensing strategy based on ECL emission of QDs

3.1. Determination of biochemical coreactant

As H_2O_2 is a substrate of multifarious enzymatic reactions, plenty of ECL biosensors for peroxidase-related analytes can be developed by combining an enzymatic reaction to change the concentration of H_2O_2 as ECL coreactant [30,31]. The first example of a QD-based ECL sensor was fabricated for detection of H_2O_2 coreactant by depositing a CdSe-QD film on a graphite electrode. A linear response of ECL to H_2O_2 was observed in the concentration range of 2.5×10^{-7}–6×10^{-5} M [13]. Afterwards, a sensor based on the ECL emission of blue-emitting ZnSe-QDs was developed to detect H_2O_2 in the pig kidney (pk-15) cell, veto cell and mineral water, respectively [32]. Under optimal conditions, the dynamic range was 6.10×10^{-7}–3.10×10^{-4} M H_2O_2, and the good reproducibility showed its potential application in real samples. For more promising applications in biological detection, less toxic ZnS nanocrystals doped with Mn^{2+} have been immobilized on a GCE and displayed a specific ECL emission at ~−1.50 V *vs* SCE for direct detection of H_2O_2 [33]. In addition, TiO_2 nanocrystals have been modified on a GCE surface with the help of Nafion for the detection of dissolved oxygen in aqueous solution. The ECL intensity is linear with the dissolved oxygen concentration in the range 0.30–10.00 mg/L with a limit of detection (LOD) of 0.12 mg/L. The method developed can be applied to detect dissolved oxygen concentration or biochemical oxygen demand [34].

To enhance the ECL emission efficiency, modifying the ECL system with functional materials has been performed to obtain high sensitivity and good detection performance [35]. For example, carbon nanotubes (CNTs) can greatly enhance the ECL of CdTe-QDs dispersed in aqueous solution. Consequently, a method for the determination of methimazole has been developed with a CNT-film-modified electrode [36]. Jie et al. found that the ECL of CdSe-QDs could be greatly enhanced by co-immobilizing CNTs and poly(diallyldimethylammonium chloride) in the CdSe-QD film, and developed a sensitive ECL immunosensor for the detection of human IgG(Ag) [37]. Ju and co-workers prepared a nanocomposite of CdSe-QDs with nitrogen-doped CNTs [38]. With hydrogen peroxide as co-reactant, the cathodic ECL emission from the nanocomposite-modified electrode was five times stronger than that from pure CdSe-QDs, and three times stronger than that from CdSe-QDs composited with CNTs.

Significantly, the ECL intensity of ZnO/CNT nanocomposites with lily-like structures is almost an order of magnitude greater than that of pure ZnO nanoflowers, and the ECL starting voltage shifts positively from −1.06 V to −0.41 V due to the presence of CNTs, which decrease the barrier of ZnO reduction during the ECL process [39]. By facilitating CdTe-QD oxidation and triggering $O_2^{\bullet -}$ generation, graphene oxide can also result in enhanced ECL for glutathione sensing [40]. The graphene-CdS nanocomposite not only enhances its ECL intensity by about 4.3-fold but also decreases its onset potential by about 320 mV [41]. In addition, a DNA

cycle device has been found to be an alternative way to improve the ECL signal for thrombin assay [42].

3.2. Analyte inhibiting ECL emission

This strategy is mainly based on the inhibition effect of the analyte on the QD ECL [43]. For example, in the ECL system of thioglycolic acid (TGA)-capped CdSe-QD film/peroxide, it is believed that the intermediate $OH^{\cdot}$ radical is the key species for producing hole-injected QDs [44]. Thus, a highly sensitive method for the detection of both scavengers and generators of $OH^{\cdot}$ has been developed. Using glutathione (GSH) and L-cysteine as models, the ECL intensity decreases linearly in the concentration ranges of 2.0–60 μM for GSH and 2.0−50 μM for L-cysteine with LODs of 1.0 μM for GSH and 2.0 μM for L-cysteine at an S/N ratio of 3, respectively.

