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博士論文題目 Study on Sensing Systems Based on Luminescent Biscyclometalated Iridium(III) Complexes
 (発光性ビスシクロメタレート型イリジウム(III)錯体を用いたセンシングシステムの研究)

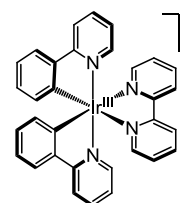
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[General Introduction]

Numerous ions and small molecules play vital roles in human life and exist within organisms or in the external environment. Consequently, selective detection of such analytes is of great significance in fields as diverse as chemistry, environment, medicine and health sciences. A wide range of analytical techniques have been employed to detect those analytes, among which luminescent sensing systems have received special attention and become dominant tools because of their rapid, convenient and sensitive properties.^[1]

Typically containing a recognition site and a luminophore, the luminescent sensing system utilizes optical property changes of the luminophore to report the recognition event occurring between the recognition site and the corresponding analyte. Based on this mechanism, a wide range of small molecular organic emitters have been chosen as luminophores for luminescent sensors. However, the undesirable photophysical properties of those organic fluorophores such as small Stokes shift, short excited-state lifetimes and susceptibility to photobleaching greatly constrain the full potential of their applications. On the other hand, phosphorescent iridium(III) complexes possess large Stokes shifts, long lifetimes, good photostabilities and optical properties sensitive to their chemical environment. In particular, cationic biscyclometalated iridium(III) complexes (Scheme 1) have been favorably used as emission units for various chemical sensors because of their easy incorporation of recognition sites and good solubility in polar solvents.^[2]



Scheme 1. Biscyclometalated iridium(III) complex

In this thesis, a series of luminescent probes for different analytes based on biscyclometalated iridium(III) complexes were designed and synthesized: (1) a switchable Cu^{2+} chemosensor; (2) a ratiometric Hg^{2+} chemodosimeter and (3) a turn-on HOCl chemodosimeter.

[Results and Discussion]

1. Luminescent Biscyclometalated Iridium(III) Complex for Selective and Switchable Cu²⁺ Ion Binding in Aqueous Media

Well known as an excellent ligand for various transition metal ions, 2,2'-bipyridine (bpy) was incorporated into a biscyclometalated iridium(III) skeleton through a simple dimethylene linker, being anticipated to interact with metal ions to influence the optical properties of the iridium(III) luminophore.

Upon addition of a wide range of metal ions including heavy, transition, and main group metal ions, the probe **Ir1** exhibits a highly selective Cu²⁺ quenching in the emission in aqueous media. (Figure 1) Among various metal ions, only Cu²⁺ quenches the emission totally. Hg²⁺ also quenches the emission to some extent, but with much less effect than that of Cu²⁺. Other metal ions cause no or only slight luminescent changes.

The binding of **Ir1** to Cu²⁺ ions was carefully investigated through titration experiments. (Figure 2) Upon the successive addition of Cu²⁺, the yellow emission of **Ir1** at 593 nm was gradually quenched. Results of the titration curve (Figure 2, inset) suggests the 1:1 binding of **Ir1** to Cu²⁺ with a large binding constant of $(1.31 \pm 0.25) \times 10^7 \text{ M}^{-1}$. The quenching of the emission band is attributed to the intrinsic quenching property caused by the paramagnetic nature of Cu²⁺ ions.

Interestingly, this sensing system shows good switchability. As shown in Figure 3, the addition of I⁻ ion to the solution containing **Ir1** and Cu²⁺ immediately restored the quenched emission to the original level of **Ir1**. The further addition of Cu²⁺ quenched the emission again. This process was able to repeat several times without any loss of the emission intensity. The switching was probably caused by the I⁻ reduction of Cu²⁺ to CuI^[3] to regenerate **Ir1**.

The sensor is of great importance as Cu²⁺ is one of the most important heavy metal ions that play critical roles in the controlling of many biological processes. Significantly, the high reversibility makes the sensor recyclable, a property favorable for practical application.

