

Control of peroxyoxalate chemiluminescence by nitrogen-containing ligand quenching: turning off and on by ligand-metal ion host-guest interactions

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Abstract:

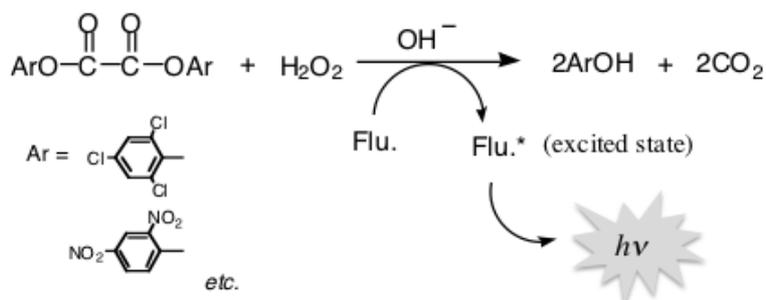
The control of peroxyoxalate chemiluminescence (PO-CL) by the coordination of nitrogen-containing ligands and metal cations was investigated. Turning the CL off and on was done by PO-CL using 15-monoazacrown-5-tethered anthracene and alkali metal ions. CL quenching and regeneration was also observed in the separated molecular system of 15-monoazacrown-5 and the fluorophores. CL quenching by a number of ligands bearing dipicolylamino groups was evaluated by these PO-CL reactions and found to be closely related to their oxidation potentials, which is dependent on the Weller rate law for electron exchange and this provides strong support for the existence of the CIEEL PO-CL process. When Zn^{2+} or Cu^{2+} are added to the PO-CL system quenched by the ligand, *N*-[2-(2,2'-dipicolylamino)ethyl]aniline, CL was turned on because the electron donating ability of the ligands was modulated. This was controlled by the coordination of the studied metal ions and, therefore, this system results in CL because of host-guest interactions.

Keywords: peroxyoxalate chemiluminescence, quenching, CIEEL, ligand, dipicolylamino group

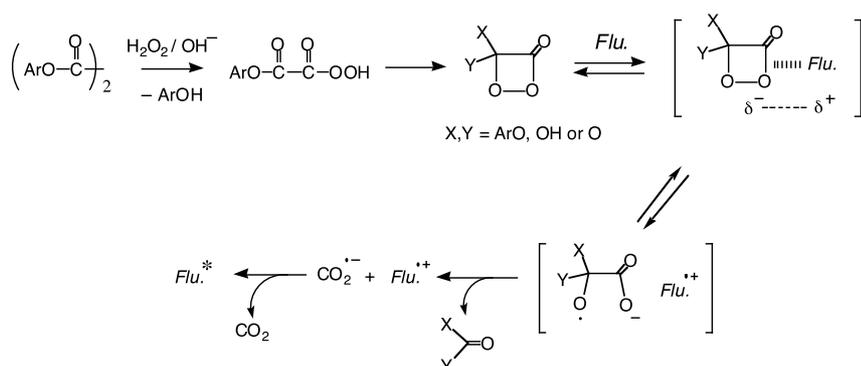
1. Introduction

Chemiluminescence (CL) is becoming important in many applications beyond chemical analyses, such as in biological diagnostics, because an electric light source is not required to decrease the background noise, which results in the detection of light only from the fluorescent target species.¹ Of all the CL reactions, peroxyoxalate chemiluminescence (PO-CL) that is

generated from the reactions of active oxalates bearing phenol moieties that contain electron withdrawing groups such as bis(2,4,6-trichlorophenyl) oxalate (TCPO) or bis(2,4-dinitrophenyl) oxalate (DNPO) and alkaline hydrogen peroxide in the presence of various fluorophores is the most effective (Scheme 1). An enormous amount of effort has been put into mechanistic studies² and into analytical applications.³



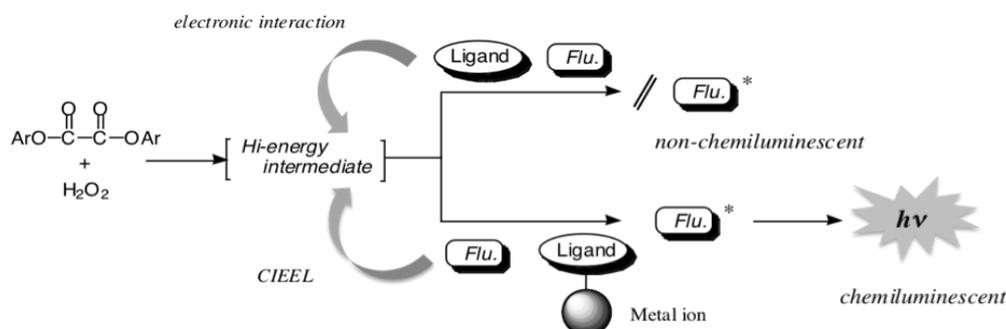
Scheme 1. Peroxyoxalate chemiluminescence (PO-CL) reaction.



Scheme 2. A plausible CIEEL process during PO-CL.

