Recent Advances in Electrochemiluminescence Analysis

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Electrochemiluminescence (ECL), also called electrogenerated chemiluminescence, refers to a light emission process in which species generated at the electrode surface undergo exergonic electron transfer reaction to form excited states that emit light.1,2 As ECL is emitted through bimolecular recombination of electrogenerated radicals, its mechanism can be divided into two categories according to the source of radicals, namely, annihilation mechanism and coreactant mechanism. As for the former, radical species are generated from a single emitter, while the latter involves a bimolecular set of electrochemical reactions between the emitter and a suitable coreactant.3 The emitter plays a key role in the transformation from electrical energy into radiative energy. Three types of luminophores, including ruthenium(II) complexes, iridium complexes, and the organic systems includes anthracenes, fluorenes, thiényltriazoles, lumilin, and their derivatives. Among these luminophores, tris(2,2′-bipyridine)ruthenium(II) (Ru(bpy)_3^2+) and lumilin are the most successful with very high efficiency in both visible and near-infrared (NIR) regions due to the electron rich core. Thus, they have been widely utilized in vast majority of ECL studies.

In a sense, ECL is the ideal combination of electrochemical and spectroscopic methods. Therefore, ECL not only holds the sensitivity and wide dynamic range inherited from conventional chemiluminescence (CL) but also exhibits several advantages of electrochemical methods including simplicity, stability, and facility.4 On the other hand, as a light emission technique, ECL possesses unique superiorities over other light emission methods, such as photoluminescence (PL) and CL. Specifically, in comparison with CL, ECL has superior temporal and spatial control on light emission. Also, the absence of excitation light in ECL promises near-zero background, while PL suffers from unselective photoexcitation induced background.5 Therefore, ECL has now become a powerful analytical technique and been widely used in a large number of environments, ranging from fundamental studies to practical applications for sensing trace amounts of target molecules.

This Review focuses on developments in ECL assays during 2015 to 2016. There have been hundreds of relevant papers published during the past 2 years. Therefore, a comprehensive review is necessary. The aim of this Review is to outline new advances in areas ranging from new ECL systems, novel sensing mechanisms, strategies for ECL signal amplification to representative sensing applications. Lastly, future prospects for the development of ECL analysis will be discussed. We recommend readers interested in the general principles of ECL methods and sensors to refer to previous excellent reviews for a broad scope in this area.1,2,6,7 We tried to be as comprehensive as possible; however, due to the explosion of publications in this active field, as with any review, it is impossible to cover all of the published works in the past 2 years. For those papers unintentionally missed, we apologize to the authors in advance.

**NOVEL ECL SYSTEMS**

According to the luminophores, ECL systems can be generally classified into three classes including inorganic systems, organic systems, and nanomaterial systems. Specifically, the inorganic systems mainly comprise ruthenium complexes and iridium complexes, and the organic systems includes anthracenes, fluorenes, thiényltriazoles, lumilin, and their derivatives. Among these luminophores, tris(2,2′-bipyridine)ruthenium(II) (Ru(bpy)_3^2+) and lumilin are the most successful with very broad applications. Since the pioneering work of Bard et al. on ECL of silicon semiconductor nanocrystals (also known as QDs) in 2002, the ECL behaviors of various nanomaterials such as QDs, noble metal clusters, and carbon nanomaterials have been extensively studied. The pioneering works concerning classic inorganic systems, organic systems, and nanomaterial systems have been reviewed comprehensively before,8 so it will not be elaborated in detail here. We will introduce some novel luminophores either emerged recently or witnessed rapid development as follows.

**Novel Organic Luminophores.** Boron-dipyrromethene (BODIPY) dyes have a peculiarly high absorption coefficient and PL quantum efficiency in both visible and near-infrared (NIR) regions due to the electron rich core. Thus, they have been...

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widely investigated and have application as fluorescent labels and laser dyes. In addition to the optical properties, BODIPY dyes also possess diverse electrochemical features with a direct correlation to the structure design. Bard and co-workers pioneered the electrochemistry and ECL research of several BODIPY dyes.\textsuperscript{8–10} However, their ECL efficiency was not as high as expected. Highly efficient ECL of a giant BODIPY dye including a biphenyl linker and two long chain (C8) arms in the meso and alpha positions was investigated by Ding et al.\textsuperscript{11} Essentially, the presence of the aromatic chains provides a high possibility for $\pi$ interaction, thus enabling intermolecular electronic transition. Blocking at alpha, beta, or meso positions of the BODIPY core is expected to stabilize the electrogenerated radicals and therefore to enhance the ECL intensity. This kind of BODIPY dye showed an ECL efficiency of $>80\%$ relative to that of Ru(bpy)$_3^{2+}$/tris-propylamine (TPrA) coreactant system, much higher than the other BODIPY dyes. While numerous derivatives of BODIPYs have been designed and their ECL has been investigated, they are often synthesized by time-consuming methods and in low yield. Formazans have gained growing interest due to their facile and low-cost synthesis as well as tunable absorption, emission, and redox properties.\textsuperscript{12} Ding and co-workers reported the first systematic study of the ECL of a formazan-derived species, specifically a boron difluoride formazanate dye. The obtained boron difluoride 3-cyanoformazanate dye was found to be robust in the presence of TPrA as a reductive coreactant, leading to maximum emission at 724 nm with three distinct, voltage dependent mechanisms.

During the past several years, perylene and its derivatives have attracted considerable attention due to their intrinsic advantages, such as good stability, functional flexibility, fast electron transfer rate, outstanding optical properties, and low-cost. Perylene and its derivatives have been proven to be ECL active, but the poor solubility and radical ion stability problems limit their applications in aqueous solution. One of the effective methods for solving this problem is to synthesize some new perylene derivatives by introducing hydrophilic groups, such as carboxyl and amido, to improve the water-solubility. Chen et al. found the cathodic ECL behavior of the ammonolysis product of 3,4,9,10-perylenetetracarboxylic dihydride (denoted as PTC–NH$_2$) in aqueous solutions with K$_2$S$_2$O$_8$ as the coreactant for the first time.\textsuperscript{13} On the basis of the fact that dopamine (DA) could efficiently quench the ECL signals of PTC–NH$_2$, the detection of DA was achieved. However, PTC–NH$_2$/ECL still suffers from imperfect luminescence efficiency due to limited water-solvability, which made it depend on exogenous reagent of K$_2$S$_2$O$_8$ as coreactant. In the same group, a novel covalently cross-linked perylene derivative (PTC–PEI) composed of polyethyleneimine (PEI) and perylenetetracarboxylic acid (PTCA) has been first investigated for cathodic ECL in an aqueous system using endogenous dissolved O$_2$ as coreactant.\textsuperscript{14} The PTC–PEI exhibited admirable physical and chemical stability and higher ECL efficiency than other perylene derivatives, which held an alternative way to construct ECL sensor with improved sensitivity.

Though thiophene molecules are capable of generating ECL, reports on donor–acceptor $\pi$-conjugated (D–$\pi$–A) systems consisting of thiophene, triazole, and electron acceptor are less common. Moreover, the synthesis of elaborated thienyl compounds is difficult to realize. Ding’s group reported the successful click coupling of 3-azidothiophene and 4-azido-2-$\text{N}^2$-bithiophene with a variety of aryl acetylenes to synthesize eight thiophene-based luminesphores intended for electrochemical and ECL study.\textsuperscript{15} Their ECL behaviors, in both annihilation and coreactant systems with benzoyl peroxide (BPO), ammonium persulfate, and TPrA were also investigated. ECL in the annihilation route confirmed the weak light-emitting nature of these thiophenes. However, with the addition of oxidizing coreactants, the efficiency could be increased. ECL spectroscopy revealed that the excimer or polymeric excited states were more favorable in formation than their monomeric excited states, which was tunable based on the applied potentials.

Star-shaped conjugated oligomers, a branched molecules comprising a central core with linear polymer arms, have received considerable attention recently since they have advantages from both the core and arms in electrical, optical, and morphological properties.\textsuperscript{16} The $\pi$-conjugated oligofluorophores have received particularly attention due to their blue electroluminescent properties. However, the electrochemistry of these compounds and ECL in solution has not been reported. Bard et al. reported ECL of three 1,3,5-tri(anthracen-10-yl)-benzene-centered starburst oligofluorophores (T1–T3) in acetonitrile–benzene solution.\textsuperscript{17} The compounds T1–T3 contain 1,3,5-tri(anthracen-10-yl)-benzene as a core with fluorene as an arm from monofluorene to trifluorene groups ($n = 1–3$), generating a rigid three-dimensional structure. The formal potentials of the sequential removal or addition of electrons from the core and the arms were evaluated. The mechanisms of multiple electron transfer were confirmed by chronoamperometry at an ultramicroelectrode, digital simulations, and DFT calculations. Strong blue ECL emission could be generated under ion annihilation condition from T1–T3, assigned as S-route. These compounds can be used as promising candidates for ECL materials.

