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Electronic Supplementary Information

High-efficiency cathodic electrochemiluminescence of tris(2,2'bipyridine)ruthenium(II)/*N*-hydroxy compounds system and its use for sensitive "turn-on" detection of mercury (II) and methyl blue

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1. Experimental section

1.1 Chemicals and materials

Ru(bpy)₃²⁺, tripropylamine (TPrA), and alizarin red S were bought from Sigma–Aldrich (USA). NHSS sodium salt, erythrosin B, methyl blue, superoxide dismutase (SOD), 5,5dimethyl-1-pyrroline N-oxide (DMPO), sodium oxalate (Na₂C₂O₄), and acridine orange were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Metal salts were supplied by Beijing Chemical Reagent Company (Beijing, China). Potassium peroxydisulfate (K₂S₂O₈) was bought from Xilong Scientific Co., Ltd. (Guangdong, China). Sodium sulfite (SS) and NHS were purchased from Alfa Aesar Chemical Reagent Co., Ltd. (China). Methylene blue was bought from Solarbio Life Sciences Company (Beijing, China). Trypan blue was purchased from Beijing Dingguo Changsheng Biotechnology Co. Ltd. (Beijing, China). Succinimide was purchased from Macklin Biochemical Co. Ltd. (Shanghai, China). The working phosphate buffer solution (pH 7.4; 0.1 M PBS) was prepared by mixing the stock solutions of Na₂HPO₄ and NaH₂PO₄. All the chemicals were analytical-reagent grade and water purification was done with a Millipore system (18.2 MΩ· cm).

1.2 Apparatus

A homemade three-electrode cell with transparent bottom was used to conduct ECL experiments on a model MPI A multifunctional electrochemical and ECL system (Xian Remex Electronics Co. Ltd., Xian, China). The voltage of photomultiplier tube (PMT) was tuned to get optimal ECL intensities. The conventional three-electrode setup was used throughout the ECL experiments. The working, counter and reference electrodes were glassy carbon electrode (GCE), Pt wire and Ag/AgCl electrode (with saturated KCl), respectively. Prior each ECL experiment, the working GCE was polished in sequential order with 0.3 µm and 0.05 µm alumina slurry on a microcloth to realize a mirrorlike surface, followed by ultra-sonication in the double-distilled water, and finally dried in air. The ECL curves were recorded by applying the electrode potential from 0 to -1.5 V with the scan rate of 0.1 V/s. A number of band-pass filters (400 to 700 nm) were utilized to capture the maximum ECL emission wavelength (ECL emission spectrum). Electron paramagnetic resonance (EPR) spectra were recorded on a Model JES-FA200 spectrometer operating at room temperature.

1.3 Procedure of ECL detections of Hg²⁺ and methyl blue

The ECL detection process was performed as follows: The three-electrode cell with transparent bottom was filled with 1 ml of PBS solution (0.1 M, pH 7.4) containing 1 mM $Ru(bpy)_{3}^{2+}$, 1 mM of NHSS, and different concentrations of Hg²⁺ and methyl blue, respectively. All cyclic voltammetry experiments and the ECL measurements were conducted using GCE and a MPI A electrochemical and ECL analyzer. The voltage of PMT was set at 700 V during detection of both targets.

2. Proposed mechanisms of the cathodic Ru(bpy)₃²⁺–NHSS ECL system (additional detail)

The maximum ECL emission was achieved at a wavelength of ~ 620 nm (**Fig. 2B**), which is consistent with that of Ru(bpy)^{2+*}.^{1, 2} The effects of SS, AA and SOD on the ECL intensity of the Ru(bpy)₃²⁺—NHSS system were further examined (**Fig. S6**). AA and SOD are effective radical scavengers of *OH, ¹O₂, H₂O₂,³ and O₂*⁻, respectively, while SS is an oxygen (O₂) scavenger.⁴ As shown in **Fig. S6**, both SS and SOD had no obvious quenching effect on ECL intensity, indicating that O₂ and O₂*⁻ were not playing critical role in the ECL generation.⁴ In contrast, the addition of AA decreased the ECL intensity greatly. These results indicated that *OH (and/or ¹O₂, H₂O₂) could be critical intermediates in the Ru(bpy)₃²⁺—NHSS ECL system. In addition, the generation of *OH was further confirmed by EPR spectrum by utilizing frequently applied DMPO as a spin trap.⁵ As shown in **Fig. S7**, the characteristic *g* factor values of EPR peaks from Ru(bpy)₃²⁺-NHSS-DMPO confirmed the generation of *OH in this ECL system.⁶

