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A Tris(bipyridine)ruthenium(II)–β-Cyclodextrin Derivative: Synthesis, Luminescent Properties, and Application in Electrochemiluminescence DNA Sensors

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In recent years, supramolecular systems based on metallocyclodextrins have increasingly attracted the interest of researchers from many fields.[1] The elegant guest-binding properties of cyclodextrins (CDs) and the electronic and photoactive characteristics of metal centers, such as ruthenium, in a single molecule make metallocyclodextrins appropriate for the development of sophisticated supramolecular and molecular sensing devices.[2] The majority of previously presented metallocyclodextrins contain only a single metal center,[3] and this configuration has a limited sensitivity. However, polynuclear metal complexes, and polypyridine–ruthenium complexes in particular, have been reported and successfully applied in the construction of photovoltaic molecular devices[4] and electrochemiluminescence (ECL) sensors[5] owing to their high photovoltaic efficiency and unique multielectron redox processes stemming from multiple chromophores of a single molecule. Taking these properties into consideration, polynuclear metallocyclodextrin materials possessing molecular-recognition functions and multiple chromophores are worthy of further investigation.

ECL using $[Ru(bpy)_3]^{2+}$ (bpy = bipyridine) is a popular and powerful analytical technique because of its high sensitivity, low background, simple instrumentation, and rapid sample analysis. It has been widely used in the clinical detection of immunoassays[6] and tumor markers.[7a] To improve the analytical sensitivity and extend its application, new luminophores with higher ECL efficiencies and techniques for the labeling of biomolecules while maintaining their specific biological activity[7] are desired.

Herein, we propose a new polynuclear metallocyclodextrin complex, a tris(bipyridine)ruthenium(II)–β-cyclodextrin derivative, which we abbreviate as tris(bpyRu)–β-CD (Scheme 1). The use of multiple ruthenium centers in a single supermolecule enables a higher level of luminescence relative to that produced using a single ruthenium center. This versatile complex, presenting excellent electronic and photoactive properties owing to its assured luminescent structure and molecular-recognition function of β-CD as the host model, is a highly useful signal supermolecule for the construction of supramolecular devices and biosensors.

The tris(bpyRu)–β-CD film on an electrode surface, a type of controllable solid-state film, is used as an ECL signal-generating unit and recognition element to further increase the luminescence intensity relative to the solution-state ECL. The characteristics of the tris(bpyRu)–β-CD film were further demonstrated through the application of an ECL DNA sensor. The determination of specific target DNAs (t-DNAs) through homogeneous hybridization offers several key advantages: 1) the attachment of ECL labels on DNA by the host–guest recognition of tris(bpyRu)–β-CD, thereby avoiding the use of expensive biolabels; 2) homogeneous hybridization without DNA immobilization, which improves the efficiency of t-DNA hybridization to probe DNA; and 3) an amplification of the ECL signals by the triruthenium center of the complex.

The synthesis of this polynuclear metallocyclodextrin complex was initiated using β-CD and a key β-CD derivative (Scheme 1). Trisubstitution of phenylamino β-CD (ligand **a**) was obtained first followed by the synthesis of the final product. Their respective structures and purities were characterized by ^1H and ^{13}C NMR spectroscopy, high-resolution MALDI-TOF MS, and elemental analysis (details of the preparation and characterization can be found in the Supporting Information).

The photoactive properties of tris(bpyRu)–β-CD were investigated further by UV/Vis and fluorescence spectroscopy as well as ECL analysis (Figure 1). Some spectroscopic data are summarized in Table 1. The UV/Vis spectra of tris(bpyRu)–β-CD and

Table 1. Spectroscopic characteristics of tris(bpyRu)–β-CD and $[Ru(bpy)_3Cl_2]$ in acetonitrile at 25 °C.

