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Photofunctional molecular materials for chemical sensing, bioimaging and electrochromic applications

Yun Ma
Hong Kong Baptist University
Photofunctional Molecular Materials for Chemical Sensing, Bioimaging and Electrochromic Applications

MA Yun

A thesis submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

Principal Supervisor: Prof. WONG Wai Yeung

Hong Kong Baptist University

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DECLARATION

I hereby declare that this thesis represents my own work which has been done after registration for the degree of Doctor of Philosophy at Hong Kong Baptist University, and has not been previously included in a thesis or dissertation submitted to this or other institution for a degree, diploma or other qualifications.

Signature:____________________

Date: July 2015
ABSTRACT

This thesis is dedicated to developing novel photofunctional molecular materials for the applications in chemical sensing, bioimaging and electrochromic.

To begin with, a brief introduction of photofunctional molecular materials and an overview of their applications in chemical sensing, bioimaging and electrochromic were presented in Chapter 1.

In chapter 2, we have synthesized a series of water-soluble phosphorescent cationic iridium(III) solvato complexes (1-7) as multicolor cellular probes for imaging in living cells. All of these complexes can be dissolved in PBS. The emission of complexes can be tuned from green to red by changing the chemical structure of cyclometalating ligands. All complexes exhibit low cytotoxicity to living cells and exhibit cell membrane permeability and specific staining of cytoplasm. They enter the cells by the mechanism of energy-independent passive diffusion mechanisms. More importantly, complex 7 can act as a two-photon phosphorescent cellular probe, and fluorescence lifetime imaging microscopy is successfully applied for bioimaging in the presence of short-lived background fluorescence.

We developed two excellent optical probes for CO₂ detection in Chapter 3. The first one for the CO₂ detection is a phosphorescent probe based on an iridium(III) complex with 2-phenylimidazo-[4,5-f][1,10]phenanthroline. After bubbling CO₂ into the detection solution, the quenched phosphorescence by the addition of CH₃COO⁻ can
be recovered. Photobleaching experiment demonstrates that this phosphorescent CO₂ probe shows higher photostability than some of the reported organic probes. More importantly, the time-resolved PL experiment demonstrates that this probe can be used to detect CO₂ in the presence of strong background fluorescence, which improves the sensitivity and signal-to-noise ratio of the sensor in complicated media. The second one is a water-soluble fluorescent probe based on tetraphenylethene derivative. After bubbling CO₂ into the detection solution, remarkable color change and fluorescence enhancement could be observed. The response of this probe to CO₂ in aqueous solution is fast and the detection limit is about 2.4 × 10⁻⁶ M. To emphasize the practical application of this probe, a porous film was successfully fabricated by mixing the dye with sodium carboxymethyl cellulose in water, which can serve as an efficient CO₂ gas sensor. More importantly, this probe exhibits low cytotoxicity towards live cells and has the ability to monitor the external CO₂ concentration changes of living cells.

Chapter 4 focused on the development of novel soft salt based phosphorescent probe. This type of probe consists of two oppositely charged ionic complexes with two distinguishable emission colors, which makes it a perfect candidate as a ratiometric probe. The emission color of 10 changes from blue to red with increasing pH value. 10 is cell-permeable and exhibits low cytotoxicity, and it has been successfully applied for ratiometric pH imaging with the use of confocal microscopy, demonstrating its great potential for intracellular environment monitoring. Furthermore, phosphorescence lifetime imaging experiments can detect intracellular
pH variations by photoluminescence lifetime measurements, which allowed for eliminating background fluorescence and selecting long-lived phosphorescence images. Quantitative measurement of intracellular pH fluctuations caused by oxidative stress has been successfully carried out for 10 based on the pH-dependent calibration curve.

A series of cationic Zn(II) complexes has been designed and synthesized in chapter 5. The photophysical properties of these Zn(II) complexes are affected by the counterions. By altering the counterions, the emission peak can be changed from 549 nm to 622 nm. Interestingly, the CIE coordinate and the emission colors can be simply tuned by adjusting the concentration of 11d in the polyether. Under an electric field of about 15 V applied onto the electrodes, the emission color of the solution of 11b-11d near the cathode changed its original emission color to sky blue. Based on this interesting electrochromic fluorescence of 11d, a quasi-solid information recording device has been successfully designed. Furthermore, data encryption has been realized by combining 1d with BODIPY, and information decoding processed has been accomplished, for the first time, by employing TPA excitation techniques, in which the large TPA cross section of 11d is differentiated from small TPA cross section of common organic dyes.

Finally, Chapters 6 and 7 present the concluding remarks and the experimental details of the work described in Chapters 2–5.
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<td>Photoluminescence</td>
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<td>PET</td>
<td>Photoinduced electron transfer</td>
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<td>D-π-A</td>
<td>Donor–π-conjugation bridge–electron acceptor</td>
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<td>ICT</td>
<td>Intramolecular charge transfer</td>
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<td>FRET</td>
<td>Förster resonance energy transfer</td>
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<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>HOMO</td>
<td>The highest occupied molecular orbital</td>
</tr>
<tr>
<td>LUMO</td>
<td>The lowest unoccupied molecular orbital</td>
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<tr>
<td>GSH</td>
<td>Glutathione</td>
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<tr>
<td>Cys</td>
<td>Cysteine</td>
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<tr>
<td>Hcy</td>
<td>Homocysteine</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>$^1$O$_2$</td>
<td>Singlet oxygen</td>
</tr>
<tr>
<td>H$_2$S</td>
<td>Hydrogen sulfide</td>
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<tr>
<td>$^1$LC</td>
<td>Spin-allowed ligand-centered transition</td>
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<tr>
<td>$^1$MLCT</td>
<td>Singlet metal-to-ligand charge-transfer transition</td>
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<tr>
<td>$^1$MLCT</td>
<td>Triplet metal-to-ligand charge-transfer transition</td>
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<tr>
<td>$^3$LC</td>
<td>Spin-forbidden ligand-centered transition</td>
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<td>MTT</td>
<td>3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
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<tr>
<td>GC-MS</td>
<td>Gas chromatography-mass spectrometry</td>
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<tr>
<td>AIE</td>
<td>Aggregation-induced emission</td>
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<tr>
<td>TRPT</td>
<td>Time-resolved photoluminescence technique</td>
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<tr>
<td>TPE</td>
<td>Tetraphenylethene</td>
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<tr>
<td>DLS</td>
<td>Dynamic light scattering</td>
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<tr>
<td>SNR</td>
<td>Signal-to-noise ratio</td>
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<td>PLIM</td>
<td>Photoluminescence lifetime imaging microscopy</td>
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<tr>
<td>PTMCs</td>
<td>Phosphorescent transition-metal complexes</td>
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<tr>
<td>Kq</td>
<td>Quenching rate constant</td>
</tr>
<tr>
<td>AcOH</td>
<td>Acetic acid</td>
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<tr>
<td>CHCl₃</td>
<td>Chloroform</td>
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<tr>
<td>CHCl₂</td>
<td>Dichloromethane</td>
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<td>DMF</td>
<td>N,N-Dimethylformamide</td>
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<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
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<tr>
<td>MeOH</td>
<td>Methanol</td>
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<tr>
<td>NEt₃</td>
<td>Triethylamine</td>
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<tr>
<td>NBS</td>
<td>N-Bromosuccinimide</td>
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<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
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<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<tr>
<td>ppm</td>
<td>Part per milion</td>
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<tr>
<td>Hz</td>
<td>Hertz</td>
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<tr>
<td>s</td>
<td>Singlet</td>
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<td>d</td>
<td>Doublet</td>
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<tr>
<td>t</td>
<td>Triplet</td>
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<tr>
<td>m</td>
<td>Multiplet</td>
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<tr>
<td>MS</td>
<td>Mass spectrometry</td>
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<tr>
<td>FAB</td>
<td>Fast atom bombardment</td>
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<tr>
<td>MALDI-TOF</td>
<td>Matrix-assisted laser desorption ionization/time-of-flight</td>
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<tr>
<td>$M^+$</td>
<td>Molecular ion</td>
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<tr>
<td>$m/z$</td>
<td>Mass to charge ratio</td>
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<tr>
<td>$M_n$</td>
<td>Number-average molecular weight</td>
</tr>
<tr>
<td>$M_w$</td>
<td>Weight-average molecular weight</td>
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<tr>
<td>$\delta$</td>
<td>Chemical shift</td>
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<tr>
<td>°C</td>
<td>Degree Celsius</td>
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<tr>
<td>$\lambda$</td>
<td>Wavelength</td>
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<tr>
<td>$\varepsilon$</td>
<td>Molar extinction coefficient</td>
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11a \( X^- = \text{AcO}^- \)
11b \( X^- = \text{BF}_4^- \)
11c \( X^- = \text{ClO}_4^- \)
11d \( X^- = \text{PF}_6^- \)
Chapter 1 Introduction

1.1 Introduction

Luminescence refers to the emission of light from other sources of energy (such as photons, electric field, chemical reaction energy, biochemical reaction energy, etc), which can take place at normal and lower temperatures. Photoluminescence (PL) means that the emission of light is triggered by absorption of photons. The primary types of photoluminescence consist of fluorescence and phosphorescence. The Jablonski diagram shows that fluorescence occurs when the molecule returns to the electronic ground state, from the excited singlet, by emission of a photon (Figure 1.1). The transition is spin-allowed and is often fast (short lifetime). On the other hand, phosphorescence occurs when the molecule returns to the electronic ground state, from the excited triplet state, by emission of a photon. The transition is spin-forbidden and is usually slow (long lifetime). Photofunctional molecular materials are materials that emit visible light due to the excitation they undergo when exposed to a light source. Over the past decades, photofunctional molecular materials play quite important roles in the research areas of chemical sensors,\textsuperscript{[1-5]} biological detectors,\textsuperscript{[6-15]} electronic devices,\textsuperscript{[16-20]} etc.
1.2 Photofunctional molecular materials for chemical sensing