**Novel Inorganic Luminophores.** To meet the increasing demand for accuracy and multiplexing in diagnostics, ECL emitters with high efficiency and different emission colors are urgently needed. In comparison with the mostly used Ru(bpy)$_3^{2+}$/tris-propylamine (TPrA) coreactant systems, cyclometalated iridium(III) complexes generally show much higher PL efficiency and easy tunability of the emission energies, thus becoming an increasingly active ECL subject in the past few years. By decorating the main ligand and changing the coordination mode, a series of cyclometalated iridium(III) complexes with 2-phenylquinoline or its derivatives were designed, synthesized, and thoroughly investigated by photophysics, electrochemistry, and ECL.\textsuperscript{18} By incorporating methyl groups into the 2-phenylquinoline, the corresponding complexes displayed lower oxidative potential and higher HOMO energy levels, resulting in considerably stronger ECL emissions than that of Ru(bpy)$_3^{2+}$ in acetonitrile solutions. The ECL of cyclometalated iridium(III) complexes are mainly studied in organic media, and only a few examples in aqueous media were reported. Cola et al. reported on the PL and ECL behavior of bis-cyclometalated Ir(III) complexes, [Ir(1,10-phenanthroline)(ppy)$_2$]+ (CN) = cyclometalated ligand, LX = picolinate (pic), acetylacetonate (acac)), in organic solvent and in the aqueous buffer solution used in commercial immunoassays.\textsuperscript{19} The results showed that modification of the CN ligand could lead to ECL efficiency higher than the commercial Ru-based labels. In particular, the complex based on phenylphenanthridine (ppht) as the CN ligand, showed a signal ~3 times higher than Ru(bpy)$_3^{2+}$ employed in commercial equipment.

Because of rich structural diversity and remarkable PL properties, coinage-metal alkynyl complexes have attracted considerable attention in the last few decades. However, ECL properties of coinage-metal alkynyl complexes were completely unexplored. Wei’s group reported ECL from Ag$_3$Cu$_8$ hetero-
metallic alkylnyl clusters for the first time. The synthesized \( \text{Ag} \), \( \text{Cu} \), \( \text{Au} \) heterometallic clusters showed a windmill-like structure that could be regarded as a hexagonal bipyramidal rotational axis (\( \text{Ag} \), \( \text{Cu} \)) and three sails of the windmill consisting of three dpppy ligands (dpppy = 2,6-bis(diphenylphosphino)pyridine). The \( \text{Ag} \), \( \text{Cu} \) heterometallic clusters displayed novel PL and ECL properties, which could be modified by changing the substituent on the alkynyl ligands.

The inherent potential initiated excitation and emission processes of ECL make it promising for multiplexed detection by generating and detecting multiple emissions simultaneously or sequentially in a single system comprising different luminophores. Previous reports have showed the possibility of selectively exciting coreactant ECL from a mixture of two or more transition metal complex luminophores with a distinct difference in their emission colors and/or oxidation potentials. However, annihilation ECL from mixed transition metal complex luminophores with a distinct difference in their emission colors and/or oxidation potentials.21,22 However, annihilation ECL from mixed transition metal complex systems in a single solution is yet to be explored. Hogan and co-workers have examined the multicolor emissions from a series of mixed annihilation ECL systems containing \( \text{Ru} \), \( \text{Os} \) and various cyclometalated iridium(III) chelates exhibiting green or blue luminescence by utilizing an electrochemical cell coupled with a CCD spectrometer for instantaneous collection of emission spectra.25 This system showed simultaneous emissions from multiple emitters, and the ratio of these emissions (and hence the overall color of the luminescence) could be tuned though the applied electrode potentials, exploiting the multiple, closely spaced reductions and oxidations of the reactants. In a later report, they investigated the detailed mechanism of annihilation ECL from mixed luminophores.25 They finally rationalized the results through several complementary mechanisms, including resonance energy transfer and various energetically favorable electron-transfer pathways.

Apart from these so-called mixed ECL systems, variable color ECL from a single molecular emitter has rarely been reported. Hogan et al. demonstrated an unusual ECL behavior of \( \text{fac-tris}[5-(4-	ext{fluoro}-3-	ext{methylphenyl})-1-	ext{methyl}-3-	ext{n-propyl}-1,2,4-	ext{triazolyl}]	ext{iridium(III)} \) complex, \( \text{Ir} \)-pmpt-.25 Several different emission colors, including red, green, blue, and near white, could be reversibly generated by changing the applied potential. The annihilation ECL mechanism was investigated using the 3D-ECL technique, where the ECL spectral profile was continuously monitored as a function of potential during voltammetric scanning. The results shown that the multicolored ECL in this system arises as a result of the formation of small traces of two highly emissive electrolysis products. At least one of the products appeared to result from oxidative dissociation of a methyl group from the triazole moiety. In another work, Sun’s group synthesized bimetallic Ru–Os complexes, \( \text{[bppy]}_2\text{Ru(bpy)}_3 \) \( \text{(CH}_3\text{)}_2\text{Osbppy}_2 \), by connecting normal red Ru- and near-infrared Os-ECL labels through a flexible saturated C chain to obtain infrared/near-infrared dual-emission ECL at around 620 nm for Ru and 730 nm for Os.26 Concerted intra- and intermolecular ECL performance and ratiometric ECL detection were investigated and utilized to reduce the amount of TPcA coreactant necessary in the system.

**Novel Nanomaterial System.** Since the first report on ECL phenomenon of silicon QDs, multifarious QDs including CdS, CdSe, CdTe, ZnS, Ag,Sn, and the corresponding alloyed or core–shell structure QDs have been reported.27–34 Besides, other miscellaneous nanomaterials have emerged and received considerable attention as effective ECL emitters in recent years, including carbon nanodots, noble metal nanoclusters, graphene-like carbon nitride, upconversion nanoparticles, and polymer dots.35–43

Gold nanoclusters (Au NCs) represent a new type of ECL nanomaterials due to their discrete electronic energy and direct electron transition. However, the application of Au NCs is limited by their relative low PL and ECL efficiency. Wei et al. localized Au NCs in to 2D layered double hydroxides (LDHs) nanosheets via a layer-by-layer assembly process to obtain Au NCs-based ultrathin films (UTFs), which exhibited an ordered structure with Au NCs anchoring onto LDH nanosheets densely and uniformly.38 Because of the host–guest interaction, Au NCs were stabilized in the confined environment of LDH nanosheets, resulting in reduced nonradiative transition and thus enhanced PL and ECL performances. The Wang group recently found a novel mechanism to drastically enhance the ECL by covalent attachment of coreactants N,N-diethylhexylenediamine (DED) onto lipoic acid stabilized Au (Au-La) clusters with matching redox activities.44 This design reduced the complication of mass transport between the reactants during the lifetime of radical intermediates. The intracal cluster reactions are highly advantageous for applications by eliminating additional and high excess coreactants otherwise needed. The multiple energy states per Au cluster and multiple DEDA ligands also contributed to the enhanced ECL efficiency, which was multifold higher than the standard Ru(bpy) \( _3 \) \( ^{2+} \) system with excess coreactants TPcA.

Since the first report on ECL of poly(9,9-dioctylfluorene-co-benzothiadiazole) dots in acetonitrile solution, polymer dots (PDs) have aroused growing interest as promising ECL nanomaterials.45 However, because of their poor water solubility, pioneering ECL systems of PDs worked in organic solvent. The toxicity of the employed organic solvent make the application of PDs in the ECL field become a great challenge. Therefore, the synthesis of hydrophilic PDs and their ECL performances in aqueous solutions are of paramount importance. Stable, uniform, and hydrophilic PDs were synthesized by capping conjugated polymer, [poly[2-methox)-5-(2-ethylhexyloxy)-1,4-phenylenevinylen] (MEH-PPV) particles, with Triton X-100.46 For the first time, the ECL emission of PDs was investigated in aqueous solution. The PDs exhibited annihilation ECL activity upon switching potential between anodic and cathodic potentials in the absence of coreactants and emitted bright anodic and cathodic ECL emission in the presence of TPcA and \( \text{S}_2\text{O}_8^{2-} \), respectively. The nonsurface state ECL mechanism and easily tunable band gap of the PDs enable the synthesis and applications of multicolor PDs. Chen et al. found the anodic ECL behavior of water-soluble PFO dots (poly(9,9-dioctylfluorenyl-2,7-diyl) with \( \text{Na}_3\text{C}_2\text{O}_4 \) as coreactant. On the basis of the quenching effect of melamine on the ECL signal of the PFO-\( \text{C}_2\text{O}_4^{2-} \) system, a new ECL sensing method for melamine was further developed.46 In another report by the same group, \( \text{H}_2\text{O}_2 \) was used as a coreactant to enhance the ECL intensity of the PFO dots.47 Afterward a novel donor–acceptor conjugated polymer backbone containing silole and 9-octyl-9H-carbazole units was further used to prepare PDs with a nanoprecipitation method.48 The resulting PDs was proved to be a low-potential ECL emitter with strong anodic ECL emission at +0.78 V (vs Ag/AgCl) in the presence of coreactant TPcA in aqueous solution, which came from the band gap emission of the excited PDs.