	λ_{abs}[a] [nm]	λ_{em}[b] [nm]	I[c]	ε[d] [$\times 10^4$ mol^{-1} cm^{-1}]	Φ[e]
tris(bpyRu)–β-CD	458	615	635	5.69	0.010
$[Ru(bpy)_3Cl_2]$	451	598	235	1.58	0.012

[a] Maximum absorption wavelengths of MLCT, ±2 nm. [b] Maximum fluorescence emission wavelengths excited at 450 nm, ±2 nm. [c] Peak value of the fluorescence intensity at 450 nm, ±2% (the concentration of the complexes was 1.0×10^{-5} mol). [d] Molar extinction coefficients of the MLCT band calculated according to the Lambert–Beer Law, ±3%. [e] Emission quantum yields (±3%), excited at 450 nm, were measured according to the literature using rhodamine B as a standard (the measurements are described in detail in the Supporting Information).[8]

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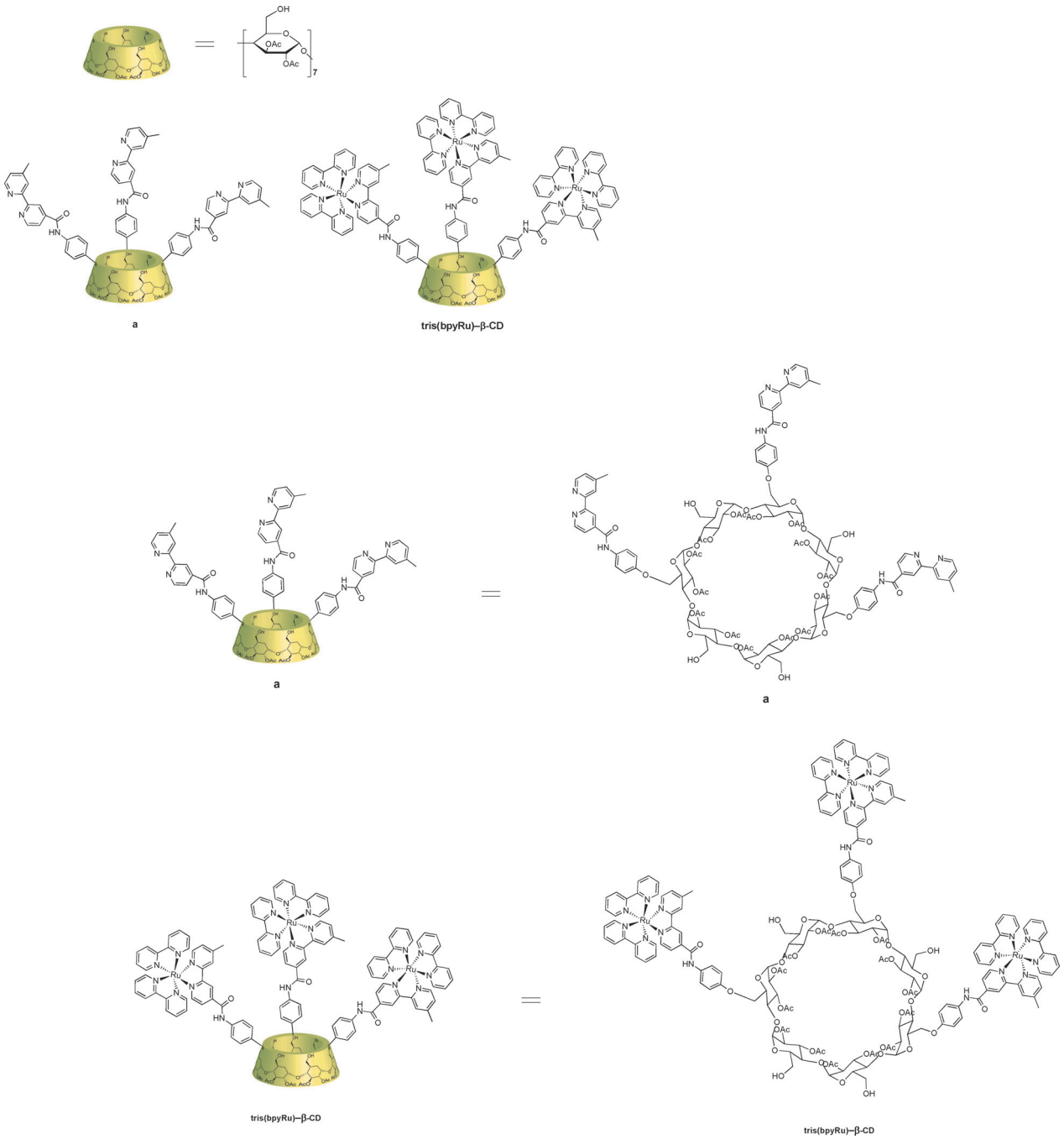