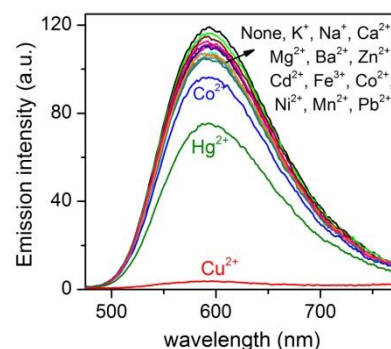
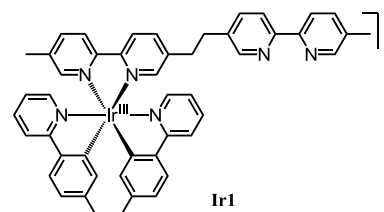


Figure 1. Emission response of **Ir1** (5 μM) to various metal ions (perchlorate salt, 2 equiv.). CH₃CN-H₂O (1:1, v/v). λ_{ex} = 400 nm.

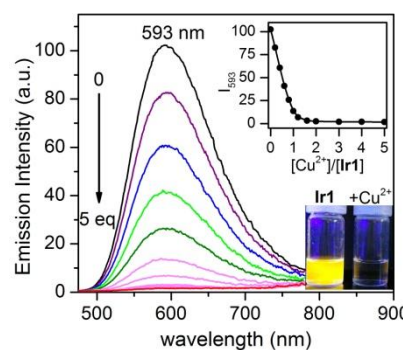


Figure 2. Luminescent spectral changes of **Ir1** (5 μM) upon increasing addition of Cu(ClO₄)₂ (0-5 equiv.). CH₃CN-H₂O (1:1, v/v). λ_{ex} = 400 nm.

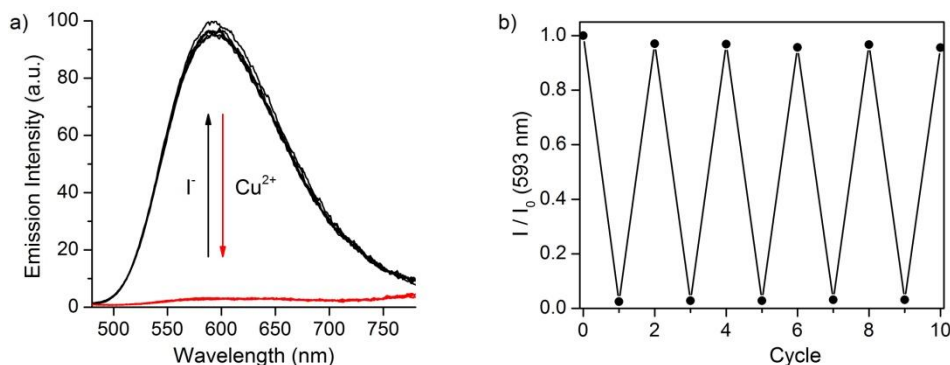


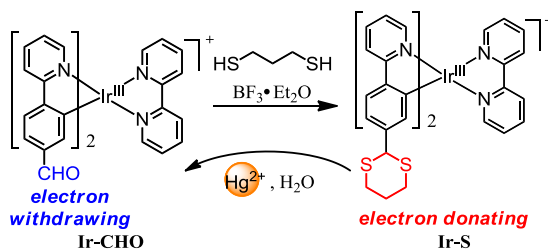
Figure 3. a) Emission spectra showing the reversible complexation between **Ir1** (5 μM) and Cu(ClO₄)₂ (2 equiv.) by introduction of I⁻ (4 equiv.). b) Stepwise complexation/decomplexation cycles carried out in CH₃CN-H₂O (1:1, v/v). λ_{ex} = 400 nm.

2. A Highly Selective and Sensitive Ratiometric Chemodosimeter for Hg²⁺ Ions Based on an Iridium(III) Complex *via* Thioacetal Deprotection Reaction

The development of various luminescent sensing systems for the monitoring of Hg²⁺ has received increasing attention in recent years due to the high toxicity of the ion. However, most sensing systems are of ON-OFF type as their emission are facilely quenched by the heavy Hg²⁺ ions through the spin orbit coupling effect. Such emission intensity based probes tend to be easily affected by many other variable environmental (pH, polarity, temperature, and so on) or instrumental factors. To eliminate these unfavorable interferences, ratiometric probes, which allow for the simultaneous measurement of the intensities at two different emission bands, are being favourably developed due to their built-in correction for the influences mentioned above.