Chemical sensor is a receptor that transforms chemical information (such as particular ions, concentration, electrical field, temperature, pressure and so on) in the environment into an analytically useful signal. Up to now, chemical sensors have been applied in various fields, including biological detecting, package, medicine, environmental pollution analysis and many others.[21-27]

Photofunctional molecular materials serve as chemical sensors to detect target analytes through highly selective and reversible/irreversible chemical reactions between the chemical sensors and the target molecules. The selectivity of chemical sensors for the target molecules is usually very high. Besides, they often exhibit high sensitivity and fast response. More importantly, chemical sensors developed by photofunctional molecular materials are based on fluorescence/phosphorescence...
“turn-on” or and emission wavelength shift. Thus, photofunctional chemical sensors provide a convenient and visible method for detecting many analytes, including cations, anions, biological molecules, and so on. Hence, the application of photofunctional molecular materials for sensing and analyzing target molecules is attracting increasing research attention. In the recent years, a large number of photofunctional sensors have been designed and developed to detect anions, cations, biomolecules and environmental stimuli, such as Cl\(^-\), F\(^-\), Cr\(^{3+}\), Fe\(^{3+}\), Zn\(^{2+}\), Hg\(^{2+}\), Cd\(^{2+}\), Cu\(^{2+}\), Cu\(^{+}\), Mg\(^{2+}\), Ca\(^{2+}\), NO, and H\(_2\)O\(_2\), cysteine and homocysteine, peroxynitrite, singlet oxygen, pH, temperature and so on.

1.2.1 Design principles of photofunctional molecular materials for chemical sensing

One basic requirement of photofunctional molecular materials for chemical sensing is that the optical properties, including emission intensity, wavelength and lifetime, of product is evidently different from that of reagent after combines with the analyte. So, any factors that will affect the luminescent process can be exploited for designing photofunctional chemical sensors.

(a) Extent of \(\pi\)-electron system

In most cases, by changing the conjugation degree of the \(\pi\)-electron of a photofunctional compound will result in the variation of the wavelength shift and/or intensities of its luminescence. When an analyte interacts with a photofunctional compound and changes its \(\pi\)-conjugation degree, this compound can be used as a photofunctional sensor for the analyte.
(b) **Effect of substituents with electron-donating or electron-withdrawing units**

Generally, by introduction of electron-donating or electron-withdrawing groups into a luminophore will result in a shift in photoluminescence spectrum. In the previous literature, the most common electron-donating groups of photofunctional chemical sensors are amines, phenol and alkoxy group, and electron-withdrawing ones are commonly ketone, carboxyl, cyano, aldehyde and nitro group. Numerous photofunctional sensors are linked with this strategy of introducing electron-donating or electron-withdrawing groups.\[28\]

(c) **Photoinduced intramolecular charge transfer (ICT)**

Mostly, intramolecular charge transfer (ICT) dominates the excited state of electron donor–π-conjugation bridge–electron acceptor (D-π-A) molecule. Intramolecular charge transfer provides the luminophore a large polarity, and exhibits strong luminescence in the visible region. Up to now, many photofunctional sensors have been designed based on this strategy of ICT process.\[29\]

(d) **Photoinduced electron transfer (PET)**

Photoinduced electron transfer (PET) is a common strategy to exploit photofunctional sensors. For photofunctional sensors based on PET process, the interaction between luminophore and target molecule should induce luminescence quenching or enhancement. Up to now, the PET effect has been utilized for fabricating photofunctional sensors.\[30\]
Fürster resonance energy transfer (FRET)

Fürster resonance energy transfer (FRET) is a mechanism describing energy transfer between two luminophores. The efficiency of FRET is dependent on the distance, and it occurs only over very short distances (within 10 nm). In addition, the FRET dye pair must also display great overlap of the donor's emission spectrum with the acceptor's absorption spectrum. To date, a few cases with FRET process have been reported in fabricating photofunctional sensors.

1.2.2 Photofunctional molecular materials for \( \text{Hg}^{2+} \) detection

**Figure 1.2** Chemical structures of photofunctional molecular materials for \( \text{Hg}^{2+} \) detection.
Mercury and most of its compounds are amongst the most toxic and pervasive contaminants. The most hazardous forms of mercury is its organic compounds, such as methylmercury species (CH$_3$HgX), which can be absorbed via skin and mucous membranes and results in severe poisoning (brain damage, cognitive disorders, vision and hearing loss, etc).

Desulfurization reaction is widely utilized in the design of fluorescent sensors to detect Hg$^{2+}$ due to its high thiophilic affinity. The thiocarbonyl group can be transformed to carbonyl through Hg$^{2+}$-induced desulfurization reaction, which leads to the alteration of the photoluminescence properties of fluorescent sensors. Recently, as shown in Figure 1.2, some fluorescent sensors have been developed to determine Hg$^{2+}$ ion in solution by desulfitation reaction (1-6). For them, the fluorescence intensities are often enhanced due to the Hg$^{2+}$-induced transformation of thiocarbonyl group to carbonyl. For example, 6 shows weak fluorescence emission at 511 nm in H$_2$O/CH$_3$CN (1:1, v/v) mixture. However, its fluorescence intensity is increased by about 26-fold after the interaction with Hg$^{2+}$. And this detection process is highly selective and quick in response.

Hg$^{2+}$-induced alteration of the thiourea group of the fluorescent sensor to an imidazoline moiety is another extensively used desulfurization and cyclization reaction for Hg$^{2+}$ detection. On the basis of this reaction, some excellent fluorescent sensors for Hg$^{2+}$ have been reported. For compounds 7-9, upon the addition of Hg$^{2+}$ into their solutions, Hg$^{2+}$-induced desulfitation and cyclization reaction of thiourea occurred, resulting in a remarkable blue-shift in photoluminescence spectrum.
Figure 1.3 Chemical structures of photofunctional molecular materials for Hg$^{2+}$ detection.

Rhodamine derivatives are one of the most popular molecules used as fluorescent sensors due to their high fluorescence quantum efficiencies and broad emission wavelengths in visible region. Rhodamine derivatives are normally non-emissive, however, ring-opening of the corresponding spirolactam will light-up the intensive fluorescence. On the basis of this spirolactam ring-opening process, there are a huge number of examples in the development of fluorescent sensors in recent years.$^{[42-46]}$

Up to now, many rhodamine derivatives have been developed as Hg$^{2+}$-selective
fluorescent sensors based on this strategy. For example, as shown in Figure 1.3, compounds 10-15 show highly sensitive and selective binding ability toward Hg$^{2+}$ and serve as “turn-on” fluorescent sensors.

Phosphorescent heavy-metal complexes show some advantageous photophysical properties as chemical sensors, such as high luminescence efficiency, tunable excitation and emission wavelengths, significant Stokes shifts for easily distinguishing emission over excitation and relatively long lifetimes. As shown in Figure 1.3, a series of heavy metal complexes have been developed as phosphorescent sensor for Hg$^{2+}$ detection.$^{[47-49]}$ The sulfur atom in the ligand of complex can easily interact with the Hg$^{2+}$, which will tune excited state properties. For instance, addition of Hg$^{2+}$ to acetonitrile solution of complex 16 will result in significant blue-shifts in phosphorescent emission peak.

1.2.3 Photofunctional molecular materials for F$^-$ detection

F$^-$ plays quite important roles in a wide range of biological, medical, and chemical processes, including dental care, etc. Therefore, the detection of F$^-$ is of great significance in daily life. Up to now, many chromophores have been designed and synthesized as chemical sensors for F$^-$ detection. Amides and imidazolium are well known functional groups for F$^-$ sensing via the formation of a hydrogen bond between the active N-H group and F$^-$. Besides, dimesitylboryl (BMes$_2$) is also a popular functional group used for F$^-$ sensing due to its high affinity. Strong interaction between boron atom and F$^-$ can interrupt the extended $\pi$ conjugation of these
compounds in the presence of $F^-$, thus leading to significant changes in the PL spectrum. However, the chemical sensors based on these mechanisms can work only in organic solvents.

![Chemical structures of photofunctional molecular materials for $F^-$ detection.](image)

**Figure 1.4** Chemical structures of photofunctional molecular materials for $F^-$ detection.

To utilize the high affinity of silicon toward $F^-$, an alternative approach has been developed for detecting $F^-$ in aqueous solution. A series of $F^-$ chemical sensors have been designed and synthesized based on the reaction of $F^-$ induced Si-O, Si-C or $\text{–C≡C–SiMe}_3$ bond cleavage. As shown in Figure 1.4, these chemical sensors show high selectivity for $F^-$ with turn-on or/and ratiometric luminescence responses.
The first example of such F⁻ chemical sensor is compound 19, which can detect F⁻ in the presence of H₂O. A drastic change in fluorescence emission was observed in CH₃CN/H₂O (50/50, v/v) with the addition of F⁻. In addition, a coumarin derivative 23 has been developed as non-cytotoxic fluorescent sensor for F⁻ detection. Upon treatment of F⁻ in PBS buffer solution of 23, about 4-fold enhancement of emission intensity was observed. More importantly, the possibility of 23 for biological application in aqueous solution was successfully demonstrated. After incubating with F⁻, the living cells displayed a remarkable enhancement in the emission intensity, implying that 23 has the ability to detect F⁻ in living systems.

1.2.4 Photofunctional molecular materials for CN⁻ detection

CN⁻ is quite toxic and hazardous to mammals, which is associated with vomiting, convulsions, loss of consciousness, etc. In accordance with the standard of World Health Organization (WHO), the concentrations of CN⁻ lower than 1.9 μM can be acceptable in drinking water. Therefore, it is of great significance for developing efficient detecting systems for the determination of CN⁻ in the environment. In the recent years, many types of reactions have been developed for sensing CN⁻, including hydrogen bonding, coordinative bonding and so on. Because of the strong nucleophilic nature of CN⁻, the detection mechanisms of such chemical sensors are mainly based on the addition reaction.^[56-65]
Figure 1.5 Chemical structures of photofunctional molecular materials for CN⁻ detection.