Scheme 1. Structures of tris(bpyRu)–β-CD and its ligand **a**.

$[Ru(bpy)_3Cl_2]$ are compared in Figure 1A. The tris(bpyRu)–β-CD trace (curve a) shows a strong characteristic metal–ligand charge transfer (MLCT) centered at 458 nm and ligand-centered (LC) absorptions at 291 nm, corresponding to those of $[Ru(bpy)_3Cl_2]$ at approximately 451 and 287 nm (curve b), respectively. These spectra confirm the similar metal–ligand interactions of these materials. However, the MLCT transition of tris(bpyRu)–β-CD at 458 nm shows a slight redshift compared with that of $[Ru(bpy)_3Cl_2]$ at approximately 451 nm, which can be attributed to the influence of the electron-withdrawing amide groups on the ligands of tris(bpyRu)–β-CD.[9] Furthermore, the maximum MLCT absorption intensity of tris(bpyRu)–β-CD is 0.569, which is significantly higher than that of $[Ru(bpy)_3Cl_2]$ ($A_{451\,nm} = 0.158$). Measurements of molar extinction coefficients centered at the MLCT band under the present experimental conditions demonstrated that tris(bpyRu)–β-CD has a remarkably high value ($5.69 \times 10^4\ mol^{-1}\,cm^{-1}$), which is 3.6 times higher than that of $[Ru(bpy)_3Cl_2]$ (Table 1). This difference can be easily explained by the fact that tris(bpyRu)–β-CD with triple ruthenium centers contains several more benzene rings, which leads to an enlarged π–π electronic conjugate system for the absorption of MLCT and LC.