Metal–organic frameworks (MOFs) are promising ECL active species because of their high mass transfer capacities and electrocatalytic efficiency. However, MOF-based electrochemical systems are less advanced, let alone ECL investigation. Yin’s
group reported the ECL from a redox-active MOF prepared from \([\text{Ru}(4,4′-(\text{HO}_2\text{C})_2\text{-bpy})_2\text{bpy}]^{2+}\) and Zn\(^{2+}\).\(^{49}\) The MOF structure was independent of its charge and is therefore stable electrochemically. ECL emission with good concentration-dependent response toward coreactant of TpPa was observed, which has not been reported previously for MOFs. The high ECL emission suggested the admirable electron transfer between the MOF and coreactants. Instead of organic molecules, the use of a metallic coreactant K\(_2\)S\(_2\)O\(_8\) was suggested as a good method to endow MOFs with excellent ECL activity. Novel luminescence-functionalized MOFs with superior ECL properties were synthesized based on transition metal centers, such as tris(4,4′-dicarboxylicacid-2,2′-bipyridyl)ruthenium(II) dichloride \((\text{[Ru(dcbpy)]}_2^{2+})\) as the ligands.\(^{50}\) As a special type of nanoporous materials, the special structure and properties of MOFs have been widely investigated. However, the ECL behavior of CD-based MOFs is less reported. We et al. reported the excellent ECL behavior of \(\text{Pb(II)-β-CD}\) MOF using K\(_2\)S\(_2\)O\(_8\) as a coreactant.\(^{51}\) Pb\(^{2+}\)-CD also showed unexpected reducing capacity toward Au\(^{3+}\) and Ag\(^{+}\). Au and silver nanoparticles \(\text{NPs})\) were in situ formed on Pb\(^{2+}\)-CD without adding any other reductant.\(^{51,52}\) The doped Au and silver nanoparticles could facilitate the ECL emission and increase the biocompatibility of Pb\(^{2+}\)-CD, which is beneficial to fabricate an ECL biosensor.

Nanostructured metal oxides possessing big surface area and good reaction activity are potential candidates for the fabrication of biosensors. Nevertheless, the investigation about ECL of nanostructured metal oxides is less well reported. CeO\(_2\) nanoparticles were first exploited as an ECL luminescent material with K\(_2\)S\(_2\)O\(_8\) as a coreactant.\(^{53,54}\) Since CeO\(_2\) suffers from low electron conductivity, graphene oxide \((\text{GO})\), multwall carbon nanotubes \((\text{MWCNTs})\), and Au NPs were adopted as carriers of CeO\(_2\) to improve the conductivity and the surface-to-volume ratio in these works. Liu et al. revealed the ECL activity of nontoxic, chemical stable, and low-cost CuO nanowires \((\text{NWs})\) for the first time.\(^{55}\) They prepared reduced graphene oxide supporting CuO NWs \((\text{CuO NWs/rGO})\) as a novel platform for ECL sensing system. The immobilization of CuO NWs on rGO could not only ensure recyclability of the ECL-based sensor but also further amplify the ECL signal of CuO NWs. The ECL sensors based on Zn-based II−VI semiconductors have rarely been investigated due to the instability and the wide band gap. Wang et al. prepared ZnO-nanocrystal-decorated nitrogen doped graphene \((\text{N-GR})\) composites via one-step thermal-treatment route. Compared with ZnO-nanocrystal-decorated undoped graphene \((\text{ZnO/GR})\), the ZnO/N-GR could not only enhance the ECL intensity by 4.3-fold but also move the onset ECL potential more positively for about 200 mV.\(^{56}\)

The detection of new highly efficient, biocompatible, and tunable ECL nanomaterials has attracted increasing interest thanks to their biocompatibility, well-defined structures, and capability of molecular recognition. Yang et al. at the first time explored the ECL properties of cationic dipetide self-assembled nanovesicles \((\text{PNVs})\).\(^{57}\) The cathodic ECL of the PNVs modified glassy carbon electrodes \((\text{GCE})\) was observed in the presence of coreactant K\(_2\)S\(_2\)O\(_8\). Furthermore, dopamine \((\text{DA})\) was chosen as a model analyte to study the potential of the PNVs in the ECL analytical application. Since the surface of the PNVs consisted of aromatic stacking arrangement, DA could be adsorbed on the PNVs due to strong π–π interaction, which could accelerate the electron transfer from the DA directly to the PNVs, finally leading to the enhancement of ECL signal.

### DETECTION METHODOLOGIES AND SIGNALING AMPLIFICATION STRATEGIES

#### General Detection Methodologies

Because of the outstanding characteristics of ECL, ECL has been considered a powerful analytical technique and growing amounts of ECL bioassays have been constructed in the past several decades for the detection of miscellaneous target analytes. The approaches for the construction of sensitive ECL assays can be generally classified into five broad categories:\(^{7,58}\) First, the inhibiting or enhancing effect of the target analytes on the ECL reaction by means of either energy transfer or electron transfer. Second, the reinforcement or decomposition of ECL emitters via either redox reaction or surface binding/detachment is also a useful sensing method. Third, a strategy to alter the emission of ECL through the generation or consumption of coreactants, which is mainly realized by enzymatic reactions. Fourth, steric hindrance from biorecognition reactions or target induced deposition has enabled the development of a signal-off ECL sensing systems. Last, the recently emerged ECL resonance energy transfer \((\text{ECL-RET})\) has been widely adopted as an efficient sensing strategy based on overlapped spectra of donors and acceptors.

Following these sensing approaches, various signal amplification strategies have been developed to further boost the sensitivity of ECL sensors. The most common amplification strategy is the usage of multifunctional nanomaterials thanks to the remarkable achievements in nanotechnology and nanoscience. Nanomaterials can serve as electrode materials or carriers for either ECL emitters or recognition elements owing to their large specific surface area. In addition, functional nanomaterials cannot only produce a synergic effect among catalytic activity, conductivity, and biocompatibility to accelerate the signal transduction but also amplify recognition events with specifically designed signal tags, leading to highly sensitive biosensing.\(^{69}\) So far different kinds of nanomaterials have been used for ECL signal amplification, such as graphene sheets,\(^{60-62}\) carbon nanotubes,\(^{63-66}\) and metal nanomaterials.\(^{54,67-70}\)

Catalytic reactions are also widely used to amplify ECL by using enzyme, enzyme mimics, or nanocatalyst signals, such as horseradish peroxidase \((\text{HRP})\) \(^{71-73}\) and DNAzyme.\(^{74,75-76}\)

Despite these burgeoning developments, there is still high demand for the development of highly sensitive ECL platforms. Novel signal amplification strategies are springing up and have now become the main driving force of innovation for ECL assays.

