Biotechnology in Agriculture, Industry and Medicine Series

BIOSENSORS: PROPERTIES, MATERIALS AND APPLICATIONS

In: Biosensors: Properties, Materials and Applications ISBN: 978-1-60741-617-3
Editors: R. Comeaux and P. Novotny

Chapter 5

ELECTROCHEMILUMINESCENT SENSORS: FABRICATIONS AND APPLICATIONS

Hui Wei and Erkang Wang*

State Key Laboratory of Electroanalytical Chemistry,
Changchun Institute of Applied Chemistry, Chinese Academy of Sciences,
Changchun, Jilin 130022, People's Republic of China, and Graduate School of the Chinese Academy of Sciences, Beijing 100039, People's Republic of China

ABSTRACT

Over the past decades, electrochemiluminescence (ECL) of tris(2,2'-bipyridyl)ruthenium [$Ru(bpy)_3^{2+}$] has received considerable attention from many researchers and been widely used to detect a variety of analytes that range from metal ions and small molecules to DNA, peptides, and proteins. Among all the areas of $Ru(bpy)_3^{2+}$ ECL, the $Ru(bpy)_3^{2+}$ based ECL sensors are of great active. In this chapter we will review the state of the art of $Ru(bpy)_3^{2+}$ ECL sensors. After a brief introduction of $Ru(bpy)_3^{2+}$ ECL and its mechanisms, the fabrications and applications of the $Ru(bpy)_3^{2+}$ ECL sensors are discussed in details. It also indicates the future outlook in this field.

Keywords: electrochemiluminescence; ECL; sensors; tris(2,2'-bipyridyl) ruthenium; fabrications; applications; bioassay.

1. INTRODUCTION

Electrochemiluminescence (also called electrogenerated chemiluminescence, abbreviated as ECL) is a means of converting electrochemical energy into radiative energy at the surface of an electrode via an applied potential.[1] Luminescent signals could be obtained from the excited states of an ECL active species generated at electrode surfaces during the electrochemical reaction. Among many organic and inorganic ECL systems studied, ECL of

* Tel: +86 431 85262003; Fax: +86 431 85689711; Email: ekwang@ciac.jl.cn (E. K. Wang)

tris(2,2'-bipyridyl) ruthenium(II) [$Ru(bpy)_3^{2+}$] has played an important role in the development of ECL and its applications, as manifested in, for example, the growing interest in clinical tests and biomolecule detection.[1-8] $Ru(bpy)_3^{2+}$ ECL offers several distinct advantages over other detection systems such as strong luminescence, good solubility in a variety of aqueous and nonaqueous solvents, inherent sensitivity, good spatial and temporal resolution, and wide linear range in the utility in different analytical areas.

Since the first detailed studies of $Ru(bpy)_3^{2+}$ ECL was realized in solution by Tokel and Bard in 1972,[9] the $Ru(bpy)_3^{2+}$ ECL has been extensively studied, from reaction mechanisms to clinical applications, synthesis of ECL-active compounds, solid-state ECL systems, and ECL instrumentation.[1-8, 10-20] Up to date, more than 2000 scientific literatures (including papers, patents and book chapters) on various topics of ECL have been published and a considerable number of excellent reviews have also appeared.[1-6, 16, 21-28] Thus, we will present the state of the art of ECL sensors, including their fabrications and applications, in this chapter and mainly focus publications appeared after 2000. The interested readers are encouraged to consult several outstanding reviews for further and deep discussions on certain specific topics.[1-3]

2. MECHANISMS OF $RU(BPY)_3^{2+}$ ECL

The mechanisms of $Ru(bpy)_3^{2+}$ ECL have been extensively studied and have been well summarized.[1-3, 10] According to how the excited $Ru(bpy)_3^{2+*}$ was produced, the $Ru(bpy)_3^{2+}$ ECL can be generally classified into two types: ion annihilation ECL and coreactant ECL. Most ECL sensors developed so far are based on coreactant ECL technology. Though several kinds of coreactants, including oxalate, peroxydisulfate, and amine, have been investigated to study the mechanisms of $Ru(bpy)_3^{2+}$ ECL, the majority of study has been focused on the coreactant tri-*n*-propylamine (TPrA) due to its highest ECL efficiency and its importance to immunoassay and DNA analysis. Therefore, the $Ru(bpy)_3^{2+}$/tri-*n*-propylamine (TPrA) system is taken as an example to elucidate the ECL mechanisms here.

As shown in figure 1, the excited $Ru(bpy)_3^{2+*}$ can be generated via three different routes: (1) $Ru(bpy)_3^{3+}$ reduction by $TPrA^{\bullet}$ free radicals (figure 1A); (2) the $Ru(bpy)_3^{3+}$ and $Ru(bpy)_3^{+}$ annihilation reaction (figure 1B); and (3) $Ru(bpy)_3^{+}$ oxidation by $TPrA^{\bullet+}$ cation radicals (figure 1C). However, from an experimental viewpoint, the mechanisms can be divided into two types according to the amount of $Ru(bpy)_3^{2+}$ used. In relatively high concentrations of $Ru(bpy)_3^{2+}$ (~millimolar) solutions, an ECL wave in 1.0-1.4 V (vs. Ag/AgCl) will be observed (Type I); while in dilute $Ru(bpy)_3^{2+}$ solutions (less than approximately micromolar) containing ~0.1 M TPrA, another ECL wave in 0.7-1.0 V (vs. Ag/AgCl) will be observed (Type II). Type I ECL wave can be explained by the mechanisms in figure 1A and figure 1B; while Type II ECL wave can be explained by the mechanism in figure 1C, respectively.

3. FABRICATIONS OF $RU(BPY)_3^{2+}$ ECL SENSORS

As shown in figure 1, the ECL signal intensity is positively related to the concentration of $Ru(bpy)_3^{2+}$ or the coreactant, so ECL can be used to detect for both. According to the two types ECL mechanisms discussed above (Type I and Type II), two corresponding kinds of

ECL sensors could be designed. For Type I ECL sensor, $Ru(bpy)_3^{2+}$ or its derivatives is usually immobilized onto an electrode surface, so the local concentration of $Ru(bpy)_3^{2+}$ is high and constant, thus the concentration of coreactants and their analogues (i.e. analytes themselves are used as coreactants) could be determined by measuring the ECL emission intensity.

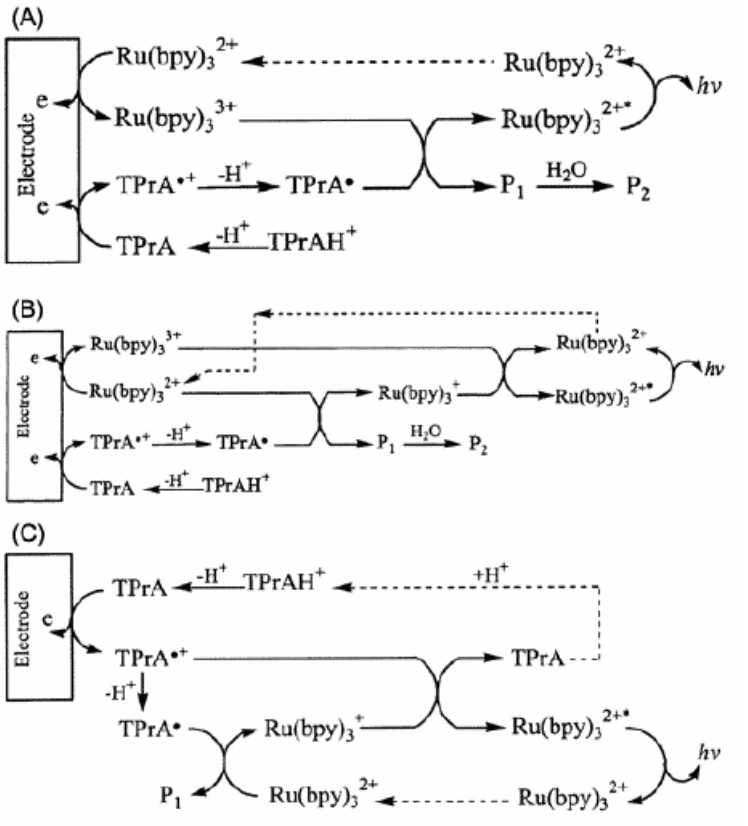

Figure 1. Proposed mechanisms of $Ru(bpy)_3^{2+}$/TPrA ECL system. (A) Electrogenerated $Ru(bpy)_3^{3+}$ reacts with TPrA as well as by direct reaction of TPrA at the electrode; (B) $Ru(bpy)_3^{2+}$ reacts with $TPrA^{\bullet}$ to form $Ru(bpy)_3^{+}$, which can then interact with $Ru(bpy)_3^{3+}$ to form ECL via annihilation; (C) generation of ECL within the potential range before the oxidation of $Ru(bpy)_3^{2+}$ at the electrode, involving formation of excited state $Ru(bpy)_3^{2+*}$ on reaction of $TPrA^{\bullet+}$ with $Ru(bpy)_3^{+}$ [formed by reaction of $Ru(bpy)_3^{2+}$ with $TPrA^{\bullet}$ free radical].[2-3, 10].

3. FABRICATIONS OF $RU(BPY)_3^{2+}$ ECL SENSORS

As shown in figure 1, the ECL signal intensity is positively related to the concentration of $Ru(bpy)_3^{2+}$ or the coreactant, so ECL can be used to detect for both. According to the two types ECL mechanisms discussed above (Type I and Type II), two corresponding kinds of ECL sensors could be designed. For Type I ECL sensor, $Ru(bpy)_3^{2+}$ or its derivatives is usually immobilized onto an electrode surface, so the local concentration of $Ru(bpy)_3^{2+}$ is high and constant, thus the concentration of coreactants and their analogues (i.e. analytes themselves are used as coreactants) could be determined by measuring the ECL emission intensity. However, not all the analytes are ECL active, especially some bio-related analytes

(such as tumor markers and antigenes). In this case, Type II ECL sensors are usually fabricated and $Ru(bpy)_3^{2+}$ derivatives are employed to label one of the species involved in an affinity binding reaction. The concentration of an analyte can be determined by measuring the emission of the $Ru(bpy)_3^{2+}$ derivative labels in the presence of an excess and constant concentration of coreactant (usually TPrA). We will discuss the fabrications and applications of these two types of ECL sensors in detailed in the following sections.

3.1. Fabrications of Type I ECL Sensors

To fabricate Type I ECL sensors, $Ru(bpy)_3^{2+}$ or its derivatives should be immobilized on an electrode surface. Up to date, a large number of different methods and matrix materials have been explored for $Ru(bpy)_3^{2+}$ immobilization.

3.1.1. Cation Ion-Exchange Approach

Nafion, a cation ion-exchange polymer, can be used as matrix to immobilize positively charged $Ru(bpy)_3^{2+}$ via cation ion-exchange approach. Since Rubinstein and Bard's seminal report of immobilization of $Ru(bpy)_3^{2+}$ on an electrode surface using Nafion,[29-31] quite a lot of extended work have been done to make robust and sensitive ECL sensors. New materials, such as carbon nanotube (CNT), titania (TiO_2), silica and V_2O_5, have been incorporated into Nafion films to accelerate the charge transfer and enhance its long-term stability.[32-42]

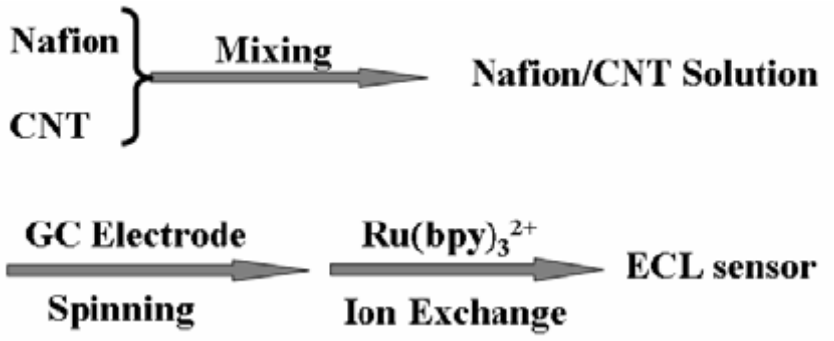

Figure 2. The schematic approach for the fabrication of $Ru(bpy)_3^{2+}$ ECL sensor on a glassy carbon (GC) electrode using Nafion and CNT.[32].

As shown in figure 2, by employing the composite film of Nafion and CNT, Dong's group has developed an ECL sensor on a glassy carbon (GC) electrode with improved sensitivity, reactivity, and long-term stability.[32] Nafion was used as the ion exchanger for $Ru(bpy)_3^{2+}$, the solvent of CNT and the membrane material, while the CNT incorporated greatly enhanced the electronic conductivity of Nafion film and played an important role in adsorbing $Ru(bpy)_3^{2+}$. By combining the merits of the CNT and Nafion, their ECL sensor exhibited a more than 3 order of magnitude higher ECL signals than the pure Nafion films modified electrode towards TPrA detection towards TPrA determination.