A chemically initiated electron exchange luminescence (CIEEL) process has been suggested to be a plausible explanation for the chemiexcitation pathway during PO-CL (Scheme 2).⁴ In this process the electron exchange between high energy intermediates (acceptors), 1,2-dioxetanones or 1,2-dioxetanedione, and fluorophores (donors) is a crucial step. Based on this convincing mechanism we hypothesize that if additives control the CIEEL process by an electronic interaction the CL might turn off and on under suitable conditions. To turn the CL off, CL quenching should be dependent on the electron donating ability of the organic additives and this electron donor additive might interrupt the CIEEL process and disrupt the electron exchange between the high energy intermediate and a fluorophore. Therefore, an electron donating additive and a fluorophore would compete during their interaction with the dioxetanones. The additive would, therefore, behave as a CL quencher. When the additive is a nitrogen-containing ligand, the complexation of a suitable metal ion to the ligand can control its

electron donating ability and the metal ion will become important in switching the CL on. If this process operates in an intermolecular manner there is a possibility that a good choice of a combination of additives will enable the visualization of various molecular interactions by the CL technique, as shown in Scheme 3.



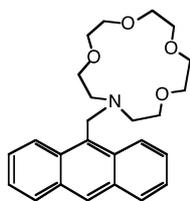
Scheme 3. Concept of CL detection during host-guest interactions.

In this paper, CL control by a host-guest interaction during PO-CL reactions was investigated using several nitrogen-containing ligands. Efforts were directed toward finding a relationship between the electronic effect of the ligands and CL quenching during the CIEEL process. This was applied to the CL detection of the host-guest interaction between the ligands and the metal ions. This system resembles a displacement indicator assay,⁵ which is a fluorescence detection method in which analyte binding leads to indicator displacement to yield optical signal modulation. However, this is the first time that such a noncovalent system has been used in a CL reaction.

2. Results and discussion

2.1. CL control with azacrown ethers in an intra- and intermolecular fashion

15-monoazacrown-5-tethered anthracene (**1**) is a well-known fluorescent compound⁶ capable of changing its fluorescence intensity upon chelation by suitable metal ions and this fluorescence modulation is due to the control of fluorescence quenching by intramolecular photoinduced electron transfer (PET). When this compound is used as a fluorophore in PO-CL the CL is also expected to be controlled by the interaction with metal ions.



1

Before the application of **1** to a chemiluminescence reaction, its fluorescence behavior upon metal ion complexation was explored. The ionophore 15-monoazacrown-5 can incorporate various alkali- and alkaline earth metal ions and among them sodium and calcium cations are most effective in increasing the fluorescence intensity of 15-monoazacrown-5-tethered fluorescent molecules by interrupting PET fluorescence quenching.⁷ The fluorescence spectra of **1** were measured under various Na⁺ concentrations because the interaction of **1** and Na⁺ has not been well documented. As shown in Fig. 1, the fluorescence intensity increased markedly as the Na⁺ concentration increased despite the required large excess of Na⁺, which led us to apply this event to the chemiluminescence system.

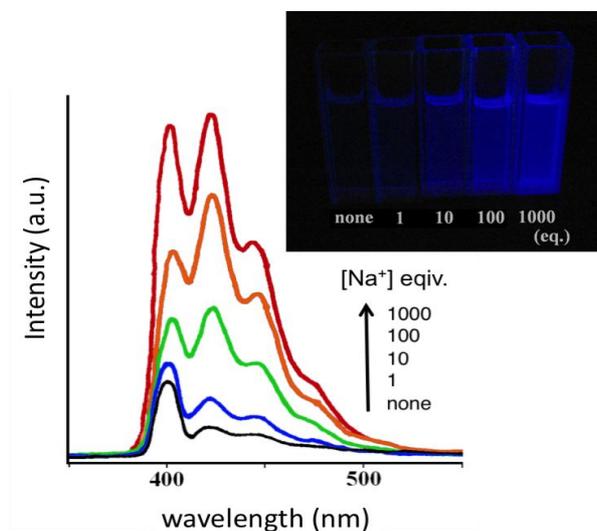


Fig. 1. Fluorescence spectra of **1** as a function of [NaClO₄].
[**1**] = 1.0 × 10⁻⁴ M in THF.

Using **1** as a fluoroionophore the CL reaction was carried out using several reagents including bis(4-chlorophenyl) oxalate (4-CPO), hydrogen peroxide and tetrabutylammonium hydroxide (TBAH) as a metal free base. The reaction without any metal cation in aqueous THF gave a weak CL emission, as expected from the fluorescence spectrum shown in Fig. 1. In contrast, the addition of NaClO₄ increased the CL quantum yield (Φ_{CL}) 10 times compared to the system without Na⁺. Among the alkali metal ions tested in this study, Na⁺ was the most effective in

increasing the Φ_{CL} , as shown in Fig. 2, which is based on the advantageous incorporation of Na^+ into the azacrown cavity.⁷

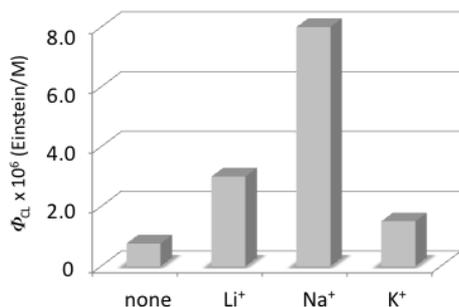


Fig. 2. PO-CL for compound **1** and the alkali metal ions. **1** ($7.5 \times 10^{-4} \text{M}$), 4-CPO ($7.5 \times 10^{-5} \text{M}$), LiClO_4 , NaClO_4 and KClO_4 ($7.5 \times 10^{-3} \text{M}$), H_2O_2 ($7.5 \times 10^{-3} \text{M}$) and TBAH ($7.5 \times 10^{-4} \text{M}$) in $\text{H}_2\text{O}/\text{THF}$ (1:3).

