Persistent generation of hydroxyl radicals in Tris-Co(II)complex-H\textsubscript{2}O\textsubscript{2} system for long-lasting multicolor chemical lights

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Experimental section

Chemicals and materials. 10 mM luminol stock solution was prepared by dissolving luminol (J&K Scientific Ltd., China) into the 0.1 M NaOH solution. 10 mM Co(NO\textsubscript{3})\textsubscript{2} stock solution and 100 mM Tris stock solution were prepared by dissolving Co(NO\textsubscript{3})\textsubscript{2}•6H\textsubscript{2}O (Beijing chemical reagent company, China) and Tris (Solarbio Science & Technology Co., Ltd., China) into ultrapure water (18.2 MU cm, Milli Q, Millipore, Barnstead, CA, USA), respectively. 5% Ti(SO\textsubscript{4})\textsubscript{2} solution was prepared by dissolving Ti(SO\textsubscript{4})\textsubscript{2} into the 3 M H\textsubscript{2}SO\textsubscript{4} solution. 30% (v/v) H\textsubscript{2}O\textsubscript{2} was obtained from Beijing chemical reagent company and the working solution was prepared fresh daily before the experiment. Acetone solution was precooled at 4 °C. Superoxide Dismutase (SOD) was obtained from Solarbio Science & Technology Co., Ltd. t-BuOH was purchased from Beijing chemical reagent company. Methocel K4M and Methocel K15M (HPMC) were purchased from Dow chemical and stored in a desiccator. All the chemicals in the experiment were ensured to be analytical grade. The solution of Tris-Co(II) complexes was prepared by mixing Co(NO\textsubscript{3})\textsubscript{2} solution with Tris solution in different ratios at room temperature.

Optimization of the chemical light system. Tris-Co(II) complex solution was prepared by mixing cobalt nitrate solution with Tris solution. After mixing Tris-Co(II) complex solution with luminol solution and H\textsubscript{2}O\textsubscript{2}, an intensive and long-persistent light emission appeared, which
could be observed by naked eye in dark environment (Fig. S1). The CL kinetic behaviour of the proposed system was then investigated by a static injection method in a BPCL luminescence analyzer.\textsuperscript{1} The optimal concentration of each component was priority considered. As shown in Fig. S2, the CL intensity and the duration time of the proposed system showed an increase with increasing the concentration of Co(II) from 0.1 mM to 0.25 mM. However, the higher concentrations of Co(II) did not favour the longer CL emission and shorten the duration time of the proposed system. In addition, the CL emission of the proposed system was also depending on the concentration of H\textsubscript{2}O\textsubscript{2} (Fig. S3). The duration time of proposed system showed an increase with the increase of H\textsubscript{2}O\textsubscript{2} concentration from 2.5 mM to 5.0 mM. When the concentration of H\textsubscript{2}O\textsubscript{2} was higher than 5.0 mM, the CL emission of proposed system became unstable. Moreover, the duration time of CL signals in the luminol-Tris-Co(II) complex-H\textsubscript{2}O\textsubscript{2} system was gradually increased in the presence of 10 mM to 30 mM Tris, but the CL inhibition was observed when the concentrations of Tris were above 30 mM (Fig. S4). This phenomenon might be ascribed to the fact that the reactive sites of Co(II) could almost be occupied in the presence of excessive chelating agents, leading to an inhibition of ·OH generation.\textsuperscript{2} Moreover, the pH value of luminol-Tris-Co(II) complex-H\textsubscript{2}O\textsubscript{2} system was also considered (Fig. S5). According to the result, the proposed system showed the longer CL duration time with an increased pH. Since an exorbitant pH (>12.5) could cause the hydrolysis of Co(II) in aqueous solution, we chose the pH=12 system as the optimal pH for further investigation.

**Properties of the chemical light system.** A 100 μL, 20 mM H\textsubscript{2}O\textsubscript{2} solution was injected into a mix solution containing 100 μL luminol (10 mM), 200 μL Tris-Co(II) solution for the long-persistent CL. Moreover, 100μL, 20 mM H\textsubscript{2}O\textsubscript{2} solution was injected into a mix solution
containing 100 μL luminol (10 mM), 100 μL Co(NO₃)₂ (1 mM) and 100 μL H₂O for the comparison. The concentration dependence of each monomer were also investigated to achieve longer and more stable CL. In addition, in the CPPO-BPEA luminescence system, 10 mM CPPO and 0.2 mg/mL BPEA were prepared by diluted each monomer into the ethyl acetate solution, respectively. As a sensitizer, sodium salicylate was dissolved in deionized water to gain a 1.0 M solution. 100 μL, 0.2 M H₂O₂ solution was injected into the two-layer system containing 100 μL CPPO (10 mM), 100 μL BPEA (0.2 mg/mL) and 100 μL sodium salicylate (1.0 M). The stability of CL in luminol tablet-Tris-Co(II)-PVP-H₂O₂ system was also conducted by periodically taking 500 μL of the reaction liquid into the CL quartz vial and monitored. All the CL signals were monitored by a BPCL luminescence analyzer. The photomultiplier tube (PMT) set a working voltage of -720 V for the CL detection and the integration time of the BPCL analyzer was set at 0.1 s per spectrum. Digital images were shoot by iPhone 8 and the photos were taken with an exposure time of 1/6 s. The luminescence spectra of luminol–Tris-Co(II) complex–H₂O₂ system and the CRET spectra were obtained by the F-7000 fluorescence spectrophotometer with its light source off. The measurements for the luminance of CPPO–BPEA–sodium salicylate–H₂O₂ system and our proposed chemical light systems were measured by Konica Minolta CS-2000 spectroradiometer.