Other ECL sensors based on Nafion have also been fabricated and studied by many researchers. For example, Zhang and co-workers have reported the simple and sensitive ECL

detection of an antihistamine chlorphenamine maleate (CPM) by using TiO_2/Nafion composite film modified GC electrode.[34] Lee's group has developed a $Ru(bpy)_3^{2+}$ ECL sensor based on sol-gel-derived V_2O_5/Nafion composite films and their sensor exhibited greatly enhanced ECL responses towards analytes compared to other types of sol-gel ceramic/Nafion composite films such as SiO_2/Nafion and TiO_2/Nafion.[39]

Although Nafion has been the one most studied to construct $Ru(bpy)_3^{2+}$ ECL sensor, other ion-exchange polymers have also been investigated.[43-49] For example, Eastman AQ55D polymers (AQ), an alternative to Nafion, have been used to make a silica/AQ/$Ru(bpy)_3^{2+}$ ECL sensor for the determination of a commonly used depressant chlorpromazine (CPZ).[43] AQ is a kind of poly(ester sulfonic acid) cation exchange polymer similar to Nafion, but has some advantages over Nafion due to its more hydrophilic characteristic, lower cost, more rapid response and anti-fouling properties. As shown in Figure 3, our group has demonstrated a simple and effective method to construct an ultra-sensitive ECL sensor by using novel platinum nanoparticles(PtNPs)/AQ/$Ru(bpy)_3^{2+}$ colloidal materials.[44] The sensor was fabricated via a two-step approach: first, the colloidal materials of PtNPs/AQ/$Ru(bpy)_3^{2+}$ were synthesized through a wet-chemical routine; and then the colloidal materials were cast onto a GC electrode surface to produce an ECL sensor. In our sensor, AQ was used not only to immobilize $Ru(bpy)_3^{2+}$ but also as the dispersant of PtNPs. Due to the electronic conductivity and electroactivity of PtNPs in composite film, this ECL sensor exhibited enhanced ECL responses and especially a very low limit of detection (1 fM) of TPrA.

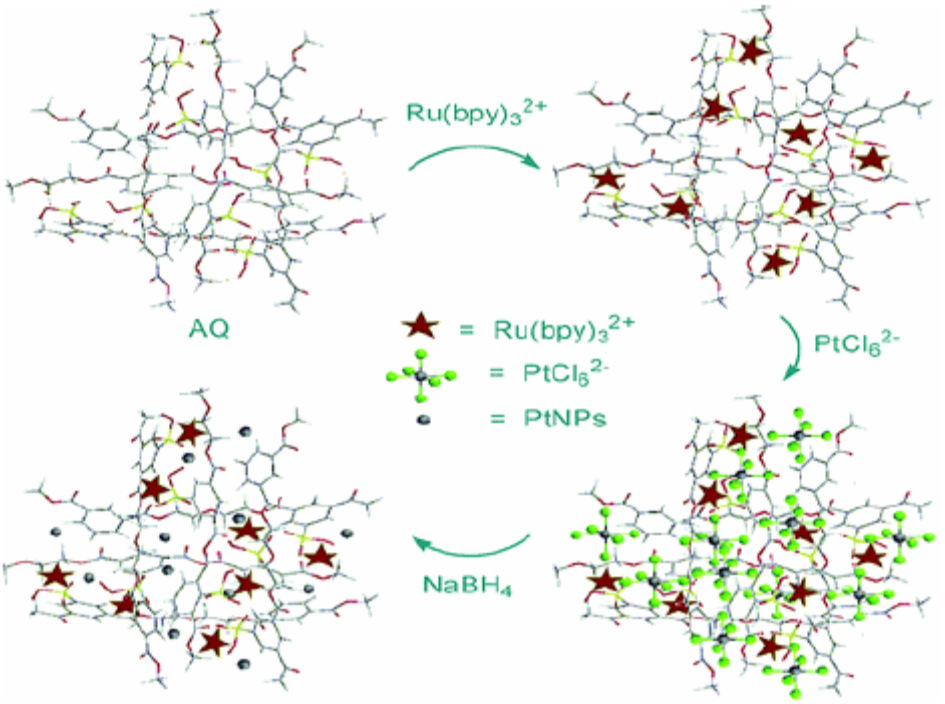

Figure 3. The schematic approach for the preparation of PtNPs/AQ/$Ru(bpy)_3^{2+}$ composite film.[44].

Poly(sodium 4-styrenesulfonate) (PSS), another ion exchange polymer, has also been used to improve the ECL sensor sensitivity.[46-49] Recently, PSS was extended to partial sulfonation of polystyrene (PSP) to develop a solid-state ECL sensor.[49] The PSP was used as the matrix to immobilize the ECL reagent $Ru(bpy)_3^{2+}$ due to the electrostatic interactions between the sulfonic acid groups of PSP and $Ru(bpy)_3^{2+}$ cations. Such a sensor has been used for the sensitive determination of 2-(dibutylamino)ethanol (DBAE) (figure 4).[49]

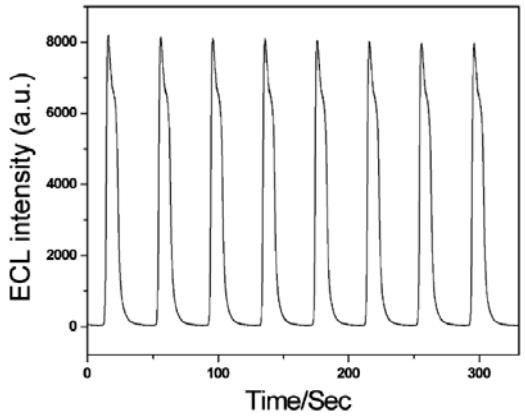

Figure 4. ECL intensity-time curve of the PSP/CNT based $Ru(bpy)_3^{2+}$ ECL sensor in 0.25 mM DBAE solution under continuous cyclic voltammetry (CVs) for 8 cycles at the scan rate of 50 mV/s.[49].

3.1.2. Covalent Approach

Covalent approach can also be employed to immobilize $Ru(bpy)_3^{2+}$ and therefore to prepare $Ru(bpy)_3^{2+}$ ECL sensors. As shown in figure 5, Dennany and co-workers have synthesized $Ru(bpy)_3^{2+}$ contained metallopolymer by covalently incorporating $Ru(bpy)_3^{2+}$ into poly(vinylpyridine) (PVP).[50-51] Then they used the metallopolymer to prepare catalytic oxidation DNA ECL sensors via the electrostatic layer-by-layer assembly of $[Ru(bpy)_2(PVP)_{10}]^{2+}$ and DNA.

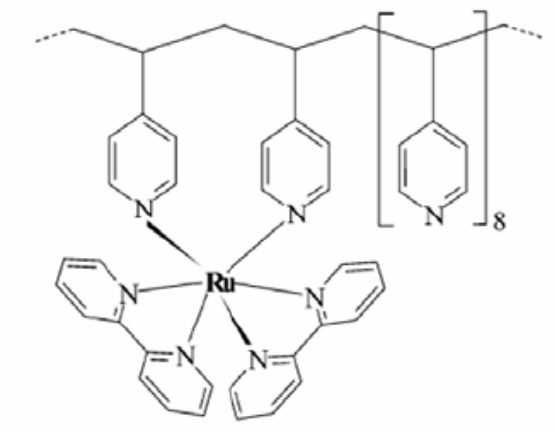

Figure 5. The chemical structure of $[Ru(bpy)_2(PVP)_{10}]^{2+}$.[50].

Lu and Whang have used the metallopolymer to perform detailed studies on the ECL and electrochemical behaviors of $[Ru(bpy)_2(PVP)_{10}]^{2+}$/oxalate system on an indium tin oxide (ITO) electrode.[52]

Besides metallopolymer, $Ru(bpy)_3^{2+}$ can also be covalently attached into silica polymers.[53-55] Through hydrolysis these silica polymers could be easily converted into sol-gel films which could be readily coated on an electrode surface. We have synthesized silica hybridized ruthenium bipyridyl complex through amidation reaction by covalent attachment of bis(bipyridyl)-4,4'-dicarboxy-2,2'-bipyridyl-ruthenium to (3-aminopropyl)-triethoxysilane (APTS) (see figure 6).[53] The hybrid complex then was coated onto an ITO electrode through acid catalytic hydrolysis and spin-coating techniques to fabricate an ECL sensor. As prepared ECL sensor possessed good stability therein with excellent ECL behavior.

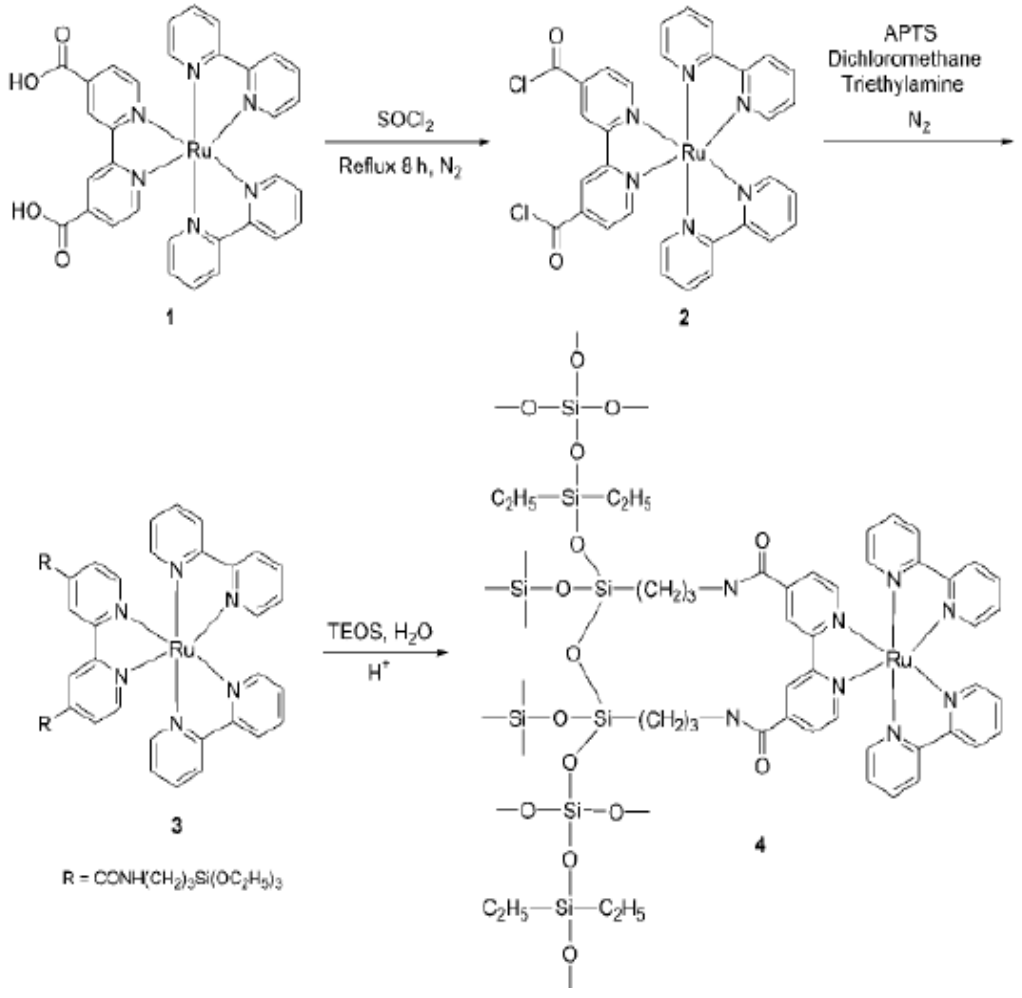

Figure 6. Synthetic procedure for formation of bis(bipyridyl)-4,4'-dicarboxy- 2,2'-bipyridyl- ruthenium-silica hybrid materials and their predicted structure.[53].

3.1.3. Electrostatic Approach

Since $Ru(bpy)_3^{2+}$ is positively charged, it can also be immobilized through electrostatic interactions.[56-65] Dong and co-workers have developed an ECL sensor based on the multilayers of silica nanoparticles/$Ru(bpy)_3^{2+}$ (figure 7).[56] Since the ITO substrate was negatively charged, positively charged poly (diallyldimethylammonium chloride) (PDDA) could be adsorbed onto the ITO surface to provide a preassembled layer. Then negatively charged silica nanoparticles and positively charged $Ru(bpy)_3^{2+}$ could be alternately assembled to fabricate the sensor. The assembly process was characterized by cyclic voltammograms and absorption spectroscopy. Their sensor showed high sensitivity and long-term stability due to the high surface area and special structure of the silica nanoparticles. The similar approach could also be extended to the clay/$Ru(bpy)_3^{2+}$ and decatungstate/$Ru(bpy)_3^{2+}$ systems where clay and decatungstate both were negatively charged.[57].

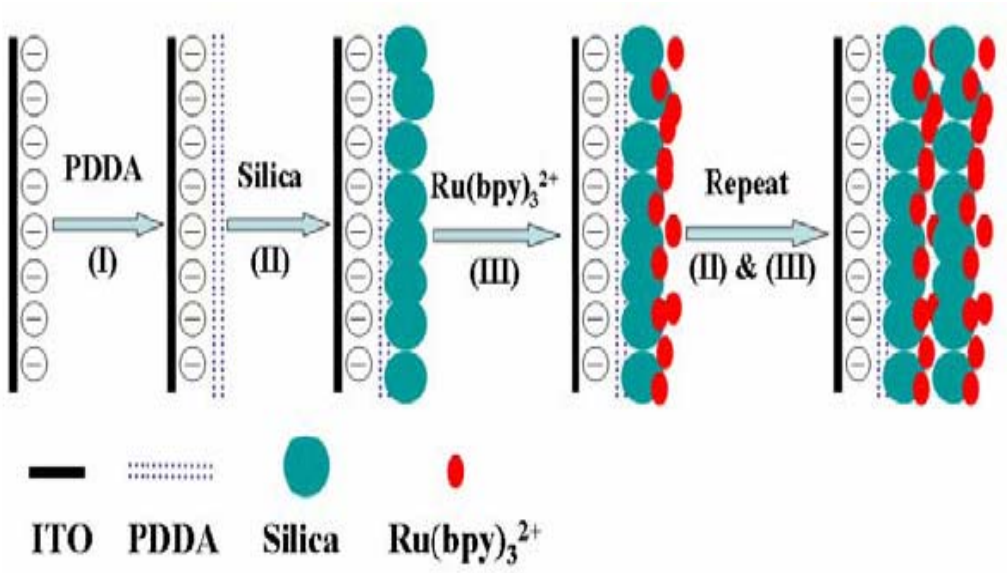

Figure 7. The schematic approach for the preparation of silica nanoparticles/$Ru(bpy)_3^{2+}$ ECL sensor by electrostatic layer-by-layer assembly.[56].