It is noteworthy that turning the CL off or on could also be done in an intermolecular fashion using molecules where a fluorophore and an ionophore are not covalently linked such as free anthracene and 15-monoazacrown-5. Turning the CL off was done by adding 15-monoazacrown-5 to the CL system containing 4-CPO, TBAH, hydrogen peroxide and anthracene as a fluorophore. The CL intensity decreased with an increase in the 15-monoazacrown-5 concentration and it finally disappeared when 50 equivalents of azacrown ether was added to the oxalate (Fig. 3), and hence 15-monoazacrown-5 acted as a CL quencher during this PO-CL reaction. Upon the addition of NaClO_4 to the non-luminescent PO-CL system that was quenched by the azacrown, Φ_{CL} recovered to a value 2.3 times higher than the system without Na^+ while a Φ_{CL} value 100 times higher was obtained when coumarin-7 was used as a fluorophore (Fig.3, inset). During CL quenching an electronic interaction takes place between the ligands and the high energy intermediates rather than fluorescence quenching between the ligands and the fluorophores by intermolecular PET because the solutions containing the fluorophores and the azacrown do not give significant fluorescence quenching even when using 100 times more azacrown at which the CL emission from the PO-CL reaction would be completely quenched. Thus, in this PO-CL reaction the azacrown is an electron donor to the high energy peroxyoxalate intermediates and it does not donate electrons to the electronically excited fluorophores. The high energy intermediates such as dioxetanones act as electron acceptors to inhibit the chemiexcitation of the fluorophores resulting in CL quenching. This process involves the CL turning off or on, and implies that the CL can be controlled by the host-guest interaction between the various ligands and metal ions.

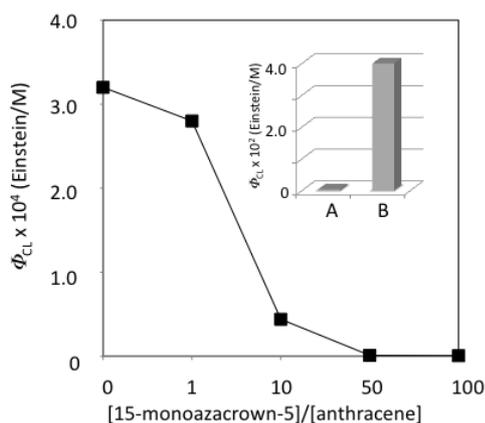


Fig. 3. PO-CL quenching by 15-monoazacrown-5 and CL recovery by the addition of coumarin-7 and Na^+ (inset). 4-CPO($1.0 \times 10^{-4} \text{M}$), anthracene($1.0 \times 10^{-3} \text{M}$), 5-monoazacrown-15($0-1.0 \times 10^{-1} \text{M}$), H_2O_2 ($1.2 \times 10^{-1} \text{M}$), TBAH($1.0 \times 10^{-3} \text{M}$) in $\text{H}_2\text{O}/\text{THF}$ (1:3). Inset: A; 4-CPO($0.75 \times 10^{-4} \text{M}$), H_2O_2 ($0.25 \times 10^{-2} \text{M}$), TBAH($0.25 \times 10^{-4} \text{M}$), 15-monoazacrown-5($0.75 \times 10^{-3} \text{M}$), coumarin-7($0.75 \times 10^{-4} \text{M}$). B; addition of NaClO_4 ($0.75 \times 10^{-1} \text{M}$).

2.2. Preparation and oxidation potentials of the ligands

Dipicolylamino groups are useful as chelators in fluorescence chemosensors⁸ for the detection of biologically important metal ions, because of their strong electron donating ability, as well as their strong coordination of particular metal ions with excellent selectivity. We applied this functionality containing multiple nitrogen atoms to the PO-CL system and its intensity was controlled by the metal ion-ligand interaction (*vide infra*). The ligands (**2-4**) shown in Fig. 4 were prepared by a modification of the established method^{8c-d, 9} using the reactions of 2-(chloromethyl)pyridine and the corresponding amines. To investigate the effect of these ligands on CL quenching their oxidation potentials (E_{ox}) were measured by cyclic voltammetry. As shown in Table 1, the E_{ox} 's of the ligands are dependent on their electronic nature as modulated by the attached substituents for ligands **2a-c** and **3a-c**. The electron donating substituents increase the electron releasing ability, which leads to a lower E_{ox} . Ligand **4** has the highest E_{ox} among all the ligands probably because of its radical cation that is generated after the release of an electron, which causes it to be less stable as a result of the lack of a directly linked aromatic ring on the nitrogen atom.

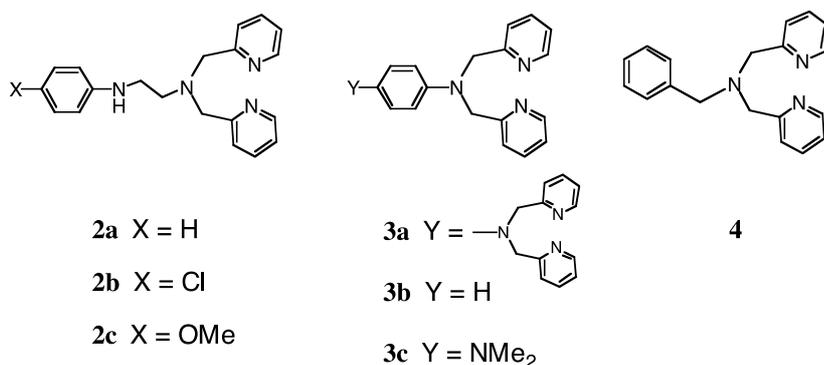


Fig. 4. Structures of the ligands used in this study.