Encouraged by the advantages of ratiometric indicators, a novel phosphorescent ratiometric probe **Ir-S** based on a biscyclometalated iridium(III) complex for Hg²⁺ sensing was designed and synthesized. (Scheme 2) The sensing took the advantage of the well known specific Hg²⁺-promoted thioacetal deprotection reaction to generate the formyl group.^[4] According to this reaction, **Ir-S** was supposed to generate **Ir-CHO** in the presence of Hg²⁺. On the basis of the fact that the optical properties of iridium(III) complexes are very sensitive to their electronic distributions, **Ir-S** and **Ir-CHO**, appended by electron-donating 1,3-dithiane groups and electron-withdrawing aldehyde groups respectively, are believed to exhibit different emission properties.

As expected, upon the titration of Hg²⁺, the emission of **Ir-S** at 590 nm gradually decreased and changed by a blue shift to 540 nm, an emission band characteristic of **Ir-CHO**. (Figure 4a) This pronounced blue shift in the maximum emission wavelength (50 nm) allowed ratiometric measurement of the Hg²⁺ ion. As shown in Fig. 4b, the emission intensity ratio of I₅₄₀/I₅₉₀ increased with the enhancement of the Hg²⁺ concentration. Interestingly, an excellent linear relationship was obtained from 0 to 10 μM of Hg²⁺ concentration, indicating that **Ir-S** was a promising tool for the quantitative determination of the Hg²⁺ concentration. Moreover, from the linear equation (inset of Figure 4b), the detection limit for Hg²⁺ was calculated to be 41 nM at the signal to noise ratio (S/N) = 3, which is much lower than many reported ratiometric fluorescent Hg²⁺



Scheme 2. Sensing mechanism of **Ir-S** for Hg²⁺.

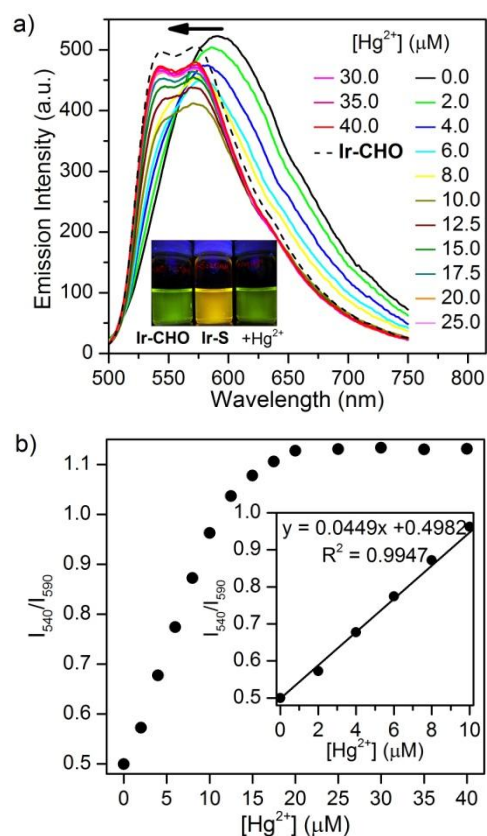


Figure 4. Luminescent spectral changes of **Ir-S** (5 μM) upon increasing addition of Hg(ClO₄)₂ (0-5 equiv.). CH₃CN-H₂O (98:2, v/v). λ_{ex} = 420 nm.

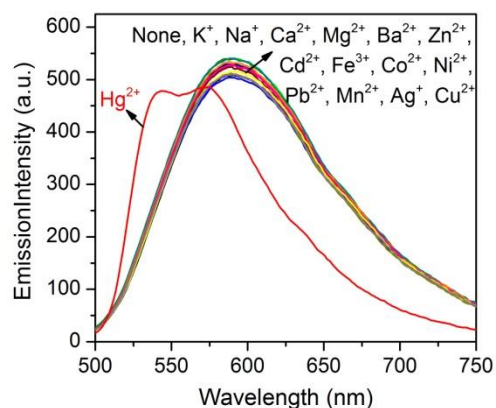


Figure 5. Luminescent responses of **Ir-S** (5 μM) upon addition of various metal ions (Hg²⁺, 5 equiv.; other, 20 equiv.) CH₃CN-H₂O (98:2, v/v). λ_{ex} = 420 nm.