Moreover, control experiments showed that succinimide (with –NH moiety) was inactive and displayed a ECL value close to that of $Ru(bpy)_3^{2+}$ alone (**Figs. S1 & S2**). In contrast, both NHS and NHSS participated in the ECL reaction and significantly enhanced the ECL of $Ru(bpy)_3^{2+}$ (**Figs. 1 & S2**), suggesting the crucial role of the –NOH moiety. Besides, NHSS showed higher ECL response than NHS, suggesting also the important role of sulfonate moiety. It has been reported that hydrophilic/ electron-withdrawing sulfonate group (–SO₃H of NHSS) can remarkably improve stability, solubility, and enhance the ECL emission.⁷ And these active anionic groups can coordinate with $Ru(bpy)_3^{2+}$ cations through electrostatic interactions at the electrode surface to further contribute to increase ECL.

3. Optimization of detection conditions

To achieve optimal performance of the ECL detection system, we investigated some crucial experimental parameters. The effect of the pH on the ECL intensity was first investigated from pH 6.5 to 11.5. As shown in Fig. S5, the ECL intensities increase with increasing pH from 6.5 to 10.5, and then decrease as the pH increases further. Therefore, the optimum pH for Ru(bpy)₃²⁺-NHS/NHSS ECL system was 10.5. However, a physiological pH of 7.4 was chosen to achieve the best performance for biological sample detections. The ECL intensity increased gradually with the increasing of concentration of Ru(bpy)₃²⁺ from 25-1000 μ M (Fig. S13A) and then slowed down (Fig. S13B). Therefore, 1000 μ M of Ru(bpy)₃²⁺ was used for further experiments. Moreover, the concentration of NHSS (coreactant) was also an important parameter to achieve high performance of the developed system. As can be seen from Fig. S13 (C&D), with increasing the concentration of NHSS, the ECL signal enhanced sharply till 1000 μ M and then decreased gradually (data not shown). Therefore, 1000 μ M concentration of NHSS was opted for subsequent experiments. Furthermore, the effect of scan rates in the range of 10-200 mV/s on the ECL intensity was examined. As shown in Fig. **S14**, the maximum ECL intensity was achieved at the scan rate of 100 mV/s and then reached nearly a constant value. Thus, 100 mV/s was chosen as an optimal scan rate for further experiments. In addition, the effect of pulse potential on ECL intensity was also optimized in the range of -0.8 V to -1.55 V. The maximum cathodic ECL intensity was recorded at -1.45 V (Fig. 2A). Besides, the effect of pH was also tested towards the detection of Hg²⁺ and methyl blue. As displayed in Fig. S15 & S16, the maximum ECL enhancement/change (ΔI) was achieved at a pH of 7.4. Therefore, a pH value of 7.4 was selected for the subsequent detection of Hg²⁺ and methyl due.



Fig. S1. (A) ECL–potential curves for different solutions: buffer only (black color), NHSS (red color), succinimide (orange color), NHS (blue color), and $Ru(bpy)_3^{2+}$ -succinimide (green color), and **(B)** corresponding CV curves for different solutions: buffer only (black color), NHSS (red color), succinimide (orange color), NHS (blue color), $Ru(bpy)_3^{2+}$ (pink color), $Ru(bpy)_3^{2+}$ -succinimide (green color), and $Ru(bpy)_3^{2+}$ -succinimide (green color), and $Ru(bpy)_3^{2+}$ -H₂O₂ (cyan color). Concentration of each coreactant: 1 mM; pH: 7.4 (0.1 M PBS); PMT voltage: 900 V.



Fig. S2. (A) ECL–potential profiles for different solutions: NHS (green color), NHSS (black color), Ru(bpy)₃²⁺ (red curve), Ru(bpy)₃²⁺-H₂O₂ (blue curve), Ru(bpy)₃²⁺-NHS (pink curve), and Ru(bpy)₃²⁺-NHSS (green curve). The inset shows CV curves for Ru(bpy)₃²⁺, Ru(bpy)₃²⁺-NHS, and Ru(bpy)₃²⁺-NHSS. **(B)** ECL– potential profiles for buffer only (black color), succinimide (red curve), and Ru(bpy)₃²⁺-Succinimide (blue curve). **(C)** DPV curves for different solutions: Ru(bpy)₃²⁺ (red curve), Ru(bpy)₃²⁺-NHS (pink curve), and Ru(bpy)₃²⁺-NHSS (green curve). The inset shows DPV curves for succinimide, NHS, and NHSS. **(D)** Corresponding CV curves for different solutions: buffer only (black color), succinimide (red color), Succinimide (red color), NHS (blue color), NHSS (green color), Ru(bpy)₃²⁺-succinimide (violet color), and Ru(bpy)₃²⁺-H₂O₂ (orange color). Concentration of each coreactant: 1 mM; pH: 10.5 (in 0.1 M PBS); PMT voltage: 800 V.



Fig. S3. Chemical structures of succinimide (A), NHS (B), and NHSS (C) employed in this study.



Fig. S4. Comparison of cathodic ECL–potential profiles of $Ru(bpy)_3^{2+}$ -NHSS system with classic $Ru(bpy)_3^{2+}$ -TPrA **(A)** and $Ru(bpy)_3^{2+}$ -C₂O₄²⁻ **(B)**. Concentration of each coreactant: 1 mM; pH: 10.5 (in 0.1 M PBS); PMT voltage: 800 V.



Fig. S5. (A) ECL emission–time curves at different pH values: 6.5, 7.0, 7.5, 8.5, 9.5, 10.5, 11.25, and 11.5. **(B)** ECL intensity plotted as a function of pH. pH study was conducted in 0.1 M PBS containing 1 mM Ru(bpy)₃²⁺ and 1 mM NHSS; PMT: 700 V.



Fig. S6. ECL intensities of $Ru(bpy)_3^{2+}$ -NHSS system in the absence and the presence of different concentrations of AA (0.15 mM), SOD (0.8 µg/mL), and SS (0.3 mM) in 0.1 M PBS (pH 7.4) containing 1 mM Ru(bpy)_3^{2+} and 1 mM NHSS; PMT: 800 V.



Fig. S7. EPR spectra of $Ru(bpy)_3^{2+}$ -NHSS-DMPO without a cyclic potential scan (**A**; black curve), in a cathodic cyclic potential scan from 0 to -1.5 V (**B**; red curve), and combined control + cathodic spectra (**C**) from magnetic field (mT) range of 0 to 800. EPR spectra were conducted with 1 mM Ru(bpy)₃²⁺, 1 mM NHSS, and 200 mM DMPO in 0.1 M PBS (pH 7.4).



Fig. S8. ECL-time curves for nine replicate detections at the Hg²⁺ concentration of 0.01 μ M.



Fig. S9. (A) ECL–potential profiles and **(B)** corresponding CV curves for different solutions: Hg^{2+} (black curve), NHSS- Hg^{2+} (red curve), $Ru(bpy)_3^{2+}-Hg^{2+}$ (pink curve), $Ru(bpy)_3^{2+}-NHSS$ (green curve), $Ru(bpy)_3^{2+}-NHSS-Hg^{2+}$ (blue curve). Concentration of $Ru(bpy)_3^{2+}$ and NHSS: 1 mM; Concentration of Hg^{2+} : 10 μ M; pH: 7.4 (in 0.1 M PBS); PMT voltage: 700 V.