Table 1.

Oxidation potentials (*Eox.*) of ligands **2,3**, and **4**

Ligand	<i>Eox.</i> (V) ^a
2a	0.66
2b	0.70
2c	0.45
3a	0.35
3b	0.87
3c	0.23
4	0.93

^a The measurements were carried out in THF/H₂O (1:1). The ligand concentration was 0.01 M and the electrolyte was Bu₄NClO₄. Platinum working electrode, Ag/AgCl reference electrode. Scan rate of 10 mVs⁻¹.

2.2. CL quenching by the ligands containing dipicolyl groups

To investigate the role of the series of ligands described above as CL quenchers systematically, the effect of ligand **2a** on PO-CL quenching was studied. PO-CL reactions that included TCPO as an oxalate, hydrogen peroxide, Na₂CO₃, four kinds of fluorophores and **2a** at various concentrations were carried out. As shown in Fig. 4, the CL intensity in the presence of fluorophores such as 9, 10-diphenylanthracene (DPA), perylene, rubrene, and coumarin-7 gradually decreased along with an increase in the amount of **2a**. The PO-CL intensity is known to depend on the electronic property as well as the fluorescent properties of the fluorophores.^{21, 4, 10} Therefore, the CL intensity decayed in different ways for each fluorophore, as shown Fig. 5. The CL was almost completely inhibited when a large excess of **2a** was added in all cases. It is important to note that **2a** might be superior to the fluorophores as an electron donor because of its much lower oxidation potentials compared to all the fluorophores used here (1.24, 0.96, 0.94,

1.11 (V) for DPA, perylene, rubrene, and coumarin-7, respectively).

For a systematic CL quenching study of the CIEEL process, a series of ligands (**2a-c**, **3a-c**, and **4**) were employed for the PO-CL reactions in the presence of 4-CPO and perylene as the common oxalate and fluorophore, respectively. Of the seven ligands investigated **2a-c**, **3a** and **3c** quenched the CL but **3b** and **4** were much less effective (Fig. 6). If the observed CL quenching involves an electronic interaction and the ligands compete with the fluorophores during the CIEEL process the effect of the ligands on CL quenching should be closely related to their E_{ox} 's. The CL ligand quenching trend agrees with this relation because **3b** and **4**, with a higher E_{ox} , are inferior to the other ligands with a lower E_{ox} during CL quenching.

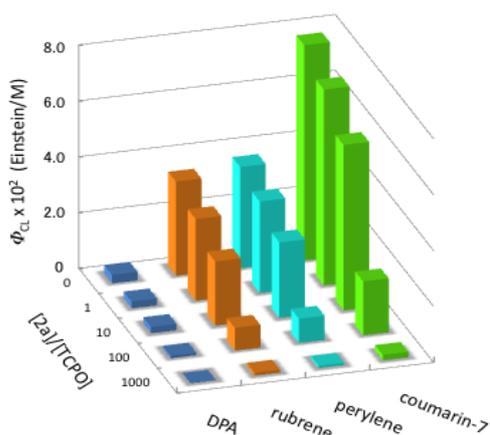


Fig. 5. CL quenching in the presence of **2a** during PO-CL using various fluorophores. TCPO (0.75×10^{-5} M), the fluorophore (0.75×10^{-4} M), H_2O_2 (0.25×10^{-2} M) and Na_2CO_3 (1.25×10^{-4} M) in THF/ H_2O (3:1) were used.

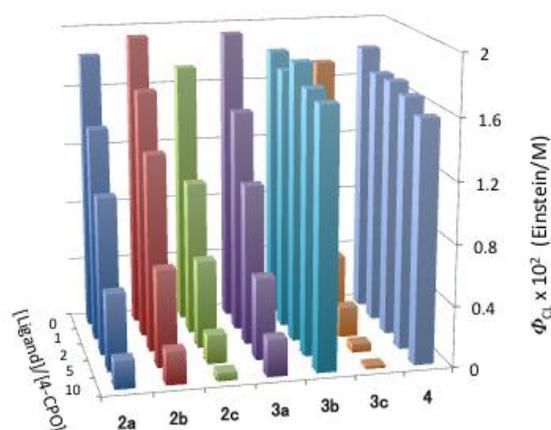


Fig. 6. Ratio of Φ_{CL} decrease because of the addition of ligands **2a**, **2b**, **2c**, **3a** and **3c** during PO-CL. 4-CPO (0.75×10^{-5} M), perylene (0.75×10^{-4} M), H_2O_2 (0.25×10^{-2} M) and Bu_4NOH (1.25×10^{-4} M) in THF/ H_2O (1:1) were used.

