

Electronic Supplementary Information

Highly Sensitive Novel Cathodic Electrochemiluminescence of Tris(2,2'-bipyridine)Ruthenium (II) using Glutathione as a co-reactant

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Experimental section:

Chemicals

Tris(2,2-bipyridine)ruthenium(II)hexahydrate, $(\text{Ru}(\text{bpy})_3\text{Cl}_2 \cdot 6\text{H}_2\text{O})$ (99.99%), disodiumhydrogenphosphate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$), Sodimdi-hydrogenphosphate mono hydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$), reduced Glutathione (GSH), oxidized Glutathione (GSSG) and hydrogen peroxide (30%) were purchased from sigma Aldrich. All the chemicals were used without any further purification. Argon and oxygen gas of 99.99% used to saturate the electrolyte solutions. Milli-Qwater (18.2Ω) used as a solvent for preparing the electrolyte solution.

Electrochemistry and ECL measurements

Commercially available Glassy carbon electrode (GCE) with 0.0732 cm^2 surface area serves as working electrode, platinum foil as the counter electrode and Ag/AgCl is used as reference electrode respectively. Cyclic voltammetry (CV) and potential step experiments were performed with an Autolab electrochemical workstation (EcoChemie, The Netherlands). The ECL along with CV signals were measured simultaneously with a photomultiplier tube (PMT, Hamamatsu H9305-04). The PMT was held at -500 V with a high-voltage power supply. The photo current generated at the PMT was converted to a voltage using an electrometer system (model 6517, Keithley, Cleveland, OH) and connected to the Autolab via an analog-to-digital converter (ADC).

ECL Spectrum:

We recorded the ECL spectrum using the optimized PMT voltage as 950 V and slit width is 20 nm in spectrofluorimeter. Also, applied constant potential pulse at -1.6 V vs Ag/AgCl on GC plate ($1 \times 1\text{ cm}^2$) in solution containing $2\text{ mM Ru}(\text{bpy})_3^{2+}$ and $100\text{ }\mu\text{M}$ of GSH in air saturated 0.1 M PBS at pH 9. The same experiments also repeated with GSSG instead of using GSH.

Cyclic voltammogram and ECL response of $\text{Ru}(\text{bpy})_3^{2+}$, GSH in 0.1M PBS (pH 9).

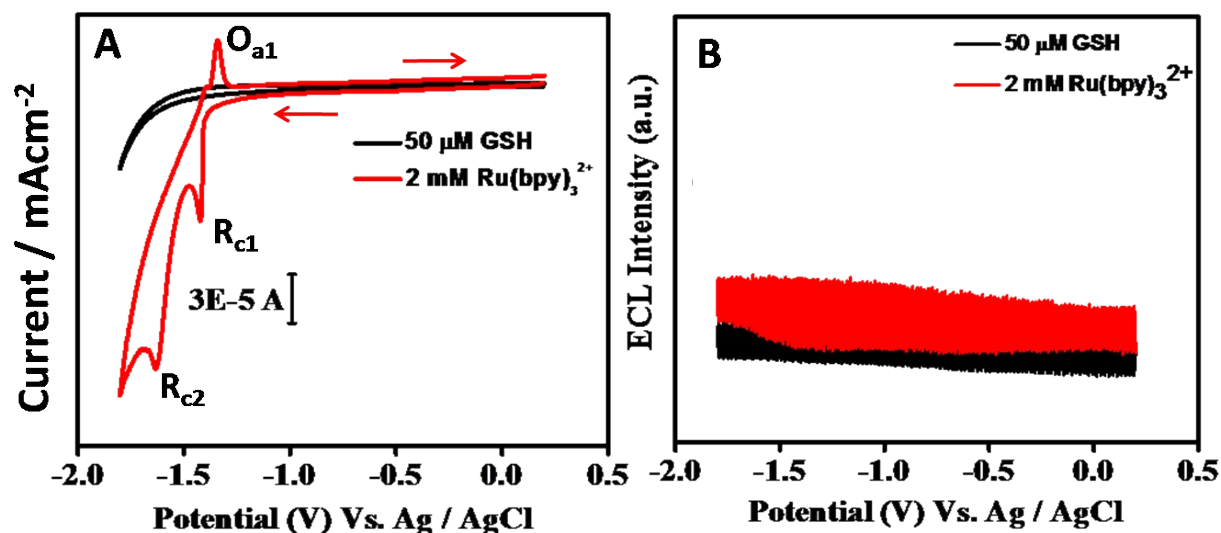


Fig. S1 (A)Cyclic voltammograms and its corresponding ECL (B) responses of 2 mM $\text{Ru}(\text{bpy})_3^{2+}$ (red), 50 μM GSH (black) on bare GC in air saturated 0.1 M PBS at pH 9 at 0.05 V/s.