#### Novel Signal Amplification Strategies

At present, the ECL detection is usually based on the single emission intensity changes. False positive or negative errors may interfere with the detection of trace level analytes due to instrumental or some environmental factors.\(^{77}\) Thus, there is an urgent need to seek an efficient ECL system to minimize and even eliminate these interference factors. Ratiometric assay, in which the quantification depends on the ratio of two signals instead of absolute values, is an ideal strategy to limit the interference factors via normalizing environmental variation by self-calibration, which has attracted widespread attention recently.\(^{40,78}\) According to mechanism of ECL, the ratiometric ECL systems should include both dual-potential and dual-wavelength signal ratiometric assays. Until now, most works adopted the dual-potential ratiometric ECL approach in biological and chemical analysis.\(^{74,79-85}\) Dual-wavelength ratiometric ECL has rarely been...
developed in analytical detection due to the restriction by the luminescence intensity and wavelengths of commonly used ECL emitters as well as the requirement of special detection instrument. Xu’s group recently reported a dual-wavelength ratiometric ECL approach based on resonance energy transfer (RET) from graphite-like carbon nitride (g-C3N4) nanosheet (460 nm) to Ru(bpy)32+ (620 nm) for sensitive detection of microRNA (miRNA). They also developed a visual color-switch ECL sensing platform for quantitative detection of HL-60 cancer cells based on different colors of luminol (blue) and Ru(bpy)32+ (red). The vast majority of ECL assays adopted coreactant mechanism since the ECL intensity of most luminophores are not strong enough. It is well known that the introduction of an appropriate coreactant into the ECL system can significantly enhance the ECL intensity and effectively improve the detection sensitivity. Most of these coreactants are water-soluble small molecules, which are usually added into the detection solution to amplify the ECL signal. However, the intermolecular interaction between the luminescent reagents and the corresponding coreactants presents defects in poor stability and low efficiency of electron transfer. Besides, the biological toxicity and volatile nature of those coreactants may facilitates the operation and increase the measurement error. Recently the self-enhanced ECL luminophore is proposed by covalently linking the ECL-active luminophore and coreactant into one molecule, which can generate enhanced ECL signal through intramolecular interaction. The intramolecular interactions between luminophore groups and coreactant groups can shorten the electronic transmission distance and improve the luminous stability, thus enhancing the luminous efficiency. These self-enhanced approaches provided a new perspective to construct sensitive ECL system for detection of target analytes.

ECL APPLICATIONS

Metal Ions Detection. Accurate and reliable detection of metal ions is thoroughly significant due to both deficiency, and overdose of them will cause the imbalance of homestasis and subsequent severe diseases. Lead ion (Pb2+), as one of them, is one of the most hazardous metal pollutants. Therefore, rapid and sensitive detection of Pb2+ is of great significance for environmental protection as well as disease prevention and treatment. A sensor for the detection of Pb2+ was constructed by immobilizing CdS QDs and capture probe on gold nanodendrites (Au NDs) modified indium tin oxide (ITO) electrode. With Pb2+-induced activation of DNAzyme, the Ag/ZnO coupled structures were close to the surface of the electrode to catalyze the reduction of H2O2, the coreactant for cathodic ECL emission, leading to a decrease of ECL intensity. Yuan et al. constructed an ECL biosensor using N doped carbon dots (N-CDs) in situ electro-polymerized on GCE as luminophores and Pd−Au hexadecrons (Pd@Au HOHs) as enhancers for the detection of intracellular Pb2+. In this work, Pd@Au HOHs−DNA dendraimers with were formed on N-CDs modified electrode, which could couple Pb2+ in the form of Pb2+-stabilized G4 structure. Therefore, the ECL intensity of the N-CDs was quenched by Pb2+. In another work, a novel ECL-RET system from O2/S2O82− to a kind of amino-terminated polymer derivative (PTC-NH2) was demonstrated and then applied to construct a ratiometric aptasensor for Pb2+ detection. A sensitive ECL-RET switch was obtained where the Pb2+ dominated the amount of PTC-NH2 by generating G-quadruplex structure. The ratio of donor/acceptor peak intensity could be regulated upon the concentrations of Pb2+, thus Pb2+ could be quantitatively detected.

Mercury exposures can cause many adverse health effects in human and wildlife, even at a low concentration level. Therefore, the sensitive detection of Hg2+ is of great importance. Liu et al. developed an ECL sensor with a high-intensity charge transfer interface for Hg2+ detection based on Hg2+-induced DNA hybridization. dsDNA with thymine-Hg2+-thymine (T-Hg2+-T) base pairs exhibited more facile charge transfer, which could accelerate the electron transfer performance and increase the ECL intensity. The increased ECL signals were found to be logarithmically linear with the concentration of Hg2+. Huang et al. prepared a sensitive ECL biosensor for the detection of Hg2+ by self-assembling mercury-specific oligonucleotide on the surface of Au NPs modified ITO electrode. The binding of Hg2+ through T-Hg2+-T coordination could induce a conformation change of the oligonucleotide from linear chain to hairpin. The dual-function oligonucleotide served as the probe to Hg2+ but also a carrier for the conjugation of multiple ECL-generating molecules. A detection limit of 5.1 pm Hg2+ was outstanding from the interference of other metal ions. On the basis of the strong and stable T-Hg2+-T interaction and the quenching effect of Hg2+ on the ECL of N-(aminobutyl)-N-(ethylisoluminol) (ABEI), an ECL aptasensor to detect Hg2+ was successfully developed.

As a key component of vitamin B12, cobalt is vital in humans and biological systems. Li et al. fabricated a dual-potential ratiometric responsive ECL sensor for Co2+ ion detection. Nitrogen-doped graphene quantum dots (NGQDs) could emit two ECL signals at both positive and negative potentials with the participation of dissolved oxygen. In the presence of Co2+ ion, the anodic ECL intensity of NGQDs increased dramatically (amplified about 15 times) while the cathodic ECL decreased obviously. On the basis of the ratio of two ECL intensities, a ratiometric sensor for Co2+ ion was developed and has been applied to detection of Co2+ in real water.

When the concentrations of interfering metal ions are several times higher than that of the target metal ion, it is almost impossible to distinguish which metal ion changes the ECL signals in real sample detection. Zhang and co-workers reported that the dual-ECL signals could be actuated by different ECL reactions merely from graphite-phase polymeric carbon nitride (GPPCN) nanosheets at anodic and cathodic potentials, respectively. They found that different metal ions exhibited distinct quenching/enhancement of the ECL signal at different driven potentials, possibly because of the different energy-level matches between metal ions and GPPCN nanosheets and catalytic interactions of the intermediate species in ECL reactions. On the basis of the preliminary “fingerprint” (ECL quenching or enhancement at cathodic and anodic potentials, respectively) and the linear relationship between the ECL intensity and different concentrations of the metal ions, the false-positive result could be largely avoided without labeling and masking reagents. Especially, Ni2+ ion showed highest quenching efficiency at the cathodic potential range due to the best energy match. Meanwhile, unlike most metal ions, Ni2+ could result in enhancement of the anodic ECL intensity of GPPCN. Thus, the proposed dual-ECL signals of GPPCN was applied for the detection of trace Ni2+ ion with a detection limit of 1 nM in tap and lake water.

Small Molecules Detection. Recently, increasing interest has been drawn to the determination of H2S because it has been recognized as an endogenous gas signal molecule. Ye et al. used a...
novel ruthenium complex, [Ru(bpy)3(bpy-DPA)]2+ (where bpy = 2,2′-bipyridine and bpy-DPA = 4-methyl-4′-[N,N-bis(2-picolyl)aminomethyl]-2,2′-bipyridine) as a recognition unit to construct a reaction-based turn-on ECL sensor for selective detection of extracellular H2S in rat brain. The ECL of [Ru(bpy)3(bpy-DPA)]2+ could be quenched by Cu2+ via the formation of [Ru(bpy)3(bpy-DPA)Cu]4+. The [Ru(bpy)3(bpy-DPA)Cu]4+/NaClon/GCE sensor demonstrated enhanced ECL signal after reacting with volatile H2S due to the high-affinity binding between sulfur and Cu2+, returning to [Ru(bpy)3(bpy-DPA)]2+/NaClon/GC. The changes of ECL signal at the sensor depended linearly on the concentration of Na2S in the range from 0.5 to 10 μM, with a detection limit of 0.25 μM.

In an ECL detection system, the amount of light generated directly depends on the concentration of the luminesphore but also of the coreactant. Sojc et al. designed and prepared a series of amine based coreactants integrating boronic acid function as receptor units. They demonstrated that the recognition of the saccharide could modify both the structure and the reactivity of the coreactant and thus the resulting ECL emission. Besides, excellent differential selectivity for d-glucose and d-fructose was achieved by tuning the linker length of a bis-boronic acid amine coreactant.

Organophosphates (OPs) have raised serious human health and environmental concerns. Therefore, effective OPs analysis is of great importance. Wang et al. designed a novel “smart” ECL switch-type OP sensor by employing the specific binding of target pesticide molecules on graphene oxide (GO) decorated by cobalt phthalocyanine (CoPc) and using ethanol as the radical scavenger on a GO-CoPc-based sensing platform, which generates an “ON1−OFF−ON2” ECL response. Yuan’s group constructed a signal on an ECL biosensor using β-cyclodextrin functionalized γ-C3N4 as the luminesphore for sensitive OPs detection based on the enzyme inhibition of OPs, showing that the consumption of coreactant triethyamine decreased with a lessening of the acetic acid in situ generated by enzymatic reaction.