Compound 25 has the ability to selectively detect CN⁻ in DMSO/water (95:5 v/v) mixture with about 14-fold decrease in emission intensity. Similarly, compound 26 is also a CN⁻ sensor based on an enhanced ICT and PET process from the excited quinoline to the pyridinium ion, which will result in emission “off-on” responses. CN⁻ also has the ability to nucleophilic attack toward an aldehyde group. The fluorescent
sensor 30 has been reported based on this mechanism for CN\(^-\) detection, which exhibited about 200-fold emission intensity enhancement by addition of CN\(^-\).

A Co(II) complex 32 was reported as a fluorescent sensor for selectively sensing CN\(^-\). Complex 32 exhibited weak emission intensity because of the PET process from the coumarin unit to the metal center. CN\(^-\) is capable of binding with Co\(^{2+}\) in 1:2 binding stoichiometry, resulting in an alteration in HOMO and LUMO energy levels and thus leading to a remarkable fluorescence intensity change. A iridium(III) complex 33 has been developed as an excellent phosphorescent sensor toward CN\(^-\) with remarkable luminescence enhancement.

1.3 Photofunctional molecular materials for bioimaging

In the past decades, with the development of life science and pathology, the research on the intracellular active species, cell signal transduction and apoptosis has become increasingly significant in the field of biomedical research and clinical diagnostics. Molecular imaging is playing quite crucial role in these research areas. Among all kinds of molecular imaging methods, fluorescent bioimaging provides the high spatial resolution of several hundreds of nanometers, which is widely used for cell and tissue imaging in biomedical research and is an effectively visualized analysis technique to solve the problems that cannot be resolved by conventional ultrasound, X-ray or magnetic resonance imaging.

In fluorescence imaging, fluorescent probes play the key role in labeling interest molecule and amplifying the fluorescent signal. To date, a series of new fluorescent
probes, including organic dyes, heavy-metal complexes, semiconductor nanocrystals, upconversion nanoparticles, and other classes of nanoparticles have been synthesized, and almost all of them can be utilized as probes in monitoring biomacromolecules (such as proteins and DNA) in living biological samples through high affinity of antibody-antigen interaction or ligand-acceptor interaction.

1.3.1 General requirements of photofunctional molecular materials for bioimaging

There are several requirements for photofunctional molecular materials as an excellent probe for bioimaging, such as Stokes shifts, photoluminescence quantum efficiency, cellular penetration and cytotoxicity, etc.\cite{66} Firstly, as fluorescent labels applied in bioimaging, the probes should exhibit high photoluminescence quantum yield under visible or even near infrared region excitation. Secondly, the probes should have good biocompatibility, which means they have the ability to cross the cell membrane at a fast rate and have low cytotoxicity. Except these general requirements of fluorescent probes for bioimaging, a good probe for bioimaging should show luminescence “turn-on” or/and significant shift of emission wavelength when reacting with the target molecule. Moreover, the probes should exhibit high selectivity and sensitivity in sensing the analyte in living cells or tissues.

1.3.2 Photofunctional molecular materials for bioimaging of Cu$^{2+}$

Cu$^{2+}$ plays a crucial role in various cellular processes, such as those in nervous system, gene expression, the functioning increase of proteins and so on.\cite{67} The lack of Cu$^{2+}$
was associated with different diseases. However, high concentration of Cu$^{2+}$ may also lead to other diseases, such as gastrointestinal disorders and kidney damage and so on. Consequently, the detection of Cu$^{2+}$ has attracted increasing attention in recent years and many fluorescent probes for sensing Cu$^{2+}$ in biological samples have been reported.

Figure 1.6 Chemical structures of photofunctional molecular materials for intracellular Cu$^{2+}$ detection.
Up to now, many Cu$^{2+}$ probes have been developed based on rhodamine derivatives, and their design strategy is on the basis of Cu$^{2+}$ triggered spirolactam ring-opening mechanism and fluorescence intensity increase.$^{[67-74]}$ For example, the chelate groups of compound 34 containing nitrogen and oxygen atoms have been used to coordinate with Cu$^{2+}$ for detection in living cells. Compound 35 is an example of switch on fluorescent probe for detecting intracellular Cu$^{2+}$ through an acetyl group by the blocking of PET process. Taking advantage of the ability of Cu$^{2+}$ to oxidize aromatic sulfur and amines, probe 40 was designed as bioprobe for intracellular Cu$^{2+}$ detection.$^{14}$ The recognition mechanism of 40 for Cu$^{2+}$ is on the basis of the fact that the electron donation process would change by Cu$^{2+}$-induced oxidation process. Probe 40 exhibited high sensitivity to Cu$^{2+}$ with a detection limit of 2.61×10$^{-7}$ M. In addition, probe 40 is cell membrane permeable and can show a fluorescence enhancement response to intracellular Cu$^{2+}$.

1.3.3 Photofunctional molecular materials for bioimaging of thiols

Intracellular thiols, such as Glutathione (GSH), play pivotal functions in physiological matrices. For instance, GSH is associated with maintaining the reducing environment in living systems. Therefore, the sensing and recognition of thiol-containing molecules in living systems are of great significance. Up to now, a number of probes for thiols detection have been exploited based on different mechanisms, such as nucleophilic addition, bond cleavage process, etc.$^{[75-78]}$
Figure 1.7 Chemical structures of photofunctional molecular materials for intracellular thiol detection.

Figure 1.7 shows some examples of thiol probes based on the nucleophilic addition process. For instance, probe 41 exhibits an emission peak at around 640 nm in acetonitrile/water (20/80, v/v) mixture, the fluorescence was quenched after the addition of thiol-containing compounds. Probe 168 was reported for the sensing of thiol-containing compounds based on the nucleophilic attack induced spiro-ring opening of fluorescein. More importantly, it can be used in detecting intracellular thiol species and imaging of thiols zebrafish for the first time.
Figure 1.8 Chemical structures of photofunctional molecular materials for intracellular thiol detection.

Another design strategy is based on the fact that a thiolate anion can easily cleave sulfonyl ether and sulfonamide. For probe 45, disulfide group can be cleaved by the thiol-containing molecules, such as GSH, which will result in a shift of emission wavelength in PL spectra. In addition, due to the relatively large two-photon cross section of probe 45, it has been used for ratiometric two-photon fluorescent imaging. In addition to the small organic dye based probes, metal based complex 46 was also exploited as thiol-selective probe. Owing to the efficient PET process from metal centre to DNS, probe 46 is non-emissive. After the thiol-containing molecule cleaves the DNS, a 90-fold luminescent enhancement at around 600 nm was observed. Confocal fluorescence imaging experiments show that probe 46 is specific for thiols over other molecules in living cells.
1.3.4 Photofunctional molecular materials for bioimaging of Cysteine (Cys) and Homocysteine (Hcy)

Cys and Hcy play vital roles in many physiological processes, such as the formation and growth of cells and tissues. The lack of Cys is associated with many diseases, such as slow growth, hair depigmentation, liver damage, skin lesions and so on. And the lack of Hcy would result in cardiovascular and Alzheimer's disease. Hence, it is quite important to develop detecting systems for imaging intracellular Cys and Hcy.

![Chemical structures of photofunctional molecular materials](image)

**Figure 1.9** Chemical structures of photofunctional molecular materials for intracellular Cys/Hcy detection.
It is well known that the aldehydes can easily react with the thiol group of Cys or Hcy to form thiazolidine or thiazinane.\(^{[81-84]}\) Figure 1.9 shows some examples of Cys/Hcy probes based on this cyclization reaction. Probe 47 is capable of selectively recognizing Cys from other thiol molecules. Similarly, probe 48 also exhibited a significant selectivity for Hcy and Cys over the other amino acids and GSH with about 50-fold emission intensity enhancement. A cationic iridium(III) complex 50 has been reported as a phosphorescent probe for the imaging of intracellular Hcy and Cys.\(^{25}\) By addition of Hcy or Cys to the solution of probe 50, a shift in emission wavelength was observed. Moreover, probe 50 is suitable for bioimaging due to its membrane permeable ability and low cytotoxicity. Subsequent confocal fluorescence imaging experiment demonstrated that probe 50 is capable of monitoring Hcy/Cys by the changes in intracellular Hcy/Cys concentration in a ratiometric way.

1.3.5 Photofunctional molecular materials for bioimaging of hydrogen peroxide (\(\text{H}_2\text{O}_2\))

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. \(\text{H}_2\text{O}_2\) is one kind of ROS. The accumulation of ROS in living systems results in a condition widely named oxidative stress which is associated with aging and a host of diseases. Also, \(\text{H}_2\text{O}_2\) can serve as both intra- and intercellular messengers. Therefore, there are increasing demands for the detection and quantitation of intracellular \(\text{H}_2\text{O}_2\) levels recently. Recently, series probes have been developed for intracellular \(\text{H}_2\text{O}_2\) detection based the \(\text{H}_2\text{O}_2\)-promoted boronate deprotection.\(^{[85-88]}\) Probe 51 is non-emissive in the solution, however, addition of \(\text{H}_2\text{O}_2\) into the solution induces a
prompt emission intensity enhancement. Similarly, probes 52 and 53 are also capable of visualizing luminescence changes after the addition of H$_2$O$_2$. Compound 248 is a ratiometric probe for imaging H$_2$O$_2$ produced in living cells. Probe 248 is membrane-permeable and is capable of imaging of intracellular H$_2$O$_2$ at natural immune response levels. In addition, the ratiometric measurement allows for detection of highly localized variations in H$_2$O$_2$ concentration and visualizing alteration in H$_2$O$_2$ concentration in the cytoplasm region.