Fig.S1A (black colour line) shows CV response of reduced form of GSH and not shown any redox active peak even at very high cathodic potential region on bare GC surface and indicate the electro inactive nature under the present experimental condition of air saturated (means normal or without any de-aeration of oxygen and argon gas condition) in 0.1M phosphate buffer (PBS) of pH 9 at the scan rate of 0.05V/s²⁸. There is no corresponding ECL emission on glassy carbon surface as shown in Fig. 1B (black colour solid line).The redox response of 2mM $\text{Ru}(\text{bpy})_3^{2+}$ alone in PBS at pH-9 in the scan rate of 0.05V/s under the same air condition is shown in Fig.S1A (red colour line). The obtained redox response of $\text{Ru}(\text{bpy})_3^{2+}$ is more consistent with reported literature by J.P. Choi and A.J. Bard. As they mentioned, the first pre-reduction peak at -1.41 V (R_{c1}) is corresponds to adsorbed or surface confine species of $\text{Ru}(\text{bpy})_3^{1+}$ on GCE, since this reduction peak current is linearly increased with scan rate and followed the zero intercept. Subsequently, the another strong reduction peak at -1.55 V (R_{c2}) is the due to the dissolved $\text{Ru}(\text{bpy})_3^{2+}$ to dissolved $\text{Ru}(\text{bpy})_3^{+}$ on GEC and this process is controlled by pure diffusion since the peak is linearly increased with increasing the square root of scan rate with zero intercept. Instead of obtaining completely reversible redox peaks for $\text{Ru}(\text{bpy})_3^{+}/\text{Ru}(\text{bpy})_3^{2+}$ only small appreciable peaks obtained at -

1.34 V (O_{a1}) because of hydrogen evolution also occurs simultaneously and that inhibits the reduction process of $Ru(bpy)_3^+/Ru(bpy)_3^{2+}$.

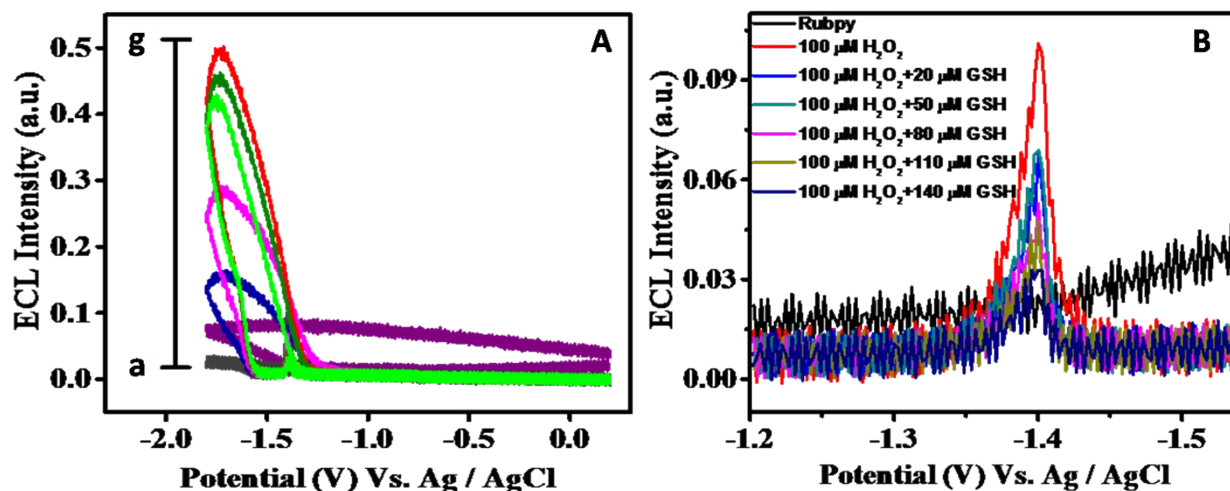


Fig. S2(A)ECL responses of 2 mM $Ru(bpy)_3^{2+}$ + 100 μM H_2O_2 with various GSH concentrations (20 to 140 μM) in PBS pH 9 at 0.05 V/s on GCE at the scan rate of 0.05 V/s (B) close up image of X (peak at -1.4 V) and Y scale of panel A).