Other negatively charged materials, especially the emerging nanomaterials, have also been explored to develop new ECL sensors.[58-65] For example, Wang and coworkers have developed a novel method to construct a stable ECL sensor (figure 8).[62] The sensor was fabricated via a two-step approach: first, $Ru(bpy)_3^{2+}$-Au nanoparticles (Ru-AuNPs) aggregates were obtained via the electrostatic interactions between $Ru(bpy)_3^{2+}$ and citrate-capped AuNPs; then the Ru-AuNPs aggregates were assembled on a mercaptopropyl triethoxysilane (MPTES) modified ITO electrode surface via Au-S interaction. The same group has also used platinum nanopartilces (PtNPs) to fabricate solid-state ECL sensors.[63] Even inorganic anions, such as $PtCl_6^{2-}$ and $Fe(CN)_6^{3-}$, have been used to develop new $Ru(bpy)_3^{2+}$ ECL sensors via the electrostatic interactions between $Ru(bpy)_3^{2+}$ and anions approach.[64-65]

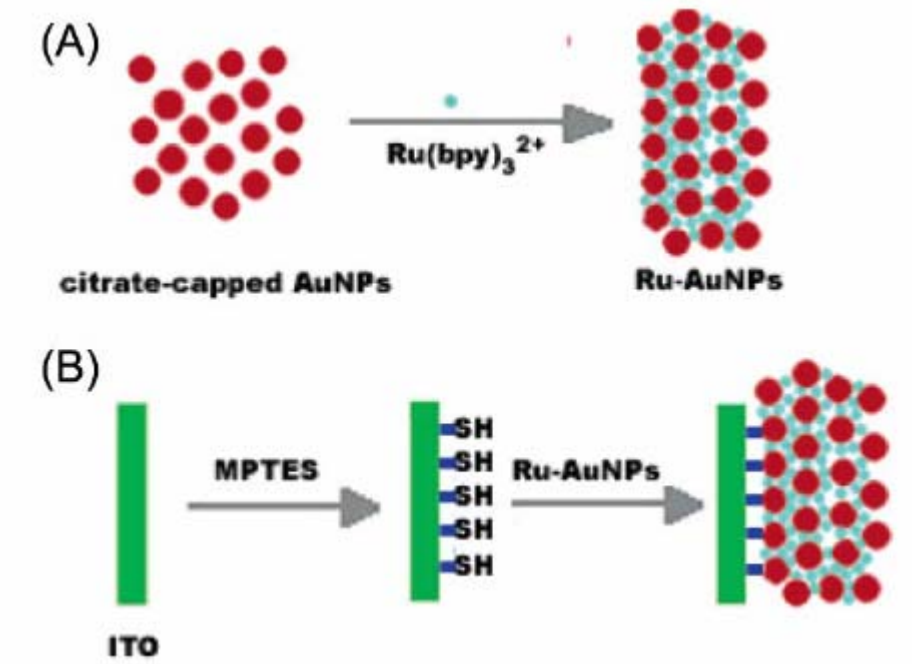

Figure 8. Scheme illustrating (A) the formation of $Ru(bpy)_3^{2+}$-AuNPs (Ru-AuNPs) in aqueous medium due to electrostatic interactions between $Ru(bpy)_3^{2+}$ and citrate-capped AuNPs and (B) the immobilization of Ru-AuNPs on a sulfhydryl-derivated ITO electrode surface.[62].

3.1.4. Physical Entrapment Approach

Physical entrapment approach can also be employed to construct type I ECL sensors. In most cases silica materials are used for physical entrapment of $Ru(bpy)_3^{2+}$ owing to their porous properties, and ease to prepare, to modify and to dope with various reagents.[66-76] Pastore and coworkers have produced an ECL sensor on K-glass conducing substrates by embedding $Ru(bpy)_3^{2+}$ inside silica glass thin films.[66] Their sensor exhibited a diffusion-controlled redox behavior of the $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}$ system. They found that the thermal treatment (i.e. annealing) could improve the sensor's performance. Collinson et al. have investigated the ECL of $Ru(bpy)_3^{2+}$ and its co-reactants entrapped within sol-gel-derived silica monoliths using an immobilized ultramicroelectrode.[67]

Dong's group has developed a novel ECL sensor based on $Ru(bpy)_3^{2+}$-doped silica (RuDS) nanoparticles conjugated with a biopolymer chitosan membrane.[68] The RuDS nanoparticles were prepared by the water/oil microemulsion method. Their sensor showed good sensitivity and reproducibility for TPrA detection.

As shown in figure 9, we have synthesized the similar RuDS nanoparticles.[69] Then the ECL behavior of the RuSi nanoparticles was investigated after deposition with biomolecules through LBL self-assembly. Our results indicated that the biopolymer coatings could improve its stability though the sensor's ECL signals were inhibited.

The ECL sensor could also be fabricated via Stöber method.[74-76] For example, Yang's group has prepared a solid-state ECL sensor with good reproducibility and stability through Stöber method.[74]

Besides all the approaches and materials mentioned above, many other approaches and materials have also been investigated to develop **Type I** ECL sensors. Very recently, a review has well summarized the recent developments in fabrication of solid-state ECL sensors and their analytical applications.[16] Interested readers are referred to it for the further information.

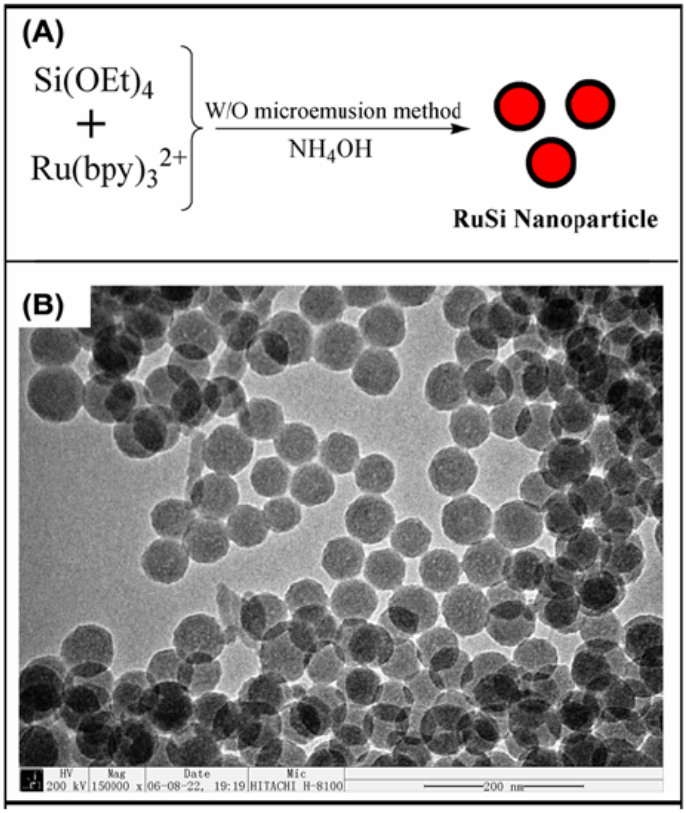

Figure 9. (A) The preparation of $Ru(bpy)_3^{2+}$-doped silica (RuSi) nanoparticles via a water/oil microemulsion method and (B) TEM image of the as-prepared RuSi nanoparticles.[69].

3.2. Fabrications of Type II ECL Sensors

As for the Type I ECL sensors described in section 3.1, the analytes of interest must be used as the ECL coreactants. Usually, the presence of an amine group on the analytes, such as alkylamines, antibiotics, antihistamines, opiates, and nicotinamide, is required. However, most of biologically and clinically important targets, such as DNA, peptides, and proteins, are not the ECL coreactants. In this case, Type II ECL sensors are usually fabricated and $Ru(bpy)_3^{2+}$ derivatives are employed as ECL labels that bind the analytes. In this section we will first discuss the $Ru(bpy)_3^{2+}$ derivatives explored as ECL labels, and then address the assay formats for Type II ECL sensors in details.

3.2.1. ECL Labels for Type II ECL Sensors

$Ru(bpy)_3^{2+}$ derivatives conjugated with suitable groups, such as N-hydroxysuccinimide (NHS) ester and phosphoramidite conjugates, have been synthesized and used as ECL labels in Type II ECL sensors. Shown in figure 10 are the chemical structures of some ECL labels mostly used. In 1991, Blackburn et al. reported the first $Ru(bpy)_3^{2+}$ NHS ester, namely [4-(N-succinamidyloxy -carbonylpropyl)-4'-methyl-2,2'-bipyridine] bis(2,2'-bipyridine) ruthenium (II) dihexa -fluorophosphates (see figure 10 for the chemical structure, Ru-Label 1).[8] Ru-Label 1 has been successfully commercialized and has been widely used to label targets with amine groups in biological assay and clinical tests. Ru-Label 1 can be used to label both proteins and DNA (RNA). Some analogues of Ru-Label 1, such as Ru-Label 2 and Ru-Label 3 in figure 10, have also been developed by researchers.

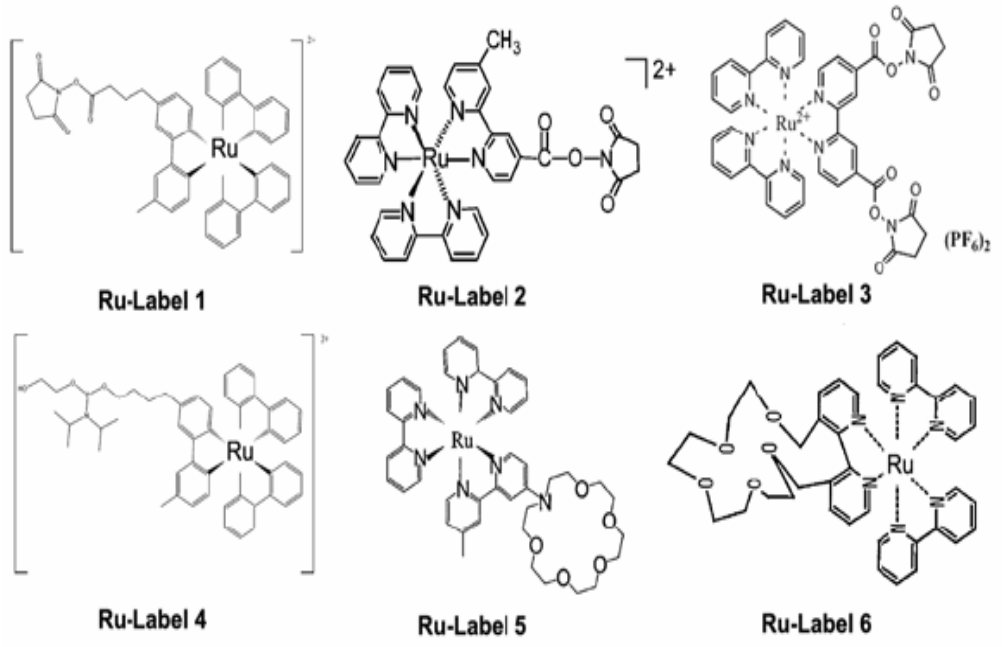

Figure 10. Chemical structures of $Ru(bpy)_3^{2+}$ derivatives as ECL labels.[8, 15, 77-79].

Though Ru-Label 1 has been demonstrated to label DNA and RNA, the target DNA and RNA must be modified with amine groups. In 1993, DiCesare et al. have developed a high-sensitivity ECL-based detection system for automated PCR product quantitation by using the ECL label Ru-Label 4 conjugated with a phosphoramidite group (figure 10).[77] Using Ru-Label 4, unmodified DNA and RNA can be directly labeled.

Other kinds of ELC labels have also been developed for specific analytical applications. For example, an ECL sensor for the determination of Pb^{2+}, Hg^{2+}, Cu^{2+}, and K^+ in solution has been developed using $Ru(bpy)_2$(AZA-bpy) (AZA-bpy =4-(N-aza-18-crown-6-methyl-2,2'-bipyridine).[78] The structure of $Ru(bpy)_2$(AZA -bpy) was given as Ru-Label 5 in figure 10. As for Na^+ ECL detection in both aqueous and nonaqueous media, Ru-Label 6 in figure 10 was used as an ECL probe.[79] Recognition of Na^+ by the crown ether moiety in Ru-Label 6 resulted in a significant increase in the ECL emission intensity of the complex.

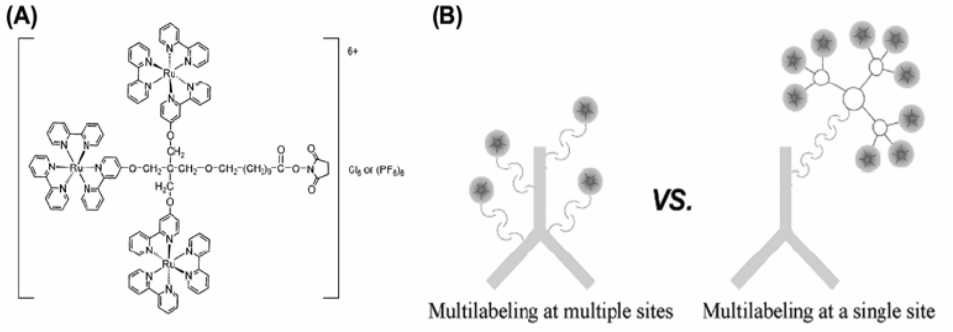

Figure 11. Chemical structures of dendritic $Ru(bpy)_3^{2+}$ derivatives as ECL labels.