![Chemical structures of photofunctional molecular materials for intracellular H$_2$O$_2$ detection.](image)

**Figure 1.10** Chemical structures of photofunctional molecular materials for intracellular H$_2$O$_2$ detection.
1.3.6 Photofunctional molecular materials for bioimaging of singlet oxygen ($^1\text{O}_2$)

Singlet oxygen ($^1\text{O}_2$) is a highly reactive molecule due to its excited state or spin-state nature, which has the potential to damage living systems. It is able to oxidize different kinds of biomolecules, including DNA, proteins, and lipids, etc. Up to now, the most common mechanism for detecting $^1\text{O}_2$ is on the basis of $^1\text{O}_2$-induced 1,4-cycloaddition of imidazole ring and the formation of endoperoxide.$^{[89-92]}$

![Chemical structures of photofunctional molecular materials for intracellular $^1\text{O}_2$ detection.](image)

**Figure 1.11** Chemical structures of photofunctional molecular materials for intracellular $^1\text{O}_2$ detection.

A representative example of probe based on $^1\text{O}_2$-induced 1,4-cycloaddition of imidazole ring is compound 55, which is a cyanine dye-based fluorescent probe containing histidine. The emission intensity of probe 55 is weak because of the PET process from histidine to the cyanine. The 1,4-cycloaddition reaction of probe 55 with
\(^1\text{O}_2\) leads to the formation of 55\(^*\) (Figure 1.11). Thus, the fluorescence emission was significantly enhanced since the electron-donating ability is not sufficient for PET process to occur. More importantly, this probe has the ability to measure the changes of concentrations of \(^1\text{O}_2\) in living cells. Another type of \(^1\text{O}_2\) probes is based on anthracene or 9,10-diphenylanthracene derivatives. These probes can react with \(^1\text{O}_2\) to form stable endoperoxide in a very short time. Probe 56 is almost non-emissive due to the PET process. The endoperoxide derivative was formed after reaction with \(^1\text{O}_2\), which is highly fluorescent. Thus, it can work as a good “turn-on” fluorescence probes for \(^1\text{O}_2\). The emission intensity of probe 58 is quite weak due to the strong triplet–triplet quenching that blocks the energy transfer. The triplet–triplet quenching disappears upon reaction with \(^1\text{O}_2\), thus the intense phosphorescence can be observed. In addition, the time-gated imaging was successfully used to detect \(^1\text{O}_2\) generation in living systems.

1.3.7 Photofunctional molecular materials for bioimaging of hydrogen sulfide (H\(_2\)S)

H\(_2\)S is associated with many physiological processes, including neuromodulation, apoptosis, and inflammation, etc. Abnormal intracellular H\(_2\)S level is relevant to some diseases, such as Alzheimer’s disease, Down’s syndrome and so on. Therefore, it is quite crucial to selectively track H\(_2\)S in living systems by luminescent probes.
Figure 1.12 Chemical structures of photofunctional molecular materials for intracellular H$_2$S detection.

Recently, two novel fluorescent probes (59 and 60) for selectively detecting intracellular H$_2$S based on H$_2$S-mediated reduction of azides to amines have been developed.[93] The azide functional group was introduced into rhodamine and the reductive reactions would induce the ring-opening process, resulting in bright fluorescence. Such fluorescence turn-on response is highly selective for H$_2$S over other sulfur species in living systems. In addition, these two probes are able to visualize alterations in H$_2$S concentration in live-cell imaging. Such kind of fluorescent probes provide an effective way for understanding the endogenous production of intracellular H$_2$S and its relationship with physiological processes.
1.4 Photofunctional molecular materials for electrochomic applications

In recent years, electrochromic materials have been intensively studied because these materials can potentially be used in various applications such as sensors, reflective-type display, memory devices, and smart mirrors, etc. In most cases, electrochromic materials are based on the change in absorption properties. In fact, luminescence is intrinsically more sensitive than absorption as a sensing technique. However, the electrochromic luminescent materials are quite rare and in view of their advantages including easy tuning of luminescence color, short response time and relatively high contrast, it is important to develop new electrochromic luminescent materials.

1.4.1 Luminescent electrochromic based on oxidation and reduction

Generally, the reversible transitions between oxidation and reduction states of π-conjugated polymers result in electrochromism. For example, an electrochemical polymer 61 has been developed by anodic oxidation and cathodic reduction. As shown in Figure 1.13, the emission colors of polymer 61 under UV light were different before and after the electrochemical reactions. Polymer 61 showed light blue-green fluorescence, however, the oxidation state of 61 exhibited a dark yellow luminescence with a emission peak at around 550 nm. In sharp contrast, polymer 61” affords a bright yellow emission in its reduction state. In addition, a multicolored gradient film has been achieved by placing polymer 61 on a BDD bipolar electrode.
Figure 1.13 (a) Chemical structure of electrochromic polymer 61. (b) Photographs of spin-coated films of 61, 61’, and 61” excited at 365 nm. (c) Photograph of the conducting polymer film under a UV lamp with a gradient composition.

1.4.2 Luminescent electrochromic based on the polarization of functional group

A series of cationic iridium complexes containing N-H unit in their ancillary ligand have designed and prepared. The various counter ions result in the alterations in the phosphorescence colors from yellow to green by the formation of hydrogen bonds. Interestingly, these complexes are sensitive to the electric field, resulting in electrochromic luminescence. On the basis of this interesting property, a quasi-solid information recording and storage device was successfully fabricated. More importantly, information encryption is demonstrated by employing an intensely
emissive short-lived fluorescent dye as interference, and the decryption is achieved with time-resolved imaging techniques. In particular, such security protection technology can be applied to the production of secret media, such as banknotes, identity cards, trademark tags and so on, and to prevent forgery, tampering or counterfeiting. Therefore, it is believed that this electrochromic phosphorescence will open up a novel method for applications in data encoding and decoding.

![Chemical structure of electrochromic complex 62.](image)

**Figure 1.14** (a) Chemical structure of electrochromic complex 62. (b) Photographs of a quasi-solid film under a UV lamp. (c) Schematic diagram of information encryption and decryption.

Similarly, as shown in Figure 1.15, complex 63 is another electrochromic phosphorescent iridium complex with an -OH unit developed for information recording, encryption, and decryption.\(^{102}\)
Figure 1.15 (a) Chemical structure of electrochromic complex 63. (b) Photographs of complex 63 in CH$_3$CN solution under UV lamp before and after electrical stimuli. (c) Schematic diagrams of data recording device and fluorescence microscope image and FLIM image of complex 63.

1.5 Scope of the thesis

To date, significant advancements have been achieved for chemical sensing and bioimaging. However, there are still many issues limit in the real application of fluorescent probes. Therefore, several aspects should be noticed to improve fluorescent probes and exploit their further application: (i) It will be important to develop water-soluble fluorescent probes with good sensing performances in vivo; (ii) It will be important to develop phosphorescent probes for application in time-resolved luminescence detection and bioimaging; (iii) It will be important to exploit
fluorescent probes with large two-photon absorption properties and apply them in two-photon excited bioimaging.

In addition, because electrochromic luminescent materials are quite rare, it would be of significance to develop novel electrochromic luminescent materials.

In this thesis, we focus on developing some novel water-soluble photofunctional molecular materials for two-photon and lifetime sensing and imaging. In addition, a series of cationic Zn(II) complexes has been designed and synthesized for electrochromic applications.
References


Biomaterials, 2011, 32, 1148.


Chapter 2 Water-Soluble Iridium(III) Complexes as Multicolor Probes for Single-Photon, Two-Photon and Fluorescence Lifetime Imaging

2.1 Introduction

Compared with organic dyes, phosphorescent heavy-metal complexes possess excellent photophysical properties, such as evident Stokes shifts for easy separation of excitation and emission, and relatively long emission lifetime (microseconds to milliseconds) of phosphorescent signals.\textsuperscript{[1-3]} The long emission lifetime allows us to avoid the interference from short-lived autofluorescence and scattered light by a time-resolved imaging technique.\textsuperscript{[4]} Thus, it is anticipated that such class of phosphorescent materials could be used as the appealing candidates for bioimaging. However, limited examples have been reported about the application of phosphorescent heavy-metal complexes in bioimaging to date.\textsuperscript{[5-11]}

Among all kinds of phosphorescent heavy-metal complexes, iridium(III) complexes are regarded as the most excellent phosphorescent materials due to their high photoluminescence efficiency and excellent color tunability. And they have exhibited promising application in the fields of biolabeling and phosphorescent chemosensing systems.\textsuperscript{[12-21]} Most recently, the application of cationic iridium(III) complexes in living cell imaging has also been reported.\textsuperscript{[22-27]}
For application in bioimaging, the phosphorescent probes should be water-soluble because the interaction media is water or buffer solution. However, the water-solubility of most reported phosphorescent heavy-metal complexes for bioimaging is very poor, and the addition of organic solvents (such as DMSO) is often required in the imaging experiments to improve the solubility of heavy-metal complexes in the aqueous media. Although bioimaging has been realized in these mixed solvents, the addition of organic solvents is often toxic to cell and destroys the cell membrane properties, which limits the real application of phosphorescent probes. Therefore, it is necessary to develop new phosphorescent probes with complete solubility in water.

![Scheme 2.1 Chemical structures of complexes 1-7.](image_url)

In this work, a series of cationic iridium(III) solvato complexes (1-7) (see Scheme 2.1) were synthesized. For them, the water-solubility was realized successfully
through the introduction of water as ancillary ligand. The emission colors of these iridium(III) complexes can be tuned evidently from green to red by changing the chemical structures of cyclometalating ligands. The cellular uptake of these complexes has been studied by confocal fluorescent microscopy. And multicolor phosphorescent imaging for cytoplasm staining of living cells has been realized.