On the basis of the competition of metal ion to the stabilizer, the quenching effect of metal ion on ECL emission leads to a sensitive chemical sensing application. Using Cu^{2+} as a model analyte, the competitive binding of Cu^{2+}, with a stronger metal-S interaction than the Cd-S bond, to the stabilizer leads to a quenching effect on ECL emission [17], so a sensor for Cu^{2+} with a linear range of 5.0 nM–7.0 μM and an LOD of 3.0 nM was successfully developed and applied in the detection of copper in human hair.

3.3. ECL biosensing based on resonance-energy transfer

ECL quenching can occur via charge transfer, which has been applied in most analytical systems. It can also occur via energy transfer for the development of photoluminescence-based biosensors. An QD-based ECL emitter comprises the excited QDs, and the possible energy transfer from excited QDs can provide a strategy for biosensing [45,46]. This strategy mainly works in two ways, as follows.

One is ECL-energy transfer directly from the excited CdTe-QDs to analyte, the quencher. For example, the intensive anodic ECL emission from MPA-capped CdTe-QDs can be observed with a peak at +1.17 V (*vs.* Ag/AgCl) in pH 9.3 PBS at an indium-tin-oxide electrode [16]. In the presence of catechol derivatives (e.g., dopamine or L-adrenalin), the energy of excited CdTe-QDs can transfer to these derivatives to decrease the ECL emission [16].

Another is the energy transfer from excited QDs to a quencher (not to analyte), which is related to the amount of analytes. For example, the energy transfer from an excited CdS:Mn-NP film to proximal Au-NPs can quench the ECL emission [45], and the quenching process depends on the distance between excited QDs and Au-NPs. When using Au-NPs to label a DNA probe, hybridization with target DNA can enlarge the distance between Au-NPs and CdS:Mn-NPs, hence enhance the ECL emission of the CdS:Mn-NP film. By changing the distance between excited QDs and quencher, an opto–magnetic interaction–induced enhancement of ECL from CdS:Mn-QDs film by superparamagnetic Fe_3O_4-NPs has also been achieved for ultrasensitive antigen detection [46].

3.4. Determination by enzymatic reaction

The QD-based ECL biosensor was first developed by coupling with a glucose-oxidase catalyzed reaction for glucose detection [14]. In this ECL-biosensing system, TGA-capped CdSe-QDs and glucose oxidase were co-immobilized on an electrode surface, and glucose was detected by measuring the ECL decrease due to the consumption of dissolved oxygen as coreactant, which resulted from the enzymatic oxidation of glucose. This strategy can be applied in more bioanalytical systems for oxidase substrates. By combining the enzymatic cycle of trace tyrosinase to produce *o*-quinone, an extremely sensitive ECL detection of tyrosine with a sub-picomolar LOD has been achieved [15].

Most recently, by coupling to the surface unpassivated QDs with HRP as a model enzyme and hydroquinone (HQ) as its substrate, an ECL biosensor for phenolic derivatives was constructed. After HRP was assembled on the QD/GCE surface, H_2O_2 produced in the cathodic reduction of disolved oxygen would be consumed by the enzymatic oxidation of HQ. Consequentially, the consumption of coreactant H_2O_2 could weaken the ECL process, as shown in Fig. 2. Based on the quenching effect, the biosensor for HQ detection showed a linear range of 0.2–10 μm with an LOD of 24.7 nM at a S/N ratio of 3 [47].

Based on the ECL emission of CdS-QDs formed in situ on CNTs, two signal-on ECL enzyme biosensors were successfully fabricated for the detection of choline and acetylcholine. Compared with pure CdS-QDs, the CNTs enhanced with CdS-QDs showed 5.3-fold higher ECL intensity, and the onset ECL potential also shifted to a ~400 mV more positive potential, which reduced H_2O_2 decomposition at the electrode surface and increased the detection sensitivity of H_2O_2 [48].

