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COMMUNICATION

Ratiometric ECL of heterodinuclear Os/Ru dual-emission labels

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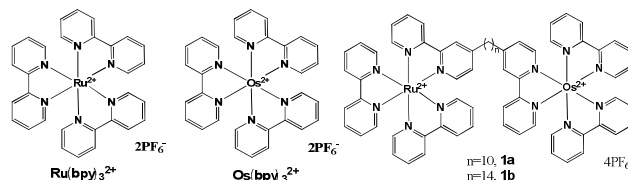
A dual emission of two heterodinuclear Os-Ru labels was first established a linear calibration curve between the ECL intensity ratio (I_{731}/I_{618}) and tripropylamine (TPA) concentration.

Electrogenerated chemiluminescence (ECL) is a method of producing light on an electrode surface. Used along with co-reactants in a solution system, this method exhibits remarkable sensitivity and versatile detection application.¹ ECL technology has been extensively used in light-emitting devices,² anion sensing,³ multiplex (microarray) immunoassays,⁴ damaged or sequence-specific DNA detection⁵ and molecular encoding–decoding⁶ etc. Commercial ECL systems are predominantly based on a [Ru(bpy)₃]²⁺-tripropylamine (TPA) co-reactant system^{7,8} (where bpy is 2,2'-bipyridyl). Although ECL technology has been widely used in several fields, several drawbacks, including the high background noise presented by TPA, because of its continuous consumption during oxidation,⁹ limit its applicability. A previous study¹⁰ reported that the background noise intensity may reach as high as 12% relative to the Ru(bpy)₃²⁺ complex ECL signal on a Pt electrode. Background light interference can affect the ECL detection limit and sensitivity and presents significant barriers to further ECL development and application.

Several methods¹¹ to solve the problems associated with ECL detection, including adjusting the pH range, adding surfactants and adopting different metal complexes, have been developed. Unfortunately, these methods cannot efficiently eliminate high background noise. Most of the reported method was simply adjust intermolecular ECL interactions between the Ru(bpy)₃²⁺ complex and the TPA co-reactant, thus, a TPA co-reactant is still necessary for ECL measurement and background interference from TPA is still observed. A potential approach to solve the background noise problem is simultaneous utilization of concerted intramolecular and intermolecular interactions and dual-ECL emissions. This technique eliminates TPA background interference by employing ratiometric ECL under different wavelengths. In our previous study,¹² the TPA

content was reduced to 20 mM and its detection limit was lowered to 0.1 fM on a glassy carbon (GC) electrode using two Ru centres with a -(CH₂)₈- bridge, this bridge connects the two Ru moieties intramolecularly. This strategy was executed without significant steric hindrance on either Ru-activating center on an electrode or in solution, the ECL intensity can be increased by combining both intermolecular and intramolecular ECL interactions,¹³ and findings provide evidence that the proposed method is feasible.

In the present study, two novel bimetallic Ru–Os complexes, [(bpy)₂Ru(bpy)(CH₂)_n(bpy)Os(bpy)₂]⁴⁺ (**1**, Scheme 1: n = 10, **1a**; n = 14, **1b**), were synthesized by connecting normal red Ru- and near-infrared Os-ECL labels through a flexible saturated C chain to obtain infrared/near-infrared dual-emission ECL labels.



Scheme 1. Structures of the bimetallic ruthenium(II) and osmium(II) tris-bipyridyl complexes **1** and the corresponding mononuclear reference molecules of Ru(bpy)₃²⁺ and Os(bpy)₃²⁺.

The different moieties emitted luminescence at different wavelengths, and emission intensity (I_{731}/I_{618}) ratios were employed to achieve a ratiometric ECL strategy. Differences between the two emission wavelengths were extremely large ($\Delta E_m = 113$ nm) and not only promoted accurate intensity measurement of the two emission peaks but also produced precise ratiometric ECL detection values. Concerted intra- and intermolecular ECL performance and ratiometric ECL detection were investigated and utilized to reduce the amount of TPA co-reactant necessary in the system and overcome the many disadvantages of commercial technology, such as utilization of only intermolecular interaction ECL, single detection wavelength and significant ECL background noise.