2.2 Synthesis

The synthesis routes of these seven iridium(III) solvato complexes [Ir(C^N)2(H2O)2]^+CF3SO3^− (1-7) are shown in Schemes 2.2–2.5. As shown in Scheme 2.2, L1–L3 was prepared by four steps. Firstly, the N-phenyl of carbazole was achieved by modified Ullmann condensation between carbazole and appropriate p-iodoarene using CuI/phen/KOH as the catalyst combination and the product can be prepared in a large scale from the easily accessible carbazole. Secondly, 1.1 equiv. of N-bromosuccimide (NBS) was used to produce the 3-bromo-N-phenylcarbazole precursors. Then, the 3-boronic acid-N-phenylcarbazole was prepared by the reaction of n-butyl lithium and trimethylborate in dry THF at −77 °C. Lastly, L1–L3 were synthesized by using Suzuki coupling reaction with N-phenyl-3-boronic acid carbazole and various monomers (2-bromopyridine, 2-chloro-5-(trifluoromethyl)pyridine and 1-chloroisquinoline).

The syntheses of L4–L6 generally involve three steps. 2-Bromofluorene was firstly reacted with bromoethane in DMSO to produce 9, 9-diethyl 2-bromofluorene. Then, 9, 9-diethyl 2-boronic acid fluorene was prepared the reaction of n-butyl lithium and
trimethylborate. Lastly, L4–L6 were synthesized by using Suzuki coupling reaction with N-pheny-3-boronic acid carbazole and various monomers (2-bromopyridine, 2-chloro-5-(trifluoromethyl)pyridine and 1-chloroisooquinoline).

**Scheme 2.2** Synthetic route of cyclometalating ligands L1–L3

**Scheme 2.3** Synthetic route of cyclometalating ligands L4–L6.
There are several steps to prepare cyclometalating ligand L7. 4-Bromotoluene was reacted with n-butyl lithium and trimethylborate in dry THF at –77 °C to prepare the 4-methylbenzeneboronic acid first, and by using the Suzuki coupling reaction with 2-chloro-5-(trifluoromethyl)pyridine to give 2-p-tolyl-5-(trifluoromethyl)pyridine. The next steps are to reflux this compound with NBS and BPO in CCl₄, the refluxing with triethylphosphine in toluene to give compound i. Finally, L7 was prepared by the Wittig reaction with compound i and N-hexyl-carbazole-3-carbaldehyde.

Scheme 2.4 Synthetic route of cyclometalating ligand L7.

The chloro-bridged dinuclear cyclometalated iridium(III) precursors [Ir(C^N)₂Cl]₂ (C^N =L1–L7) were synthesized according to the method reported by Nonoyama. Then, the iridium(III) complexes were synthesized by the reactions of [Ir(C^N)₂Cl]₂ and AgOTf in the mixture of EtOH/H₂O (v : v = 9 : 1). All complexes can be soluble in water completely. The obtained complexes were characterized by ¹H NMR, ¹³C
NMR and MALDI-TOF.

\[
\begin{align*}
\text{L1 - L7} & \\
\text{Scheme 2.5 Synthetic route of complexes 1-7.}
\end{align*}
\]

2.3 Photophysical properties

The UV/vis absorption spectra of complex 1-7 in PBS buffer at room temperature are displayed in Figure 2.1, and the electronic absorption spectral data are summarized in Table 2.1. All of the complexes exhibit intense absorption bands below 350 nm, which are attributed to the spin-allowed ligand-centered transition \(^1\text{LC}\). The moderately intense absorption bands at about 350-400 nm are attributed to the singlet metal-to-ligand charge-transfer transition \(^1\text{MLCT}\) and the weak bands above 400 nm are assigned to the triplet metal-to-ligand charge-transfer transition \(^3\text{MLCT}\) and the spin-forbidden ligand-centered transition \(^3\text{LC}\).

The photoluminescence (PL) spectra of complex 1-7 in PBS buffer at room temperature are shown in Figure 2.2, and the corresponding PL data for different medium are given in Table 1. The emission wavelengths of these complexes can be tuned significantly from 504 to 659 nm in PBS by changing their cyclometalatating ligands. And their luminescent emission colors were also changed from green to red.
Figure 2.1 UV-visible spectra of complexes 1-7.

Figure 2.2 Photoluminescence spectra of complexes 1-7.

Table 2.1 Photophysical properties of complexes 1–7

<table>
<thead>
<tr>
<th>Complexes</th>
<th>solvent</th>
<th>$\lambda_{\text{abs}}$ nm (log $\varepsilon$)</th>
<th>$\lambda_{\text{PL}}$ (298 K), nm</th>
<th>$\tau$, ns</th>
<th>$\Phi_{\text{em}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PBS</td>
<td>299 (3.79), 383 (3.29)</td>
<td>504</td>
<td>322</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>PBS</td>
<td>302 (3.92), 398 (3.53)</td>
<td>559</td>
<td>413</td>
<td>0.001</td>
</tr>
<tr>
<td>3</td>
<td>PBS</td>
<td>254 (4.12), 299 (4.02), 409 (3.64)</td>
<td>598</td>
<td>229</td>
<td>0.002</td>
</tr>
<tr>
<td>4</td>
<td>PBS</td>
<td>306 (3.48)</td>
<td>556</td>
<td>389</td>
<td>0.001</td>
</tr>
<tr>
<td>5</td>
<td>PBS</td>
<td>334 (3.61)</td>
<td>589</td>
<td>474</td>
<td>0.002</td>
</tr>
<tr>
<td>6</td>
<td>PBS</td>
<td>301 (3.52), 398 (3.27)</td>
<td>659</td>
<td>371</td>
<td>0.003</td>
</tr>
<tr>
<td>7</td>
<td>PBS</td>
<td>297 (3.52), 403 (3.27)</td>
<td>654</td>
<td>275</td>
<td>0.002</td>
</tr>
</tbody>
</table>
The luminescence responses of complexes 3, 6 and 7 to various natural amino acids were investigated. Upon addition of histidine, the emission intensities of complexes 3, 6 and 7 were increased approximately by 4 to 10 times (Figure 2.3). However, no detectable change in phosphorescent emission of complexes 3, 6 and 7 was observed upon the addition of other amino acids, such as alanine (Ala), arginine (Arg), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr) and valine (Val), demonstrating that complexes exhibited high selectivity for His over other amino acids. According to the previous report, living cells contain a large amount of histidine, which might be beneficial to increase emission intensity of complexes 1-7 inside cells.

**Figure 2.3** Luminescent responses of complexes 3, 6 and 7 in PBS buffer to various amino acids. 1, Blank; 2, Ala; 3, Arg; 4, Asp; 5, Cys; 6, Gln; 7, Glu; 8, Gly; 9, His; 10, Leu; 11, Lys; 12, Met; 13, Phe; 14, Pro; 15, Ser; 16, Thr; 17, Trp; 18, Tyr; 19, Val.
2.4 Cytotoxicity

Generally, for application in living cells, a cellular probe should have low cytotoxicity and good cell permeability. The cytotoxicity of complexes 3, 6 and 7 toward the Hela cells has been determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The results are shown in Figure 2.4. The cellular viabilities were estimated to be over 90% for complexes 3, 6 and 7 with concentration of 5-200 μM for 24 h. For complex 3 and 6, even after the concentration was increased to 400 μM, cell viability was still greater than 90%. The cellular viabilities of complex 7 with concentration of 400 μM were assessed to be greater than 85%. These data imply that complexes 3, 6 and 7 are excellent probes for living cell imaging, showing low cytotoxicity to the living Hela cells.

Figure 2.4 Cell viability values (%) assessed by MTT proliferation test versus incubation concentrations of complexes 3, 6 and 7. Hela cells were cultured in the presence of 10-400 μM complexes 3, 6 and 7 at 37 °C for 24 h.
2.5 Single-photon living cell imaging

Practical application of complexes 1-7 in single-photon luminescence imaging of live Hela cells was performed using a confocal fluorescence microscopy. The confocal fluorescence microscopy images of live Hela cells incubated with complexes 1-7 (20 μM) for 30 min at 37 °C are shown in Figure 2.5. All complexes exhibited intense intracellular luminescence, and the overlays of confocal luminescence and bright-field images confirmed that the luminescence was obvious in the cytoplasm, not in the membrane and nucleus. Additionally, 3D luminescence imaging of the Hela cells (Figure 2.6a) further demonstrates that the complexes were internalized into the cells. Further quantization of the luminescent signal from the complex 7 along the line showed large signal ratios (I₂/I₃) between the cytoplasm (region 1) and nucleus (region 2), indicating that extremely weak nuclear uptake occurred for 7 (Figure 2.6b).

Figure 2.5 Confocal Luminescence, bright-field and overlay images of live Hela cells.

Hela cells incubated solely with 20 μM 1 (or 2-7) in PBS for 30 min at 37 °C.
Figure 2.6 (a) 3D luminescence image of live Hela cells. (b) Luminescence images (insert) and luminescence intensity profile of live Hela cells.

In order to investigate the cellular uptake mechanism of complexes, temperature dependence experiments have been carried out. Figure 2.7 shows the image of live Hela cells treated with complexes 3, 6 and 7 (20 μM) for 30 min at 4 °C. Interestingly, compared with incubation at 37 °C, incubation at 4 °C did not result in any observable changes in the cellular uptake with complexes 3, 6 and 7. As displayed in Figure 2.8, complexes 3, 6 and 7 were incubated with fixed Hela cells for 30 min at 37 °C, no notable luminescence intensity change can be observed. These results suggest that complexes 3, 6 and 7 entered the cells by energy-independent passive diffusion mechanism.
**Figure 2.7** Confocal luminescence images of live Hela cells: cells incubated solely with 20 μM 3 (or 6 and 7) in PBS for 30 min at 4 °C.

**Figure 2.8** Confocal luminescence images of fixed Hela cells: cells incubated solely with 20 μM 3 (or 6 and 7) in PBS for 30 min at 37 °C.
2.6 Two-photon living cells imaging

Application of complexes 3, 6 and 7 in two-photon luminescence imaging of live Hela cells was carried out on a laser scanning confocal microscope. As shown in Figure 2.9, when a semiconductor laser was served as the excitation at 800 nm, Hela cells exhibited very weak intracellular luminescence after incubation with complexes 3 and 6 for 30 min at 37 °C. However, complex 7 exhibits strong two photon induced emission and shows bright intracellular luminescence after treatment for 30 min at 37 °C, which might be due to the fact that the introduction the double bond into complex increases the two-photon cross section.