A new ruthenium(II) complex based multisignal chemosensor, Ru-Fc, was reported for the highly sensitive and selective detection of lysosomal hypochlorous acid (HOCl) in living cell and laboratory animal samples. Ru-Fc was weakly luminescent because the MLCT (metal-to-ligand charge transfer) state was suppressed by the efficient PET (photoinduced electron transfer) process from Fc (ferrocene) moiety to Ru(II) center. The cleavage of the luminescence quencher moiety Fc by a HOCl-induced specific reaction led to elimination of PET, which re-established the MLCT state of the Ru(II) complex, accompanied by remarkable PL and ECL enhancements.

Sensitive and accurate detection of antibiotics plays a paramount role in various fields including environment and food safety. A sensitive sensor for the determination of selected nitrofurans in animal feed samples, including furaltadone, furazolidone, and nitrofuratoin, was proposed with use of CdTe QDs enhanced ECL of the Ru(bpy)32+ system. It was found that the induced ECL from the Ru(bpy)32+ CdTe-QDs system was inhibited by the presence of selected nitrofurans. This quenching effect of nitrofurantin antibiotics was found to be selective and concentration dependent and was observed to have a linear relationship over a wide concentration range. In addition, the proposed ECL method was successfully applied to detect the total residuals of selected nitrofuran residues in animal feed samples with satisfactory results. A novel triple-amplification ECL assay was designed for detecting chloramphenicol (CAP) based on single-strand DNA-binding protein (SSB) and EnVision reagent (EV) labeled on Au NPs (EV–Au–SSB) as nanotracer and exonuclease-assisted target recycling. In the EV–Au–SSB, Au NPs could effectively enhance the ECL intensity of CdS NCs by surface plasmon resonance. Moreover, the combination of Au NPs and EV could further oxidize CdS NCs for the ECL signal enhancement via the catalysis of H2O2 to generate a large number of reactive oxygen species. The developed aptasensor exhibited the linear response range from 0.0001 and 10 nM with a detection limit of 0.03 pM (S/N = 3) for CAP.

**ECL Immunossay.** ECL is powerful and promising for sensitive and selective determination of analytes in clinical samples owing to the integration of high affinity of antigen–antibody interaction with the intrinsic properties of ECL. The determination of cancer markers associated with certain tumors plays an important role in diagnosing cancer diseases. Prostate-specific antigen (PSA) is the best serum marker currently available for the diagnosis and targeting of prostate cancer at an early stage. Wei et al. used Ag NPs doped Pb(II)–β-CD (Ag@Pb(II)–β-CD) as a substrate material to construct a new type of label-free immunosensor for detecting PSA. Wei et al. developed a CeO2-matrical enhancing ECL sensing platform for PSA based on the B12S-labeled inverted quenching system. In this work, amidogen graphene (NH2-Gr) and Au NPs functionalized CeO2 NCs (NH2-Gr/Au@CeO2) exhibited strong ECL activity which could be quenched efficiently by bismuth sulfide Bi4S3. By using NH2-Gr/Au@CeO2 as the ECL response substrate layer and Ag NP functionalized Bi4S3 as carrier of the secondary antibody, a novel sandwich ECL immunosensor was constructed for the detection of PSA with a low detection limit of 0.3 pg mL−1. Du and co-workers proposed a label-free immunosensor for PSA by using ECL active EuPO4 nanowire.

Self-enhanced signal amplification strategy has been successfully adopted for the construction of ECL immunosensors by Yuan’s group. An intramolecular self-enhanced ECL immunosensor based on palladium nanowires (PdNWs) was constructed for carcinoembryonic antigen (CEA). PdNWs, with high specific surface area and superior electrocatalytic activity, were synthesized with a green procedure by using Lentinan (LNT) as a stabilizer and reducing agent. The obtained PdNWs were applied to immobilize an enhanced amount of tris(4,4′-dicarboxylicacid-2,2′-bipyridyl) ruthenium(II) dichloride (Ru(dcbpy)2+) functionalized polyamidoamine (PAMAM) dendrimer to form a new electrochemiluminescent derivative (PdNWs–PAMAM–Ru). In this way, the Ru(II) luminesphore and its coreactive groups (amine groups in PAMAM) existed in the same complex. The obtained complex (PdNWs–PAMAM–Ru), as a new self-enhanced ECL derivative with enhanced luminous efficiency, was applied to construct a “signal on sandwiched ECL immunosensor by using Au NPs as ECL substrate and PdNWs–PAMAM–Ru as a signal tracer. In the following report, a new manganese ions doped zinc oxide porous cubes as a mimic peroxidase for signal amplification.
PdIr cubes could effectively catalyze coreactant $\text{H}_2\text{O}_2$ decomposition and thus enhance the ECL intensity of ABEL. The developed strategy resulted in a significantly enhanced ECL signal output. By covalently linking luminescent $[\text{Ru}((\text{dcbpy})_2\text{dppz})_{2^+}]$ with $N,N$-disopropylethylendiamine (DPEA) through amidation reaction, Yuan et al. also designed a novel “light-switch” molecule ($[\text{Ru}((\text{dcbpy})_2\text{dppz})_{2^+}]$-DPEA) with self-enhanced ECL property, which was almost nonemissive in aqueous solution but brightly luminescent after intercalating into the DNA duplex. As shown in Figure 1, biotin labeled DNA dendrimer (the fourth generation, G4) was prepared from $\text{Ru}((\text{dcbpy})_2\text{dppz})_{2^+}$-DPEA; (B) schematic diagram of the construction of the immunosensor and the response mechanism. Reproduced from Wang, H.; Yuan, Y.; Zhuo, Y.; Chai, Y.; Yuan, R. Anal. Chem. 2016, 88, 5797–5803 (ref 94). Copyright 2016 American Chemical Society.

Jiang et al. reported a QDs based potential-resolved ECL immunosensor to realize simultaneous detection the model molecules of alpha fetoprotein (AFP) and its AFP-L3 isoform. AFP-L3%, a novel biomarker for hepatocellular carcinoma laboratory diagnosis, was calculated accordingly. Because of different surface microstructures, dimercaptosuccinic acid stabilized CdTe (DMSA-CdTe) QDs and TiO$_2$ NPs-glutathione stabilized CdTe (TiO$_2$-GSH-CdTe) QDs showed a large difference of ECL peak potential ($\sim$360 mV). Two separate interfaces of ITO electrodes were modified to specifically recognize AFP and AFP-L3, respectively. By immobilizing the DMSA-CdTe QDs-anti-AFP and TiO$_2$-GSH-CdTe QDs-anti-AFP-L3 on ITO electrodes surface, combined with the enzymatic amplification strategy, on-step label-free test of AFP-L3% could be realized.

Ugo et al. reported the design of a novel immunosensor for anti-transglutaminase type-2 antibody (anti-tTG) IgG determination based on an ECL readout, using membrane-templated gold nanoelectrode ensembles (NEEs) as a detection platform. A major originality of this approach was the physical separation between the location of the initial electrochemical reaction at the Au NEEs (i.e., oxidation of the coreactant) from the ECL-emitting region where the luminophore label was immobilized on the polycarbonate (PC) substrate, as shown in Figure 2. Specifically, the capturing agent tTG was at first bound onto the PC of a NEE to react with the target analyte anti-tTG IgG antibody and then realize the immobilization of a streptavidin-modified ruthenium-based ECL label via reaction with a suitable biotinylated secondary antibody. Thanks to the customized architecture of the platform, the TPrA coreactant was oxidized at the nanoelectrodes, and the resulting radicals diffused all over the geometric area of the NEEs to reach the Ru(bpy)$_3^{2+}$ complex embedded DNA dendrimer “light-switch” molecule $[\text{Ru}((\text{dcbpy})_2\text{dppz})_{2^+}]$-DPEA. The self-enhanced nano-composite (G4-$[\text{Ru}((\text{dcbpy})_2\text{dppz})_{2^+}]$-DPEA) could well bind with streptavidin labeled detection antibody (SA-Ab$_2$) due to the existence of abundant biotin. Through sandwiched immuno-reaction, an ECL immunosensor was fabricated for sensitive determination of N-acetyl-$\beta$-D-glucosaminidase (NAG). These works all utilized nanomaterials to hold luminophore and coreactive agent. They also used self-enhanced ECL reagent, synthesized by covalently linking bis(2,2-bipyridyl)(4’-methyl-2,2′bipyridinyl-4-carboxylic acid) ruthenium(II) (Ru(bpy)$_2$(mcbpy)$_2$)$^{2+}$ with TPrA, as a precursor to prepare nanorods ($[\text{Ru}((\text{bpy})_2(\text{mcbpy})_{2^+}]$-TPrA)NRs) with high luminous efficiency. Then Pt NPs functionalized $[\text{Ru}((\text{bpy})_2(\text{mcbpy})_{2^+}]$-TPrA)NRs were used to load the detection antibody (Ab$_2$) while the Au/Pd dendrimers (DRs) with hierarchically branched structures were used to immobilize capture antibody (Ab$_1$). On the basis of sandwiched immuno-

the ECL emission is obtained at much lower operative potential, thus reducing significantly the possible interference from side reactions in samples containing oxidizable species. It can also minimize possible electrochemical damage of sensitive biomolecules and reduce the oxide formation on Au or Pt electrode surfaces.