With the goal of realizing the ultrasensitive ECL bio assays, multiple labels at multiple accessible sites of a target molecule has been investigated. However, such multilabeling at multiple sites could affect the bioreactivity of biomolecules and could even cause the precipitation of target proteins. Therefore, multilabeling at a single site strategy by using dendritic ECL labels has been studied. As shown in figure 11, using a dendritic prototype label with three $Ru(bpy)_3^{2+}$ linked to a NHS group, Zhou et al. have demonstrated multilabeling a model protein at a single NH_2 position.[80]

Other amplification methods have also been developed to enhance the sensitivities of Type II ECL sensors. By entrapping $Ru(bpy)_3^{2+}$ into silica particles, polystyrene beads or liposomes, or loading $Ru(bpy)_3^{2+}$ onto CNT, enhanced ECL labels for Type II ECL sensors could be prepared (figure 12).[68-69, 81-84] Since thousands of $Ru(bpy)_3^{2+}$ molecules entrapped in a single enhanced ECL label, the detection sensitivity for the biological recognition event can be amplified using these kinds of new labels. When the surfaces of these enhanced ECL labels are conjugated with suitable groups, such as NH_2, COOH and biotin conjugates, they can be readily used to label the analytes of interest via proper bioconjugation reactions.

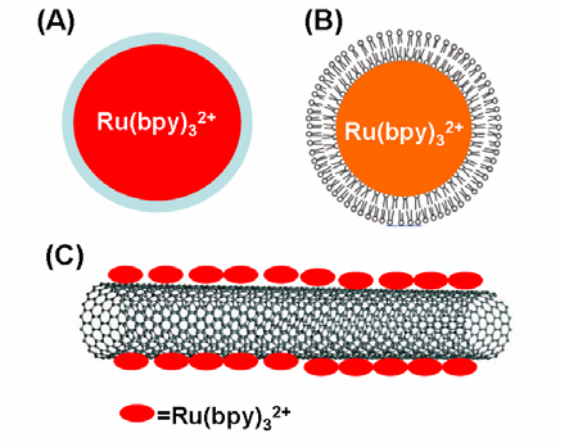

Figure 12. Enhanced ECL labels for Type II ECL sensors prepared by entrapping $Ru(bpy)_3^{2+}$ into silica particles (A) or liposomes (B), or by loading $Ru(bpy)_3^{2+}$ onto CNT (C).[68-69, 81-84].

Recently, we have studied the ECL properties of $Ru(bpy)_3^{2+}$-doped silica nanoparticles within layer-by-layer (LBL) biomolecular coatings and investigated their possible usage as ECL label materials in details.[69] We found that the biomolecular coatings could improve their biocompatibility and prevent the leaking of the $Ru(bpy)_3^{2+}$ ions, the $Ru(bpy)_3^{2+}$-doped silica nanoparticles within the LBL biomolecular coatings could be readily used as stable and efficient ECL tag materials.

3.2.2. Assay Formats for Type II ECL Sensors

In their seminal work, Blackburn and coworkers have demonstrated that Type II ECL sensors were suitable for immunoassays and DNA probe assays in clinical diagnostics.[8] In a recently review, Miao has well summarized the assay formats for Type II ECL sensors.[3] As shown in figure 13, eight typical assay formats are given where most of biologically important analytes, such as DNA, antibody-antigen, and peptide-related, could be detected.

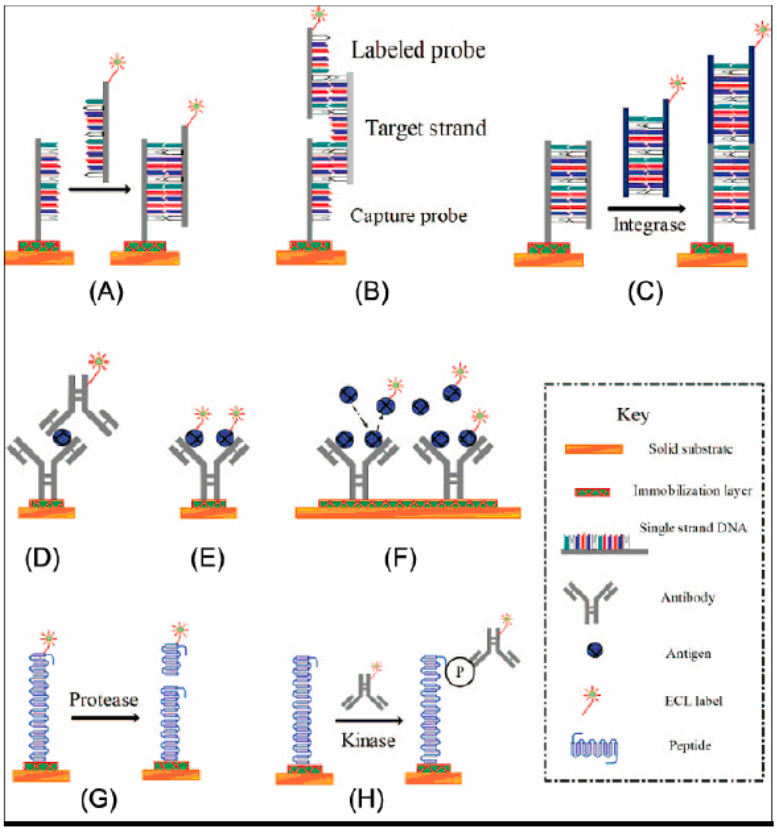

Figure 13. Eight examples of ECL assay formats: (A) DNA hybridization assay based on an immobilized ssDNA hybridizes with a labeled target ssDNA; (B) sandwich type DNA biosensor; (C) assay used for integrase activity test with immobilized and free labeled dsDNA; (D) sandwich type immunoassay; (E) direct immunoassay; (F) competitive assay in which analyte competes with labeled analyte for antibody binding sites on immobilized antibody; (G) protease activity assay in which cleavage of the immobilized peptide results in the decrease in ECL emission due to the removal of the ECL label; (H) kinase activity assay using a labeled antibody to recognize the phosphorylated product.[3].

For example, the concentrations of C-reactive protein (CRP), a so-called “acute phase protein”, in unknown human plasma/serum specimens have been measured by the ECL

sensor based on the method depicted in figure 13D.[85] For this sandwich type immunoassay, biotinylated anti-CRP species were first immobilized onto the Au (111) substrate pre-modified with an avidin layer; then CRP and anti-CRP tagged with $Ru(bpy)_3^{2+}$ labels were conjugated to the surface layer. Finally, the ECL signals were obtained from the sandwich sensing electrode by immersing them in a TPrA containing electrolyte solution.

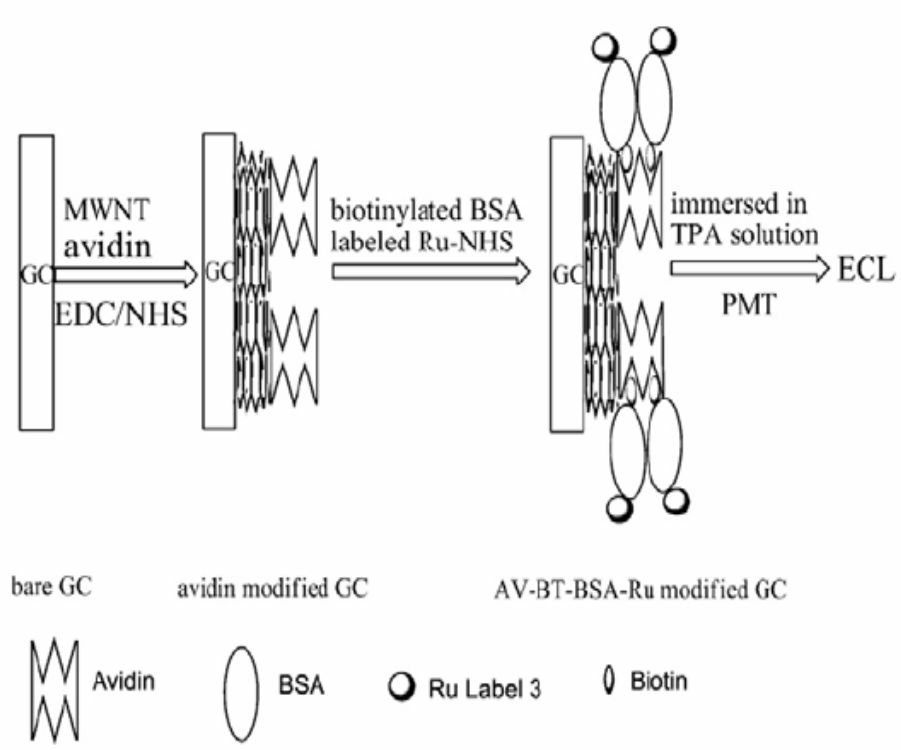

Figure 14. The schematic diagram of direct ECL quantification of BSA by using Ru-Label 3.[86].

Recently, we have reported a quantitative ECL detection of bovine serum albumin (BSA) via biotin-avidin interaction using an avidin-based sensor and Ru-Label 3 as ECL labels and TPrA as coreactant (figure 14).[86] To detect BSA, first, an avidin layer was first immobilized onto a glassy carbon electrode pretreated with CNT; then, biotinylated BSA tagged with Ru-Label 3 was attached to the as-prepared avidin surface; finally, ECL response was generated when the self-assembled modified electrode was immersed in a TPrA-containing electrolyte solution.

Cao et al. developed an interesting approach for quantitative DNA detection via quenching $Ru(bpy)_3^{2+}$ ECL by ferrocene.[87] As shown in figure 15, the ssDNA with a $Ru(bpy)_3^{2+}$ label gave intense ECL signal; however, when it was hybridized with its complementary DNA sequence labeled with ferrocene, an intramolecular ECL quenching occurred and the ECL signal was inhibited. This quenching sensor provided a promising approach for application to sequence specific DNA detection.

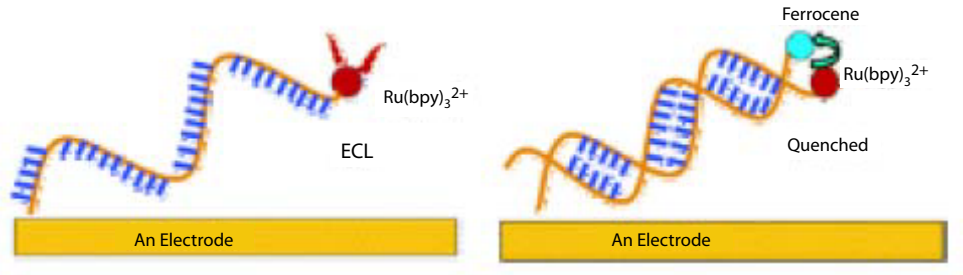

Figure 15. Quantitative DNA detection via quenching $Ru(bpy)_3^{2+}$ ECL by ferrocene.[87].

Beyond the traditional immunoassay, novel recognition elements, especially aptamers, have also been involved to construct Type II ECL sensors. Aptamers are in vitro selected DNA or RNA sequences that are capable of binding to specific targets.[88-89] As far back as in 1999, Bruno and Kiel have reported the in vitro selection of DNA aptamers to anthrax spores with ECL detection.[90] Recently, a lot of aptamer-based ECL sensors have been developed to detect small molecules and proteins.[91-94] We have designed an aptamer-based biosensor combined with gold nanoparticles amplification for the determination of lysozyme with ECL method (figure 16).[94] The lysozyme detection was realized with a competition assay format. In this format, unlabeled lysozyme in the test sample could replace the labeled lysozyme from the labeled lysozyme/aptamer complexes due to its higher affinity to the aptamer than the labeled lysozyme.

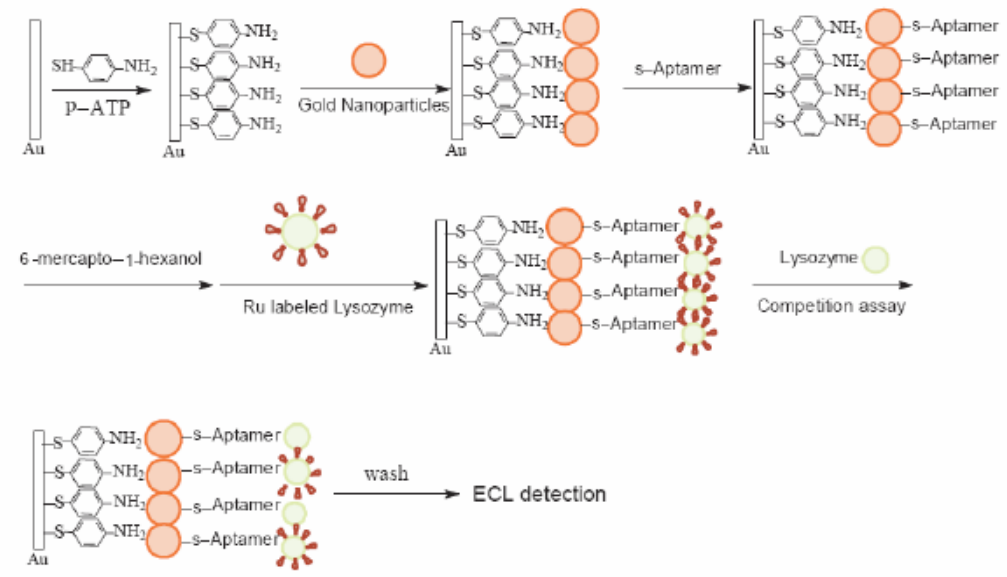

Figure 16. Schematic diagrams for the fabrication of an aptamer-based ECL sensor for lysozyme detection.[94].

4. APPLICATIONS OF $RU(BPY)_3^{2+}$ ECL SENSORS

Due to their intrinsic advantages mentioned above, $Ru(bpy)_3^{2+}$ ECL sensors have shown great promise in analytical application. With the $Ru(bpy)_3^{2+}$ ECL sensors developed, quite a lot of analytes including amines, oxalate, amino acids, drugs, alcohol, phenols, glucose and biomolecules, etc. have been detected in varieties of matrix, ranging from assay buffer to urine, serum, plasma, blood, and raw water samples.

4.1. Applications of Type I ECL Sensors

For Type I ECL Sensors, the concentration of $Ru(bpy)_3^{2+}$ immobilized on an electrode surface is constant, thus the ECL signals can be used to detect the concentration of coreactants and their analogues. Both non-biological and biological analytes could be detected with Type I ECL Sensors.

4.1.1. The ECL Assay of Non-Biomolecules

Since the $Ru(bpy)_3^{2+}$/TPrA system has been most extensively studied and exhibits the highest ECL efficiency, TPrA is usually used as a typical coreactant to validate the Type I $Ru(bpy)_3^{2+}$ ECL sensors. For most sensors, the detection limit for TPrA is from 1 μM to 1 nM. However, by using novel Pt nanoparticles/AQ as matrix to construct the $Ru(bpy)_3^{2+}$ ECL sensor, as low as 1 fM detection limit for TPrA has been finished by Wang and coworkers.[44]

Xu et al. have developed a regenerable ECL sensor by incorporating $Ru(bpy)_3^{2+}$ in ceramic carbon electrode.[95] They then combined the ECL detection with a solid-phase extraction strategy to detect dioxopromethazine in urine sample. Their sensor exhibited good selectivity for dioxopromethazine over other 17 interference species (such as phenylalanine and tryptophan). Also, the sensor improved the sensitivity for the determination of dioxopromethazine by ~3 orders of magnitude.

By immobilizing $Ru(bpy)_3^{2+}$ and alcohol dehydrogenase in the same sol-gel hybrid film, Dong's group has developed an ethanol biosensor based on ECL detection.[96] Their sensor showed a linear response to ethanol from 2.5×10^{-5} M to 5.0×10^{-2} M with a detection limit of 1×10^{-5} M.

The ECL detection of other analytes (including positively charged, neutral and negatively charged analytes) has also been finished by using Type I $Ru(bpy)_3^{2+}$ ECL sensors.[55] More interesting, gas samples could also be analyzed with Type I ECL sensor. Egashira et al. have reported a trimethylamine gas ECL sensor based on $Ru(bpy)_3^{2+}$/Nafion gel.[97] The sensor was successfully applied to monitor the seafood as practical samples. The ECL response for a squid was almost consistent with a profile curve obtained from the classic K value measurement.

4.1.2. The ECL Assay of Biomolecules

Many biomolecules could also be analyzed using Type I ECL sensors. An ECL sensor for practically important polyamines, spermidine and spermine, has been fabricated by using bifunctional nanopartices of magnetic core luminescent shell.[75] The sensor showed an

improved performance with the linear ranges of spermidine from 4×10^{-6} M to 5×10^{-3} M (R=0.988) and of spermine from 5.5×10^{-7} M to 10^{-4} M (R=0.999), respectively. The sensor reported gave 2 orders of more sensitive detection than the ultraviolet method. Other biologically important molecules, such as glucose, coenzyme NADH, and neurotransmitter dopamime, have also been analyzed using the Type I ECL sensors developed.[33, 40, 98-99]

Guanine (and adenine sometimes) bases in DNA can be catalytically oxidized by $Ru(bpy)_3^{2+}$. Thus direct ECL detection of DNA could be finished by using $Ru(bpy)_3^{2+}$ immobilized onto an electrode surface. We reported one-electron catalytic oxidation of guanine bases in DNA using a $Ru(bpy)_3^{2+}$-doped silica nanoparticles modified ITO electrode.[76] In the following work, we have distinctly discriminated native salmon testes DNA (ST-DNA) and its thermally denatured counterpart via label free ECL protocol.[100] As shown in figure 17, though both electrochemical and ECL signals could distinct native ds-ST-DNA from its thermally denatured counterpart, ECL signals gave a more efficient discrimination than electrochemical signals did. Moreover importantly, sensitive single-base mismatch of p53 gene segment has been realized with 39.3 nM.

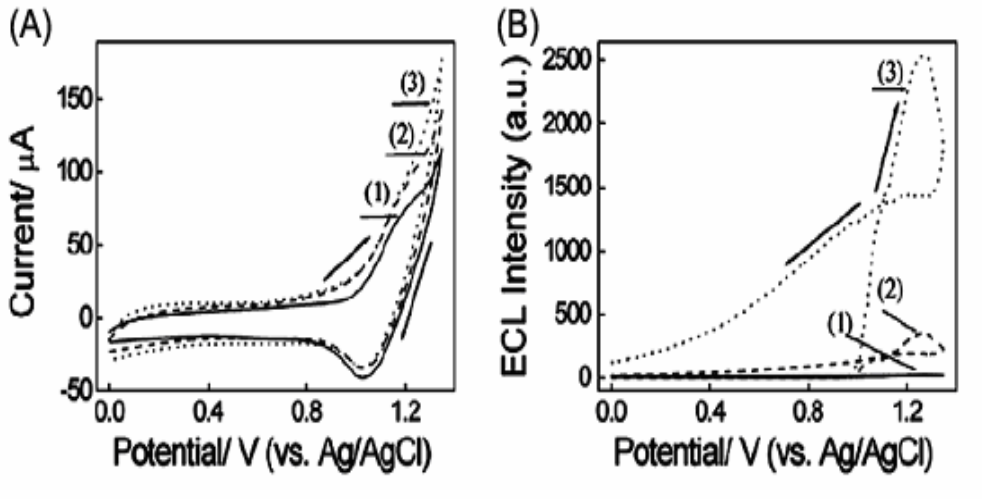

Figure 17. CVs (a) and corresponding ECL intensity-potential curves (b) of the $Ru(bpy)_3^{2+}$ modified electrode in the absence (line 1) and presence of 3.04×10^{-8} mol/L ST-DNA (ds) (line 2) and 3.04×10^{-8} mol/L thermally denatured ST-DNA (ds) (line 3) in 10 mM acetate buffer containing 50 mM NaCl (pH=5.50).[100].

Rusling's group has employed ECL sensors to study the DNA damage caused by chemical or biological routes.[50, 101-104] In their design, the ultrathin films of $[Ru(bpy)_2(PVP)_{10}]^{2+}$, DNA and enzyme were assembled onto an electrode surface (figure 18A). These ultrafilms can mimic a major toxicity pathway in the human liver, and thus are being used to develop toxicity sensor arrays (figure 18B). In a recently report, they have demonstrated ECL arrays for high throughput in vitro genotoxicity screening.[104] The ECL sensors were constructed by assembling various human cytochrome P450 enzymes, DNA and $[Ru(bpy)_2(PVP)_{10}]^{2+}$ onto the electrode surface as shown in figure 18. The sensors were then exposed to H_2O_2 to activate the enzymes. Using benzo[α]pyrene as a test substrate, enzyme activity for producing DNA damage was tested and was found in the same order as their metabolic activity.

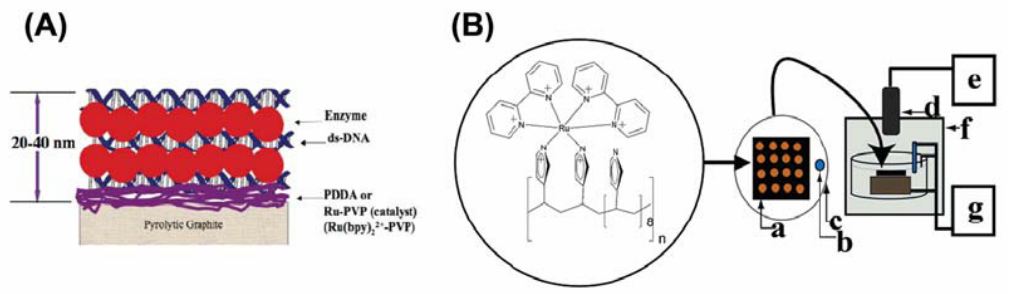

Figure 18. (A) Idealized representations of film configurations used in ECL sensors for detection of DNA damage; and (B) conceptual diagram of an ECL array instrumentation.[101, 104].

The Type I ECL sensors developed usually bear a relatively poor selectivity. By coupling with separation techniques (such as high performance liquid chromatography, capillary electrophoresis and microchip capillary electrophoresis), the selectivity of the $Ru(bpy)_3^{2+}$ ECL sensors could be greatly improved. However, these discussions are out of the scope of this chapter. So interested readers are referred to several reviews for further information.[26, 28]

4.2. Applications of Type II ECL Sensors

Most of Type II ECL sensors are developed for immunoassays and biomolecular assays. In these assays, targets (such as proteins, DNAs, and peptides) having no "coreactant functionalities" are tagged with ECL labels, then ECL signals associated with the target concentration are produced in the presence of a high and constant concentration of TPrA solution. Until now, biologically important targets from tumor markers to cardiac markers, vascular markers, growth factors, fertility markers, Alzheimer's disease markers and HIV related genes, have been successfully studied using various assay formats for Type II ECL sensors (figure 13).[3]

Miao and Bard have reported the ECL determination of immobilized DNA on Au(111) electrodes using $Ru(bpy)_3^{2+}$ labels (figure 19).[85] An amino-modified 23-mer ssDNA derived from the *Bacillus anthracis* as probe was first attached to the Au(111) substrate, then the target ssDNA (complementary or noncomplementary ssDNA) tagged with $Ru(bpy)_3^{2+}$ labels was hybridized to form the duplex. When a potential is applied to the electrode in TPrA solution, an ECL signal was generated and detected. As shown in figure 19B, the complementary DNA hybrid gave a much higher ECL response with respect to that for the noncomplementary DNA. Note that the CV response could not distinct the complementary DNA from the noncomplementary one due to the CV signals were mainly originated from TPrA oxidation.

To further improve the sensitivity for DNA detection, polymerase chain reaction (PCR) amplification could be coupled.[8, 105-110] For example, Blackburn et al. have coupled ECL with PCR amplification and lowered the detection limit of HIV 1 gag DNA to <10 copies.[8] Other amplification approaches have also been explored.[81-82, 111-114] Miao and co-workers have finished DNA hybridization detection at high amplification with $Ru(bpy)_3^{2+}$-containing microspheres.[81] The same group has extended the amplification approach to

CRP determination (figure 20).[82] With this amplification approach, as low as 0.010 μg/mL CRP has been detected, which was better than those obtained from most of the presently available automated high-sensitivity CRP assay systems. This method has also been validated to detect CRP concentration of an unknown human plasma specimen by the standard addition method.

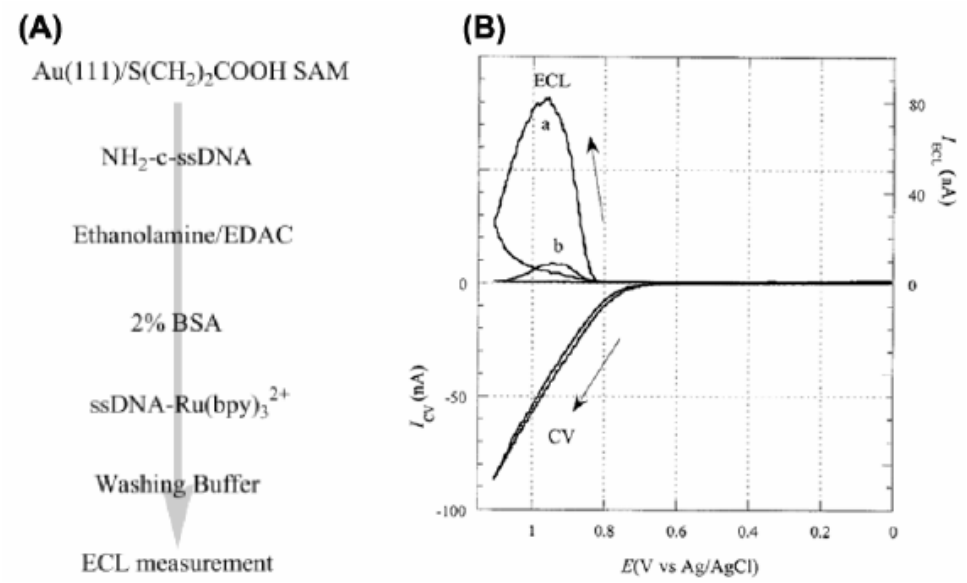

Figure 19. (A) Flowchart showing all of the processes involved in the electrode treatment for the determination of immobilized DNA. (B) ECL intensity vs potential profiles along with the CV obtained for the detection of immobilized DNA using $Ru(bpy)_3^{2+}$ labels. (a) Complementary DNA hybridization and (b) noncomplementary DNA hybridization. The CVs for both (a) and (b) were essentially the same; only one is displayed here.[85].

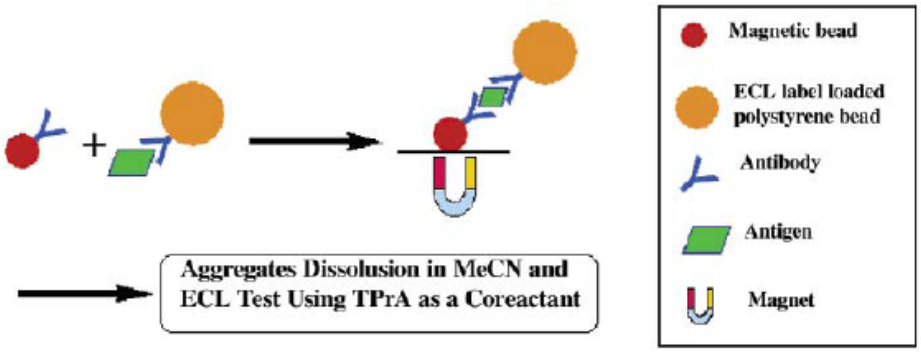

Figure 20. Schematic diagram showing the formation of a sandwich-type aggregate between an antibody-coated magnetic bead (MB) and an antibody-coated polystyrene bead (PSB) containing entrapped ECL labels in the presence of the antigen species, and the separation of the newly formed aggregate with a magnet as well as the subsequent dissolution and ECL detection in MeCN using TPrA as the coreactant.[82].