Especially, application of ratiometric ECL eliminated the background interference of TPA. All of the results obtained may be used to tune ECL efficiency using bimetallic complexes linked by flexible saturated C chains and contribute to the understanding of dual emission and ratio ECL detection.

Figure 1 shows the typical absorption and luminescence properties of Ru (II) and Os (II) tris-bipyridyl complexes in bimetallic complexes **1a** and **1b** in acetonitrile.¹⁴ The absorption intensities of the complexes nearly overlapped with those of mononuclear reference complexes Ru(bpy)₃²⁺ and Os(bpy)₃²⁺ (Figure 1a) because of the coexistence of bimetallic Ru and Os centres. The absorption observed at 240–350 nm represents $\pi-\pi^*$ transitions of the bipyridine ligand, and the absorption observed at 400–550 nm results from metal-to-ligand charge transfer (MLCT) excited states.

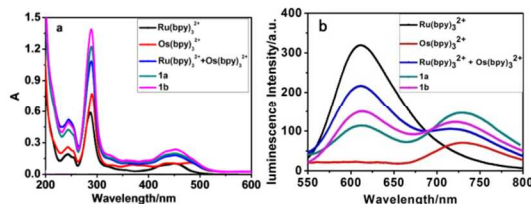


Figure 1. (a) UV-Vis absorption and (b) the luminescence spectra of Ru(bpy)₃²⁺, Os(bpy)₃²⁺, Ru(bpy)₃²⁺+Os(bpy)₃²⁺ (1:1), **1a** and **1b** in CH₃CN. The complex concentration was 10⁻⁵ M.

As shown in Figure 1b, complexes **1a** and **1b** exhibited two well-resolved emission bands centred at 615 and 720 nm (λ_{exc} , 455 nm), representing the emissions of Ru and Os moieties, respectively. Complex **1** exhibited emission peaks similar to those of the corresponding mononuclear reference complexes of Ru(bpy)₃²⁺ and Os(bpy)₃²⁺. This finding suggests that flexible intramolecular saturated C chain linkages do not significantly affect emission. The dual-emission intensity of **1b** differed from that of **1a** and may be attributed to electrostatic repulsion and phosphorescence resonance energy transfer¹⁵ between the two moieties. In the 1:1 mixture of Ru(bpy)₃²⁺ and Os(bpy)₃²⁺, the intensity of Ru(bpy)₃²⁺ was lower than that of the corresponding mononuclear Ru(bpy)₃²⁺, whereas, the intensity of Os(bpy)₃²⁺ was higher than that of the corresponding mononuclear Os(bpy)₃²⁺. This finding may be explained by phosphorescence resonance energy transfer happened from Ru(bpy)₃²⁺ to Os(bpy)₃²⁺.¹⁶ The corresponding absorption maxima, emission maxima and molecular extinction coefficients are listed in Table S1.

Table 1. The redox potentials of complexes **1**.

	Oxidation (V)		Reduction (V)	
	Os(bpy) ₃ ^{3+/2+}	Ru(bpy) ₃ ^{3+/2+}	Os(bpy) ₃ ^{2+/+}	Ru(bpy) ₃ ^{2+/+}
Ru(bpy) ₃ ²⁺	-	1.06	-	-1.79
Os(bpy) ₃ ²⁺	0.54	-	-1.32	-
1a	0.49	0.93	-1.18	-1.55
1b	0.50	0.94	-1.17	-1.54

As shown in Table 1, electrochemical data of complexes **1a** and **1b** with variable lengths of C chain linkages did not exhibit significant differences. Compared with the mononuclear reference complexes of Ru(bpy)₃²⁺ and Os(bpy)₃²⁺, the oxidation potentials of Ru(II/III) and Os(II/III) in **1a** and **1b** decreased, and the corresponding reduction potentials increased. This behaviour

confirms that the two moieties are easily oxidized because of the C chain linkage intramolecular interactions.