During the enhanced (indirect) CL, with PO-CL being typical, the high-energy intermediates interact with the fluorophores to generate excited fluorophores resulting in CL emission from

their fluorescence process. The kinetics of this process have been well established.^{2a} The double reciprocals of Φ_{CL} and the fluorophore concentration [F] are correlated by a single straight line. On the other hand, the Stern-Volmer equation has been applied to metal ion-induced CL quenching in a few cases with regards to their quantification.¹¹ Independently, we applied the kinetics of the enhanced CL to the present ligand-induced CL quenching by exploiting the elementary reaction of the CL quenching process involving the interaction between the high energy intermediate and the ligands. The obtained equation shown below resembles the Stern-Volmer equation, and it has a linear relationship between the double reciprocals of Φ_{CL} and [F],

$$1 / \Phi_{CL} = 1 / \Phi_r \Phi_F (1 + k_Q [L]/k_{ss}[F])$$

where Φ_r and Φ_F are the reaction yield and the fluorescence quantum yield of the fluorophore, respectively. k_{ss} is the rate constant for energy transfer from the high energy intermediate to the fluorophores and k_Q is a conditional CL quenching constant that is estimated as a measure of the CL quenching ability of the ligands. This equation indicates that the ligands behave as quenchers and a plot of the ligand concentration [L] vs. the reciprocal Φ_{CL} should give a straight line when using a constant [F]. In addition, the slope $k_Q/\Phi_r\Phi_Fk_{ss}[F]$ provides a relative k_Q for each ligand. Using various concentrations of **2a-c** and **3c** and by keeping [F] constant the Φ_{CL} 's of perylene-enhanced PO-CL from the reaction of 4-CPO and alkaline hydrogen peroxide were determined. As shown in Fig. 7, the plots of [L] and $1/\Phi_{CL}$

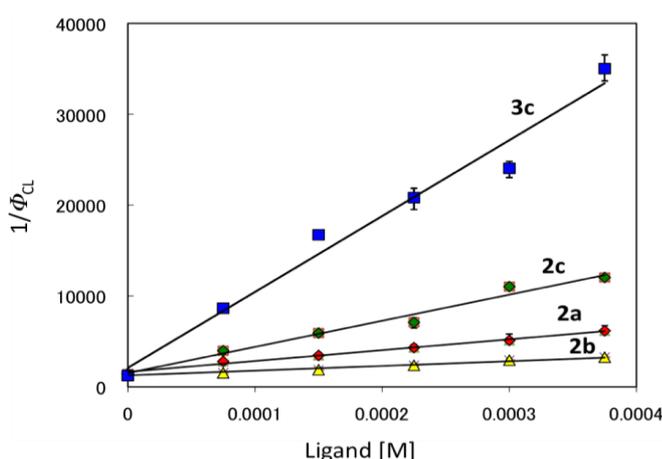


Fig. 7. Plot of CL quenching according to equation (1). 4-CPO (0.75×10^{-5} M), perylene (0.75×10^{-4} M), H_2O_2 (0.25×10^{-2} M) and Na_2CO_3 (5.0×10^{-3} M) in THF/ H_2O (1:1) were used. $R^2=0.975$ for **3c**, 0.979 for **2c**, 0.992 for **2a** and 0.962 for **2b**.

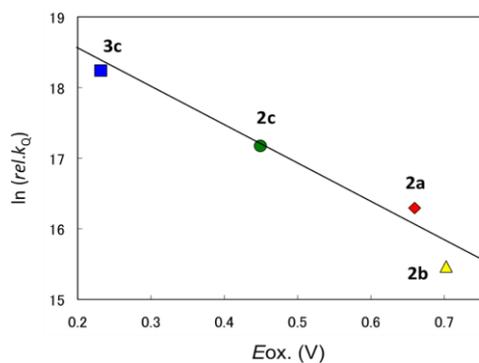


Fig. 8. Plot of $\ln(\text{rel. } k_Q)$ against the E_{ox} of the ligands.

form straight lines, which implies that the ligands interact with the high energy intermediates in a bimolecular manner and behave as CL quenchers during enhanced PO-CL. It is noteworthy that the linear relationship between $\ln(\text{rel. } k_Q)$ and the E_{ox} of the ligands is consistent with the Weller rate law¹² for electron exchange (Fig. 8), except for the weakly fluorescent ligand **3a** and the ligands **3b** and **4**, which were not effective CL quenchers. These results provide evidence for the involvement of an electron exchange process, the CIEEL process, during PO-CL from a different point of view as has been reported previously in the literature.⁴

2.3. Turning CL on by the interaction between ligands and metal cations

Because of the strong chelating effect of dipicolyl groups,¹³ ligands **2a-c** coordinate to suitable metal ions to modulate their electron donating ability, which affects the modulation of the CL intensity of PO-CL. Since Zn^{2+} is known to coordinate with the 2-(dipicolylamino)ethylamino ligand and this favorable complexation has been widely used for fluorescent chemosensors¹⁴ the Zn-salt was employed to turn CL on by its interaction with **2a-c**. As described before, these ligands quench the CL from the PO-CL reactions in the presence of various fluorophores (Fig. 6 for the TCPO-PO-CL reaction). The effect of Zn^{2+} on CL regeneration was examined using various Zn^{2+} concentrations. In the rubrene-enhanced TCPO-PO-CL reaction, Φ_{CL} increased sharply at an equivalent concentration between **2a** and Zn^{2+} and at this point the maximum CL was observed. The Φ_{CL} recovered to 20% of its initial value compared to the system without **2a** and Zn^{2+} . The use of perylene as a fluorophore instead of rubrene led to the regeneration of CL at up to 56%, 44%, and 40% at the maximum points by the coordination of **2a**, **2b** and **2c** with Zn^{2+} in 1:1 ratios (Fig. 9). However, the much higher concentration of the Zn-salt decreased the CL intensity probably because of a change in the pH of the solutions, which unfortunately restricts the application of this system to the quantification of Zn^{2+} . On the other hand, the effect of highly fluorescent coumarin-7 in turning the CL on was

studied using a lower concentration of **2a** (10^{-4} M), and 54% CL recovery was obtained. Therefore, the metal ion-ligand complexation between these additives can be determined at 10^{-4} M by this CL system when suitable conditions are used. Attempts to find the host-guest interaction between **2a** and other metal ions were also made in the presence of Li^+ , Ca^{2+} , Mn^{2+} , Co^{2+} and Cu^{2+} and we found that Cu^{2+} with an ionic radius similar to Zn^{2+} also responded to **2a** (Fig. 10). Here, we show only a few examples of CL regeneration by host-guest interactions and these results show that host-guest interactions can be determined using CL, as shown in Scheme 3.