We have recently employed novel aptamers as recognition elements to detect protein.[115] Sensitive and selective determination of α-thrombin, a kind of serine protease

that plays important role in thrombosis and haemostasis, has been realized by using an aptamer-based sandwich ECL sensing format. Since α-thrombin has two anti-α-thrombin aptamers (one is 15-mer and the other is 29-mer), the sandwich ECL sensing format could be fabricated as shown in figure 21, where gold nanoparticles functionalized with the 15-mer aptamer were immobilized onto the ITO electrode surface as capturing layer and the 29-mer aptamer tagged with $Ru(bpy)_3^{2+}$ labels as signaling layer would be captured to form the "15-mer aptamer/α-thrombin /29-mer aptamer" sandwich in the presence of α-thrombin. As shown in figure 21B, the relationship between ECL intensity and thrombin concentration was a typical behavior of sandwich assay.

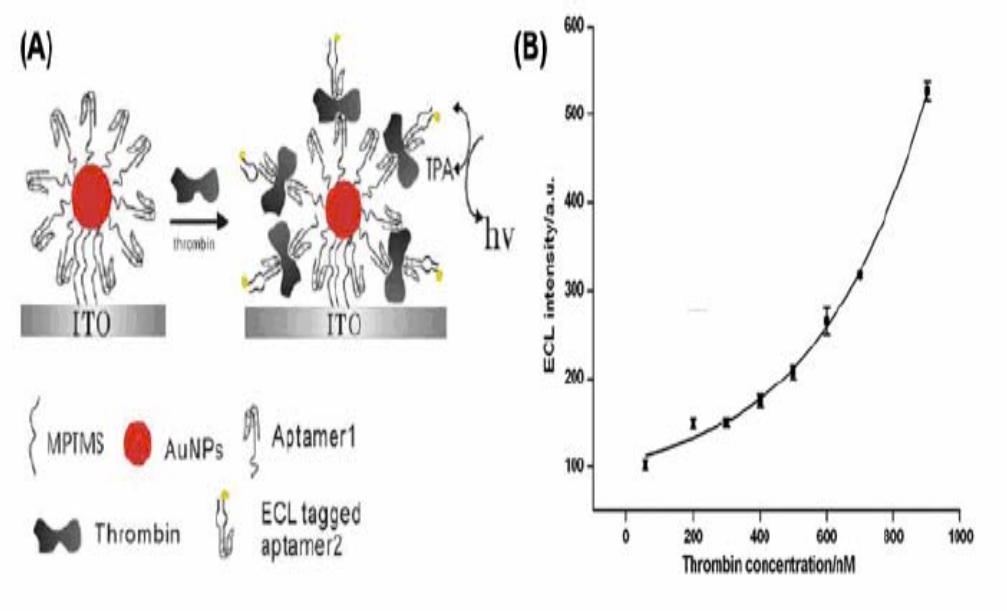

Figure 21. (A) The schematic for the determination of α-thrombin using anti-α-thrombin aptamer tagged with $Ru(bpy)_3^{2+}$ labels. (B) α-thrombin concentration-ECL intensity response curve.[115].

As alternatives to $Ru(bpy)_3^{2+}$ labels, researchers have also studied the possibility of using coreactants as ECL labels. Using 4-(dimethylamino)butyric acid, an analogue of TPrA, as an ECL label and combining with GNPs amplification, Yin et al. have fabricated an sandwich type ECL sensing system and detected BSA and IgG when the sensing electrode was in contact with 1 mM $Ru(bpy)_3^{2+}$ solution.[116] To further improve the sensing performance of this coreactant labels, the coreactants have been attached onto nanoparticles' surface to prepare nanoscale enhanced ECL labels.[84, 117] Wang's group have synthesized 2-(dimethylamino) ethanethiol (DMAET), one of the most efficient coreactants of $Ru(bpy)_3^{2+}$ system, capped CdTe semiconductor nanoparticles through a simple one-pot synthesis method in aqueous media. The DMAET capped CdTe particles have exhibited enormous signal amplification of $Ru(bpy)_3^{2+}$ ECL. Thus the particles were further used as ECL labels to lysozyme via a competitive assay format using anti-lysozyme aptamer as recognition element

(figure 22). A detectable limit of 0.5 nM lysozyme can be obtained by this "aptamer/DMAET capped CdTe particles" based biosensing system.[117]

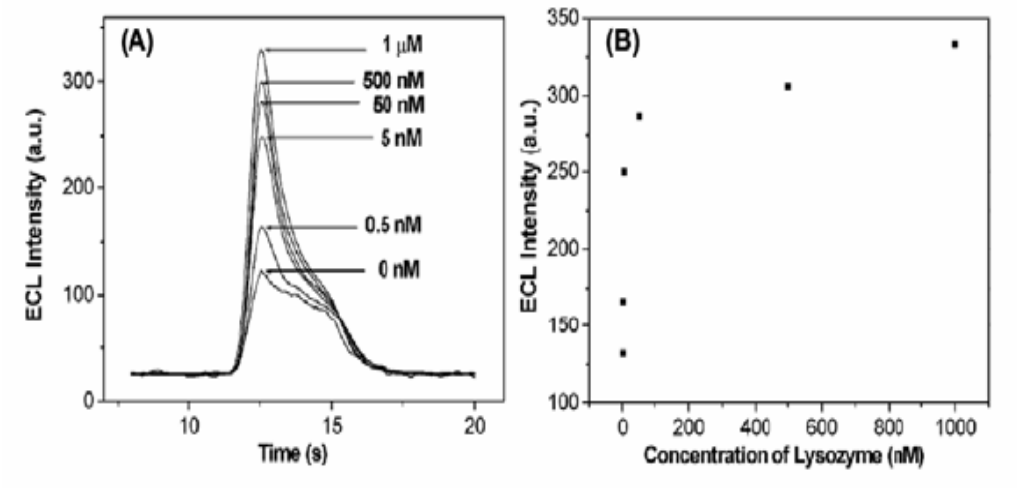

Figure 22. The ECL responses of the nanoscale enhanced ECL biosensing system to a series of the concentration of lysozyme solutions (A) and the corresponding calibration plot (B).[117].

5. CONCLUSIONS AND OUTLOOK

Owning to their intrinsic advantages, various kinds of $Ru(bpy)_3^{2+}$ ECL sensors have been developed as rapid, sensitive and selective analytical platforms for the determination of a number of biologically and nonbiologically important molecules. Though much progress has been made in both scientific and practical fields, there is still a plenty of room to further improve the $Ru(bpy)_3^{2+}$ ECL sensors' performance. By introducing functional materials, such as ionic liquids and novel nanomaterials, enhanced ECL sensors will be fabricated.[62, 118-119] For simple, reliable, and robust high-throughout bioassay, miniaturized sensing arrays based on ECL technology will certainly be the area of active research.[42, 120-121] Besides $Ru(bpy)_3^{2+}$, other ECL reagents (such as ruthenium complexes with other ligands and even coordination complexes with Os, Ir, etc. as cores) should be developed and be used to construct new ECL sensors.[78, 122-124] Though very sensitive ECL sensors have been developed, the detection of single biomolecules is still a great challenge and is the Holy Grail of ECL.

6. ACKNOWLEDGMENTS

This work is supported by the National Natural Science Foundation of China with Grant 20675078, 863 Project No. 2006AA020701, 973 Project No. 2007CB714500 and the Chinese Academy of Sciences KJCX2. YW. H11.

7. REFERENCES

[1] Electrogenerated Chemiluminescence, Ed. Bard, A. J., Marcel Dekker, New York, 2004.

[2] Richter, M. M., Electrochemiluminescence (ECL). *Chem. Rev.* 2004, 104, (6), 3003-3036.

[3] Miao, W. J., Electrogenerated Chemiluminescence and Its Biorelated Applications. *Chem. Rev.* 2008, 108, (7), 2506-2553.

[4] Lee, W. Y., Tris (2,2'-bipyridyl)ruthenium(II) electrogenerated chemiluminescence in analytical science. *Mikrochim. Acta* 1997, 127, (1-2), 19-39.

[5] Knight, A. W., A review of recent trends in analytical applications of electrogenerated chemiluminescence. *Trac-Trend Anal. Chem.* 1999, 18, (1), 47-62.

[6] Yin, X. B.; Dong, S. J.; Wang, E., Analytical applications of the electrochemiluminescence of tris (2,2'-bipyridyl) ruthenium and its derivatives. *Trac-Trend Anal. Chem.* 2004, 23, (6), 432-441.

[7] Deaver, D. R., A New Nonisotopic Detection System for Immunoassays. *Nature* 1995, 377, (6551), 758-760.

[8] Blackburn, G. F.; Shah, H. P.; Kenten, J. H.; Leland, J.; Kamin, R. A.; Link, J.; Peterman, J.; Powell, M. J.; Shah, A.; Talley, D. B.; Tyagi, S. K.; Wilkins, E.; Wu, T. G.; Massey, R. J., Electrochemiluminescence Detection for Development of Immunoassays and DNA Probe Assays for Clinical Diagnostics. *Clin. Chem.* 1991, 37, (9), 1534-1539.

[9] Tokel, N. E.; Bard, A. J., Electrogenerated chemiluminescence. IX. Electrochemistry and emission from systems containing tris(2,2'-bipyridine)ruthenium(II) dichloride. *J. Am. Chem. Soc.* 1972, 94, (8), 2862-2863.

[10] Miao, W. J.; Choi, J. P.; Bard, A. J., Electrogenerated chemiluminescence 69: The tris(2,2'-bipyridine)ruthenium(II), (Ru(bpy)(3)(2+))/tri-n-propylamine (TPrA) system revisited - A new route involving TPrA(center dot+) cation radicals. *J. Am. Chem. Soc.* 2002, 124, (48), 14478-14485.

[11] van Ingen, H. E.; Chan, D. W.; Hubl, W.; Miyachi, H.; Molina, R.; Pitzel, L.; Ruibal, A.; Rymer, J. C.; Domke, I., Analytical and clinical evaluation of an electrochemiluminescence immunoassay for the determination of CA 125. *Clin. Chem.* 1998, 44, (12), 2530-2536.

[12] Kyriatsoulis, A.; Waechter, N.; Kraus, W.; Pappert, G., A electrochemiluminescence immunoassay for the quantitative determination of Cyfra 21-1 using the random access analyzer Elecsys(R). *Clin. Chem.* 1996, 42, (6), 656-656.

[13] Liu, J. F.; Cao, W. D.; Qiu, H. B.; Sun, X. H.; Yang, X. R.; Wang, E. K., Determination of sulpiride by capillary electrophoresis with end-column electrogenerated chemiluminescence detection. *Clin. Chem.* 2002, 48, (7), 1049-1058.

[14] Golla, R.; Seethala, R., A sensitive, robust high-throughput electrochemiluminescence assay for rat insulin. *J. Biomol. Screen.* 2004, 9, (1), 62-70.

[15] Zhou, M.; Robertson, G. P.; Roovers, J., Comparative study of ruthenium(II) tris-(bipyridine) derivatives for electrochemiluminescence application. *Inorg. Chem.* 2005, 44, (23), 8317-8325.

[16] Wei, H.; Wang, E., Solid-state electrochemiluminescence of tris(2,2'-bipyridyl) ruthenium. *Trac-Trend Anal. Chem.* 2008, 27, (5), 447-459.
[17] Ying, T.; Lin, Z. J.; Chen, X. M.; Xi, C., Solid-state electrochemiluminescence on tris (2,2 '-bipyridine) ruthenium(II) modified electrode. *Progress in Chemistry* 2008, 20, (2-3), 362-367.
[18] Discovery, M. S., www.mesoscale.com.
[19] Corp., R. D., www.roche.com. Note: The initial Igen International and then BioVeris Corp. have now been acquired by Roche Diagnostics Corp.
[20] Xian Remax Electronics Co., www.xaremex.com.
[21] Pyati, R.; Richter, M. M., ECL-Electrochemical luminescence. *Annu. Rep. Prog. Chem., Sect. C: Phys. Chem.* 2007, 103, 12-78.
[22] Gorman, B. A.; Francis, P. S.; Barnett, N. W., Tris(2,2'-bipyridyl)ruthenium(II) chemiluminescence. *Analyst* 2006, 131, (5), 616-639.
[23] Gerardi, R. D.; Barnett, N. W.; Lewis, S. W., Analytical applications of tris(2,2'-bipyridyl)ruthenium(III) as a chemiluminescent reagent. *Anal. Chim. Acta* 1999, 378, (1-3), 1-41.
[24] Greenway, G. M., Analytical applications of electrogenerated chemiluminescence. *Trac-Trend Anal. Chem.* 1990, 9, (6), 200-203.
[25] Knight, A. W.; Greenway, G. M., Occurrence, Mechanisms and Analytical Applications of Electrogenerated Chemiluminescence - Review. *Analyst* 1994, 119, (5), 879-890.
[26] Du, Y.; Wang, E. K., Capillary electrophoresis and microchip capillary electrophoresis with electrochemical and electrochemiluminescence detection. *J. Sep. Sci.* 2007, 30, (6), 875-890.
[27] Yin, X. B.; Yang, X. R.; Wang, E. K., Some aspects in capillary electrophoresis-electrochemical/electrochemiluminescence with microchip technique. *Progress in Chemistry* 2005, 17, (2), 181-185.
[28] Yin, X. B.; Wang, E., Capillary electrophoresis coupling with electro-chemilurninescence detection: a review. *Anal. Chim. Acta* 2005, 533, (2), 113-120.
[29] Rubinstein, I.; Bard, A. J., Polymer films on electrodes. 4. Nafion-coated electrodes and electrogenerated chemiluminescence of surface-attached tris(2,2'-bipyridine)ruthenium(2+) *J. Am. Chem. Soc.* 1980, 102, (21), 6641-6642.
[30] Rubinstein, I.; Bard, A. J., Electrogenerated chemiluminescence. 37. Aqueous ecl systems based on tris(2,2'-bipyridine)ruthenium(2+) and oxalate or organic acids *J. Am. Chem. Soc.* 1981, 103, (3), 512-516.
[31] Rubinstein, I.; Bard, A. J., Polymer films on electrodes. 5. Electrochemistry and chemiluminescence at Nafion-coated electrodes *J. Am. Chem. Soc.* 1981, 103, (17), 5007-5013.
[32] Guo, Z. H.; Dong, S. J., Electrogenerated chemi luminescence from Ru(BPY)(3)(2+) ion-exchanged in carbon nanotube/perfluorosulfonated ionomer composite films. *Anal. Chem.* 2004, 76, (10), 2683-2688.
[33] Guo, Z. H.; Dong, S. J., Electrogenerated chemiluminescence determination of dopamine and epinephrine in the presence of ascorbic acid at carbon nanotube/nafion-Ru(bpy)(2+)(3) composite film modified glassy carbon electrode. *Electroanalysis* 2005, 17, (7), 607-612.

[34] Song, H. J.; Zhang, Z. J.; Wang, F., Electrochemiluminescent determination of chlorphenamine maleate based on Ru(bpy)(2+)(3) immobilized in a nano-titania/nafion membrane. *Electroanalysis* 2006, 18, (18), 1838-1841.

[35] Choi, H. N.; Yoon, S. H.; Lyu, Y. K.; Lee, W. Y., Electrogenerated chemiluminese ethanol biosensor based on carbon nanotube-titania-nafion composite film. *Electroanalysis* 2007, 19, (4), 459-465.

[36] Choi, H. N.; Lee, J. Y.; Lyu, Y. K.; Lee, W. Y., Tris(2,2 '-bipyridyl)ruthenium(II) electrogenerated chemiluminescence sensor based on carbon nantube dispersed in sol-gel-derived titania-Nafion composite films. *Anal. Chim. Acta* 2006, 565, (1), 48-55.

[37] Khramov, A. N.; Collinson, M. M., Electrogenerated chemiluminescence of tris(2,2 '-bipyridyl)ruthenium(II) ion-exchanged in nafion-silica composite films. *Anal. Chem.* 2000, 72, (13), 2943-2948.

[38] Choi, H. N.; Cho, S. H.; Lee, W. Y., Electrogenerated chemiluminescence from tris(2,2 '-bipyridyl)ruthenium(II) immobilized in titania-perfluorosulfonated lonomer composite films. *Anal. Chem.* 2003, 75, (16), 4250-4256.

[39] Choi, H. N.; Lyu, Y. K.; Lee, W. Y., Tris (2,2 '-bipyridyl) ruthenium (II) electrogenerated chemiluminescence sensor based on sol-gel-derived V2O5/Nafion composite films. *Electroanalysis* 2006, 18, (3), 275-281.

[40] Zhuang, Y. F.; Ju, H. X., Determination of reduced nicotinamide adenine dinucleotide based on immobilization of tris(2,2 '-bipyridyl) ruthenium(II) in multiwall carbon nanotubes/Nafion composite membrane. *Anal. Lett.* 2005, 38, (13), 2077-2088.

[41] Ding, S. N.; Xu, J. J.; Chen, H. Y., Tris(2,2'-bipyridyl)ruthenium(Il)- zirconia-nafion composite films applied as solid-state electrochemiluminescence detector for capillary electrophoresis. *Electrophoresis* 2005, 26, (9), 1737-1744.

[42] Ding, S. N.; Xu, J. J.; Zhang, W. J.; Chen, H. Y., Tris(2,2'-bipyridyl)ruthenium(II) -zirconia-nafion composite modified electrode applied as solid-state electro-chemiluminescence detector on electrophoretic microchip for detection of pharma-ceuticals of tramadol, lidocaine and ofloxacin. *Talanta* 2006, 70, (3), 572-577.

[43] Wang, H. Y.; Xu, G. B.; Dong, S. J., Electrochemiluminescence sensor using tris(2,2'-bipyridyl)ruthenium(II) immobilized in Eastman-AQ55D-silica composite thin-films. *Anal. Chim. Acta* 2003, 480, (2), 285-290.

[44] Du, Y.; Qi, B.; Yang, X. R.; Wang, E. K., Synthesis of PtNPs/AQ/Ru(bpy)(3)(2+) colloid and its application as a sensitive solid-state electrochemiluminescence sensor material. *J. Phys. Chem. B* 2006, 110, (43), 21662-21666.

[45] Zhang, L. H.; Guo, Z. H.; Xu, Z. A.; Dong, S. J., Highly sensitive electrogenerated chemiluminescence produced at Ru(bpy)(3)(2+) in Eastman-AQ55D-carbon nanotube composite film electrode. *J. Electroanal. Chem.* 2006, 592, (1), 63-67.

[46] Wang, H. Y.; Xu, G. B.; Dong, S. J., Electrochemiluminescence of tris(2,2 '-bipyridine)ruthenium(II) immobilized in poly(p-styrenesulfonate)-silica-Triton X-100 composite thin-films. *Analyst* 2001, 126, (7), 1095-1099.

[47] Wang, H. Y.; Xu, G. B.; Dong, S. J., Electrochemiluminescence of tris(2,2'-bipyridyl)ruthenium (II) ion-exchanged in polyelectrolyte-silica composite thin-films. *Electroanalysis* 2002, 14, (12), 853-857.

[48] Zhang, L. H.; Xu, Z. A.; Dong, S. J., Electrogenerated chemiluminescence biosensor based on Ru(bpy)(3)(2+) and dehydrogenase immobilized in sol-gel/chitosan-

/poly(sodium 4-styrene sulfonate) composite material. *Anal. Chim. Acta* 2006, 575, (1), 52-56.

[49] Li, J.; Xu, Y. H.; Wei, H.; Huo, T.; Wang, E. K., Electrochemiluminescence sensor based on partial sulfonation of polystyrene with carbon nanotubes. *Anal. Chem.* 2007, 79, (14), 5439-5443.

[50] Dennany, L.; Forster, R. J.; Rusling, J. F., Simultaneous direct electrochemiluminescence and catalytic voltammetry detection of DNA in ultrathin films. *J. Am. Chem. Soc.* 2003, 125, (17), 5213-5218.

[51] Dennany, L.; Hogan, C. F.; Keyes, T. E.; Forster, R. J., Effect of surface immobilization on the electrochemiluminescence of ruthenium-containing metallopolymers. *Anal. Chem.* 2006, 78, (5), 1412-1417.

[52] Lu, M. C.; Whang, C. W., The role of direct oxalate oxidation in electrogenerated chemiluminescence of poly(4-vinylpyridine)-bound Ru(bpy)(2)Cl+/oxalate system on indium tin oxide electrodes. *Anal. Chim. Acta* 2004, 522, (1), 25-33.

[53] Wei, H.; Du, Y.; Kang, J. Z.; Xu, G. B.; Wang, E. K., Tris(2,2 '-bipyridyl) ruthenium(II) doped silica film modified indium tin oxide electrode and its electrochemiluminescent properties. *Chin. J. Chem.* 2007, 25, (2), 159-163.

[54] Greenway, G. M.; Greenwood, A.; Watts, P.; Wiles, C., Solid-supported chemiluminescence and electrogenerated chemiluminescence based on a tris(2,2 '-bipyridyl)ruthenium(II) derivative. *Chem. Commun.* 2006, (1), 85-87.

[55] Lee, J. K.; Lee, S. H.; Kim, M.; Kim, H.; Kim, D. H.; Lee, W. Y., Organosilicate thin film containing Ru(bpy)(3)(2+) for an electrogenerated chemiluminescence (ECL) sensor. *Chem. Commun.* 2003, (13), 1602-1603.

[56] Guo, Z. H.; Shen, Y.; Wang, M. K.; Zhao, F.; Dong, S. J., Electrochemistry and electrogenerated chemiluminescence of SiO2 nanoparticles/tris(2,2 '-bipyridyl)-ruthenium(II) multilayer films on indium tin oxide electrodes. *Anal. Chem.* 2004, 76, (1), 184-191.

[57] Guo, Z. H.; Shen, Y.; Zhao, F.; Wang, M. K.; Dong, S. J., Electrochemical and electrogenerated chemiluminescence of clay nanoparticles/Ru(bpy)(3)(2+) multilayer films on ITO electrodes. *Analyst* 2004, 129, (7), 657-663.

[58] Ding, S. N.; Xu, J. J.; Chen, H. Y., Electrogenerated chemiluminescence of tris(2,2 '-bipyridyl) ruthenium(II) immobilized in humic acid-silica-poly(vinyl alcohol) composite films. *Electroanalysis* 2005, 17, (17), 1517-1522.

[59] Bi, L. H.; Wang, H. Y.; Shen, Y.; Wang, E. K.; Dong, S. J., Multifunctional organic-inorganic multilayer films of tris(2,2'-bipyridine)ruthenium and decatungstate. *Electrochem. Commun.* 2003, 5, (11), 913-918.

[60] Bi, L. H.; Zhou, W. H.; Wang, H. Y.; Dong, S. J., Preparation of nanoparticles of bipyridineruthenium and silicotungstate and application as electrochemiluminescence sensor. *Electroanalysis* 2008, 20, (9), 996-1001.

[61] Wang, H. Y.; Xu, G. B.; Dong, S. J., Electrochemistry and electrochemiluminescence of stable tris(2,2'-bipyridyl)ruthenium(II) monolayer assembled on benzene sulfonic acid modified glassy carbon electrode. *Talanta* 2001, 55, (1), 61-67.

[62] Sun, X. P.; Du, Y.; Dong, S. J.; Wang, E. K., Method for effective immobilization of Ru(bpy)(3)(2+) on an electrode surface for solid-state electrochemiluminescene detection. *Anal. Chem.* 2005, 77, (24), 8166-8169.

[63] Sun, X. P.; Du, Y.; Zhang, L. X.; Dong, S. J.; Wang, E. K., Pt nanoparticles: Heat treatment-based preparation and Ru(bpy)(3)(2+)-mediated formation of aggregates that can form stable films on bare solid electrode surfaces for solid-state electrochemiluminescence detection. *Anal. Chem.* 2006, 78, (18), 6674-6677.

[64] Sun, X. P.; Du, Y.; Zhang, L. X.; Dong, S. J.; Wang, E. K., Luminescent supramolecular microstructures containing Ru(bpy)(3)(2+): Solution-based self-assembly preparation and solid-state electrochemiluminescence detection application. *Anal. Chem.* 2007, 79, (6), 2588-2592.

[65] Sun, X. P.; Du, Y.; Zhang, L. X.; Doug, S. J.; Wang, E. K., Formation of [Ru(bpy)(3)](2+)-containing microstructures induced by electrostatic assembly and their application in solid-state detection of electrochemiluminescence. *Chem.- Asian J.* 2007, 2, (9), 1137-1141.

[66] Armelao, L.; Bertoncello, R.; Gross, S.; Badocco, D.; Pastore, P., Construction and characterization of Ru(II)tris(bipyridine)-based silica thin film electrochemiluminescent sensors. *Electroanalysis* 2003, 15, (9), 803-811.

[67] Collinson, M. M.; Novak, B.; Martin, S. A.; Taussig, J. S., Electrochemiluminescence of ruthenium(II) tris(bipyridine) encapsulated in sol-gel classes. *Anal. Chem.* 2000, 72, (13), 2914-2918.

[68] Zhang, L. H.; Dong, S. J., Electrogenerated chemiluminescence sensors using Ru(bpy)(3)(2+) doped in silica nanoparticles. *Anal. Chem.* 2006, 78, (14), 5119-5123.

[69] Wei, H.; Liu, J.; Zhou, L.; Li, J.; Jiang, X.; Kang, J.; Yang, X.; Dong, S.; Wang, E., [Ru(bpy)(3)](2+)-doped silica nanoparticles within layer-by-layer biomolecular coatings and their application as a biocompatible electrochemiluminescent tag material. *Chem.-Eur. J.* 2008, 14, (12), 3687-3693.

[70] Wei, H.; Zhou, L. L.; Li, J.; Liu, J. F.; Wang, E. K., Electrochemical and electrochemiluminescence study of Ru(bpy)(3)(2+)-doped silica nanoparticles with covalently grafted biomacromolecules. *J. Colloid Interf. Sci.* 2008, 321, (2), 310-314.

[71] Hun, X.; Zhang, Z. J., Electrogenerated chemiluminescence sensor for itopride with Ru(bpy)(3)(2+)-doped silica nanoparticles/chitosan composite films modified electrode. *Sens. Actuators, B* 2008, 131, (2), 403-410.

[72] Hun, X.; Zhang, Z. J., Electrogenerated chemiluminescence sensor for metoclopramide determination based on Ru(bpy)(3)(2+)-doped silica nanoparticles dispersed in Nafion on glassy carbon electrode. *J. Pharmaceut. Biomed.* 2008, 47, (4-5), 670-676.

[73] Li, M. J.; Chen, Z. F.; Yam, V. W. W.; Zu, Y. B., Multi functional ruthenium(II) polypyridine complex-based core-shell magnetic silica nanocomposites: Magnetism, luminescence, and electrochemiluminescence. *Acs Nano* 2008, 2, (5), 905-912.

[74] Qian, L.; Yang, X. R., One-step synthesis of Ru(2,2 '-bipyridine)(3)Cl-2-immobilized silica nanoparticles for use in electrogenerated chemiluminescence detection. *Adv. Funct. Mater.* 2007, 17, (8), 1353-1358.

[75] Zhang, L. H.; Liu, B. F.; Dong, S. J., Bifunctional nanostructure of magnetic core luminescent shell and its application as solid-state electrochemiluminescence sensor material. *J. Phys. Chem. B* 2007, 111, (35), 10448-10452.

[76] Wei, H.; Wang, E., Electrochemiluminescence-based DNA detection using guanine oxidation at electrostatic self-assembly of Ru(bpy)(3)(2+)-doped silica nanoparticles on indium tin oxide electrode. *Chem. Lett.* 2007, 36, (2), 210-211.

[77] Dicesare, J.; Grossman, B.; Katz, E.; Picozza, E.; Ragusa, R.; Woudenberg, T., A High-Sensitivity Electrochemiluminescence-Based Detection System for Automated Pcr Product Quantitation. *Biotechniques* 1993, 15, (1), 152-157.

[78] Muegge, B. D.; Richter, M. M., Electrochemiluminescent detection of metal cations using a ruthenium(II) bipyridyl complex containing a crown ether moiety. *Anal. Chem.* 2002, 74, (3), 547-550.

[79] Lai, R. Y.; Chiba, M.; Kitamura, N.; Bard, A. J., Electrogenerated chemiluminescence. 68. Detection of sodium ion with a ruthenium(II) complex with crown ether moiety at the 3,3 '-positions on the 2,2 '-bipyridine ligand. *Anal. Chem.* 2002, 74, (3), 551-553.

[80] Zhou, M.; Roovers, J.; Robertson, G. P.; Grover, C. P., Multilabeling biomolecules at a single site. 1. Synthesis and characterization of a dendritic label for electro-chemiuminescence assays. *Anal. Chem.* 2003, 75, (23), 6708-6717.

[81] Miao, W. J.; Bard, A. J., Electrogenerated chemiluminescence. 77. DNA hybridization detection at high amplification with [Ru(bpy)(3)](2+)-containing microspheres. *Anal. Chem.* 2004, 76, (18), 5379-5386.

[82] Miao, W. J.; Bard, A. J., Electrogenerated chemiluminescence. 80. C-reactive protein determination at high amplification with [Ru(bpy)(3)](2+)-containing microspheres. *Anal. Chem.* 2004, 76, (23), 7109-7113.

[83] Zhan, W.; Bard, A. J., Electrogenerated chemiluminescence. 83. Immunoassay of human C-reactive protein by using Ru(bpy)(3)(2+)-encapsulated liposomes as labels. *Anal. Chem.* 2007, 79, (2), 459-463.

[84] Li, Y.; Qi, H. L.; Fang, F.; Zhang, C. X., Ultrasensitive electrogenerated chemiluminescence detection of DNA hybridization using carbon-nanotubes loaded with tris(2,2 '-bipyridyl) ruthenium derivative tags. *Talanta* 2007, 72, (5), 1704-1709.

[85] Miao, W. J.; Bard, A. J., Electrogenerated chemluminescence. 72. Determination of immobilized DNA and C-reactive protein on Au(111) electrodes using Tris(2,2 '-bipyridyl)ruthenium(II) labels. *Anal. Chem.* 2003, 75, (21), 5825-5834.

[86] Fang, L. Y.; Lu, Z. Z.; Wei, H.; Wang, E. K., Quantitative electrochemiluminescence detection of proteins: Avidin-based sensor and tris(2,2 '-bipyridine) ruthenium(II) label. *Biosens. Bioelectron.* 2008, 23, (11), 1645-1651.

[87] Cao, W. D.; Ferrance, J. P.; Demas, J.; Landers, J. P., Quenching of the electrochemiluminescence of tris(2,2 '-bipyridine) ruthenium(II) by ferrocene and its potential application to quantitative DNA detection. *J. Am. Chem. Soc.* 2006, 128, (23), 7572-7578.

[88] Bock, L. C.; Griffin, L. C.; Latham, J. A.; Vermaas, E. H.; Toole, J. J., Selection of Single-Stranded-DNA Molecules That Bind and Inhibit Human Thrombin. *Nature* 1992, 355, (6360), 564-566.

[89] Famulok, M.; Hartig, J. S.; Mayer, G., Functional aptamers and aptazymes in biotechnology, diagnostics, and therapy. *Chem. Rev.* 2007, 107, (9), 3715-3743.

[90] Bruno, J. G.; Kiel, J. L., In vitro selection of DNA aptamers to anthrax spores with electrochemiluminescence detection. *Biosens. Bioelectron.* 1999, 14, (5), 457-464.

[91] Wang, X. Y.; Zhou, J. M.; Yun, W.; Xiao, S. S.; Chang, Z.; He, P. G.; Fang, Y. Z., Detection of thrombin using electrogenerated chemiluminescence based on Ru(bpy)(3)(2+)-doped silica nanoparticle aptasensor via target protein-induced strand displacement. *Anal. Chim. Acta* 2007, 598, (2), 242-248.

[92] Li, Y.; Qi, H. L.; Peng, Y.; Yang, J.; Zhang, C. X., Electrogenerated chemiluminescence aptamer-based biosensor for the determination of cocaine. *Electrochem. Commun.* 2007, 9, (10), 2571-2575.

[93] Wang, X. Y.; Yun, W.; Zhou, J. M.; Dong, P.; He, P. G.; Fang, Y. Z., Ru(bpy)(3)(2+)-doped silica nanoparticle aptasensor for detection of thrombin based on electrogenerated chemiluminescence. *Chin. J. Chem.* 2008, 26, (2), 315-320.

[94] Bai, J. G.; Wei, H.; Li, B. L.; Song, L. H.; Fang, L. Y.; Lv, Z. Z.; Zhou, W. H.; Wang, E. K., Ru(bpy)2(dcbpy)NHS Labeling/Aptamer-Based Biosensor for the Detection of Lysozyme by Increasing Sensitivity with Gold Nanoparticle Amplification. *Chem.-Asian J.* in press.

[95] Shi, L. H.; Liu, X. J.; Li, H. J.; Xu, G. B., Electrochemiluminescent detection based on solid-phase extraction at tris(2,2 '-bipyridyl)ruthenium(II)-modified ceramic carbon electrode. *Anal. Chem.* 2006, 78, (20), 7330-7334.

[96] Xu, Z. A.; Guo, Z. H.; Dong, S. J., Electrogenerated chemiluminescence biosensor with alcohol dehydrogenase and tris(2,2 '-bipyridyl)ruthenium (II) immobilized in sol-gel hybrid material. *Biosens. Bioelectron.* 2005, 21, (3), 455-461.

[97] Egashira, N.; Kumasako, H.; Uda, T.; Ohga, K., Fabrication of a trimethylamine gas sensor based on electrochemiluminescence of Ru(bpy)(2+)(3)/Nafion gel and its application to a freshness sensor for seafood. *Electroanalysis* 2002, 14, (12), 871-873.

[98] Liang, P. Y.; Chang, P. W.; Wang, C. M., Can clay emit light? Ru(bpy)(3)(2+)-modified clay colloids and their application in the detection of glucose. *J. Electroanal. Chem.* 2003, 560, (2), 151-159.

[99] Jameison, F.; Sanchez, R. I.; Dong, L. W.; Leland, J. K.; Yost, D.; Martin, M. T., Electrochemiluminescence-based quantitation of classical clinical chemistry analytes. *Anal. Chem.* 1996, 68, (8), 1298-1302.

[100] Wei, H.; Du, Y.; Kang, J. Z.; Wang, E. K., Label free electrochemiluminescence protocol for sensitive DNA detection with a tris(2,2'-bipyridyl)ruthenium(II) modified electrode based on nucleic acid oxidation. *Electrochem. Commun.* 2007, 9, (7), 1474-1479.

[101] Rusling, J. F.; Hvastkovs, E. G.; Hull, D. O.; Schenkman, J. B., Biochemical applications of ultrathin films of enzymes, polyions and DNA. *Chem. Commun.* 2008, (2), 141-154.

[102] Rusling, J. F.; Hvastkovs, E. G.; Schenkman, J. B., Toxicity screening using biosensors that measure DNA damage. *Current Opinion in Drug Discovery & Development* 2007, 10, (1), 67-73.

[103] Krishnan, S.; Hvastkovs, E. G.; Bajrami, B.; Jansson, I.; Schenkman, J. B.; Rusling, J. F., Genotoxicity screening for N-nitroso compounds. Electrochemical and electrochemiluminescent detection of human enzyme-generated DNA damage from N-nitrosopyrrolidine. *Chem. Commun.* 2007, (17), 1713-1715.

[104] Hvastkovs, E. G.; So, M.; Krishnan, S.; Bajrami, B.; Tarun, M.; Jansson, I.; Schenkman, J. B.; Rusling, J. F., Electrochemiluminescent arrays for cytochrome P450-activated genotoxicity screening. DNA damage from benzo[a]pyrene metabolites. *Anal. Chem.* 2007, 79, (5), 1897-1906.

[105] Kenten, J. H.; Gudibande, S.; Link, J.; Willey, J. J.; Curfman, B.; Major, E. O.; Massey, R. J., Improved Electrochemiluminescent Label for DNA Probe Assays - Rapid

Quantitative Assays of Hiv-1 Polymerase Chain-Reaction Products. *Clin. Chem.* 1992, 38, (6), 873-879.

[106] Boom, R.; Sol, C.; Gerrits, Y.; De Boer, M.; Wertheim-van Dillen, P., A highly sensitive assay for detection and quantitation of human cytomegalovirus DNA in serum and plasma by PCR and electrochemiluminescence. *J. Clin. Microbiol.* 1999, 37, (5), 1489-1497.

[107] Zhu, D. B.; Xing, D.; Shen, X. Y.; Yan, G. H., High sensitive detection of presenilin-1 point mutation based on electrochemiluminescence. *Chin. Sci. Bull.* 2003, 48, (16), 1741-1744.

[108] Zhu, D. B.; Xing, D.; Shen, X. Y.; Liu, J. F., A method to quantitatively detect H-ras point mutation based on electrochemiluminescence. *Biochem. Biophys. Res. Commun.* 2004, 324, (2), 964-969.

[109] Liu, J. F.; Xing, D.; Shen, X. Y.; Zhu, D. B., Detection of genetically modified organisms by electrochemiluminescence PCR method. *Biosens. Bioelectron.* 2004, 20, (3), 436-441.

[110] Liu, J. F.; Xing, D.; Shen, X. Y.; Zhu, D. B., Electrochemiluminescence polymerase chain reaction detection of genetically modified organisms. *Anal. Chim. Acta* 2005, 537, (1-2), 119-123.

[111] Zhou, X. M.; Xing, D.; Zhu, D. B.; Jia, L., Magnetic beads-based electrochemiluminescence assay for rapid and sensitive detection of telornerase activity. *Electrochem. Commun.* 2008, 10, (4), 564-567.

[112] Zhu, D. B.; Tang, Y. B.; Xing, D.; Chen, W. R., PCR-free quantitative detection of genetically modified organism from raw materials. An electrochemiluminescence-based bio bar code method. *Anal. Chem.* 2008, 80, (10), 3566-3571.

[113] Lo, W. Y.; Baeumner, A. J., Evaluation of internal standards in a competitive nucleic acid sequence-based amplification assay. *Anal. Chem.* 2007, 79, (4), 1386-1392.

[114] Lo, W. Y.; Baeumner, A. J., RNA internal standard synthesis by nucleic acid sequence-based amplification for competitive quantitative amplification reactions. *Anal. Chem.* 2007, 79, (4), 1548-1554.

[115] Fang, L. Y.; Lv, Z. Z.; Wei, H.; Wang, E. K., Novel electrochemiluminescence aptasensor for sensitive and cost-effective detection of protein. *Anal. Chim. Acta* revised.

[116] Yin, X. B.; Qi, B.; Sun, X. P.; Yang, X. R.; Wang, E. K., 4-(Dimethylamino)butyric acid labeling for electrochemiluminescence detection of biological substances by increasing sensitivity with gold nanoparticle amplification. *Anal. Chem.* 2005, 77, (11), 3525-3530.

[117] Guo, W. W.; Yuan, J. P.; Li, B. L.; Du, Y.; Yin, E. B.; Wang, E. K., Nanoscale-enhanced Ru(bpy)32+ electrochemiluminescence labels and related aptamer-based biosensing system. *Analyst* 2008, 133, (9), 1209-1213.

[118] Xu, Y. H.; Gao, Y.; Li, T.; Du, Y.; Li, J.; Wang, E. K., Highly efficient electro-chemiluminescence of functionalized tris(2,2 '-bipyridyl)ruthenium(II) enrichment of its co-reactants. *Adv. Funct. Mater.* 2007, 17, (6), 1003-1009.

[119] Yu, J. G.; Fan, F. R. F.; Pan, S. L.; Lynch, V. M.; Omer, K. M.; Bard, A. J., Spontaneous formation and electrogenerated chemiluminescence of tris(bipyridine) Ru(II) derivative nanobelts. *J. Am. Chem. Soc.* 2008, 130, (23), 7196-+.

[120] Du, Y.; Wei, H.; Kang, J. Z.; Yan, J. L.; Yin, X. B.; Yang, X. R.; Wang, E. K., Microchip capillary electrophoresis with solid-state electrochemiluminescence detector. *Anal. Chem.* 2005, 77, (24), 7993-7997.

[121] Du, Y.; Wang, E., Separation and detection of narcotic drugs on a microchip using micellar electrokinetic chromatography and electrochemiluminescence. *Electroanalysis* 2008, 20, (6), 643-647.

[122] Wei, H.; Yin, J. Y.; Wang, E. K., Bis(2,2'-bipyridine)(5,6-epoxy-5,6-dihydro-[1,10] phenanthroline)ruthenium: Synthesis and Electrochemical and Electrochemiluminescence Characterization *Anal. Chem.* 2008, 80, (14), 5635-5639.

[123] Dennany, L.; Forster, R. J.; White, B.; Smyth, M.; Rusling, J. F., Direct electrochemiluminescence detection of oxidized DNA in ultrathin films containing [Os(bpy)(2)(PVP)(10)](2+). *J. Am. Chem. Soc.* 2004, 126, (28), 8835-8841.

[124] Muegge, B. D.; Richter, M. M., Multicolored electrogenerated chemiluminescence from ortho-metalated iridium(III) systems. *Anal. Chem.* 2004, 76, (1), 73-77.