![Luminescence TPM images in Hela cells: cells incubated with 20 μM 7 in PBS for 30 min at 37 °C.](image)

**Figure 2.9** Luminescence TPM images in Hela cells: cells incubated with 20 μM 7 in PBS for 30 min at 37 °C.

2.7 Fluorescence lifetime imaging microscopy

Having demonstrated phosphorescent intracellular imaging with complex 7 by
confocal laser scanning microscopy, we carried out fluorescence lifetime imaging experiments for live Hela cells to further prove the attractive advantage of the long emission lifetime of phosphorescent signal in bioimaging. We expected that FLIM can isolate the phosphorescence signal from other contributions to the total photoluminescence. Complex 7 ($\lambda_{\text{max}} = 654$ nm) and Hoechst ($\lambda_{\text{max}} = 460$ nm, a commercial dye for staining the nuclei of cells) were chosen to stain the cytoplasm and nuclei of live Hela cells, respectively, to distinguish their luminescent wavelengths and staining regions. Figure 2.10d shows that the phosphorescence signals from complex 7 could be distinguishable from the fluorescence signals of Hoechst because of their significant differences in their lifetimes. These results further confirmed that 7 as phosphorescent probe is very beneficial for removing the short-lived fluorescence interference in bioimaging.

**Figure 2.10** (a-c) Confocal luminescence images of live Hela cells stained with complex 7 (red luminescence) and Hoechst (blue luminescence). (d) Fluorescence lifetime images of live Hela cells.
2.8 Conclusions

In summary, we have synthesized a series of water-soluble phosphorescent cationic iridium(III) solvato complexes (1-7) as multicolor cellular probes for imaging in living cells. All of these complexes can be dissolved in PBS. The emission of complexes can be tuned from green to red by changing the chemical structure of cyclometalating ligands. All complexes exhibit low cytotoxicity to living cells and exhibit cell membrane permeability and specific staining of cytoplasm. They enter the cells by the mechanism of energy-independent passive diffusion mechanisms. More importantly, complex 7 can act as a two-photon phosphorescent cellular probe, and fluorescence lifetime imaging microscopy is successfully applied for bioimaging in the presence of short-lived background fluorescence.
References


Chapter 3 Photoluminescent Materials for Carbon Dioxide Detection

3.1 A charged iridophosphor for time-resolved luminescent CO\textsubscript{2} gas identification

3.1.1 Introduction

Carbon dioxide gas detection is of great importance in various fields, such as public health, food packaging, agricultural production and medicine.\textsuperscript{[1,2]} Quantification of environmental release of CO\textsubscript{2} also needs significant attention because of its role in controlling global climate change. Many analytical methodologies have been developed for CO\textsubscript{2} detection, including infrared (IR) spectroscopic, electrochemical and gas chromatography-mass spectrometry (GC-MS) techniques.\textsuperscript{[3-5]} However, these methods are generally complicated, time-consuming, and require expensive and bulky equipment, and some of them are tolerating the interference of carbon monoxide and water vapor.\textsuperscript{[6-9]} Compared with other traditional methods, the optical CO\textsubscript{2} sensors have attracted considerable interest due to their low cost, simple construction, quick response, high tolerance to interfering agents, and ease of visibility to the naked eyes.\textsuperscript{[10-13]}

Recently, optical sensors have proved useful for the studies of many analytes because of their inherent ease of manufacture and use and relatively high chemical and mechanical stability. It is not uncommon to make use of the acidic properties of
CO\textsubscript{2} to design such optical sensors, but there are only few fluorescent pH indicators in the literature that can meet the requirements of p\textsubscript{K}\textsubscript{a}, photostability and brightness simultaneously.\textsuperscript{[14,15]} Up to now, several alternative detection approaches have been developed. By bubbling CO\textsubscript{2} into amine solution, it can generate carbamate ionic liquid, which is accompanied by an increase in viscosity, and such changes can be detected with the help of aggregation-induced emission (AIE).\textsuperscript{[16]} Recently, an optical CO\textsubscript{2} sensor that utilizes tetrapropyl benzobisimidazolium salts has been reported for both fluorescence and colorimetric detection,\textsuperscript{[17,18]} but this fluorescent CO\textsubscript{2} sensor gets excited and emit in the near UV region only, which limits its real application.

Although excellent CO\textsubscript{2} sensing results have been realized by exploiting fluorescent sensors, the interference of background fluorescence is always a thorny problem, which might come from some organic dyes in the solvents. Phosphorescent heavy-metal complexes typically with long emission lifetimes (in the microsecond to millisecond range) offer an effective way to solve this problem by using time-resolved photoluminescence technique (TRPT).\textsuperscript{[19-22]} Besides, their excellent photophysical properties, such as evident Stokes shifts and high photostability, etc, make them a good candidate for sensing purposes.\textsuperscript{[23-25]} Because of these, many chemical sensors based on heavy-metal complexes, such as iridium(III), ruthenium(II) and platinum(II), have been reported for targeting small molecules, anions, and metal ions.\textsuperscript{[26-29]} Among all, iridium(III) complexes are regarded as one of the most promising phosphorescent materials due to their high photoluminescence quantum yields and excellent color tunability. Utilization of these iridium(III) dyes in chemosensing systems has been
well highlighted in the literature.\textsuperscript{[30-33]}

It is well known that CO\textsubscript{2} can easily react with the amine (-NH) group and -NH unit on 2-phenylimidazo-[4,5-f][1,10]phenanthroline can be deprotonated by the addition of a strong alkali, leading to a quenching effect on the fluorophore. Therefore, we expect that CO\textsubscript{2} could react with the deprotonated -NH group, resulting in phosphorescence recovery. Here, we report the first example of a phosphorescent CO\textsubscript{2} sensor 8 in which a 2-phenylimidazo-[4,5-f][1,10]phenanthroline unit is introduced as an ancillary ligand in the cyclometalated iridium(III) complex (Scheme 3.1.1).

![Scheme 3.1.1](image)

**Scheme 3.1.1** Chemical structure of phosphorescent CO\textsubscript{2} sensor.

### 3.1.2 Synthesis

The 2-phenylimidazo-[4,5-f][1,10]phenanthroline ligand and 1-phenylisoquinoline were synthesized according to previous reports.\textsuperscript{[34,35]}
Complex 8 was prepared in two steps, firstly, the chloro-bridged dinuclear cyclometalated iridium(III) precursor [Ir(piq)₂Cl]₂ (piq = 1-phenylisoquinoline) was synthesized according to published method.⁹ Then, the complex 8 was synthesized through the bridge-splitting reaction of [Ir(piq)₂Cl]₂ and subsequent complexation with the ligand 2-phenylimidazo-[4,5-f][1,10]phenanthroline. The obtained complex was characterized by ¹H NMR, ¹³C NMR and MALDI-TOF.

3.1.3 Photophysical properties

The UV-vis absorption spectrum of 8 in CH₃CN displayed an intense band at 281 nm (ε = 3.2 × 10⁴ M⁻¹ cm⁻¹), which is attributed to the spin-allowed ligand-centered
transition (\(^1\)LC). And weaker broad bands at 382 nm (\(\varepsilon = 4.75 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}\)) and 444 nm (\(\varepsilon = 2.4 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}\)) are attributed to the singlet metal-to-ligand charge-transfer transition (\(^1\)MLCT), triplet metal-to-ligand charge-transfer transition (\(^3\)MLCT) and the spin-forbidden ligand-centered transition (\(^3\)LC) (Figure 3.1.1).

![Absorption Spectrum](image)

**Figure 3.1.1** UV-visible spectra of complexes 8 in CH\(_3\)CN.

![Emission Spectrum](image)

**Figure 3.1.2** Photoluminescence spectra of complex 8 in different solvents.

The photoluminescence spectra of 8 in different solutions (CH\(_2\)Cl\(_2\), EtOH, CH\(_3\)OH, CH\(_3\)CN and THF) are shown in Figure 3.1.2. The emission maximum of 8 is located
at ~596 nm which is rather independent of the solvent environment changes, suggesting that the emission mainly comes from the ligand-centered (LC) excited state.

3.1.4 CO$_2$ detection

![Scheme 3.1.4 Proposed sensing mechanism of CO$_2$ gas by complex 8.](image)

We first investigate the photoluminescence changes of 8 with different anions. Upon addition of CH$_3$COO$^-$ and F$^-$, the emission of 8 was quenched significantly. It is suggested that the quenching of 8 is due to an electron transfer process from the nitrogen atom of imidazolyl group, which possesses a partial negative charge after the formation of hydrogen bond (Scheme 3.1.4), to the excited iridium center. However, there are no obvious spectral variations for 8 upon addition of other anions (such as
Cl, Br, I, NO$_3^-$, ClO$_4^-$, HSO$_4^-$) (Figure 3.1.3).

![Figure 3.1.3 Photoluminescence responses of complex 8 (10 μM) with the addition of 3.0 equiv of various anions, Cl$^-$, Br$^-$, I, NO$_3^-$, ClO$_4^-$, HSO$_4^-$ in CH$_3$CN. The black bars represent the I$_0$/I ratios at 596 nm after addition of anions to solutions of complex 8.]

Because of the relatively low toxicity of CH$_3$COO$^-$ to the environment as compared to F$^-$, it was chosen to interact with complex 8 to quench its luminescence. As shown in Figure 3.1.4, when 3 equiv. of CH$_3$COO$^-$ was added to the CH$_3$CN solution of 8 (10 μM), the absorption band at 281 nm was red shifted to 295 nm and a new band appeared at 346 nm, indicating that a strong bond between (N-H)$^+$$\cdots$CH$_3$COO$^-$ has been formed and the phosphorescence emission was quenched significantly (Figure 3.1.4).
Figure 3.1.4 Changes in UV/Vis spectra of complex 8 CH$_3$CN solution (10 μM) containing CH$_3$COO$^-$ (3 equiv) with different volumes of CO$_2$ gas (0–15mL).