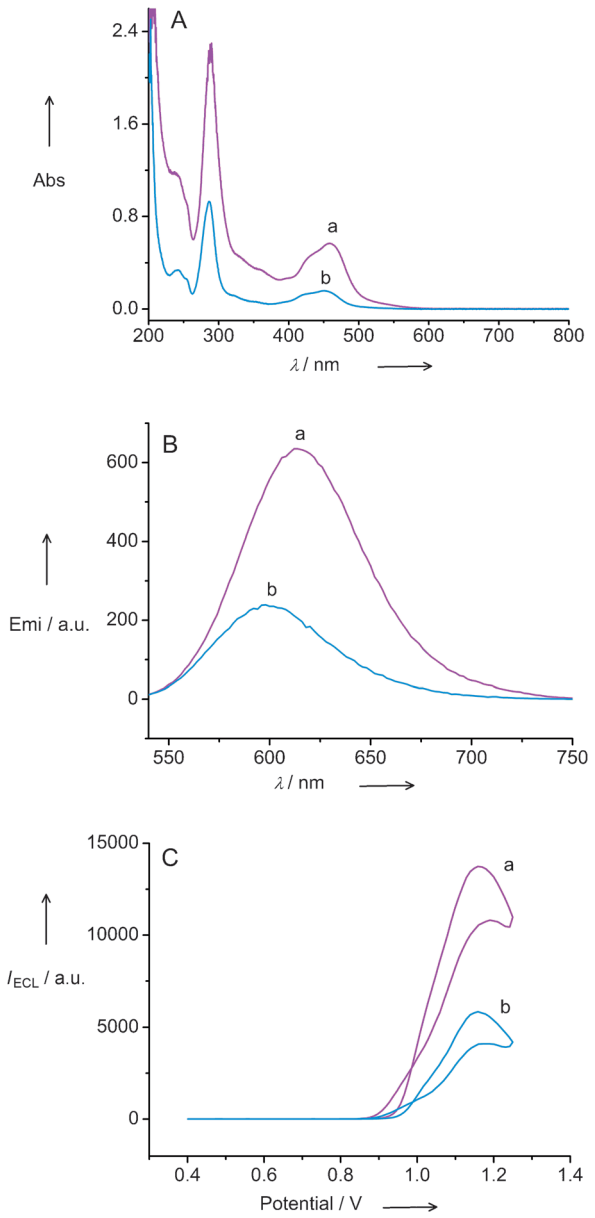


Figure 1. (A) UV/Vis absorption spectra of (a) tris(bpyRu)–β-CD and (b) [Ru(bpy)$_3$Cl$_2$] (1.0×10^{-5} mol) in acetonitrile at 25 °C. (B) Fluorescence emission spectra of (a) tris(bpyRu)–β-CD and (b) [Ru(bpy)$_3$Cl$_2$] (1.0×10^{-5} mol) in acetonitrile at 25 °C ($\lambda_{EX}=450$ nm). (C) ECL spectra of (a) tris(bpyRu)–β-CD and (b) [Ru(bpy)$_3$Cl$_2$] (1.5×10^{-7} mol) in the presence of tripropylamine (TPrA; 0.01 mol) with phosphate-buffered saline (PBS) buffer (0.10 mol, pH 7.5) on a glassy carbon electrode at a scan rate of 100 mV s^{-1}; scan range: 0.4–1.25 V (vs. Ag/AgCl).

The fluorescence spectrum of tris(bpyRu)–β-CD excited in the MLCT band is collected in Figure 1B. The obtained fluorescence spectrum displayed the same tendencies as the UV/Vis spectrum. The new complex shows a maximum emission at 615 nm (curve a), which is close to the characteristic peak of [Ru(bpy)$_3$Cl$_2$] at approximately 598 nm (curve b), thereby serving as a proof that the optical feature of ruthenium is retained in tris(bpyRu)–β-CD. Furthermore, the emission intensity of tris(bpyRu)–β-CD is 635 nm, which is 2.7 times that of [Ru(bpy)$_3$Cl$_2$] ($I_{max}=235$) and is caused by the triple ruthenium centers having a high emission efficiency. In addition, the emission quantum yield can further quantify the efficiency of the emission. The calculated emission quantum yield of 0.010 for tris(bpyRu)–β-CD is similar to that of [Ru(bpy)$_3$Cl$_2$] at approximately 0.012 (Table 1). Although there is a tiny decrease in the emission quantum yield for tris(bpyRu)–β-CD relative to [Ru(bpy)$_3$Cl$_2$], this new polynuclear metallocyclodextrin complex exhibited a remarkably high fluorescence intensity, which indicates a great potential for use in the design of photovoltaic supramolecular devices.

The ECL behavior of tris(bpyRu)–β-CD was then monitored over a scan range of 0.4–1.25 V, as shown in Figure 1C. The measured intensity of tris(bpyRu)–β-CD is 1.33-fold greater than that of [Ru(bpy)$_3$Cl$_2$] (14000 vs. ≈6000) produced by multi-metal-center oxidation. This result indicates that tris(bpyRu)–β-CD is not only endowed with the specialty for host–guest recognition but also exhibits excellent electrochemiluminescence performance, providing new possibilities for the development of ECL sensors.

In the present study, the tris(bpyRu)–β-CD film was electropolymerized on an electrode surface to construct a solid-state ECL sensor, which displayed several advantages over solution-phase ECL, including a simplified experimental design and a reduced consumption of expensive reagents. More importantly, enhanced ECL signals can be obtained from a well-designed surface-confined ECL sensing platform.[10] As shown in Figure 2, the ECL intensity of the tris(bpyRu)–β-CD film (curve a) is approximately 50500, which is a considerable improvement over the solution-state ECL, and thus proves the substantial superiority of the solid-state ECL.