4. ECL bioanalytical applications of QDs

4.1. DNA analysis

Of the diverse DNA-detection techniques, ECL-based biosensing has received considerable attention due to its versatility, simplified optical set-up, and good temporal and spatial control [49]. A common approach to ECL DNA sensing is to use QDs as ECL emitters to label bio-recognition molecules (e.g., DNA strand, avidin, or antibody). For example, a QD-labeled avidin has been used for ECL monitoring of biotin-modified target DNA by recognizing avidin and hybridizing target DNA with a thiol-modified DNA probe pre-immobilized on an Au electrode [50]. Based on the ECL increase, the biosensor

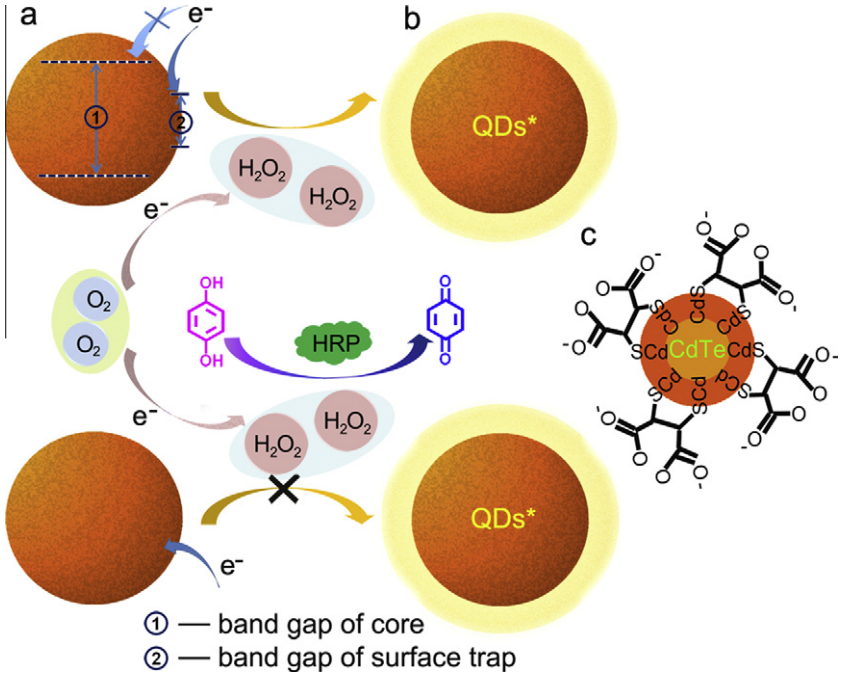

Figure 2. Cathodic electrochemiluminescence (ECL) mechanism of graphite-carbon electrode (GCE)/quantum dot (QD)/chitosan and biosensing mechanism of the biosensor for hydroquinone (HQ). (a) Electron injection to surface trap of QDs, (b) ECL emission from excited QDs, and (c) chemical banding structure of QDs. The quenching results from the consumption of H_2O_2 formed during the cathodic scan. (Reprinted from [47] with permission from Wiley).

shows a wide linear range of 5 nM−5 μM and a detectable concentration of 10 pM.

A simple ECL-sensing platform has been constructed for highly sensitive, specific detection of DNA targets with CdS:Mn-QDs as the ECL luminophores and Au-NPs functioning as both ECL quencher and enhancer (Fig. 3). Upon hybridization with target DNA, ECL enhancement occurs due to the interactions of the excited CdS:Mn-QDs with ECL-induced surface-plasmon resonance in Au-NPs at large separation, providing great sensitivity for detection of DNA. The good linearity is in the target DNA-concentration range 50 aM–5.0 fM [45].

Meanwhile, a QD ECL biosensor for the detection of lysozyme has been developed by forming aptamer-lysozyme bioaffinity complexes at an Au electrode [51]. Its ECL signal is responsive to the amount of QDs bonded to the *c*DNA oligonucleotides, which is indirectly inversely proportional to the combined target protein. Similarly, a QD ECL biosensor has been constructed for the detection of thrombin in the range 0–20 μg/mL [52].