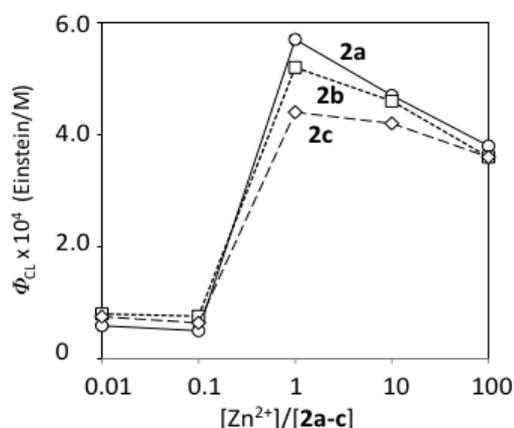


Fig. 9. Turning CL on by the interaction between **2a-c** and Zn^{2+} . TCPO (1.0×10^{-4} M), Na_2CO_3 (1.0×10^{-4} M), H_2O_2 (1.2×10^{-2} M), Perylene (1.0×10^{-4} M) and ligands (1.0×10^{-3} M) were used.

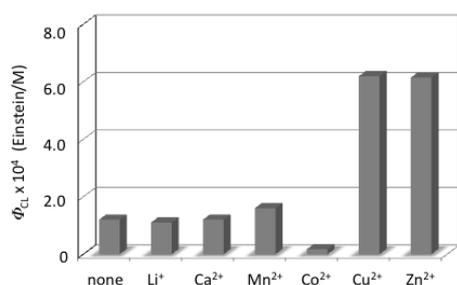


Fig. 10. Metal ion selective turning on of CL. TCPO (0.75×10^{-5} M), perylene (0.75×10^{-4} M), **2a** (0.75×10^{-3} M), metal ion (0.75×10^{-3} M, LiClO_4 , $\text{Ca}(\text{ClO}_4)_2$, MnSO_4 , CoSO_4 , CuSO_4 , ZnSO_4), H_2O_2 (0.25×10^{-2} M) and Na_2CO_3 (0.25×10^{-4} M) in THF/ H_2O (1:1) were used.

3. Conclusions

A new aspect of PO-CL reactions as a result of the combination of various fluorophores, ligands bearing dipicolylamino groups and metal ions is demonstrated. The ligands act as CL quenchers by an electronic effect, but the CL turns on in the presence of suitable metal ions because of the control of the electronic effect by the complexation of metal ions with the ligands. Strong evidence for the CIEEL process was also found based on the relationship between the CL quenching and the oxidation potentials of the ligands. With a good choice of ligands and fluorophores various combinations are possible as long as well designed electron donating ligands that are suitable for target molecules are available, and an investigation is currently underway in our laboratory.

4. Experimental

4.1. General

Melting points were determined using a hot stage microscope apparatus and were uncorrected. ^1H and ^{13}C NMR spectra were recorded on a FT-NMR spectrometer at 400 MHz and 100 MHz, respectively. The chemical shifts (δ) of the ^1H and ^{13}C NMR spectra are reported in ppm downfield from TMS as an internal standard or from the residual solvent peak. Coupling constants (J) are reported in Hz. Fluorescence quantum yields were estimated using 9,10-diphenylanthracene ($\Phi_{\text{F}} = 0.91$ in benzene) as a standard. Chemiluminescence quantum yields (Φ_{CL}) were measured by a photon-counting method using a Hamamatsu Photonics R464 photomultiplier connected to a photon-counting unit (C3866) and a photon-counting board M8784 according to a previously reported procedure.²¹ Luminol chemiluminescence was used as a standard in DMSO for the calibration of the photomultiplier tube. Compound (**1**) was prepared by an established method according to the literature. TCPO and 4-CPO were prepared by reacting oxalyl chloride and the corresponding phenols in the presence of triethylamine in benzene and were used after recrystallization.

4.2. Preparation of azacrown-tethered anthracene (**1**) and ligands **2a-c**, **3a-c** and **4**

4.2.1. *N*-(9-anthylmethyl)monoaza-15-crown-5 (**1**). The reaction between 9-bromomethylanthracene (1.23 g, 4.6 mmol), 15-monoazacrown-5 (1.0 g, 4.6 mmol) and Na_2CO_3 (0.92 g, 8.7 mmol) was carried out in CH_3CN by heating at 80 °C for 20 h. After filtration and concentrating the filtrate purification was carried out by column chromatography (SiO_2 , ethyl acetate as an eluant) and compound **1** was obtained (0.58 g, 31%) as a viscous

yellowish oil. ^1H NMR (400 MHz, CDCl_3) δ 2.91(t, 4H), 3.57-3.66(m, 16H), 4.61(s, 2H), 7.42-7.52(m, 4H), 7.98(d, 2H), 8.93(s, 1H), 8.54(d, 2H).