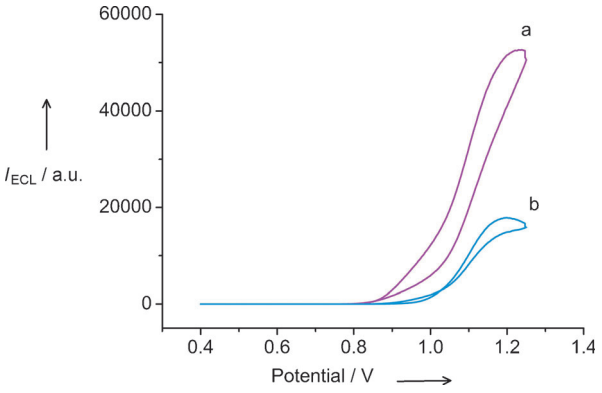
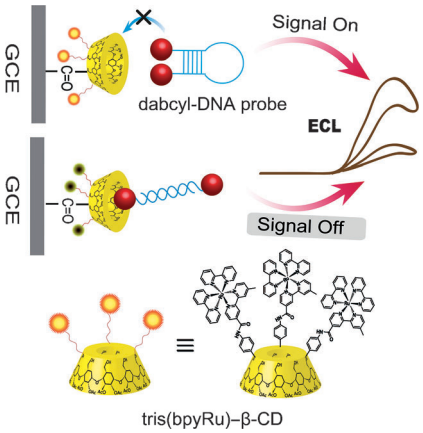


Figure 2. ECL responses of the electrode immobilized with the tris(bpyRu)–β-CD film (a) before and (b) after the addition of dabcyl (5.0×10^{-6} mol). ECL experiments were performed in the presence of TPrA (0.01 mol) with PBS buffer (0.10 mol, pH 7.5) at a scan rate of 100 mV s^{-1}; scan range: 0.4–1.25 V (vs. Ag/AgCl).

The organic guest 4-dimethylaminoazobenzene-4′-carboxylic acid (dabcyl) was selected[11] to investigate the potential participation of the tris(bpyRu)–β-CD film in the host–guest systems. An efficiently quenched ECL response was clearly observed with the addition of dabcyl (5.0×10^{-6} mol) on the film (curve b). The dabcyl guest can be included in the cavity of β-CD from the host complex, which facilitates a photoinduced

electron transfer from the excited ruthenium moiety to the guest,[12] thereby leading to the quenching of ECL signals. The relative difference between the ECL intensity before and after host–guest recognition was 67.6% for the tris(bpyRu)–β-CD film, which indicated that the host–guest recognition exhibits highly efficient ECL quenching.

The outstanding luminescent characteristics of the tris(bpyRu)–β-CD film were demonstrated by the direct detection of specific t-DNA through homogeneous hybridization with a dabcyl-labeled hairpin DNA probe (dabcyl–DNA probe) (Scheme 2). Prior to hybridization, the dabcyl–DNA probe re-



Scheme 2. Illustration of the t-DNA detection.

tains its stem-loop structure, and two guest molecules of dabcyl remain in close proximity. This configuration prevents the guest molecules from entering the β-CD cavity of the host complex owing to steric effects, and consequently, only a small amount of ECL quenching occurs. In such a case, the tris(bpyRu)–β-CD film maintains a "signal on" state. In contrast, after hybridization with t-DNA in a homogeneous solution, the stem-loop structure of the dabcyl–DNA probe is opened to form a rigid double-strand DNA (ds-DNA), and dabcyl is readily captured by the ECL film. Owing to the energy transfer between the guest and the host, the ECL signal of the tris(bpyRu)–β-CD film is quenched. In this case, the film switches to a "signal off" state.

This strategy is confirmed by the experimental results showed in Figure 3. Curve a is the ECL response of the tris(bpyRu)–β-CD film and curves b and c correspond to the "signal on" and "signal off" states, respectively. It is clear that the dabcyl–DNA probe with an intact stem-loop structure has only a minor quenching effect on the ECL signal. However, the opened probe, resulting from the hybridization with t-DNA, shows clear ECL quenching. This quenching is closely related to the concentration of t-DNA.