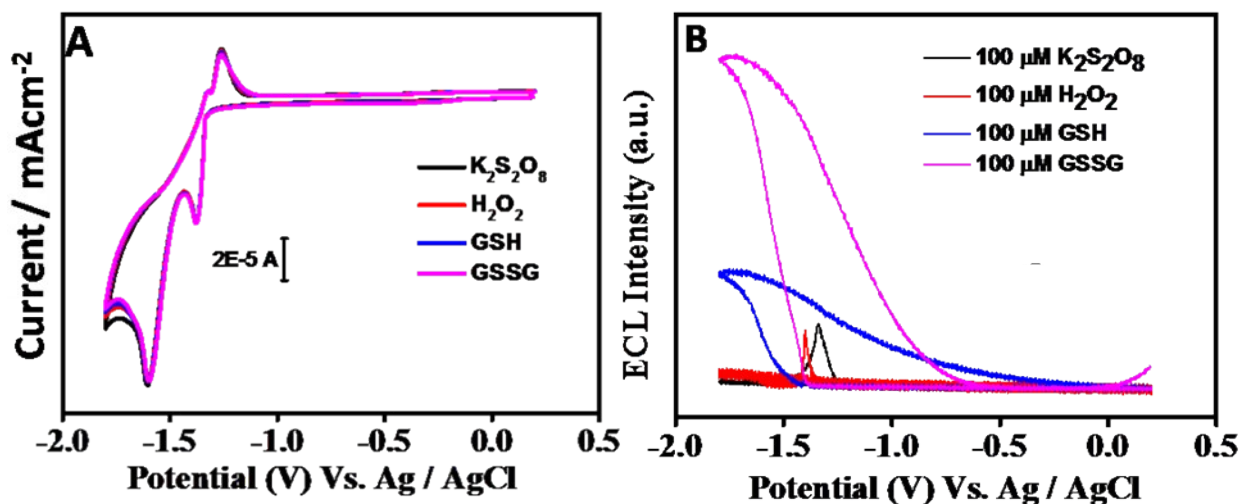


Fig. S3CV (A) and corresponding ECL (B) responses of 2 mMRu(bpy) $_3^{2+}$ at various co-reactants (100 μM) in PBS pH 9 on GCE at the scan rate of 0.05 V/s.

Comparison of $Ru(bpy)_3^{2+}$ ECL with various co-reactants.

Fig. S3 indicates the ECL responses of 2 mM $\text{Ru}(\text{bpy})_3^{2+}$ with various cathodic co- reactants such as $\text{K}_2\text{S}_2\text{O}_8$, H_2O_2 , GSH and GSSG on GCE surface in 0.1 M PBS (pH 9). We observed that $\text{Ru}(\text{bpy})_3^{2+}$ -GSH system shows two times higher ECL intensity than H_2O_2 and $\text{K}_2\text{S}_2\text{O}_8$. In the case of GSSG, we observed almost six times higher intense ECL than H_2O_2 and $\text{K}_2\text{S}_2\text{O}_8$ (See the table-S1). This means that the generation of $\text{GS}\cdot$ are highly stable and sufficient life time when compared with sulphate and hydroxyl radicals which are produced from peroxydisulphate and hydrogen peroxide reductions respectively. Also, confirms that GSH and GSSG molecules are best cathodic co-reactant for $\text{Ru}(\text{bpy})_3^{2+}$ system to generate ECL through reductive oxidation mechanism.

Coreactant(100 μM)	ECL Intensity (a.u.)
H_2O_2	0.09
$\text{K}_2\text{S}_2\text{O}_8$	0.10
GSH	0.21
GSSG	0.56

Table S1 comparison of ECL emission intensity for $\text{Ru}(\text{bpy})_3^{2+}$ with various cathodic co-reactants in argon saturated 0.1 M PBS (pH 9).