In a typical ECL detection process, TPA is normally added to the detection system at high concentrations (100 mM). Unfortunately, high concentrations of TPA in an ECL system increase the background noise. As shown in Figure 2a, adding 5 mM TPA to the detection system increased the background noise around the ECL of Ru(bpy)₃²⁺. This noise affects ECL measurement, especially in common ECL instruments where only the ECL intensity is measured and the emission wavelength is not considered.

Figure 2b shows the ECL performance of 5 × 10⁻⁵ M complexes **1a** and **1b** with 5 mM TPA co-reactant on a Pt electrode. The ECL maxima of the complexes were similar to the ECL behaviour of the mononuclear reference complexes Ru(bpy)₃²⁺ and Os(bpy)₃²⁺, however, the red emission of the Ru label (about 620 nm) and the near-infrared emission of the Os label (about 730 nm) were observed simultaneously. The ECL spectra of complexes **1a** and **1b** were similar to those obtained during photoexcitation. This result indicates that ECL is due to the MLCT-excited state and that emission characteristics are not affected by the double intramolecularly bound active centres.

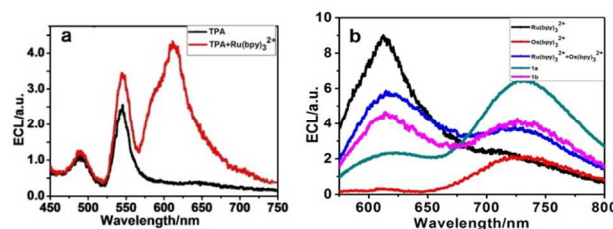


Figure 2. (a) ECL spectra of 5 mM TPA (black line) in the absence or presence of 5 × 10⁻⁵ mM Ru(bpy)₃²⁺ (b) ECL spectra of **1**, Ru(bpy)₃²⁺, Os(bpy)₃²⁺, Ru(bpy)₃²⁺+Os(bpy)₃²⁺ (1:1) with the presence of 5 mM TPA in 0.1 M (n-Bu)₄NPF₆ acetonitrile, the concentration was 5 × 10⁻⁵ M.

Complex **1b** exhibited a more ideal dual ECL than **1a** because of its unique C chain linkage. The same conclusion can be reached by using 2-(dibutylamino) ethanol (DBAE) and melamine as the coreactant (Figure S12). The shorter C chain linkage of **1a** exhibits greater steric hindrance, which affects the dual ECL intensity of complex **1a**. Increased C chain linkages cause a decrease in electrostatic repulsion between the two centres. Furthermore, phosphorescence resonance energy transfer from the Ru moiety to the Os moiety decreases the ECL intensity of the Ru(bpy)₃²⁺ moiety, this process favours the 10-C chain linkage in **1a**.¹⁵

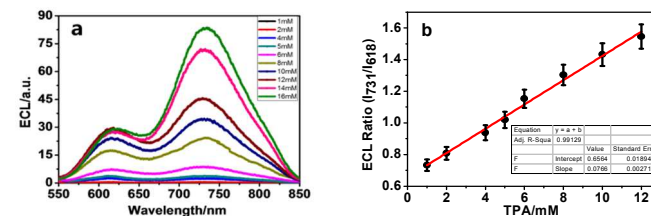


Figure 3. (a) ECL spectra changes of **1b** (5 × 10⁻⁵ M) along with the titration of TPA from 1 mM to 12 mM, 0.1 M (n-Bu)₄NPF₆ in acetonitrile. (b) A calibration curve between the ECL ratio I_{731}/I_{618} and TPA added over a concentration range of 1 mM to 12 mM in acetonitrile.