4.2.2. *N*-(2-(2,2'-dipicolylamino)ethyl)aniline (**2a**). A solution of 2-bromoethylamine hydrobromide (1.1 g, 5.4 mmol) and aniline (3.0 g, 32 mmol) in toluene (8 ml) was stirred for 1 day at 120 °C under an argon atmosphere and the precipitated product was collected by filtration. The salt was then treated with a 20% aqueous NaOH solution and extracted with dichloromethane. The organic phase was dried over anhydrous Na_2SO_4 . After the Na_2SO_4 was filtered out and the solvent was distilled the crude product was further purified by column chromatography on silica gel (eluant: methanol) to afford *N*-phenylethylenediamine as a red-brown liquid. Yield: 87% (0.64 g); ^1H NMR (400 MHz, CDCl_3) δ 1.51 (br s, 3H), 2.93 (t, 2H), 3.17 (t, 2H), 6.63 (d, 2H), 6.70 (t, 1H), 7.17 (t, 2H). 2-(Chloromethyl)pyridine hydrochloride (1.8 g, 11 mmol) was treated with a 20% aqueous NaOH solution and extracted with dichloromethane and the organic phase was dried over anhydrous Na_2SO_4 . After Na_2SO_4 was filtered out and the solvent was evaporated off, a solution of 2-(chloromethyl)pyridine in benzene (3 ml) was added to a solution of *N*-phenylethylenediamine (0.69 g, 5.1 mmol), which was prepared as described above, and triethylamine (5.1 g, 51 mmol) in benzene (4 ml) and stirred for 17 h at 80 °C under an argon atmosphere. After being cooled to room temperature and the addition of diethyl ether, the inorganic by-products were removed by washing with a saturated NaHCO_3 solution and with brine. The organic phase was dried over anhydrous Na_2SO_4 . After the Na_2SO_4 was filtered out and the solvent was removed the crude product was purified by column chromatography on silica gel (eluant: ethyl acetate) to afford **2a** as a red-brown viscous liquid. Yield: 69% (1.1 g); ^1H NMR (400 MHz, CDCl_3) δ 2.86 (t, 2H), 3.17 (t, 2H), 3.88 (s, 4H), 6.58 (d, 2H), 6.65 (t, 1H), 7.07-7.18 (m, 4H), 7.41 (d, 2H), 7.60 (t, 2H), 8.54 (d, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ 41.80, 53.26, 60.73, 113.22, 117.34, 122.50, 123.54, 129.53, 136.84, 149.04, 149.53, 159.66; HRMS (EI): Calcd for $\text{C}_{20}\text{H}_{22}\text{N}_4$: 318.1844, Found: 318.1846.

4.2.3. *N*-[2-(2,2'-dipicolylamino)ethyl]-*p*-chloroaniline (**2b**). *N*-(chlorophenyl)ethylenediamine was prepared in a similar manner to the procedure described above using 2-bromoethylamine hydrobromide (1.1 g, 5.4 mmol) and *p*-chloroaniline (3.4 g, 27 mmol). Yield: 81% (0.75 g) as a brown liquid; ^1H NMR (400 MHz, CDCl_3) δ 1.45 (brs, 3H), 2.95 (t, 2H), 3.15 (t, 2H), 6.55 (d, 2H), 7.11 (d, 2H). Compound **2b** was prepared using 2-(chloromethyl)pyridine hydrochloride (1.5 g, 9.2 mmol), *N*-(4-chlorophenyl)ethylenediamine (0.75 g, 4.5 mmol) and triethylamine (4.5 g, 45 mmol) in a similar manner to that described above by heating for 3 h at 80 °C. Yield : 43% (0.69 g) as a brown viscous liquid; mp 86-88 °C; ^1H NMR (400 MHz, CDCl_3) δ 2.87 (t, 2H), 3.15 (t, 2H), 3.88 (s, 4H), 6.49 (d, $J=8.8\text{Hz}$, 2H), 7.07 (d, $J=8.8\text{Hz}$, 2H), 7.11-7.19 (m, 2H), 7.38 (d, 2H), 7.61 (t, 2H),

8.55 (d, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ 41.95, 53.00, 60.72, 114.22, 121.72, 122.54, 123.55, 129.29, 136.83, 147.68, 149.56, 159.55; HRMS (EI): Calcd for $\text{C}_{20}\text{H}_{21}\text{ClN}_4$: 352.1455, Found: 352.1468.

4.2.4. *N*-(2-(2,2'-Dipicolylamino)ethyl)-*p*-anisidine (**2c**). *N*-(methoxyphenyl)ethylenediamine was prepared in a similar manner to the procedure described above using 2-bromoethylamine hydrobromide (2.0 g, 9.9 mmol) and *p*-anisidine (4.0 g, 33 mmol). Yield: 88% (1.4 g) as a brown solid; ^1H NMR (400 MHz, CDCl_3) δ 1.45 (br s, 3H), 2.94 (t, 2H), 3.14 (t, 2H), 3.75 (s, 3H), 6.61 (d, 2H), 6.78 (d, 2H). Compound **2c** was prepared from 2-(chloromethyl)pyridine hydrochloride (2.1 g, 12 mmol), *N*-(4-methoxyphenyl)ethylenediamine (0.79 g, 4.7 mmol) and triethylamine (4.5 g, 45 mmol) in a manner similar to that described above by heating for 3 h at 80 °C. Yield: 26% (0.77 g) as a brown viscous solid; mp 37-41 °C; ^1H NMR (400 MHz, CDCl_3) δ 2.87 (t, 2H), 3.14 (t, 2H), 3.73 (s, 3H), 3.88 (s, 4H), 6.55 (d, $J=8.9$ Hz, 2H), 6.75 (d, $J=8.9$ Hz, 2H), 7.11-7.16 (m, 2H), 7.42 (d, 2H), 7.61 (t, 2H), 8.54 (d, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ 42.83, 53.45, 56.26, 60.80, 114.50, 115.26, 122.45, 123.49, 136.79, 143.40, 149.54, 152.30, 159.73; HRMS (EI): Calcd for $\text{C}_{21}\text{H}_{24}\text{N}_4\text{O}$: 348.1950, Found: 348.1984; Anal. Calcd for $\text{C}_{21}\text{H}_{24}\text{N}_4\text{O}$: C 72.39, H 6.94, N 16.08. Found: C 72.04, H 6.79, N 15.89.