4.2. Immunoassay

The ECL-immunosensing methods developed mainly include two mechanisms:

(1) steric hindrance produced from the formation of immunocomplex [53]; and,
(2) consumption of ECL coreactant in an enzymatic reaction [54].

These effects lead to a target-concentration-dependent decrease of ECL emission. The steric hindrance mechanism can be used for design of a label-free QD ECL immunoassay strategy. For example, based on co-immobilization of antibody with CdSe-QD/CNT-chitosan/3-aminopropryl-triethoxysilane on the electrode surface and increasing resistance upon formation of immunocomplex between the antibody and the target antigen, a label-free ECL immunoassay strategy has been developed for sensitive detection of human IgG (HIgG) [55].

With self-assembly and Au-NP-amplification techniques, a label-free ECL biosensor for low-density lipoprotein (LDL) has been developed [56]. The ECL peak intensity of the biosensor decreased linearly with LDL concentration in the range 0.025–16 ng/mL with an LOD of 0.006 ng/mL. Another ECL immunosensor based on amplification of polyelectrolyte-protected graphene also shows a highly sensitive response to HIgG in the linear range 0.02–2000 pg/mL with an LOD of 0.005 pg/mL [57].

The consumption of ECL coreactant for QD-based immunosensing results from an enzymatic reaction, which can be triggered by combination of the immobilized antigen with enzyme-labeled antibody on the electrode surface. For example, a highly sensitive competitive immunosensor based on the ECL of meso-2,3-dimercaptosuccinic acid (DMSA)-stabilized CdTe-QDs immobilized on an antigen-modified electrode