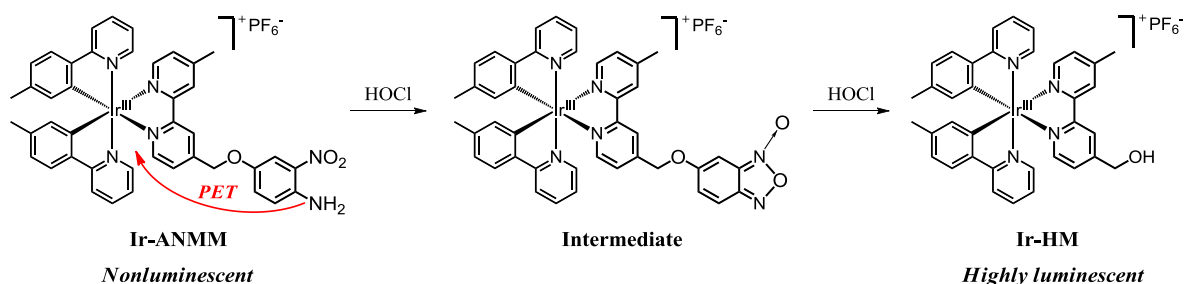
sensors and thus represents the high sensitivity of **Ir-S** for Hg^{2+} sensing.

To verify the specific response of **Ir-S** to Hg^{2+} , emission spectroscopic changes of **Ir-S** upon the addition of various metal cations were examined. As shown in Figure 5, only the addition of Hg^{2+} ions caused the blue shift in the emission of **Ir-S**. Other metal ions, even in the case of a large excess, induced no or only slight variations without any change in the emission maximum. Significantly, in the presence of other metal ions, Hg^{2+} induced similar spectral changes. So the coexistence of other metal ions in the detection system exerted a negligible influence on the signal response of Hg^{2+} , demonstrating the high selectivity of **Ir-S** toward Hg^{2+} .

3. A Highly Selective and Sensitive Turn-on Chemodosimeter for Hypochlorous Acid Based on an Iridium(III) Complex and Its Application in Bioimaging

Hypochlorous acid (HOCl), one of the reactive oxygen species (ROS), is widely employed in daily life and plays significant roles in many vital biological processes. However, concentrated hypochlorite solutions can pose a great threat to health because excessive HOCl may cause tissue damage and abnormal levels of HOCl are associated with the progression of numerous disorders. Consequently, sensitive and selective probes for the monitoring of HOCl both in vivo and in vitro are highly required.

To date, there is only one iridium(III) complex utilized as hypochlorite sensor, which, however, suffers very poor water solubility and unsatisfied detection limit. To take the full advantage of the favorable optical properties of iridium(III) complex, a novel HOCl chemodosimeter based on a biscyclometalated iridium(III) complex was designed, which possesses sufficient water solubility and is therefore suitable for the intracellular imaging of HOCl. (Scheme 3) The sensing is based on the specific oxidation of the *o*-nitroanilino group by HOCl.^[5] The complex **Ir-ANMM** has no emission due to photo-induced electron transfer (PET) from the electron-rich 4-amino-3-nitrophenyl moiety to the luminescent iridium(III) center. Nevertheless, through reacting with HOCl, the 4-amino-3-nitrophenyl moiety was cleaved and the corresponding PET effect was eliminated, generating a highly luminescent complex **Ir-HM**.



Scheme 3. Sensing mechanism of **Ir-ANMM** for HOCl.

Luminescent response of **Ir-ANMM** to HOCl was investigated by titration of different concentrations of HOCl to a solution of **Ir-ANMM**. As shown in Figure 6, with increasing addition of HOCl, the luminescent intensity at 620 nm enhanced significantly. Notably, the emission intensity has a good linear relationship with the concentration of HOCl in the range from 0 to 15 μM , which is remarkably essential for the quantitatively determination of HOCl concentration. More importantly, the detection limit for Hg^{2+} was calculated to be 16 nM at the signal to noise ratio (S/N) = 3, a value much lower than many reported luminescent HOCl sensors.

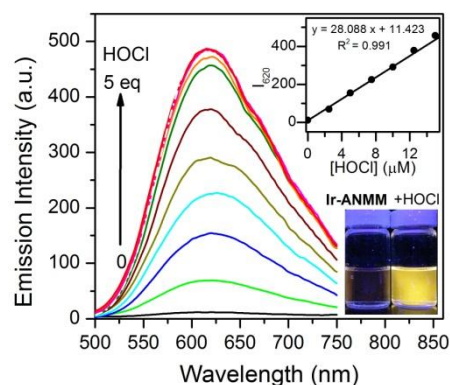


Figure 6. Luminescent spectral changes of **Ir-ANMM** (5 μM) upon titration of HOCl (0-5 equiv.). CH_3CN -0.1 M phosphate buffer (4:1, v/v, pH 7.4). $\lambda_{\text{ex}} = 400 \text{ nm}$.

To investigate the specific luminescent response of **Ir-ANMM** to HOCl, various reactive oxygen species (ROS) and reactive nitrogen species (RNS) were added to the solution of **Ir-ANMM** to see their influence on the emission. As shown in Figure 7, among diverse ROS/RNS, only HOCl resulted in a dramatic emission enhancement. In contrast, all the other species such as H₂O₂, •OH, ¹O₂, O₂^{•-}, ^tBuOOH, ROO•, NO, NO₂, NO₃ and ONOO⁻, exerted negligible or slight impact on the emission of **Ir-ANMM**. The superior selectivity for HOCl is attributed to its strong oxidability and highly specific reaction with the *o*-nitroanilino group.

The high sensitivity, superior selectivity for HOCl, together with the good water solubility, make the sensor **Ir-ANMM** favorable for intracellular imaging. Accordingly, the applicability of **Ir-ANMM** for imaging HOCl in living cells was examined, for which Hela cells were used as a model. As depicted in Figure 8, almost no luminescence could be observed from the **Ir-ANMM**-loaded Hela cells. However, after further coincubation with HOCl, a striking enhancement in the red luminescence was observed.

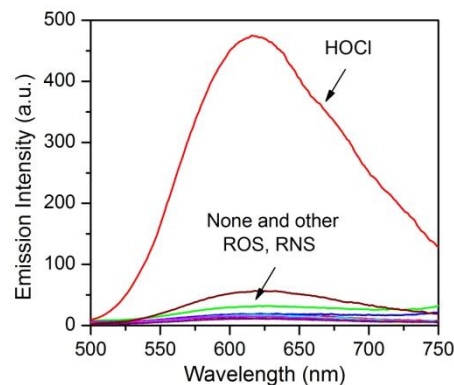


Figure 7. Emission responses of **Ir-ANMM** (5 μM) to various ROS/RNS (HOCl, 25 μM; other, 200 μM). CH₃CN-0.1 M phosphate buffer (4:1, v/v, pH 7.4). λ_{ex} = 400 nm.

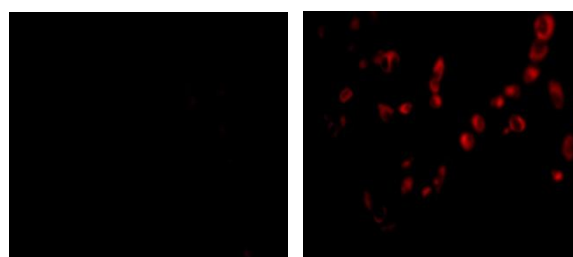


Figure 8. Luminescent image of Hela cells treated with left) **Ir-ANMM** (20 μM) and right) both **Ir-ANMM** (20 μM) and HOCl (50 μM).

[Conclusion and Perspectives]

In conclusion, a series of luminescent probes based on biscyclometalated iridium(III) complexes were successfully constructed. With easy accessibility, these indicators are effective for the highly selective sensing of Cu²⁺, Hg²⁺ and HOCl, respectively. More significantly, the systems present the fabulous means of employing the advantageous luminescent iridium(III) complexes as remarkable luminophores in sensing systems, which, hopefully, will stimulate the emergence of new profitable sensing systems for targeting analytes of environmental and biological importance.

[References]

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[Publication list]

- “Luminescent Biscyclometalated Iridium(III) Complex for Selective and Switchable Cu²⁺ Ion Binding in Aqueous Media”
Lu, F.; Yamamura, M.; Nabeshima, T. *Tetrahedron Lett.* **2013**, *54*, 779-782.
- “A Highly Selective and Sensitive Ratiometric Chemodosimeter for Hg²⁺ Ion Based on an Iridium(III) Complex via Thioacetal Deprotection Reaction”
Lu, F.; Yamamura, M.; Nabeshima, T. *Dalton Trans.* **2013**, *42*, 12093-12100. **Cover Picture**
- “A Highly Selective and Sensitive Turn-on Chemodosimeter for Hypochlorite Acid Based on an Iridium(III) Complex and Its Application to Bioimaging”
Lu, F.; Nabeshima, T. *in preparation.*