The results of the specific experiments are displayed in Figure 4, in which the ECL quenching responses for different sequences of DNA through hybridization with a dabcyl–DNA probe are compared. Bar 3 represents the absolute difference (ΔI, I_0–I) of the ECL intensity for the tris(bpyRu)–β-CD film after

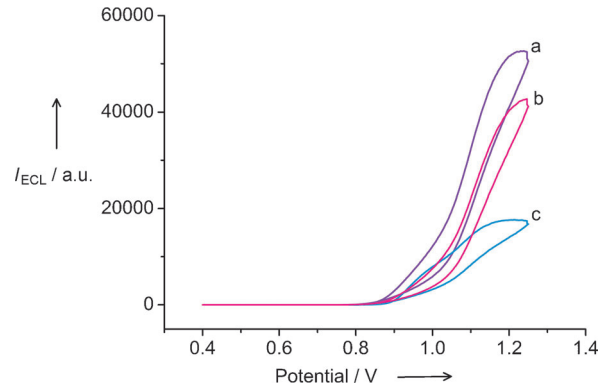


Figure 3. ECL intensities of the tris(bpyRu)–β-CD film: (a) no DNA addition; (b) after adding the dabcyl–DNA probe (5.0×10^{-6} mol); (c) after adding ds-DNA formed by the dabcyl–DNA probe (5.0×10^{-6} mol) and t-DNA (5.0×10^{-6} mol). ECL experiments were performed in the presence of TPrA (0.01 mol) with PBS buffer (0.10 mol, pH 7.5) at a scan rate of 100 mV s^{-1}; scan range: 0.4–1.25 V (vs. Ag/AgCl).

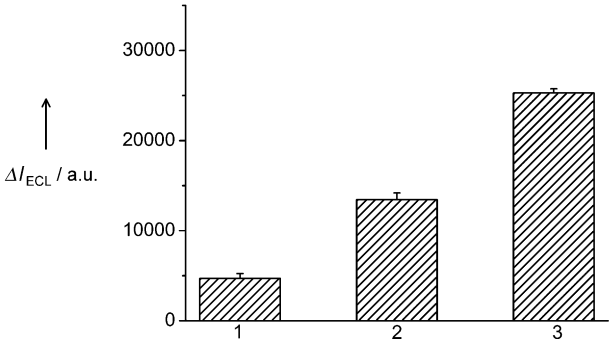


Figure 4. The absolute difference of the ECL intensities for the tris(bpyRu)–β-CD film: (1) after adding ds-DNA formed by the dabcyl–DNA probe (5.0×10^{-6} mol) and triple-base-mismatch t-DNA (5.0×10^{-6} mol); (2) after adding ds-DNA formed by the dabcyl–DNA probe (5.0×10^{-6} mol) and single-base-mismatch t-DNA (5.0×10^{-6} mol); (3) after adding ds-DNA formed by the dabcyl–DNA probe (5.0×10^{-6} mol) and complementary t-DNA (5.0×10^{-6} mol). ECL experiments were performed in the presence of TPrA (0.01 mol) with PBS buffer (0.10 mol, pH 7.5) at a scan rate of 100 mV s^{-1}; scan range: 0.4–1.25 V (vs. Ag/AgCl).

adding ds-DNA (5.0×10^{-6} mol) formed by the dabcyl–DNA probe and complementary t-DNA. Under the same experimental conditions, single-base-mismatch DNA yielded a partial quenching response (bar 2), approximately 53% that of complementary t-DNA. Furthermore, the triple-base-mismatch DNA showed an even smaller ECL intensity decrease (bar 1), which was only 18% that of complementary t-DNA. These results demonstrate that the prepared novel ECL DNA sensor with specific DNA detection is highly selective to the target DNA sequence and could be used to differentiate the DNA sequence from a mismatched DNA sequence.