Next, we expect that bubbling of CO$_2$ gas into the solution of 8 containing CH$_3$COO$^-$ would destroy the hydrogen bond by interacting CO$_2$ with the -NH unit on the imidazolyl group and then recover the luminescence. With the increasing volume of CO$_2$ (as governed by a mass flow controller) bubbled into the solution, the absorbance at 295 and 346 nm decreased gradually (Figure 3.1.4), which indicated that CO$_2$ reacted with the lone electron pair in 8. Furthermore, a dramatic increase in the phosphorescence intensity at 596 nm was observed upon exposure to an increasing volume of CO$_2$ gas (Figure 3.1.5). As shown in Figure 3.1.5, after being bubbled with about 15 mL of CO$_2$ gas, the mixture emitted bright orange light, and no additional change in the emission spectrum was observed.
**Figure 3.1.5** (a) Changes in phosphorescence spectra of 8 in CH$_3$CN solution (10 μM) containing CH$_3$COO$^-$ (3 equiv.) with different volumes of CO$_2$ gas (0–15 mL). Inset: titration curve of 8 solution (10 μM) containing CH$_3$COO$^-$ (3 equiv.) with CO$_2$ gas (0–15 mL). (b) Photos of the emission of 8, 8+CH$_3$COO$^-$, and 8+CH$_3$COO$^-$ with CO$_2$ bubbling in CH$_3$CN.

**Figure 3.1.6** Changes in phosphorescence emission spectra of complex 8 in CH$_3$CN solution (10 μM) containing F$^-$ (3 equiv) with 15 mL CO$_2$ gas.

The same phenomenon can be observed by the addition of F$^-$ followed by bubbling with CO$_2$ gas (Figure 3.1.6). The selectivity of complex 8 to CH$_3$COO$^-$ for CO$_2$ is
extremely high as there are no apparent changes in phosphorescence intensity upon treatment with CO, N₂, O₂ or argon gas. The CO₂ detection limit of this complex was calculated to be ca. 6.45 × 10⁻⁶ M (Figure 3.1.7).

**Figure 3.1.7** Changes in phosphorescence intensity of a solution of complex 8 in CH₃CN (10 μM) containing CH₃COO⁻ (3 equiv.) with various quantities CO₂ gas. Iₘᵢₙ represents the phosphorescence intensity (at 596 nm) of 8 with 3 equiv CH₃COO⁻. Iₘₐₓ represents the phosphorescence intensity (at 596 nm) of 8 with 3 equiv CH₃COO⁻, followed by bubbling with 15 mL CO₂ gas.

The CO₂/N₂ mixtures with different percentage of CO₂ were bubbled into identifying solution to check how the PL of 8 responses to variations in fraction of CO₂ (f_{CO₂}). As shown in Figure 3.1.8, PL spectrum of 8 was intensified with increasing percentage of CO₂, which further indicates the high sensitivity of 8. In addition, the plot shows a characteristic linear signal over the whole concentration range (Figure 3.1.8). According to this calibration curve, the amount of CO₂ in gas mixtures can be quantified.
Figure 3.1.8 Plots of PL intensities of 8 versus fractions of CO$_2$ ($f_{CO_2}$) in CO$_2$/N$_2$ mixtures.

To check if complex 8 can be reversibly switched by treatment with CH$_3$COO$^-$ followed by CO$_2$ bubbling, we conducted experiments with on–off cycling between 8 and 8-OOCCH$_3$. It was observed that complex 8 was not degraded after five cycles of treatment with CH$_3$COO$^-$ followed by CO$_2$ bubbling, indicating the good cyclability of our probe (Figure 3.1.9).
Figure 3.1.9 Reversible phosphorescence intensity ($I_{596nm}$) change between 8-OOCCH$_3$ (bottom points) and 8 (top points), which corresponds to the repeated treatment of 8 by the addition of CH$_3$COO$^-$ followed by CO$_2$ bubbling.

3.1.5 Time-resolved phosphorescence assay

One of the most important features of 8 is on its long phosphorescence lifetime ($\tau$), which is beneficial for avoiding the interference of short-lived background fluorescence emission. Therefore, complex 8 is envisioned to be useful for the detection of CO$_2$ gas in the presence of short-lived fluorescence emission by employing the time-resolved photoluminescence technique. Here, rhodamine B with the $\tau$ value of 1.67 ns (Figure 3.1.10) was selected as a typical source of short-lived background fluorescence because its broad emission band can overlap with that of 8. The emission lifetime of 8 in CH$_3$CN solution was measured to be $\tau = 227$ ns (Figure 3.1.10), which is long enough to carry out the TRPT experiment.
Figure 3.1.10 Fluorescence lifetime decay profiles of (a) rhodamine B and (b) complex 8 with CH$_3$COO$^{-}$ and then treatment with CO$_2$ gas in CH$_3$CN using the TCSPC method with an excitation wavelength of 365 nm.

The steady-state photoluminescent spectrum for the mixture of complex 8 with CH$_3$COO$^{-}$ and rhodamine B is dominated by the strong emission of rhodamine B and we could barely see the emission band from 8 with CH$_3$COO$^{-}$ (Figure 3.1.11, red line). Then, 15 mL CO$_2$ gas was bubbled into the mixture to allow reaction with complex 8 containing CH$_3$COO$^{-}$. However, very slight change from the steady-state spectrum (Figure 3.1.11, black line) was observed, which revealed that the detection probe suffered from severe interference due to the background fluorescence. Then, the time-resolved emission spectra of the mixture were recorded. As portrayed in Figures 3.1.11 a and b, by bubbling 15 mL CO$_2$ gas into the mixture, the emission intensity was enhanced significantly in the time-resolved emission spectra. The time-gated photoluminescence spectrum acquired after 100 ns delay of the mixture completely removed the background fluorescence from rhodamine B (Figure 3.1.11d). This result
highlights the promising application of long-lived phosphorescent probes in time-resolved assays.

**Figure 3.1.11** (a) Time-resolved emission spectra of the mixture of complex 8 solution containing CH$_3$COO$^-$ and rhodamine B. (b) Time-resolved emission spectra of the mixture of complex 8 solution containing CH$_3$COO$^-$ and rhodamine B with bubbling of 15 mL CO$_2$ gas. (c) Photoluminescence spectrum (0 ns) of the mixture of complex 8 solution containing CH$_3$COO$^-$ with bubbling of 15 mL CO$_2$ gas. (d) Time-gated photoluminescence spectrum acquired after 100 ns delay of the mixture of complex 8 solution containing CH$_3$COO$^-$ with bubbling of 15 mL CO$_2$ gas.

### 3.1.6 Photostability

Considering that continuous excitation often results in the photobleaching of
fluorescent sensors, and thus high photostability becomes a very important issue for fluorescent sensors in real applications, which is highly beneficial for the long-term observation. Hence, the photobleaching experiments were carried out between the complex 8 and pyrene under the same excitation conditions (405 nm, laser as the excitation source). The pyrene has a very similar chemical structure as 8-hydroxy-1,3,6-trisulfonate (HPTS) (Figure 3.1.12), which is the most famous state-of-the-art sensor for CO$_2$ gas. As shown in Fig. 3a, after continuous excitation at 405 nm for 600 seconds, the emission intensity of complex 8 only decreased to 90% of its initial value. In contrast, the emission intensity of pyrene decreased to 29% of the original value due to photobleaching. This result demonstrates that our phosphorescent sensor shows a higher photostability and reduced photobleaching than that of a purely organic pyrene.

**Figure 3.1.12** (a) Emission intensity of complex 8 solution containing CH$_3$COO$^-$ with bubbling of CO$_2$ gas and pyrene under continuous laser excitation (at 405 nm). (b) Chemical structure of HPTS and pyrene.
3.1.7 Detection mechanism of CO$_2$ gas

In order to confirm the detection mechanism of CO$_2$ gas by 8, the interactions of 8 with CH$_3$COO$^-$ and CO$_2$ gas were investigated by $^1$H NMR technique. Figure 3.1.13 shows the change of chemical shifts of 8 upon addition of 3 equiv. of CH$_3$COO$^-$. As we can see from the $^1$H NMR spectra, the resonance signals of protons on the cyclometalated ligands showed little change. In contrast, the partial negative charge on nitrogen, which was generated by the interaction between CH$_3$COO$^-$ and -NH unit, led to the proton signals on 2-phenylimidazo-[4,5-f][1,10]phenanthroline ligand exhibiting an obvious upfield shift. After bubbling the CO$_2$ gas into a solution of complex 8 containing CH$_3$COO$^-$, the chemical shifts of protons on the ancillary ligand were recovered to some extent due to the destruction of hydrogen bond. But, this restoration was not complete, which might be caused by the influence of the presence of acetate, acidic condition and the increase of viscosity after bubbling of CO$_2$ gas into the solution. As a control study, 2-phenylimidazo-[4,5-f][1,10]phenanthroline ligand was used to react with CH$_3$COO$^-$ and CO$_2$ gas to further confirm the detection mechanism. Compared with complex 8, the same variation trend in chemical shifts was observed in DMSO-$d_6$ after addition of CH$_3$COO$^-$ in the $^1$H NMR spectra. The exposure of L-OOCCH$_3$ to CO$_2$ gas induced almost complete recovery of the chemical shifts of all protons (Figure 3.1.14).
Figure 3.1.13 $^1$H NMR spectra of $8$, $8+\text{CH}_3\text{COO}^-$, and $8+\text{CH}_3\text{COO}^-$ treated with $\text{CO}_2$ in $\text{CD}_3\text{CN}-d_3$.