ECL performance of **1b** was investigated by titration of TPA. As shown in Figure 3a, adding more TPA increased the ECL intensities at 618 and 731 nm. The ratio of ECL intensities (I_{731}/I_{618}) was

linearly dependent on the TPA used. A linear calibration curve between the ECL intensity ratio (I_{731}/I_{618}) and the TPA concentration was established over a TPA concentration range of 1–12 mM (Figure 3b). The mechanism of ratiometric ECL spectral change of **1** is proposed to follow the classic route (details in Supporting Information). The results of this study provide a general methodology for eliminating the ECL background noise caused by TPA during ratiometric ECL. Differences between the two emission wavelengths were extremely large ($\Delta E_m = 113$ nm), thus, ECL intensity measurement of the two emission peaks was accurate and the ratiometric ECL detection value obtained was precise.

The same trend was observed during titration of TPA with the corresponding mononuclear reference complexes of $\text{Ru}(\text{bpy})_3^{2+}$ and $\text{Os}(\text{bpy})_3^{2+}$ (Figure S8) under the same conditions. However, the ECL of $\text{Ru}(\text{bpy})_3^{2+}$ was affected by TPA because of emission overlap, which affects the accuracy of the measurement results. Details of the results are presented in Table S3.

Considering that there are two active centres present in complexes **1a** and **1b**, a control experiment was performed using a 5×10^{-5} M 1:1 monometallic mixed solution of $\text{Ru}(\text{bpy})_3^{2+}$ and $\text{Os}(\text{bpy})_3^{2+}$ placed on a Pt electrode under the same conditions (Figure S9a). Dual ECL of red and near-infrared light was simultaneously observed with increasing amounts of TPA. Unfortunately, the relation between ECL ratio (I_{731}/I_{618}) and TPA concentration could not be established based on the results obtained (Figure S9b) because of phosphorescence resonance energy transfer between the $\text{Ru}(\text{bpy})_3^{2+}$ and $\text{Os}(\text{bpy})_3^{2+}$ complexes and emission overlaps between the Ru moiety and TPA, no overlap was observed between the Os moiety and TPA. To further assess the accuracy of the proposed ratiometric ECL detection method, it was applied to the determination of three TPA samples spiked into the system of complex **1b**. The values of percentage relative standard deviation (RSD) for intra and inter-day variation are given in Table S2. The relative standard deviations were less than 0.71% for TPA detection showed the fine accuracy and repeatability. In comparison, only the inter-day data of the corresponding mononuclear reference complexes of $\text{Ru}(\text{bpy})_3^{2+}$ and $\text{Os}(\text{bpy})_3^{2+}$ were obtained (Table S3), and no meaningful conclusion could be drawn from the results. Poorer results were obtained from the 1:1 mixed solution of $\text{Ru}(\text{bpy})_3^{2+}$ and $\text{Os}(\text{bpy})_3^{2+}$ (Figure S9b). All of the findings thus far confirm the advantages of the proposed ratio-ECL method.

In conclusion, the synthesized bimetallic Ru–Os complex **1** exhibited excellent ECL performance in acetonitrile. The Ru and Os labels simultaneously exhibited dual-emission and dual-ECL properties at around 620 nm for Ru and 730 nm for Os. The ratiometric ECL of complex **1b** (I_{731}/I_{618}) fitted a reasonably linear relationship ($R^2 = 0.991$) with TPA added into the detection system over a concentrations range of 1–12 mM, and good accuracy and repeatability were obtained during sample detection. This study presents a ratiometric ECL-detection method that further refines or tunes the efficiency of ECL systems. Excellent ECL efficiency was achieved by utilizing bimetallic Ru–Os complexes to eliminate the background noise contributed by TPA.

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Notes and references

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Electronic Supplementary Information (ESI) available: experimental details and additional figures. See DOI: 10.1039/c000000x/

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