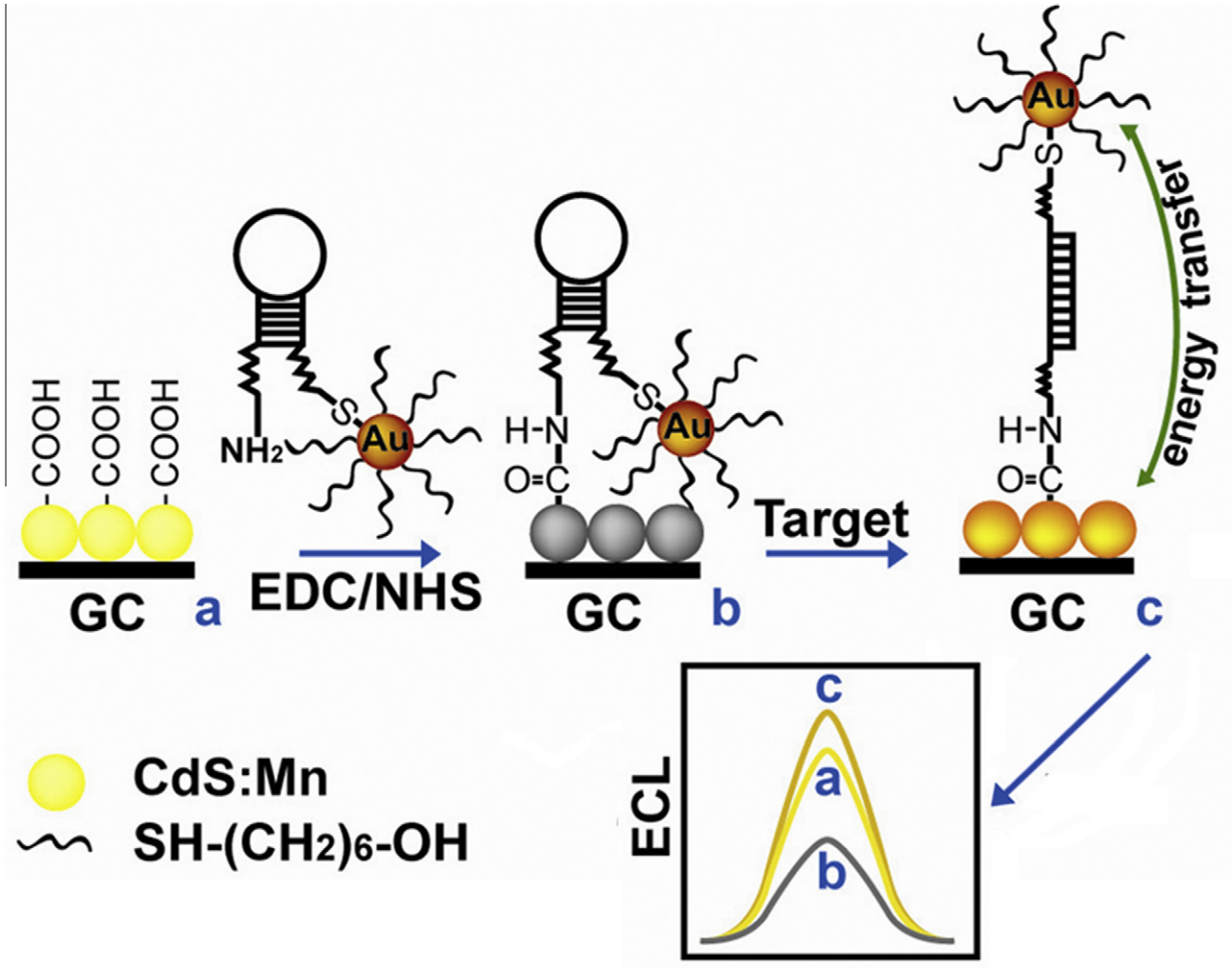

Figure 3. Electrochemiluminescence (ECL) sensing strategy with energy transfer between CdS:Mn quantum dots (QDs) and gold (Au) nanoparticles. (Reprinted from [45] with permission from RSC Publishing).

surface was proposed (Fig. 4). Upon immuno-recognition of the immobilized antigen to horseradish peroxidase-labeled antibody, the ECL intensity decreases. The decrease is due to not only the steric hindrance of the proteins to electron transfer but also an enzymatic reaction consuming the self-produced coreactant, which greatly amplifies the detection signal (ECL change), leading to a wide calibration range of 0.05 ng/mL–5 μg/mL and a low LOD for the competitive immunoassay of human IgG [54].

Recently, a versatile immunosensor using a CdTe-QD-coated silica nanosphere as a label was proposed for ultrasensitive detection of a biomarker [58]. The high loading of CdTe-QDs on a silica nanosphere produced a signal amplification of ECL emission. Compared to that using CdTe-QDs as label, a 6.6-fold enhancement in ECL was achieved. The proposed method can detect IgG down to 1.3 pg/mL. This simple, specific strategy has vast potential to be used in other biological assays.

In addition, magnetic QDs were also important materials for highly efficient immunosensing of a cancer biomarker [59].

4.3. Cytosensing and dynamic monitoring of carbohydrate expression

The initial work on ECL cytosensing and dynamic monitoring of cell-surface carbohydrate expression was designed by Ju's group to analyze cell-membrane carbohydrates by combining the ECL behavior of QDs with specific recognition of lectins to carbohydrates [60]. The specific recognition of QD-bound lectins to cell-surface carbohydrates can capture cells onto the electrode surface and thus decrease the ECL emission due to the steric hindrance of the captured cells to the electron transfer, which depends on the amount of captured cells. The ECL change thus provides a simple, highly sensitive way for ECL cytosensing and dynamic monitoring of cell-surface carbohydrate expression.