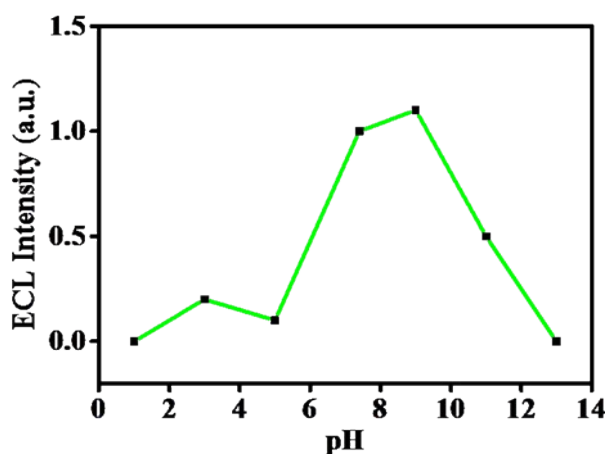


Fig. S4 Derivative plot of ECL response of 2 mM Ru(bpy)₃²⁺ with 50 μM GSH at various pH of PBS at the scan rate of 0.05 V/s.

Fig. S4 shows the effect of PBS pH on ECL of 2 mM Ru(bpy)₃²⁺ and 50 μM GSH at 0.05 V/s scan rate. As can be from Fig. S4, at lower pH value, the intensity of ECL emission is very low and gradually increases with increase in higher pH value and attains maximum ECL emission at pH-9. After that, again ECL emission decreases with increase higher pH value. This is because of producing reactive OH radicals are very low otherwise release H⁺ ions are excess which always limits the reduction and oxidation of Ru(bpy)₃²⁺ during cathodic scan potential at lower pH values and the same time excess OH radical produces at higher pH values which also quenches the intensity of ECL emission. The emission of ECL intensity of Ru(bpy)₃²⁺ and GSH system were studied by changing the concentrations of Ru(bpy)₃²⁺ and GSH. For that, we performed the experiments by keeping 50 μM GSH constant with increasing of Ru(bpy)₃²⁺ concentration on glassy carbon electrode surfaces at 0.05 V/s under argon saturated PBS at pH-9 (Fig. S5). At low concentrations of Ru(bpy)₃²⁺, no ECL emission observed due to the adsorption and precipitation of Ru(bpy)₃¹⁺ ²⁰. However, the observed ECL was significantly increased up to 2mM of Ru(bpy)₃²⁺ with 50 μM GSH and after that there is no significant increase of ECL emission peak at 1.5V.

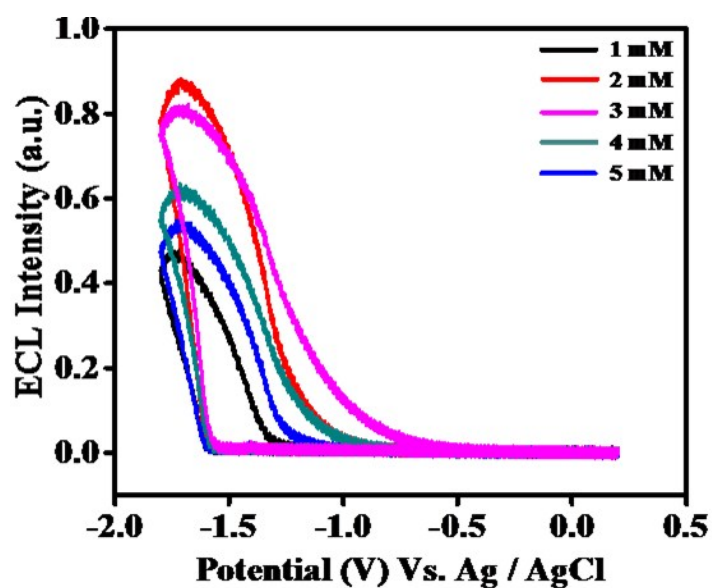


Fig. S5 ECL response of various concentrations of $\text{Ru}(\text{bpy})_3^{2+}$ (1 to 5 mM) with constant 50 μM GSH at in PBS at 0.05 V/s on GCE.