Fig. S10. ECL-time curves for nine replicate detections at the methyl blue concentration of 1.0 μ M.



Fig. S11. (A) ECL–potential profiles and **(B)** corresponding CV curves for different solutions: methyl blue (black curve), NHSS-methyl blue (red curve), Ru(bpy)₃²⁺-methyl blue (pink curve), Ru(bpy)₃²⁺-NHSS (blue curve), Ru(bpy)₃²⁺-NHSS-methyl blue (green curve). Concentration of Ru(bpy)₃²⁺ and NHSS: 1 mM; Concentration of methyl blue: 10 μ M; pH: 7.4 (in 0.1 M PBS); PMT voltage: 700 V.



Fig. S12. Chemical structure of methyl blue.



Fig. S13. Dependence of the ECL intensity of the developed system on **(A)** $Ru(bpy)_{3}^{2+}$ concentration, **(C)** NHSS concentration, and corresponding calibration plots for $Ru(bpy)_{3}^{2+}$ (25-1000 μ M) **(B)**, and NHSS (50-1000 μ M) **(D)**, respectively. The ECL responses were recorded in the solution of 1 mM NHSS and 1 mM $Ru(bpy)_{3}^{2+}$ (pH 7.4, in 0.1 M PBS); PMT: 700 V.



Fig. S14. (A) Cyclic voltammograms of 1 mM Ru(bpy)₃²⁺ and 1 mM NHSS at different scan rates: 10, 25, 50, 100, 150, and 200 mV/s. **(B)** the linear relationship between the ECL intensity and the square root of the scan rate ($v^{1/2}$) up to 100 mV/s in 0.1 M PBS (pH 7.4); PMT voltage: 900 V.



Fig. S15. (A) pH effects on the ECL enhancement towards the detection of Hg²⁺, and **(B)** change in ECL intensity plotted as a function of the pH. The measurments were performed in 0.1 M PBS (with pHs varied from 6.5 to 11.5) containing 1 mM Ru(bpy)₃²⁺ and 1 mM NHSS; PMT: 700 V.



Fig. S16. (A) pH effects on the ECL enhancement towards the detection of methyl blue (MB), and **(B)** change in ECL intensity plotted as a function of the pH. The measurments were performed in 0.1 M PBS (with pHs varied from 6.5 to 11.5) containing 1 mM Ru(bpy)₃²⁺ and 1 mM NHSS; PMT: 700 V.

Analytical Method	Materials	LOD	Ref.	
		(nM)		
Colorimetric	Au NPs	50	8	
Fluorescent	Molecular Beacon	19	9	
Fluorescent	phthalocyanine-T conjugate	32	10	
SERS	Fe ₃ O ₄ @SiO ₂ -Au	10	11	
Electrochemistry	Au NPs	1000	12	
Electrochemistry	Hemin/ oligonucleotide strands	50	13	
Photoelectrochemical	TiO ₂ -modified composite	20	14	
CL	thymine-rich DNA and hemin	12	15	
CL	Pt NPs	8.6	16	
ECL	magnetic beads/ complementary DNA probes	5.0	17	
ECL	logic gates based on MCNTs	10	18	
ECL	Au–Ag bimetallic NCs	5.0	19	
ECL	Materials Free	0.1	Present Work	

Table S1. Comparison of different reported methods for the detection of Hg^{2+}

Table S2. Recoveries of Hg^{2+} in lake and tap water real samples

Samples	Concentrations of Hg ²⁺		Recovery	RSD
	Amount	Amount found ^a	(%)	(n=3:%)
Lake Water	0.5	0.483	96.6	2.18
	1.0	1.021	102.1	1.83
	5.0	5.095	101.9	2.07
Tap Water	0.5	0.507	101.4	1.91
	1.0	0.982	98.2	2.36
	5.0	5.160	103.2	2.55

^aThe average of three replicate determinations.

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