As the concentration of t-DNA can be quantified based on the decrease of the ECL intensity, the quantitative t-DNA detection through hybridization with the dabcyl–DNA probe (5.0×10^{-6} mol) is shown in Figure 5. With an increase of the t-DNA concentration, the ECL intensity of the tris(bpyRu)–β-CD film decreased, as expected (Figure 5A). There is a good linear

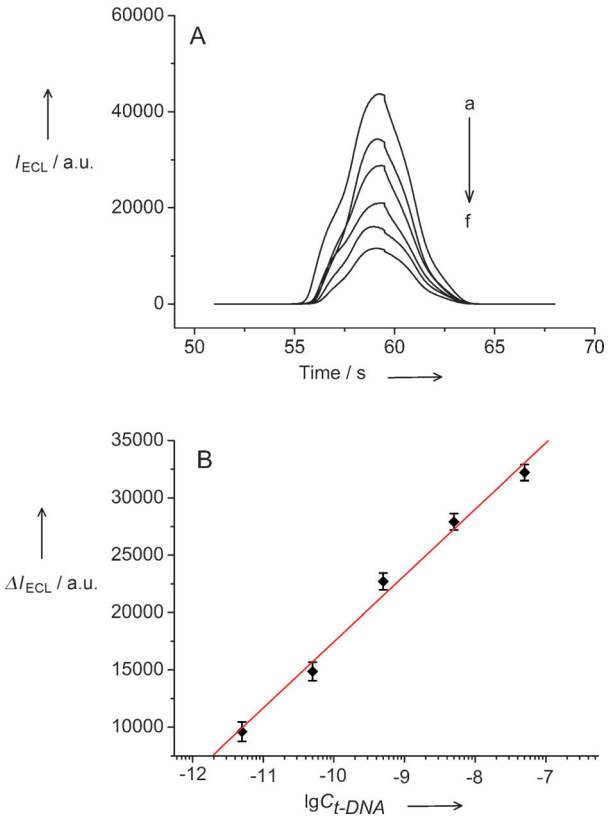


Figure 5. (A) The ECL DNA sensor responses for various t-DNA concentrations: (a) 0, (b) 5.0×10^{-12}, (c) 5.0×10^{-11}, (d) 5.0×10^{-10}, (e) 5.0×10^{-9}, and (f) 5.0×10^{-8} mol. (B) Calibration curve for the absolute difference of the ECL intensity as a function of the logarithm of the t-DNA concentration. ECL experiments were performed in the presence of TPrA (0.01 mol) with PBS buffer (0.10 mol, pH 7.5) at a scan rate of 100 mV s^{-1}; scan range: 0.4–1.25 V (vs. Ag/AgCl).

relationship between the absolute differences of the ECL intensity and the logarithm of the t-DNA concentrations (Figure 5B). The regression equation is $\Delta I_{ECL} = (7.57\times10^4) + (5.83\times10^3)\lg C_{t\text{-}DNA}$, and the regression coefficient (R) of the linear curve is 0.9950. Moreover, the minimum detecting concentration of the t-DNA is 5.0×10^{-12} mol, which demonstrates the significant potential of the ECL DNA sensor.

In conclusion, we synthesized a new polynuclear metallocyclodextrin complex that can serve as a platform for the synthesis of multinuclear complexes. This complex exhibited highly efficient fluorescence and ECL properties. Moreover, we described the fabrication of a controllable solid-state ECL film by the electropolymerization of tris(bpyRu)–β-CD on an electrode surface, which can enhance the efficiency of ECL analyses and maintain its stability. Based on such properties, a novel ECL DNA sensor was successfully constructed using the tris(bpyRu)–β-CD film as an ECL signal-generating unit in which DNA was indirectly labeled by means of host–guest recognition. All the results indicate that this polynuclear metallocyclodextrin complex is a new type of high-efficiency and cost-effective ECL luminophore that provides significant advantages over previously reported DNA detection technologies. Furthermore, owing to the high luminescent intensity and supramolecular structure of this new complex, it will have a significant potential to be applied in a broad range of domains.

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Keywords: cyclodextrins · host–guest systems · luminescence · ruthenium · sensors

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