**ECL Genosensors.** The past 2 years has witnessed substantial advances toward the development of high-performance ECL genosensors. Multifarious sensing strategies have been developed for the achievement of good sensitivity and selectivity.

Designed DNA can be used to generate DNAzyme, which is a kind of sequence-specific nuclease catalyst for certain biochemical reactions. DNAzyme is very popular in ECL genosensors for important signal amplification strategies such as target recycling amplification (TRC) and rolling circle amplification (RCA).\textsuperscript{114,115} Yuan et al. developed an ECL platform for microRNA using the target-cycling synchronized RCA strategy and in situ generated electrochemiluminescent silver nanoclusters (Figure 3).\textsuperscript{37} They designed a DNA circular template consisting of a guanine-rich region and a binding region for target-cycling synchronized RCA. A "P" junction structure was formed after the binding region hybridized with target microRNA and the primer which could trigger the RCA process. Meanwhile, target microRNA was released and acted as another trigger of RCA. The product DNA possessed tandem periodic cytosine-rich sequences which served as ligands for electrochemiluminescent silver nanoclusters generation. Thus, the ECL intensity from silver nanoclusters was positively related to target microRNA concentration. By subtly designing DNA sequences, they also developed a DNA walking machine for ECL genosensor.\textsuperscript{116} The designed DNA nanostructure tracks had four overhang sequences complementary to the walker (target DNA) and modified with ECL labels, and the tracks were self-assembled on the electrode surface. The target DNA hybridized with the complementary tracks and formed specific recognition sites for a restriction enzyme (Nt.AlwI) which could cleave the overhang sequences, lose ECL labels, and drive directional movement of the target DNA. Along with the target DNA walking through the track, all the ECL labels were released and provided a "signal-off" ECL platform. By replacing the ECL labels modified on the overhang sequences with quenching molecule ferrocene, they also built a "signal-on" ECL platform.\textsuperscript{117}

By embedding or labeling ECL emitters such as Ru(bpy)\textsubscript{3} to DNA sequences, advanced ECL genosensors are designed.\textsuperscript{118,119} For example, a Ru complex tagged thiolated shared-stem hairpin DNA was designed and self-assembled onto GO/Au NPs modified electrode surface to build an "signal-on" ECL biosensor for the detection of specific DNA sequence.\textsuperscript{120} Without target DNA, the ECL of Ru complex was quenched by GO due to the short distance between them. While, once the target DNA hybridized with hairpin DNA, the hairpin structure was opened and the tagged Ru complex became far away from the graphene oxide, leading to the increase of ECL intensity. Chen’s group reported a dual-wavelength ECL ratiometric platform for microRNA detection using the ECL-RET between g-C\textsubscript{3}N\textsubscript{4} nanosheets and Ru(bpy)\textsubscript{3}.\textsuperscript{24,40} The Au NPs modified g-C\textsubscript{3}N\textsubscript{4} nanosheets were coated on the electrode surface to give strong and stable ECL emissions, which was well matched with the absorption peak of Ru(bpy)\textsubscript{3} and could stimulate its ECL emission RET effect. Then Ru(bpy)\textsubscript{3} was labeled on DNA to form probe DNA-Ru(bpy)\textsubscript{3} and was further introduced by target microRNA to hybridize with capture DNA on electrode and realize the RET process. On the basis of the quenching of ECL signal at 460 nm and increasing at 620 nm, a dual-wavelength ratiometric platform was built.

In recent years, certain designed DNA has been applied for building biomolecular Boolean logic gates in ECL biosensors. Biomolecular Boolean logic gate is a type of molecular computing device which has aroused great interest among researchers. Chen and co-workers reported an ECL sensing strategy for protease and nuclease using biomolecular Boolean logic gates.\textsuperscript{121} First, they regulated the diffusive flux of coreactant by the target-triggered desorption of programmable polyelectrolyte film on the ECL emitting g-C\textsubscript{3}N\textsubscript{4} film, which induced the recovery of ECL signal. Different substrates programmed in the polyelectrolyte film responded to protease and nuclease, respectively. By programming OR and AND DNA logic gates in the polyelectrolyte film, this biosensor could simultaneously analyze proteases and nucleases in one sample. Another biomolecular logic device was established on the molecularly imprinted polymer (MIP) film electrodes.\textsuperscript{122} The MIP film was electropolymerized with chloromphenicol (CP) as the template molecule on the surface of Au electrodes. After CP removal, DNA acted as an enhancer to the CV and ECL peaks from Ru(bpy)\textsubscript{3} solution, while ferrocene methanol (FcMeOH) acted as a quencher. Thus, DNA, CP, and FcMeOH were three inputs and the corresponding CV and ECL signals were outputs, achieving the 3-input/3-output and 3-input/5-output logic gates. Similarly, a 3-input/4-output logic gate system based on the damage of natural DNA was also reported.\textsuperscript{123}

Nanomaterials with different chemical components, sizes, shapes, and unique properties have been adopted in ECL genosensors, bringing considerable improvement in sensitivity and stability. In the recent 2 years, a number of advanced ECL genosensors based on multifunctional nanomaterials have been reported.\textsuperscript{67,124} Wang et al. reported an ECL and PL dual detection channeled aptasensor based on N-doped GQDs@SiO\textsubscript{2} nanoparticles as a signal indicator.\textsuperscript{125} Fe\textsubscript{3}O\textsubscript{4}/Au magnetic beads were used as nanocarriers for N-doped GQDs@SiO\textsubscript{2} NPs through specific DNA hybridization between an aptamer and capture DNA. Upon target molecules incubation, N-doped GQDs@SiO\textsubscript{2} NPs were released from the magnetic electrode surface into solution. Because of the good ECL and fluorescence...
properties of N-doped GQDs@SiO$_2$ NPs, the dual detection channeled aptasensor was thus fabricated. In another ECL aptasensor, CdTe QDs were used as the ECL emitters and semicarbazide (Sem) was used as coreaction accelerator to promote the ECL reaction of CdTe QDs/S$_2$O$_8^{2−}$ system.$^{126}$ The CdTe QDs were modified onto C$_60$ nanoparticles to get CdTe QDs@C$_60$NPs nanocomposites, which were coated on the electrode surface. For the accelerator probe, hollow Au nanocages were prepared and functionalized with semicarbazide and Au nanoparticles via layer-by-layer assembly to get multi-layered nanomaterials of (AuNPs-Sem)$_x$-AuNCs which could immobilize a great deal of detection aptamers. With the good performance of two kinds of nanocomposites, this aptasensor showed great sensitivity with a detection limit of 0.03 fM. On the basis of this strategy, they subsequently reported a similar ECL aptasensor with graphene surface in situ generated CdTe as ECL emitter and a kind of perylene derivative as the coreaction accelerator.$^{127}$ Zhou et al. reported an ECL method for DNA methyltransferase (M.Ss1 MTase) activity detection based on the ECL emission of CdS QDs and the glucose oxidase mimicking effect of gold nanoparticles for co-reactant generation.$^{128}$ In this method, dsDNA containing special sequence was immobilized on CdS QDs modified electrode surface, followed by methyltransferase treatment to catalyze DNA methylation. Once the special sequence was methylated, the restriction endonuclease could not recognize and cut off the special sequence. Then Au NPs were combined with the dsDNA which were not cut off and catalyzed the oxidation of glucose to produce ECL coreactant hydrogen peroxide for CdS QDs, giving ECL signal corresponding to the DNA methyltransferase activity. Conventional ECL luminophores like Ru(bpy)$_3^{2+}$ and luminol have the advantage of strong ECL emission. Some researchers combined nanomaterials with these conventional ECL luminophores to get nanocomposites with advantages from both.$^{60,90,129}$ For example, Zhao and co-workers reported a one-pot synthesis method of GO/Ag NPs/luminol composite and used it to build an ECL biosensor for the detection of DNA methyltransferase activity.$^{130}$ This nanocomposite could assemble large amount of luminol and the Ag NPs could further enhance luminol to give strong ECL signal. The nanocomposites were immobilized on azide-terminated dsDNA modified electrode, and once the DNA hybrid was methylated and cleaved by Dpn I endonuclease, GO/Ag NPs/luminol composites were released and caused significant reduction of the ECL signal. Similar composites consisting of luminol, Ag NPs, and graphene oxide were applied in another ECL aptasensor.$^{131}$ Yuan’s group developed a Ru complex and hollow gold nanoparticles branched-poly(N-(3-aminopropyl)-methacrylamide) hydrogel composites (pNAMA-Ru-HGNPs) as an ECL label in an aptasensor for thrombin detection.$^{132}$ The pNAMA-Ru-HGNPs hydrogel composites served as effective carriers for a thrombin binding aptamer to form ECL probes. On the other hand, dendritic gold nanoparticles modified carbon nanotube-nafion were coated on the electrode surface as an enhancer to amplify the ECL signal and matrix for capture aptamers immobilization.