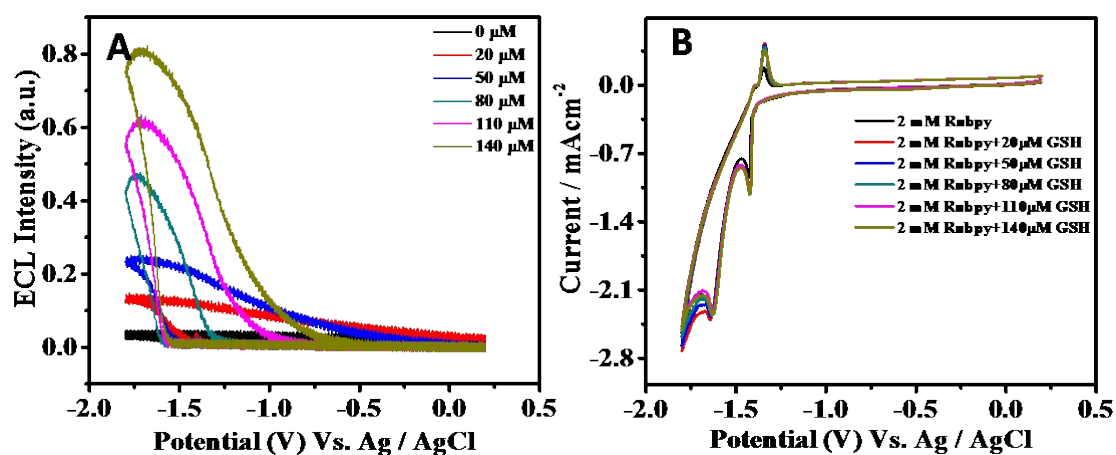


Fig. S6(A) ECL and (B) CV responses of 2 mM $\text{Ru}(\text{bpy})_3^{2+}$ with various concentration of GSH (0 to 140 μM) in PBS pH 9 on GCE at 0.05 V/s.

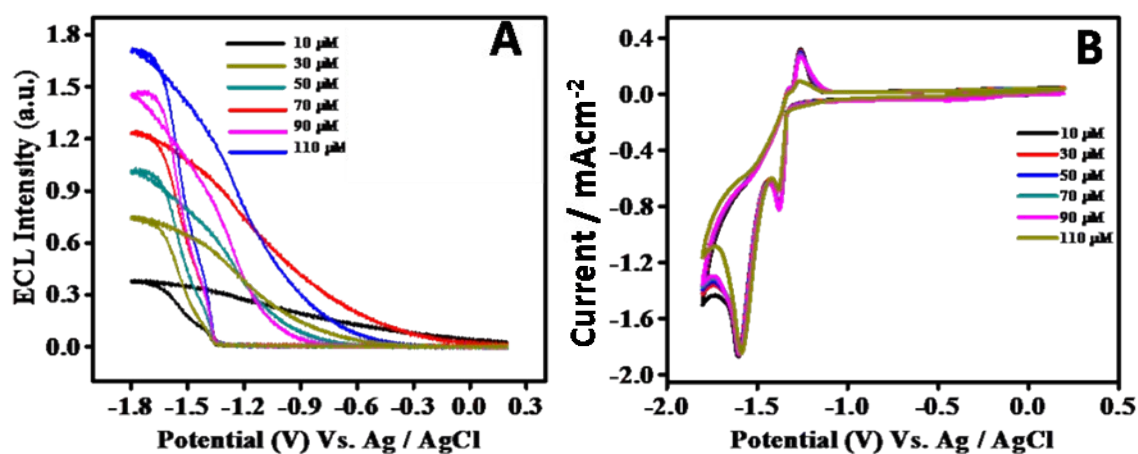


Fig. S7(A) ECL, (B) CV responses of 2 mM Ru(bpy)₃²⁺ at various concentration of GSSG (10 to 110 μM) in PBS pH 9 on GCE at 0.05 V/s.

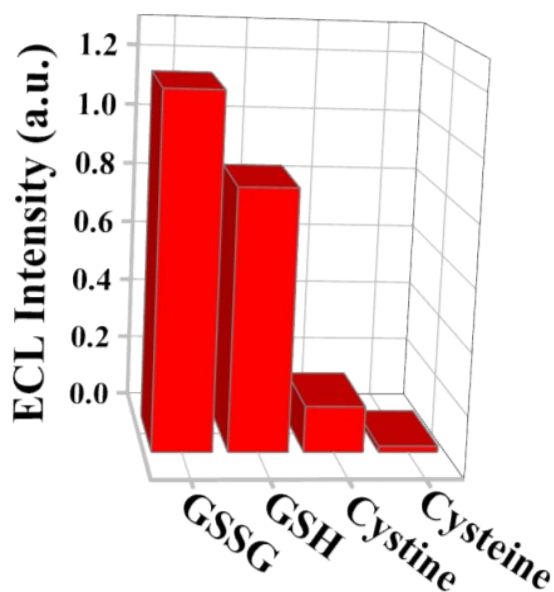


Fig. S8Plot of ECL intensity vs 50 μM of various bio-thiol molecules like GSSG, GSH, Cystine and Cysteine using 2 mM Ru(bpy)₃²⁺ in argon saturated 0.1 M PBS (pH 9).