4.2.5. *p*-Bis(2,2'-dipicolylamino)benzene (**3a**). This compound was prepared from 2-(chloromethyl)pyridine hydrochloride (2.0 g, 12 mmol), *p*-phenylenediamine (0.26 g, 2.4 mmol), and triethylamine (5.8 g, 57mmol) in a manner similar to that described above by heating for 2 h at 80 °C. The crude product was purified by recrystallization from methanol, ethyl acetate and *n*-hexane to afford a brown crystal. Yield: 48% (0.55 g); mp 196-197 °C (dec); ^1H NMR (400 MHz, CDCl_3) δ 4.70 (s, 8H), 6.58 (s, 4H), 7.11-7.15 (m, 4H), 7.29 (d, 4H), 7.58 (t, 4H), 8.54 (d, 4H); ^{13}C NMR (100MHz, CDCl_3) δ 58.38, 114.73, 121.49, 122.25, 137.06, 141.23, 149.93, 159.95; HRMS (EI): Calcd for $\text{C}_{30}\text{H}_{28}\text{N}_6$: 472.2375, Found: 472.2360.

4.2.6. 2,2'-Dipicolylaminobenzene (**3b**). This compound was prepared from 2-(chloromethyl)pyridine hydrochloride (9.5 g, 58 mmol), aniline (1.8 g, 19 mmol) and triethylamine (5.8 g, 57mmol) in a similar manner to that described above by heating for 2 h at 80 °C. Yield: 55% (2.9 g) as a brown crystal; mp 111-112 °C; ^1H NMR (400 MHz, CDCl_3) δ 4.83 (s, 4H), 6.67-6.75 (m, 3H), 7.11-7.21 (m, 4H), 7.27 (d, 4H), 7.61 (t, 4H), 8.59 (d, 2H); ^{13}C NMR (100MHz, CDCl_3) δ 57.73, 112.94, 117.62, 121.22, 122.41, 129.70, 137.20, 148.63, 150.13, 159.28; HRMS (EI): Calcd for $\text{C}_{18}\text{H}_{17}\text{N}_3$: 275.1422, Found: 275.1401; Anal. Calcd for $\text{C}_{18}\text{H}_{17}\text{N}_3$: C 78.52, H 6.22, N 15.26. Found: C 78.44, H 6.19, N 15.37.

4.2.7. *p*-(*N,N*-Dimethylamino)- 2,2'-dipicolylaminobenzene (**3c**). This compound was prepared from 2-(chloromethyl)pyridine hydrochloride (3.6 g, 22 mmol), *N,N*-dimethyl-*p*-phenylenediamine (1.0 g, 7.4 mmol) and triethylamine (2.3 g, 22 mmol) in a similar manner to that described above by heating for 2 h at 80 °C. Yield: 14% (0.34 g) as a brownish crystal; mp 98-100 °C; ¹H NMR (400 MHz, CDCl₃): δ 2.80 (s, 6H), 4.75 (s, 4H), 6.69 (m, 4H), 7.14 (m, 2H), 7.31 (d, 2H), 7.60(d, 2H), 8.57 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 42.31, 58.47, 114.72, 115.83, 121.52, 122.26, 137.09, 141.25, 144.12, 149.93, 160.00; HRMS (EI): Calcd for C₂₀H₂₂N₄: 318.1844, Found: 318.1868.

4.2.8. 2,2'-Dipicolylaminomethylbenzene (**4**). This compound was prepared from 2-(chloromethyl)pyridine hydrochloride (3.7 g, 22 mmol), benzylamine (1.0 g, 9.3 mmol) and triethylamine (4.7 g, 47 mmol) in a similar manner to that described above by heating for 4 h at 80 °C. The crude product was purified by column chromatography on silica gel (eluant: *n*-hexane/ethyl acetate = 2/1 (v/v)) to afford a yellow viscous liquid. yield: 54% (1.5 g); ¹H NMR (400 MHz, CDCl₃) δ 3.69 (s, 2H), 3.81 (s, 4H), 7.10-7.17 (m, 2H), 7.23 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 7.42 (d, *J* = 7.0 Hz, 2H), 7.59 (dt, 2H), 7.66 (t, 2H), 8.52 (d, 2H); ¹³C NMR (100MHz, CDCl₃) δ 58.94, 60.41, 122.33, 123.16, 127.45, 128.71, 129.25, 136.81, 139.39, 149.37, 160.23; HRMS (EI): Calcd for C₁₉H₁₉N₃: 289.1579, Found: 289.1582.

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Graphical Abstract:

