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$[Ru(bpy)_3]^{2+}$-Doped Silica Nanoparticles within Layer-by-Layer Biomolecular Coatings and Their Application as a Biocompatible Electrochemiluminescent Tag Material

Hui Wei, Jifeng Liu, Lingling Zhou, Jing Li, Xiue Jiang, Jianzhen Kang, Xiurong Yang, Shaojun Dong, and Erkang Wang*[a]

Abstract: $[Ru(bpy)_3]^{2+}$-doped silica (RuSi) nanoparticles were synthesized by using a water/oil microemulsion method. Stable electrochemiluminescence (ECL) was obtained when the RuSi nanoparticles were immobilized on a glassy carbon electrode by using tripropylamine (TPA) as a coreactant. Furthermore, the ECL of the RuSi nanoparticles with layer-by-layer biomolecular coatings was investigated. Squential self-assembly of the polyelectrolytes and biomolecules on the RuSi nanoparticles gave nanocomposite suspensions, the ECL of which decreased on increasing the number of bilayers. Moreover, factors that affected the assembly and ECL signals were investigated. The decrease in ECL could be assigned to steric hindrance and limited diffusion of the coreactant molecules in the silica matrix after they were attached to the biomolecules. Since surface modification of the RuSi nanoparticles can improve their biocompatibility and prevent leaking of the $[Ru(bpy)_3]^{2+}$ ions, the RuSi nanoparticles can be readily used as efficient and stable ECL tag materials in immunoassay and DNA detection.

Keywords: biomolecules · bipyridine · electrochemiluminescence · nanoparticles · ruthenium · silica

Introduction

Electrochemiluminescence (ECL; also called electrogenerated chemiluminescence) has received considerable attention from many researchers over recent decades as a result of its great scientific and technological importance to, for example, clinical tests and biomolecule detection.[1–2] ECL is a means of converting electrochemical energy into radiative energy at the surface of an electrode through an applied potential. Luminescent signals could be obtained from the excited states of an ECL luminophore generated at the electrode surfaces during the electrochemical reaction. Among many organic and inorganic ECL systems, ECL-active inorganic compounds containing Ru, Os, Ir, Cu, Ag, Au, Eu, Al, Cd, Cr, Pt, and Pd have been extensively studied.[1] However, ECL based on ruthenium(II) tris(2,2′-bipyridine) ($[Ru(bpy)_3]^{2+}$) has proven to be the most valuable since its discovery[3] owing to its strong luminescence and good solubility in a variety of aqueous and nonaqueous solvents and its inherent sensitivity, selectivity, and wide linear range in different analytical areas.[1–2,4–6]

ECL of $[Ru(bpy)_3]^{2+}$ ions has been used to develop sensors for a variety of analytes that range from metal ions and small molecules to DNA, peptides, and proteins.[7–9] For example, the determination of sodium ions in aqueous buffered solution using $[Ru(bpy)_3]^{2+}$ ions containing a crown ether moiety covalently bonded to a bpy ligand has been demonstrated.[7a] For small-molecule assays, the presence of an amine group on the analyte molecules, such as alkylamines, antibiotics, antihistamines, opiates, and nicotinamide, is usually required.[8] To realize the detection of biologically interesting molecules, such as DNA, peptides, and proteins, $[Ru(bpy)_3]^{2+}$ derivatives conjugated with suitable groups, such as *N*-hydroxysuccinimide (NHS) ester and phosphoramidite conjugates, are needed as ECL tags that bind the analytes,[9] although direct ECL involving DNA has also been

[a] H. Wei, Dr. J. Liu, L. Zhou, J. Li, Dr. X. Jiang, Dr. J. Kang, Prof. X. Yang, Prof. S. Dong, Prof. E. Wang
State Key Laboratory of Electroanalytical Chemistry
Changchun Institute of Applied Chemistry
and
Graduate School of the Chinese Academy of Sciences
Chinese Academy of Sciences
Changchun, Jilin, 130022 (China)
E-mail: ekwang@ciac.jl.cn

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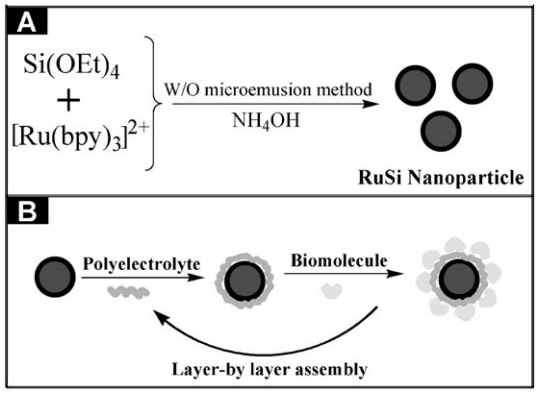
demonstrated using guanine residues as the coreactant.[10] As shown in the previous reports,[9] these ECL tags possess some limitations: directly labelling the biomolecules with the ECL tags may result in the loss of biological activity of the target analytes. Moreover, the synthesis of these ECL tags is complex, time-consuming, and labour intensive, and their ECL efficiency is low compared with $[Ru(bpy)_3]^{2+}$ ions. Therefore, the development of efficient ECL tag materials that can provide biological compatibility and enhance the sensitivity of the biological detection beyond that of the NHS ester and phosphoramidite-linked $[Ru(bpy)_3]^{2+}$ conjugate is important and promising. Recently, much effort has been devoted to developing new ECL tag materials through bionanotechnology. Zhan and Bard reported a novel approach to ECL labeling in which $[Ru(bpy)_3]^{2+}$-encapsulated liposomes and their application in a sandwich-type immunoassay of human C reactive protein.[11]

Silica-based materials, especially silica nanoparticles, offer promise for application in many research areas, such as bionanomaterials and biomolecule detection, because of their easy surface modification, chemical stability, and biocompatibility.[12–14] Doped silica nanoparticles as tag materials have shown great promise in ultrasensitive bioassays.[15] Silica nanoparticles doped with quantum dots have been intensively studied as functional materials and used for protein detection and cell imaging.[16] Silica nanoparticles doped with dyes have also been prepared and investigated for fluorescence imaging and biosensors. Rosenzweig and co-workers synthesized monodispersed $[Ru(phen)_3]^{2+}$-doped silica nanoparticles (phen = phenanthroline) based on the method developed by Stöber.[17a] Tan and co-workers prepared uniform $[Ru(bpy)_3]^{2+}$-doped silica (RuSi) nanoparticles for biomolecular conjugation by using a water-in-oil (W/O) microemulsion method.[18] The same group extended this approach to dual luminophore-doped silica nanoparticles for multiplexed signalling in bioanalysis.[19] To the best of our knowledge, however, there are few reports on the use of RuSi nanoparticles as ECL tag materials. We recently fabricated biosensors based on RuSi nanoparticles.[12c,20] Fang et al. used RuSi nanoparticles as ECL tags to detect DNA hybridization.[21] However, RuSi nanoparticles without further surface modification suffered from leaking of the $[Ru(bpy)_3]^{2+}$ ions. Thus, it is interesting and important to develop stable ECL tags based on RuSi nanoparticles.

Layer-by-layer (LBL) assembly[22] provides an effective and versatile approach to the construction of advanced, ordered, and well-defined nanostructured materials at the molecular level as a result of its simplicity, versatility, and robustness.[23–24] We successfully prepared a surface-enhanced Raman scattering active substrate on indium tin oxide by using the LBL assembly of silver nanoparticles and poly(sodium 4-styrenesulfonate).[25] Besides 2D flat substrates, LBL films can actually be fabricated on substrates of any shape, including 3D colloids. Lakowicz and co-workers reported that the fluorescence emission of $[Ru(bpy)_3]^{2+}$ ions entrapped in the RuSi nanoparticles could be enhanced when the RuSi nanoparticles were coated with silver in the LBL method to generate porous but continuous metal nanoshells.[26]

The potential use of RuSi nanoparticles in ECL analysis and detection and as ECL tag materials is significant: First, these nanoparticles have large surface areas and high surface free energies. Second, a single biological recognition event can be amplified using tens of thousands of $[Ru(bpy)_3]^{2+}$ dye molecules entrapped in the nanoparticles; therefore, this method is more sensitive than conventional ECL detection schemes. Third, if the surfaces of the RuSi nanoparticles are further modified with biopolymers by using the LBL technique, we assume that the biocompatibility of the particles could be improved and leaking of the $[Ru(bpy)_3]^{2+}$ ions might be inhibited. Previously, we developed an ECL sensor from RuSi nanoparticles prepared by using the W/O microemulsion method (Scheme 1).[20] The



Scheme 1. a) The preparation of RuSi nanoparticles and b) LBL assembly of the polyelectrolytes and biomolecules on the RuSi nanoparticles.

sensor was fabricated on glassy carbon (GC) electrodes through conjugation with a biopolymer chitosan membrane. The sensor showed high sensitivity and reproducibility toward the detection of tripropylamine (TPA).

Herein, we further extended the study of the interaction between RuSi nanoparticles and some biomacromolecules. The ECL behavior of the RuSi nanoparticles was investigated after deposition with biomolecules through LBL self-assembly (Scheme 1). When the surface of the RuSi nanoparticles was modified with biomolecules, a decrease in ECL with surface modification was observed. Moreover, factors that affect the LBL assembly and ECL signals were investigated (i.e., the concentration of NaCl, the pH value of the polyelectrolyte and biomolecules, and coreactants of different size and charge). The ECL signals of RuSi nanoparticles modified with polyelectrolyte and biomolecules constructed in different sequence was investigated. The inhibition of the leakage of $[Ru(bpy)_3]^{2+}$ ions was also studied. Since the surface modification could improve the biocompatibility of the nanocomposites and prevent leaking of the $[Ru(bpy)_3]^{2+}$ ions, the RuSi nanoparticles within LBL biomolecular coatings could be readily used as stable and efficient ECL tag materials.

Results and Discussion

Preparation and characterization of the RuSi nanoparticles: Spherical RuSi nanoparticles were prepared by using a W/O microemulsion approach.[19] The diameter of the as-prepared RuSi nanoparticles was 60 nm, as characterized by transmission electron microscopy (TEM) (Figure 1). The ECL stability of the RuSi nanoparticles fabricated on a GC electrode in the presence of 10 mM TPA was studied.[27] Figure 2 shows the ECL intensity/time curve of the GC electrode modified with RuSi nanoparticles in 150 mM phosphate buffer solution (pH 7.5) containing 10 mM TPA under continuous cyclic voltammetry (CV) for nine cycles. The relative standard deviation of the peak height was 2.64%, which reflected the good stability of the GC electrode modified with RuSi nanoparticles.

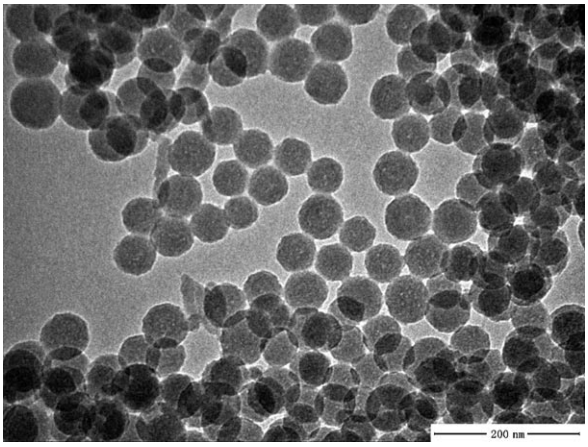


Figure 1. TEM image of the as-prepared RuSi nanoparticles.

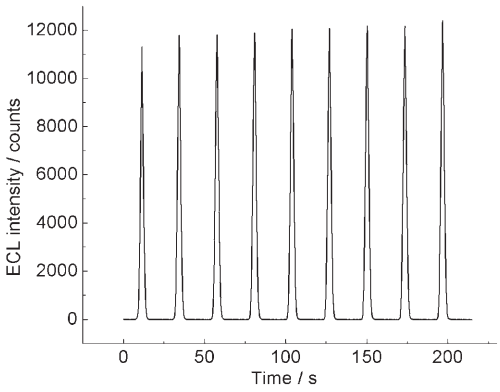


Figure 2. ECL emission of the GC electrode modified with RuSi nanoparticles in 10 mM TPA. Potential range: 0–1.3 V, scan rate: 100 mV s^{-1}.

ECL of the RuSi nanoparticles coated with biomolecules by LBL assembly: The LBL self-assembly of the biomolecular multilayers on colloidal particles has been shown to be a promising approach to the fabrication of functionalized colloids for biocatalysis, bionanoreactors, and decomposable hollow biocapsules.[28] The polyelectrolytes (i.e., poly(dimethyldiallylammonium chloride) (PDDA), poly(ethyleneimine) (PEI), and poly(styrenesulfonate) (PSS)) and biomolecules (i.e., bovine serum albumin (BSA), lysozyme, and calf thymus DNA (ctDNA)) were deposited onto the as-prepared 60-nm RuSi nanoparticles by electrostatic LBL self-assembly. Since the RuSi nanoparticles are negatively charged, the positively charged polyelectrolyte PDDA (or PEI) and negatively charged biomolecules BSA (or ctDNA) could be assembled on the surfaces of the RuSi nanoparticles by using electrostatic LBL assembly as [RuSi/(PDDA/BSA)$_n$], [RuSi/(PEI/BSA)$_n$], and [RuSi/(PEI/ctDNA)$_n$] (Scheme 1). As for the positively charged lysozyme, the positively charged PEI was first absorbed on the RuSi nanoparticles as precoating, then the negatively charged polyelectrolyte PSS and lysozyme were assembled by LBL as [PEI/(PSS/lysozyme)$_n$].

As shown in Figure 3, the sequential self-assembly of the polyelectrolytes and biomolecules on the RuSi nanoparticles could cause a decrease in ECL. The more polyelectrolytes and biomolecules deposited on the RuSi nanoparticles, the more obvious the decrease in ECL was observed to be. The decrease in ECL varied with different polyelectrolytes and biomolecules, and such a decrease could be attributed to the change in steric hindrance, limited diffusion of the coreactant, and electrostatic interaction induced by the surface modifications.

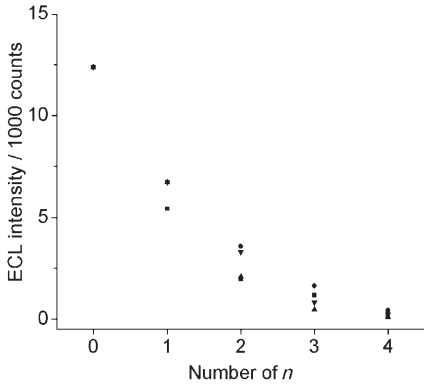


Figure 3. ECL intensity of RuSi nanoparticles coated with polyelectrolyte and biomolecule layers. (▽) [RuSi/(PDDA/BSA)$_n$], (●) [RuSi/(PEI/BSA)$_n$], (△) [RuSi/(PEI/ctDNA)$_n$], and (■) [RuSi/PEI/(PSS/lysozyme)$_n$].

Factors responsible for the decrease in the ECL of the RuSi nanoparticles coated with biomolecules through the LBL process: ECL coreactants of different size and charge were studied to investigate the diffusion behavior affected by biomolecules deposited on the RuSi nanoparticles. As previously reported, the size and charge of the coreactants were key factors that affected the diffusion behavior.[29] Figure 4 shows the ECL response of different coreactants (i.e., TPA,

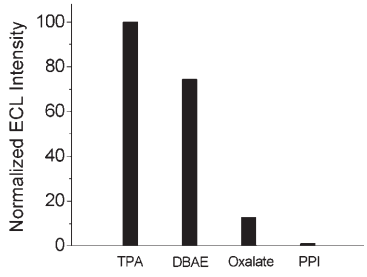


Figure 4. Dependency of the ECL intensity of [RuSi/(PDDA/BSA)$_1$] on the coreactant in 150 mM PBS (the concentration of all the coreactants was 10 mM).

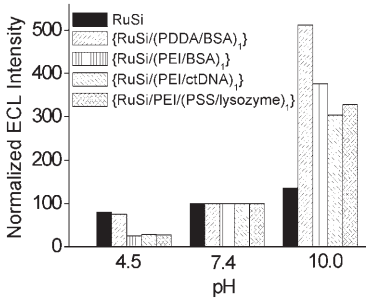


Figure 5. Dependency of the ECL intensity of the RuSi nanoparticles coated with different biomolecules on the pH value in the presence of 10 mM TPA.

2-(dibutylamino)ethanol (DBAE), poly(propylenimine)tetraamine dendrimer (PPI), and oxalate). As the coreactant became larger, the decrease in ECL became more obvious in the following trend: PPI $\gg$ DBAE $>$ TPA. Note that PPI was a dendrimer that was much larger than DBAE and TPA (see the Supporting Information for the molecular structures). This result suggests that the biomolecules could inhibit the diffusion of the coreactants to the internal sites of the RuSi nanoparticles, thus causing the decrease in ECL. As for the oxalate species, which has the opposite charge to TPA, a larger decrease in ECL was observed compared with TPA. This finding might be attributed to the electrostatic repulsion between the coreactant oxalate and BSA at the outermost assembled layer.

Since the ECL efficiencies were different for different coreactants, to further confirm the above conclusion that the size and charge of the coreactants were key factors that affected the diffusion behavior, the ECL efficiencies of TPA, DBAE, PPI, and oxalate were investigated in a solution of $[Ru(bpy)_3]^{2+}$ in 150 mL phosphate buffer solution (pH 7.5) (see the Supporting Information). Thus, in this solution, DBAE and PPI exhibited higher ECL efficiencies than TPA. Although, the oxalate species exhibited a lower ECL efficiency than TPA (oxalate gave 11.6% of the ECL signal of TPA), the ECL signal of [RuSi/(PDDA/BSA)$_1$] in the presence of the oxalate species was much lower than that in the presence of TPA (oxalate gave 0.96% of the ECL signal of TPA). These data further verified that the size and charge of the coreactants were key factors that affected the diffusion behavior (see Figure 4 and the Supporting Information).

Previous studies indicated that the pH value of the polyelectrolytes and biomolecules and the concentration of the supporting salt could modulate the pore size and film density of the multilayer film.[30] Herein, the effect of the pH value of the polyelectrolytes and biomolecules and the concentration of the supporting salt on the TPA transport and ECL intensity were investigated. After immersion in buffer solutions of different pH values, both the RuSi nanoparticles coated with and without biomolecules inhibited the increase in ECL according to the trends: pH 10.0 $>$ 7.4 $>$ 4.5 (Figure 5). However, at high pH value, a greater effect on the RuSi nanoparticles coated with biomolecules than on the uncoated RuSi nanoparticles was observed. Considering the fact the $[Ru(bpy)_3]^{2+}$/TPA system gave stronger ECL signals at high pH values,[31] the results indicated that the RuSi nanoparticles coated with biomolecules had more open structures at high pH values than at neutral or low pH values. Therefore, TPA could be transported more easily into the RuSi nanoparticles after treatment at high pH values and higher ECL signals were obtained.

The effect of the concentration of the supporting salt (NaCl herein) on the ECL signals is shown in Figure 6. When the LBL self-assembly was realized in the presence of

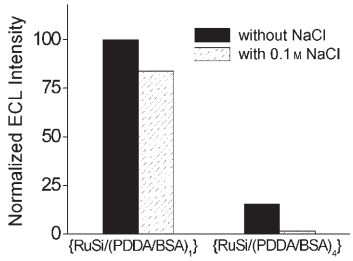


Figure 6. Dependency of the ECL intensity of [RuSi/(PDDA/BSA)$_1$] and [RuSi/(PDDA/BSA)$_4$] on the salt in the presence of 10 mM TPA.

0.1 M NaCl, more obvious decreases in the ECL were observed compared with the LBL self-assembly in the absence of NaCl for both [RuSi/(PDDA/BSA)$_1$] and [RuSi/(PDDA/BSA)$_4$]. This result hinted that the LBL multilayers have more a compact structure when assembled in the presence of NaCl, which is in accordance with previous results.[30c,32] The compact LBL multilayers should have greater obstacles for the ECL coreactant TPA, thus causing a more significant decrease in ECL.

Further control experiments were conducted to investigate the influence of the sequence of the LBL multilayers on the ECL signals. When the LBL multilayers were constructed in the manner of [RuSi/PDDA/(BSA/PDDA)$_n$], the same tendency toward a decrease in ECL was obtained compared with the multilayers constructed in the manner of [RuSi/(PDDA/BSA)$_n$] (Figure 7), though the former had a greater decrease in ECL. The greater decrease in ECL of

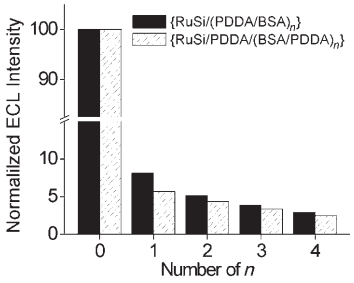


Figure 7. Dependency of the ECL intensity of the RuSi nanoparticles on the structure of the LBL multilayers in the presence of 10 mM TPA.

[RuSi/PDDA/(BSA/PDDA)$_n$] than [RuSi/(PDDA/BSA)$_n$] could be assigned to the thicker multilayers of [RuSi/PDDA/(BSA/PDDA)$_n$] and electrostatic repulsion between coreactant TPA and the outermost assembled PDDA layer of [RuSi/PDDA/(BSA/PDDA)$_n$].

Based on the results mentioned above, we suggest that steric hindrance and limited diffusion of the coreactant play dominant and cooperative roles in the decrease in the ECL of RuSi nanoparticles after LBL coating with biomolecules. In addition, electrostatic interaction might also cause such a decrease in ECL.

Possible use of the RuSi nanoparticles within LBL coatings as ECL tag materials: To develop efficient, biocompatible, and stable ECL tag materials that can be used to enhance the sensitivity of biological detection beyond that of the NHS ester and phosphoramidite-linked $[Ru(bpy)_3]^{2+}$ conjugate is important and still challenging.[1e,11,21] When the RuSi nanoparticles within LBL coatings presented herein are used as ECL tags, possible leaking of $[Ru(bpy)_3]^{2+}$ ions must be considered. The ECL behavior of the $[Ru(bpy)_3]^{2+}$ ions leaked from the particles during the study of the ECL of the RuSi nanoparticles deposited with biomolecules was inspected (see the Experimental Section). Figure 8 shows the leaking of the $[Ru(bpy)_3]^{2+}$ ions from the RuSi nanoparticles coated with and without biomolecules. For both the RuSi nanoparticles coated with and without biomolecules, leaking of $[Ru(bpy)_3]^{2+}$ ions was observed. However, functionalized nanocomposites had a smaller amount of leaked $[Ru(bpy)_3]^{2+}$ ions than bare silica nanoparticles (Figure 8).

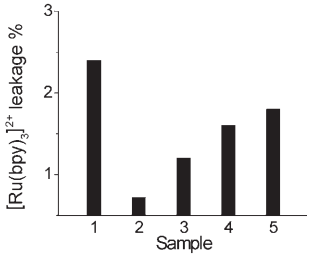


Figure 8. Leakage of $[Ru(bpy)_3]^{2+}$ ions from the uncoated RuSi nanoparticles (sample 1), [RuSi/(PDDA/BSA)$_1$] (sample 2), [RuSi/(PEI/BSA)$_1$] (sample 3), [RuSi/(PEI/ctDNA)$_1$] (sample 4), and [RuSi/PEI/(PSS/lysozyme)$_1$] (sample 5).

These results indicated that the surface functionalization of the RuSi nanoparticles with biomolecules and polyelectrolytes could prevent leaking of the $[Ru(bpy)_3]^{2+}$ ions and improve the stability of the nanocomposites in aqueous solution.

Therefore, the RuSi nanoparticles presented herein could be used as ECL tags for the following reasons: First, a single biological recognition event could be amplified using tens of thousands of $[Ru(bpy)_3]^{2+}$ dye molecules contained in the silica particles; therefore, this method is more method than conventional ECL detection schemes. Second, surface modification with biomolecules and polyelectrolytes can prevent redox leaking and improve the biocompatibility of the nanocomposites.

Conclusion

$[Ru(bpy)_3]^{2+}$-doped silica nanoparticles were synthesized by using a W/O microemulsion method. The as-prepared nanoparticles were further functionalized with biomolecules assembled by LBL. A decrease in ECL with the surface modification of the RuSi nanoparticles was observed. Factors that affected the ECL signals were investigated. It is supposed that steric hindrance and limited diffusion of the coreactant play dominant and cooperative roles in the decrease in the ECL of the RuSi nanoparticles with biomolecular coating. Since the surface modification could improve the biocompatibility of the nanocomposites and prevent the leaking of the $[Ru(bpy)_3]^{2+}$ ions, the RuSi nanoparticles within the LBL biomolecular coatings could be readily used as stable and efficient ECL tag materials.

Experimental Section

Chemicals and materials: Tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate $[Ru(bpy)_3Cl_2]\cdot 6H_2O$, poly(dimethyldiallylammonium chloride) (PDDA; $M_w = 400\,000 \sim 500\,000$], poly(ethyleneimine) (PEI; $M_w = 55\,000$], 2-(dibutylamino)ethanol (DBAE), poly(styrenesulfonate) (PSS; $M_w = 70\,000$), polystyrene (PS; $M_w = 280\,000$), tetraethyloxysilane (TEOS), and poly(propylenimine)tetraamine dendrimer (PPI) were obtained from Aldrich (WI, USA). Bovine serum albumin (BSA) was purchased from Randox (Antrim, UK). Calf thymus DNA (ctDNA) and lysozyme were obtained from Sigma (WI, USA). Tripropylamine (TPA) was obtained from Acros (NJ, USA). Other reagents and chemicals were at least of analytical-reagent grade. The water used throughout all experiments was purified by a Milli-Q system (Millipore, MA, USA).

Synthesis of $[Ru(bpy)_3]^{2+}$-doped silica nanoparticles (RuSi): The RuSi nanoparticles were prepared according to a previous method with a minor change.[19,20] Triton X-100 (5.31 mL), cyclohexane (22.5 mL), *n*-hexanol (5.4 mL), and water (1.02 L) were mixed together to form a water/oil microemulsion. A concentrated solution of $[Ru(bpy)_3]^{2+}$ in water was then added into the microemulsion system to a final concentration of 1.2 mM. After addition of TEOS (300 μL) and NH_4OH (180 μL), the hydrolysis reaction was allowed to continue for 24 h. Acetone was then added to destroy the emulsion and isolate the deep orange nanoparticles, which were centrifuged, washed with ethanol and water, and treated by ultrasonication to remove any surfactant molecules. Finally, the orange RuSi nanoparticles were obtained and stored in a refrigerator (4 °C) until use.

Preparation of the modified electrode: The immobilization of the RuSi nanoparticles on electrodes was similar to a previously reported procedure.[27] A suspension of RuSi nanoparticles (2 mg mL^{-1}) in THF (10 μL) containing PS (1 mg mL^{-1}) was spread on a GC electrode (diameter: 3 mm), and the solvent was allowed to evaporate.

Deposition of proteins and ctDNA on RuSi nanoparticles by the LBL process: For the LBL deposition, RuSi nanoparticles (2 mg) were added to a 1.5-mL centrifuge tube followed by the addition an aqueous solution of polyelectrolyte (1 mL, 2 mg mL^{-1}) or aqueous solutions (1 mL) of BSA (2 mg mL^{-1}), lysozyme (2 mg mL^{-1}), and ctDNA (0.4 mg mL^{-1}) to give shell architectures of the following sequence (classified as four groups of samples): [RuSi/(PDDA/BSA)$_n$], [RuSi/(PEI/BSA)$_n$], [RuSi/(PEI/ctDNA)$_n$], and [RuSi/PEI/(PSS/lysozyme)$_n$]. As for [RuSi/PEI/(PSS/lysozyme)$_n$], PEI was used as the precursor layer. After adding each species, 15 min was allowed for the components on the RuSi nanoparticles to reach saturated adsorption. The deposited RuSi nanoparticles were then centrifuged at 9800 *g* for 10 min, and the supernatant containing the starting material was removed. The particles were washed with water, and the centrifugation/washing cycle was repeated twice to avoid admixing of the sequentially deposited components. To determine the amount of adsorbed proteins or ctDNA, UV/Vis spectra of the supernatant were compared with those of the standard solutions of the biomolecule prior to exposure to the RuSi nanoparticles.

ECL detection system: The electrochemical measurements were performed with a CHI 832 workstation (CH Instruments, Austin, TX, USA) using a three-electrode system. A KCl-saturated Ag/AgCl electrode and a platinum-wire electrode were used as the reference and auxiliary electrodes, respectively. To inspect the ECL response of the RuSi nanoparticles on the electrode, a GC electrode coated with the RuSi nanoparticles was used as the working electrode. To investigate the ECL behavior of the RuSi nanoparticles coated with biomolecules, an unmodified GC electrode was used as the working electrode.

To investigate the ECL responses of the RuSi nanoparticles coated with biomolecules, coated RuSi nanoparticles (50 μL, 2 mg mL^{-1}; dispersed in 150 mM PBS, pH 7.4) were mixed with 10 mM TPA solution (750 μL). The mixed solutions were used as an ECL test solution to record the signals in a homemade ECL cell using above-mentioned three-electrode system (referred to as ECL_{RuSi}).

The ECL signals of the $[Ru(bpy)_3]^{2+}$ ions leaked from the RuSi nanoparticles were recorded as follows (referred to as ECL_{leak}): After centrifugation, the supernatant (50 μL) of the RuSi nanoparticles coated with biomolecules put through the LBL process was mixed with 10 mM TPA/PBS solution (750 μL). The mixed solutions were used as an ECL test solution to record the signals. The percentage ratio of ECL_{leak}/ECL_{RuSi} was used to analyse the leaking of the $[Ru(bpy)_3]^{2+}$ ions.

The ECL emissions were recorded with a Model BPCL Ultra-weak Luminescent Analyzer (Institute of Biophysics, Chinese Academy of Sciences, Beijing, China). The intensities of the ECL peaks were used for quantitative analysis.

Instrumentation: The absorption spectra were recorded on a Cary 500 Scan UV/Vis/NIR Spectrophotometer (Varian, Harbor City, CA, USA) at room temperature. TEM measurements were made on a Hitachi H-8100 transmission electron microscope operated at an accelerating voltage of 200 kV. The samples for TEM characterization were prepared by placing a drop of the colloidal solution on a carbon-coated copper grid and dried at room temperature.

Acknowledgement

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