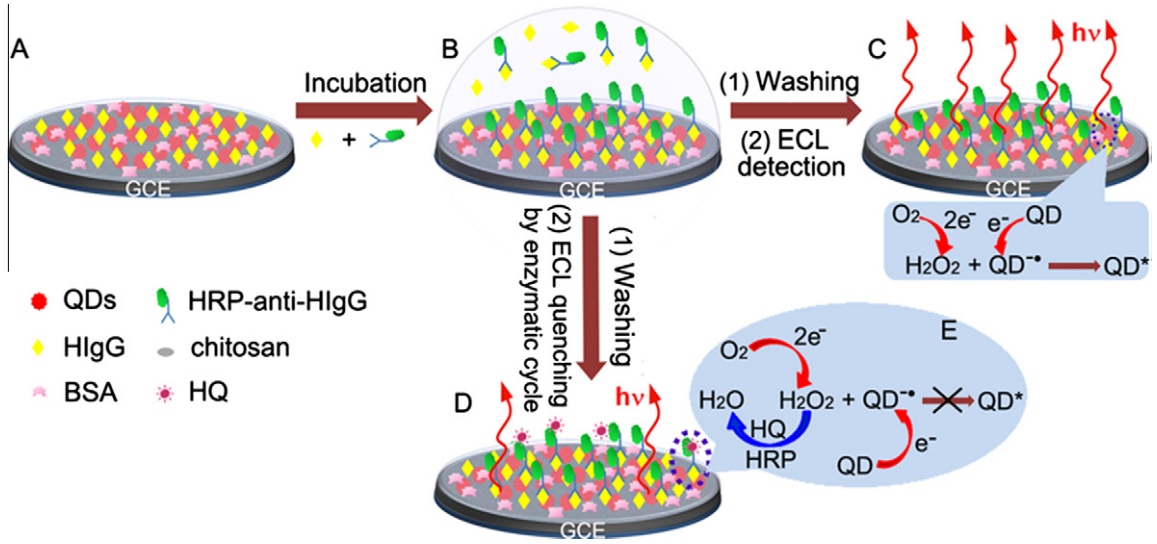

Figure 4. Construction (A) and incubation (B) of the immunosensor, and electrochemiluminescence (ECL) detection without (C) and with (D) the enzymatic amplification by consumption of H_2O_2 as coreactant. (E) Schematic representation of ECL quenching of DMSA-CdTe quantum dots (QDs). (Reprinted from [54] with permission from ACS Publications).

Furthermore, an easier ECL strategy for in situ label-free monitoring of carbohydrate expression on living cells was designed by integrating the competitive recognition of lectin to cell-surface carbohydrate with a carbohydrate-functionalized CdS-nanocomposite-modified electrode [61]. The combination of lectin to electrode surface led to the steric hindrance to the electron transfer, thus decreasing the ECL emisson of QDs. A wide linear response to cells in the range 2×10^3–1×10^7 cells/mL was obtained with an LOD of 1.2×10^3 cells/mL. The average number of mannose moieties on single living BGC cell was detected to be 8.7×10^7. The proposed approach could be expanded to high-throughput detection with the addition of more specific glycan–lectin pairs to the repertoire.

5. Conclusion and outlook

As a new kind of ECL emitter, the QD shows excellent advantages (e.g., high fluorescence quantum yields, size or surface trap-control luminescence, and good stability against photobleaching). Functionalized QDs with multitudinous biomolecules offer excellent platforms for ECL-signal transduction for ultrasensitive biomolecule detection and quantification.

To meet the demand for the fabrication of various nano-scaled electronic and optoelectronic devices, great efforts have been made to prepare these QD nanomaterials with various shapes and special properties (e.g., well-shaped nanotubes, nanowires, and hollow spheres). The highly efficient, low applied potential and tunable QD-ECL systems (emitters and coreactants), especially near-infrared QDs [62], show favorable biocompatibility as good ECL emitters for further improving the analytical performance of QD-based ECL biosensors. Moreover, because metal-based QDs are toxic, it is of great interest to use low-toxicity, eco-friendly alternatives (e.g., silicon and carbon QDs) to develop QD-ECL probes and to realize ECL detection of single molecules in biological systems.

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