The development of a simple, fast, portable, and cost-effective ECL device is the main challenge for the industrialization of ECL biosensors. Rusling’s group developed an ECL microfluidic array featuring a 64-nanowell chip with polymer [Ru(bpy)$_2$(PVP)$_6$]$_{30}$ as an ECL emitter for measuring DNA damage.$^{133}$ This microfluidic chip had 64 printed toner ink nanowells which could capture 1 μL of solution by virtue of a hydrophilic bottom and hydrophobic wall. Films of enzymes, DNA, and [Ru(bpy)$_2$(PVP)$_6$]$_{30}$ were achieved via alternative electrostatic layer-by-layer fabrication. ECL activation and detection were run by passing reaction solutions through the array chamber inside a dark box with a charge coupled device (CCD) camera. Besides, the LC–MS/MS technique was used as a companion method to detect the nucleobase metabolite adducts. Moreover, a digital microfluidics ECL device was built for microRNA analysis by Shamsi et al.$^{134}$ In this device, ECL detectors were fabricated into the top plates of digital microfluidics with specially designed ITO working electrodes to allow optical detection and digital microfluidics operation. Ru(Phen)$_3^{2+}$ was used as ECL label to be intercalated into the double strands formed by targets and aptamers on magnetic particles and subsequently measured by this device. Jiang and co-workers developed a kind of homemade screen-printed electrodes chip based aptasensor for simultaneous detection of malachite green with chloramphenicol.$^{135}$ The screen-printed electrodes chip consisted of two parallel carbon working electrodes, an Ag/AgCl reference electrode, and a carbon counter electrode. CdS QDs and luminol-gold nanoparticles (L-Au NPs) labeled ssDNA complementary with two kinds of aptamers were modified on the two working electrodes as a cathode and anode ECL emitters, respectively. Then, ECL quenchers linked aptamers were introduced to electrode surfaces and caused ECL decreases, which would recover in the presence of targets. Khoshfetrat et al. reported a wireless ECL bipolar electrode (BPE) array device for the visualized genotyping of single nucleotide polymorphisms (SNPs).$^{136}$ In the BPE array, signals from each individual anodic pole were controlled by two driving electrodes, the driving potential was applied through an electrolyte solution and potential drop in the solution induced a potential difference along the length of the BPE. After the hybridization of targets to the DNA probes modified on the anodic poles of BPE array, genotyping of different SNPs was monitored by exposing to different monobase modified luminol-platinum nanoparticles (M-L-PtNPs), which hybridized to mismatch sites and gave ECL emission with the simultaneous O$_2$ reduction at the cathodic poles. Recently, Liu and co-workers developed a paper-based BPE ECL device for genetic detection of pathogenic bacteria (Figure 4).$^{137}$ They used wax-screen printing to form hydrophilic channels on filter paper and screen-printed the carbon ink-based BPE and driving electrodes into the channels. Then they designed a “light-switch” molecule [Ru(phen)$_2$(dppz)$_2$]$_2^{2+}$ as ECL reporter. The ECL of this molecule was quenched by the protonation of the phenazine N atoms in aqueous solution while enhanced after intercalated into the base pairs of dsDNA, which were products from target induced polymerase chain reaction (PCR) amplification. Down to 10 copies/μL of the genomic DNA of Listeria monocytogenes was detected by this device.

**ECL Cytosensors.** Considering the great contribution to early stage cancer diagnosis, the cytosensor is another important application field of ECL platforms. Cytosensor is one of the most rapidly growing class of biosensors. In recent years, ECL methods are proved to be selective, sensitive, and cost-effective for the detection of cancer cells concentration, the distribution study of biomolecules on cancer cells surface, the monitoring of cell apoptosis and even single cell analysis. On the other hand, new developments in nanomaterials and analysis devices have advanced the progress of the improvement for ECL cytosensors.

Using the ECL nanolabel is a typical strategy in many different kinds of ECL biosensing platforms including cytosensors. By
introducing different kinds of functional nanomaterials and aptamers for efficient labels and novel cyto-devices, advanced ECL cytosensors keep being developed. For example, Yu et al. reported an origami ECL cyto-device with porous AuPd alloy as catalytically promoted nanolabels for multiple cancer cells detection. In this microfluidic paper-based ECL origami cyto-device named as μ-PECLOC, cell-targeting aptamers modified 3D macroporous Au paper electrodes were used as both working electrodes and cells capture platforms. As for the nanolabels, they loaded concanavalin-A conjugated porous AuPd alloy nanoparticles (AuPd@Con-A), which could catalytically promote the peroxydisulfate ECL system, onto the cancer cell surface via the specific recognition of cancer cell surface mannose with Con-A. Excellent analytical performance was achieved toward the cytosensing of four kinds of cancer cells. This microfluidic paper-based cyto-device or so-called lab-on-paper device contributed to the development of facile, portable, disposable, and cost-effective cytosensing platforms. Furthermore, they developed a similar microfluidic paper-based cyto-device with GQDs loaded surface villous Au nanocages as ECL nanolabels for in situ determination of CA153 at MCF-7 cell surface. Recently, they further improved the bimetallic AuPd nanoparticles based lab-on-paper cyto-device to detect two antigens at the MCF-7 cell surface.

Ratioometric ECL platforms exhibit improved sensitivity, stability, and reproducibility during cell analysis. He et al. developed a reusable and dual-potential responsive ECL platform for synchronously cytosensing and dynamic evaluation of cell surface N-glycan. They used cancer cell recognized aptamer hybridized with capture DNA for cell capture. The anodic ECL label Ru(phen)$_3^{2+}$ were intercalated into the grooves of double-strand DNA. With the presence of target cells, aptamer would specifically interact with target cells and release the capture DNA and ECL probes Ru(phen)$_3^{2+}$. On the other hand, concanavalin A conjugated gold nanoparticle modified graphene-C$_3$N$_4$(Con A@Au–C$_3$N$_4$) was used as negative ECL label for cell surface N-glycan recognition owing to the excellent cathodic ECL properties of g-C$_3$N$_4$. Meanwhile, electrochemically reduced MoS$_2$ nanosheets were chosen as electrode modification material for signal amplification. In this strategy, the negative signals from Con A@Au–C$_3$N$_4$ nanoprobes were associated with both cell concentration and N-glycan expression, and the positive ECL signals from Ru(phen)$_3^{2+}$ were closely related with the cells captured on the electrode. Thus, the dynamic evaluation of the N-glycan expression on the cell surface could be realized with high sensitivity and excellent selectivity based on the ratio of ECL intensity from the negative and positive potential signals.

Recently, Chen’s group developed a ratiometric ECL cytosensor with graphite-C$_3$N$_4$ nanosheets and Ag–PAMAM–luminol nanocomposites as ECL labels. The ECL-RET effect was also used in this system. They prepared the Ag-PAMAM-luminol nanocomposite (Ag-PAMAM-luminol) and functionalized it with DNA probe to hybridize with the aptamers on magnetic microbeads. Once the target cells got captured by the aptamers, nanocomposites were released and hybridized on the capture DNA modified g-C$_3$N$_4$ nanosheets coated ITO electrode. Because of the RET effect from g-C$_3$N$_4$ nanosheets to Ag NPs, the ECL signal from g-C$_3$N$_4$ at −1.25 V (vs SCE) decreased and the ECL signal of luminol at +0.45 V (vs SCE) increased. The ratio of these two signals would change corresponding to the change of target cell concentration.

Other cytosensors based on novel ECL nanolabels have been reported recently, such as the cytosensor with multibranched DNA hybridization chain reaction linked CdSe/ZnS quantum dot and gold nanoparticles nanocomposites as ECL labels and the cytosensor using hemin–graphene–Au nanoparticle ternary composite as catalyst for ECL co-reactant reduction.