**ESR measurements.** ESR signals of ·OH were measured on an ESR spectrometer (JEOL, JES-FA 200 spectrometer, Tokyo) at 22 °C. DMPO was used as a spin-trap agent, and 10 μL DMPO (~8.97 M) was added into 990 μL solution containing Tris-Co(II)-H₂O₂ system or Co(II)-H₂O₂ system at different concentrations. The reaction media was periodic collected by capillary tube and detected by ESR spectrometer.
**Luminol tablets and the release efficiency.** The luminol tablets were prepared by direct compression process. HPMC (containing Methocel K4M and Methocel K15M in different mass ratio), Mgst and luminol were mixed with a mass ratio of 200: 2: 5. The mixed powder was fully grinded and tableted by the punching machine. The net weight for each tablet was controlled to 0.5 g. For the release efficiency test, the prepared tablets were placed into a 20 mL solution containing 0.025 M NaOH, respectively. 10 μL solution was periodic collected and diluted to 1000 μL for the fluorescence measurements. Fluorescence of luminol solutions were measured by an F-7000 fluorescence spectrophotometer by setting 375 nm as the excitation wavelength. The slit width of the excitation and the emission were set at 5.0 nm and the scanning rate was 1200 nm/min. The calculation of luminol concentration was based on the standard curve (Fig. S15).

**GC-MS analysis**

GC-MS analysis were performed on a system consisting of the Thermo ISO single quadruple MS and the Thermo TRACE 1300 Gas chromatograph to determine the existence of formaldehyde in Tris-Co(II) complex-H$_2$O$_2$ system. TG-WAXMS column and EI source was used for GC-MS analysis. Initially, 20 mL reaction media containing 30 mM Tris, 0.25 mM Co(II) and 5 mM H$_2$O$_2$ was placed in a head space bottle. After 40 min reaction, 0.2 μL sample from the reaction media was injected into the gas chromatograph.

**H$_2$O$_2$ encapsulation and the release of H$_2$O$_2$.** PVP-H$_2$O$_2$ complex solution were prepared through the method which was previous reported. PVP (2.22 g) with different MW were dispersed into a 5 M H$_2$O$_2$ solution and stirred for 6 h at 4 °C. The colorless solution was transformed to a slightly transparent yellowish solution after the stirring process was finished. The as-prepared PVP-H$_2$O$_2$ solutions were then encapsulated in to a tube by using
semipermeable membrane as the parafilm and stored at 4 °C. In order to prove the sustained-release of H$_2$O$_2$ in the proposed tube, a traditional titanium sulfate method was carried out to quantify the H$_2$O$_2$ in the encapsulated PVP-H$_2$O$_2$- H$_2$O system.\(^3\) After the encapsulated PVP-H$_2$O$_2$ tube was placed into 20 mL H$_2$O solution, 10 μL solutions was periodic collected from the reaction media and diluted into a solution containing 990 μL acetone, 0.1 mL 5 % Ti (SO$_4$)$_2$ and 0.2 mL ammonia, respectively. For the accurate measurement of the absorbance at 415 nm, the turbid solutions were further diluted by 3 M H$_2$SO$_4$ to achieve a transparent state. After 5 minutes reaction, light yellow solutions were transferred to a quartz cell and measured on a Shimadzu UV−3600 spectrophotometer (Tokyo, Japan), respectively. Finally, the variation of H$_2$O$_2$ concentration in the reaction media was calculated based on the standard curve of H$_2$O$_2$ (Fig. S16).
Supporting Figures

**Fig. S1** The CL emission of luminol-Tris-Co(II)-H$_2$O$_2$ system.
**Fig. S2** Influence of Co(II) concentration on the luminol (2.5 mM) CL reactions in the presence of 30 mM Tris and 5.0 mM H₂O₂ under alkaline condition, PMT Voltage = -720 V.
Fig. S3 Influence of H$_2$O$_2$ concentration on the luminol (2.5 mM) CL reactions in the presence of Tris (30 mM)-Co(II) (0.25 mM) complexes under alkaline condition, PMT Voltage = -720 V.
Fig. S4 Influence of Tris concentration on the luminol (2.5 mM) CL reactions in the presence of 5.0 mM H₂O₂ and 0.25 mM Co(II) under alkaline condition, PMT Voltage = -720 V.
Fig. S5 Influence of pH value in the luminol (2.5 mM)-Tris (30 mM)-Co(II) (0.25 mM)-H$_2$O$_2$ (5.0 mM) system, PMT Voltage = -720 V.
Fig. S6 Effects of NaN$_3$ (100 mM), t-BuOH (50 mM) and SOD (0.5 mM) as radical scavengers on the CL reactions of luminol–Tris-Co(II) complex–H$_2$O$_2$ system under alkaline condition, PMT Voltage = -720 V.
**Fig. S7** (A) ESR spectra of DMPO-·OH adduct in the Co(II) (0.25 mM)-H₂O₂ (5.0 mM) system. DMPO-·OH peaks are depicted by stars. (B) Relative FL kinetic curve of fluorescein sodium salt (10 μM) in the Co(II) (0.25 mM)-H₂O₂ (5.0 mM) or Tris (30 mM)-Co(II) (0.25 mM) complex-H₂O₂ (5.0 mM) system under alkaline condition. F₀ is the initial fluorescence emission of 10 μM FSS solution, F is the fluorescence emission of 10 μM FSS at different time during the reaction process. Ex = 490 nm, PMT Voltage = 700 V. (C) ESR spectra of DMPO-·OH adduct in the Tris (30 mM)-Co(II) (0.25 mM) complex-H₂O₂ (5.0 mM) system under alkaline condition. DMPO-·OH peaks are depicted by stars. (D) UV-vis spectra variation of Rh B (10 μM) in Tris (30 mM)-Co(II) (0.25 mM) complex-H₂O₂ (5.0 mM) system under alkaline condition.
**Fig. S8** GC chromatography and the mass spectra (inset) of the media sample in Tris-Co(II)-H$_2$O$_2$ system.
Fig. S9 (a) Influence of 0–0.25 mM ethanolamine on the luminol (2.5 mM) CL reactions in the presence of 5.0 mM H$_2$O$_2$ and 0.25 mM Co(II) under alkaline condition. (b) Influence of 12.5–50.0 mM ethanolamine on the luminol (2.5 mM) CL reactions in the presence of 5.0 mM H$_2$O$_2$ and 0.25 mM Co(II) under alkaline condition. PMT Voltage = -720 V.
Fig. S10 (a) Influence of 0–0.025 mM EDTA on the luminol (2.5 mM) CL reactions in the presence of 5.0 mM H₂O₂ and 0.25 mM Co(II) under alkaline condition. (b) Influence of 0.05–0.25 mM EDTA on the luminol (2.5 mM) CL reactions in the presence of 5.0 mM H₂O₂ and 0.25 mM Co(II) under alkaline condition. PMT Voltage = -720 V.
Fig. S11 Influence of excess adding luminol (2.5 mM, final concentration) and H₂O₂ (5 mM, final concentration) on the proposed CL system after the proposed system was reacted for ~30 min.
Fig. S12 The release efficiency of the prepared luminol-HPMC-Mgst tablets in 0.025 M NaOH solution.
Fig. S13 The cumulative concentration of released H₂O₂ in PVP (10 K)-H₂O₂ system, PVP (40 K)-H₂O₂ system and PVP (150 K)-H₂O₂ system.
Fig. S14 The stability of CL intensity in eosin (100 μg/mL) based CRET system and acriflavin (25 μg/mL) based CRET system recorded by BPCL luminescence analyzer.
Fig. S15 The standard curve of FL intensity at 415 nm of luminol versus the concentration of luminol
**Fig. S16** The standard curve of absorbance at 415 nm of Titanium peroxide versus the concentration of H$_2$O$_2$. 

The equation of the line is $y = 3.0599x + 0.1609$ with $R^2 = 0.9924$. 

[Graph showing the line and data points]
**Fig. S17** The image of luminol tablet, Tris-Co(II) complexes solution and encapsulated $\text{H}_2\text{O}_2^-\text{PVP}$ tube (From left to right).
Table S1 CRET efficiency of luminol-Tris-Co(II)-H$_2$O$_2$ system in the presence of different concentrations of eosin and acriflavin.

<table>
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<th>$C_{\text{acriflavin}}$ (µg/mL)</th>
<th>CRET efficiency (%)</th>
<th>$C_{\text{eosin}}$ (µg/mL)</th>
<th>CRET efficiency (%)</th>
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**Table S2** Luminance characteristics for CPPO–BPEA–sodium salicylate–H$_2$O$_2$ system and luminol–Tris-Co(II) complex–H$_2$O$_2$ system in the presence of different fluorophores under alkaline condition.

<table>
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<th>System</th>
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<td>CPPO–BPEA–sodium salicylate–H$_2$O$_2$</td>
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<td>Luminol–Tris-Co(II) complex–eosin–H$_2$O$_2$</td>
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References