Cell apoptosis detection can be achieved by nanolabel based ECL cytosensors. One of the noteworthy works about ECL cytosensor for cell apoptosis monitoring and efficient drug screening was reported by Yuan et al. This platform used annexin V modified Ru(dcbpy)$_3^{2+}$-silica composite NPs as ECL labels and concanavalin A modified gold NPs as signal amplification material and capture agent. It was successfully applied to investigate the efficiency of paclitaxel upon breast cancer cell apoptosis. Recently, Zhu’s group developed an ECL cytosensor for the sensitive detection of cancer cells secreted caspase-3 activity. Caspase-3 is commonly treated as the biomarker for apoptosis because it is frequently activated during cancer cell apoptosis process. Ru(bpy)$_3^{2+}$ doped silica NPs acted as ECL labels with TPrA as coreactant, and the nanocomposites consisting of MWCNTs and gold NPs acted as electrode modification material. The biotinylated DEVD-peptides were further immobilized on the nanocomposites to capture the streptavidin-modified ECL labels onto electrode by the specific interaction between biotin and streptavidin, which would give strong ECL signal. With the cell secreted caspase-3 specifically cleaving the N-terminus of DEVD, ECL labels were released from electrode surface and led to the decrease of ECL signal. Thus, this biosensor could achieve effective application for monitoring caspase-3 activity.

The insulation from cells and steric hindrance from biorecognition reactions can cause severe suppression of the ECL signal from the electrode surface, providing a simple and classic method to build label-free ECL cytosensors. In recent years, novel kinds of multifunctional nanomaterials have been developed to improve the performance of label-free ECL cytosensors. A good example is the cytosensing application of the superparamagnetic functionalized graphene/
Fe₃O₄@Au nanocomposites reported by Wang’s group. This kind of multifunctional nanocomposites were formed by integrating poly(ethyleneimine) functionalized graphene/iron oxide hybrids (BGNs/Fe₃O₄) and luminol functionalized gold nanoparticles to give good ECL emission and magnetic control as well as promote electron transfer. This cytosensor showed good stability, sensitivity, and reproducibility in HeLa cells determination. Another example is the ECL cytosensor for HepG2 cells reported by Liu et al. A novel kind of nanocomposites consisting of highly oriented CdS-coated ZnO nanorod arrays were developed for electrode modification. This nanocomposites arrays had excellent ECL property, good stability, and fast response speed during detection. They also applied gold nanoparticles onto the nanocomposites arrays for signal amplification and cell capture antibodies modification. This nanocomposites arrays based label-free ECL cytosensor showed sensitive response to HepG2 cells in a linear range of 300–10 000 cells per mL.

Meanwhile, advanced devices have been developed in label-free ECL cytosensing platforms. Chen’s group developed a visual color-switch ECL cytosensor on a multichannel bipolar electrode chip. The bipolar electrode (BPE) was developed in their previous work of an ECL biosensor for cell surface protein detection. The BPE-ECL platform was built based on a microchannel chip with a BPE embedded in it. When sufficiently high potential applied through the microchannel chip, reduction and oxidation reactions would occur on the ends of BPE with the same rate. In this case, the ECL reaction at anode would be highly influenced by the cathodic reduction reaction. In their color-switch ECL cytosensor, the microchannel chip had three separated reservoirs with buffer, luminol, Ru(bpy)₃²⁺/TPrA solutions, respectively, and two arrays of BPEs. After voltage applied, the orange ECL emission from Ru(bpy)₃²⁺/TPrA system was observed at the anode of one BPE. By adding H₂O₂ and DNAzyme, the orange ECL was quenched, while the blue ECL signal from luminol was observed at the anode of the other BPE. With the fact that H₂O₂ could be produced by stimulating cancer cells, this cytosensor was applied to quantitatively detect HL-60 cancer. Recently, they reported another ECL cytosensing platform with bipolar electrode chip. The anode of BPE acted as a reporting pole with Au NPs assembled by the DNA double strand. Au NPs worked as a catalyst for the ECL reaction of the luminol system as well as seeds for the reduction reaction of the Ag layer which could amplify a slight conductivity change during detection. Also because of the formation of Ag@Au, ECL emission of luminol would be completely quenched while the ECL recovery could reflect the extent of anodic dissolution. Down to 5 cells/cm² of cancer cells such as MCF-7 and A549 could be quantified due to the difference of conductivity by monitoring the ECL recovery time before and after cells incubation.

Cellular heterogeneity analysis is a crucial issue in bioanalysis fields, especially in cancer analysis. It is a central challenge to understand how individual cell process and respond to various information. Single cells analysis can give distinct and significant study for cellular heterogeneity at high resolution, providing valuable information such as chemical composition and surface local activities of cells. Jiang’s group reported a series of ECL platforms for single cell monitoring and analysis in recent years. After the investigation of active cholesterol at the single cell level with the photomultiplier tube (PMT) based ECL platform, they further improved their detecting device by building an ECL imaging platform with a charge-coupled device (CCD) to collect the ECL signal (Figure 5). This ECL imaging platform could collect the signal from the entire electrode surface, achieving simultaneous analysis of active membrane cholesterol on multiple single HeLa cells. Furthermore, with this ECL imaging platform they achieved the visualization of intracellular hydrogen peroxide at the single cell. In this system, a comprehensive Au-luminol-microelectrode was developed as the working electrode. This microelectrode was made of a capillary filled with the mixture of chitosan and luminol and coated with thin layers of polypyrrole chloride/nitrophenyloctyl ether (PVC/NPOE) and gold with a tip opening of 1–2 μm. Thanks to the small diameter of the tip, this microelectrode could be inserted into one signal cell and contact with the intracellular hydrogen peroxide, leading to ECL emission from the luminol inside the microelectrode tip. Recently, they further studied the intracellular glucose at the single cell level using the ECL imaging platform with a gold coated ITO slide as the working electrode. This gold coated ITO slide had cell-sized microwells on the surface to retain individual cells. Upon treating with luminol, triton X-100, and glucose oxidase, intracellular glucose would be released into the microwell and generate hydrogen peroxide which were further involved in the ECL emission of luminol. Large deviations of glucose concentration from tested single cells were observed, revealing high cellular heterogeneity in intracellular glucose. Their research of this series provides important information on cellular heterogeneity study and give a potential ECL platform for single cell analysis.

CONCLUSIONS AND OUTLOOKS

Inherent sensitivity, negligible background, simplicity, controllability continue to be strong driving forces for the development of ECL assays. ECL has established itself as a powerful tool for ultrasensitive detection of a wide range of analytes. Multifarious strategies are used to improve the efficacy of ECL assays. There have been hundreds of relevant papers published during the past 2 years. These achievements containing new variations of ECL emitters and devices, have widened ECL sensing strategies, advanced the development of high-throughput and portable ECL assays especially immunoassays and genoassays, and even offered a new form of bioimaging method. Some high-throughput ECL immunoassays have been commercialized such as the Elecsys technology from Roche and the MULTI-ARRAY Technology from Meso Scale Diagnostics. These commercialized ECL immunoassays have high sensitivity, broad dynamic range, and low background. These systems are easy to use and quicker than conventional enzyme-linked immunosorbent assay (ELISA).
They can achieve clinical data in a variety of sample types, including serum, plasma, cell supernatant, and even whole blood. However, these commercialized ECL immunoassays are mainly based on the ECL reaction of the ruthenium-complex and TPrA. Therefore, wavelength-based ECL may be the key to the future. Wavelength of most emitters can be easily tuned spanning from near ultraviolet to infrared region. Consequently, wavelength-based ECL may give the commercialized ECL assays with low-cost ECL materials and the paper-based portable devices mentioned above may be commercially available.

Despite these burgeoning developments, there is still great potential for novel e-fluorescent sensing methodologies. The development of ECL high-throughput point-of-care assays based on nanoelectrodes and paper-based ECL devices mentioned above may be commercially available. However, a fully automated and equipment-free, and deliverable to end-users) remains a challenge. In this regard, many laboratories are working on making ECL more affordable, sensitive, specific, user-friendly, rapid and robust. The development of ECL high-throughput point-of-care assays based on microfluidic technologies, bipolar electrochemistry, and a wireless system to meet the criteria of being ASSURED (i.e., affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users) remains a substantial challenge.

On the other hand, current ECL assays mainly based on potential resolved signal changes by using PMT. Since PMT only measures the global number of photons emitted, a fully quantitative measurement allowing a deconvolution of ECL contributions was not possible.158 Besides, in comparison with immunoassays, many laboratories including serum, plasma, cell supernatant, and even whole blood. They can achieve clinical data in a variety of sample types, including serum, plasma, cell supernatant, and even whole blood. Therefore, wavelength-based ECL may give the commercialized ECL assays with low-cost ECL materials and the paper-based portable devices mentioned above may give the commercialized ECL technology a brilliant future.

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