Figure 3.1.14 $^1$H NMR spectra of $L$, $L-\text{CH}_3\text{COO}^-$, and $L-\text{CH}_3\text{COO}^-$ treatment with $\text{CO}_2$ in $\text{DMSO}-d_6$. 
Figure 3.1.15 shows the $^{13}$C NMR spectrum of 2-phenylimidazo-[4,5-f][1,10]phenanthroline that was first exposed to CH$_3$COO$^-$ and then bubbled with CO$_2$ in DMSO-$d_6$. The chemical shifts of carbon atoms due to the formation of hydrogen bond can be well recovered by the exposure of 2-phenylimidazo-[4,5-f][1,10]phenanthroline to CO$_2$ gas. In addition, a new characteristic signal at ~160 ppm was observed, which can be assigned to HCO$_3^-$ anion based on the literature results. These results are consistent with the observed photoluminescence changes and support the proposal above that hydrogen bond is formed upon CH$_3$COO$^-$ treatment which is followed by destruction of the bond via the interaction with CO$_2$.

![Partial $^{13}$C NMR spectra of L, L+CH$_3$COO$^-$ and L+CH$_3$COO$^-$ treated with CO$_2$ in DMSO-$d_6$.]
Figure 3.1.16 (a) Proposed formation of the CS$_2$ adduct of 2-phenylimidazo-[4,5-f][1,10]phenanthroline. (b) $^{13}$C NMR spectra of L and L-CS$_2^-$ K$^+$ in DMSO-$d_6$.

As a further control study, 2-phenylimidazo-[4,5-f][1,10]phenanthroline ligand was used to prepare the corresponding $N$-dithiolate derivative by interaction with carbon disulfide, which is an isoelectronic analogue of CO$_2$. The $N$-dithiolate derivative was prepared by using 2-phenylimidazo-[4,5-f][1,10]phenanthroline ligand which was treated with KOH for 4 h followed by reaction with CS$_2$ in DMSO solution (Figure 3.1.16). The $^{13}$C NMR spectroscopic technique shows that a new carbon signal at ~216 ppm appeared (Figure 3.1.16), indicating that the stable $N$-dithiolate adduct was formed. Collectively, these results provide support for the conclusions that complex 8 reacted with CO$_2$ gas to form the intermediate complex 8b, and then the original
complex 1 was recovered after the decomposition of 8b.

3.1.8 Conclusions

In summary, we have developed a novel phosphorescent probe based on an iridium(III) complex with 2-phenylimidazo-[4,5-f][1,10]phenanthroline for CO$_2$ detection. After bubbling CO$_2$ into the sensing solution, the quenched phosphorescence by addition of CH$_3$COO$^-$ can be recovered. This phosphorescent CO$_2$ sensor shows higher photostability than some of the reported organic probes. More importantly, the time-resolved PL experiment demonstrates that 8 is able to detect CO$_2$ in the presence of strong background fluorescence.
3.2 A water-soluble tetraphenylethene based probe for luminescent carbon dioxide detection and its biological application

3.2.1 Introduction

CO₂ concentration is critically important in promoting the growth and normal function of living cells. To create an environment suitable to support living cells, an atmospheric condition of 5% CO₂ is needed. In order to detect the external/internal CO₂ concentration of living cells, water-soluble luminescent probes are highly desirable. Although excellent CO₂ detection results have been obtained by exploiting luminescent sensors, most of them can only detect CO₂ gas in organic solvents. Therefore, the research on water-soluble luminescent CO₂ probes for the detection of external/internal CO₂ concentration of living cells is still quite rare. Thus, it motivates us to further explore new water-soluble chromophores for CO₂ gas sensing. It is not uncommon to make use of the acidic properties of CO₂ to design water-soluble fluorescent probe. However, there are only few fluorescent pH indicators in the literature that can meet the requirements of pKₐ and brightness simultaneously.

Tang’s group discovered the phenomenon of aggregation-induced emission (AIE), and many molecules with AIE properties have been developed in recent years. Tetraphenylethene (TPE) is a typical AIE-active molecule, which possesses the advantages of facile synthesis and flexible structural modification. Up to now, a large number of TPE based molecules have been developed for chemical sensing and
bioimaging.\cite{39-41} In this work, we present a TPE based probe for sensing CO$_2$ in aqueous solution and measuring the external CO$_2$ concentration of living cells. Scheme 3.2.1 describes the design concept of the detection. Sodium phenolic salt (−O’Na$^+$), as a hydrophilic group, is incorporated into the TPE moiety and a new compound (9) is obtained. Compound 9 is soluble and non-emissive in the aqueous solution. After bubbling CO$_2$ gas into the aqueous solution, it would be in a chemical equilibrium with the carbonic acid produced. Consequently, the water insoluble and AIE-active TPE-OH can be generated by the reaction between 9 and carbonic acid. Then, strong fluorescence could be observed in the sensing solution (Scheme 3.2.1).

\begin{center}
\textbf{Scheme 3.2.1} Proposed detection mechanism of CO$_2$ gas in aqueous solution by 9.
\end{center}
3.2.2 Synthesis

As depicted in Scheme 3.2.2, compound 9 was synthesized in three steps. Firstly, TPE-OCH₃ was prepared through a McMurry coupling of 4,4'-dimethoxybenzophenone. This was followed by demethylation to give TPE-OH. Finally, the target compound 9 was prepared by the reaction of TPE-OH with CH₃ONa in methanol. Compound 9 has good water solubility due to the incorporation of sodium phenolic salt. The desired compounds were characterized by ¹H and ¹³C NMR spectroscopy, MALDI-TOF mass spectrometry, and elemental analysis.

Scheme 3.2.2 Synthetic routes of 9.
3.2.3 AIE properties

Figure 3.2.1 (a) PL spectra of TPE-OH in THF–water mixtures (50 μM) with different water fractions. (b) Plots of the relative PL intensity (I/I₀) of TPE-OH at 434 nm versus the composition of THF–water mixtures of TPE-OH (I₀ is the PL intensity in pure THF solution).
We first studied the photoluminescence (PL) properties of TPE-OH in solution and aggregated states. As shown in Figure 3.2.1, TPE-OH is almost non-emissive when dissolved in THF solution, and the blue fluorescence at 434 nm is constantly intensified with increasing water volume fraction in the THF-water mixed solution, clearly demonstrating the AIE effect of TPE-OH. However, for the 9, it is non-emissive in both solution and aggregated states, which may be attributed to the low-lying charge transfer states of the anionic compound.\textsuperscript{43}

3.2.4 CO\textsubscript{2} detection

![Figure 3.2.2 Plot of $I_{434\,\text{nm}}$ versus pH value.](image)

Firstly, pH response properties of 9 were studied in aqueous solution. As shown in Figure 3.2.2, the response of 9 is described by a typical sigmoidal dependence, and
the pKa value is calculated to be 9.7. This value is high and shows potential suitability of 9 for highly sensitive carbon dioxide probes.

**Figure 3.2.3** (a) Changes in the UV-vis spectra of 9 in aqueous solution (50 μM) with 0.25 mL of CO₂ gas. Inset: photos of the emission of 9 and 9 with CO₂ bubbling in aqueous solution. (b) Changes in the fluorescence spectra of 9 in aqueous solution (50 μM) with different volumes of CO₂ gas (0–0.32 mL). Inset: titration curve of 9 solution (50 μM) with different volumes of CO₂ gas (0–0.32 mL) and photos of the emission of 9 and 9 with CO₂ bubbling in aqueous solution.

Next, we expect that bubbling of CO₂ gas into the aqueous solution would transform negatively charged 9 to neutral TPE-OH, thus leading to the spectral changes. As shown in Figure 3.2.3a, with the 0.25 mL of CO₂ (as governed by a mass flow controller) bubbled into the solution, the absorbance at 580 nm disappeared and a new peak at 324 nm emerged, suggesting that CO₂ reacted with 9 in aqueous solution. Consequently, the solution color was changed significantly from purple black to straw yellow. As shown in Figure 3.2.3b, analogous effects were observed in the corresponding fluorescent emission spectra. A dramatic increase in the
fluorescence intensity at 434 nm was observed upon exposure to an increasing volume of CO₂ gas, and it can be increased by up to 91-fold upon further bubbling of CO₂ gas. After being bubbled with about 0.32 mL of CO₂ gas, the mixture emitted bright blue light, and no additional change in the emission spectrum was observed. Other sour gas, such as NO₂, SO₂ and HCl are pungent or colored, which makes them quite distinguishable from CO₂. Besides, there are no apparent changes in the fluorescence intensity upon treatment with colorless and odorless CO, Ar, O₂ and N₂. Thus, we consider that the selectivity of 9 towards CO₂ is extremely high.

![Dynamic light scattering results for 9 (50 μM) with CO₂ bubbling in aqueous solution.](image)

**Figure 3.2.4** Dynamic light scattering results for 9 (50 μM) with CO₂ bubbling in aqueous solution.

To understand the excellent detection performance, dynamic light scattering (DLS), a powerful tool to study aggregate formation, was employed to investigate the size change of 9 with CO₂ addition. Compound 9 is well dissolved as an isolated molecule
in aqueous solution, so its particle sizes could not be measured by DLS. Upon bubbling of CO₂ gas, the insoluble compound TPE-OH was generated by the reaction of 9 and CO₂ in aqueous solution. Thus, the aggregated state was formed, with an average diameter of 458 nm from DLS as displayed in Figure 3.2.4. Consequently, an obvious emission enhancement at 434 nm was found for 9 after bubbling CO₂.

![Image](image_url)

**Figure 3.2.5** Time-dependent fluorescence spectra of 9 in aqueous solution with different amounts of CO₂ gas (0.25, 0.5, 1.0 mL).

The fluorescence spectra of 9 in aqueous solution with different amounts of CO₂ gas (0.25, 0.5, 1.0 mL) were recorded. Figure 3.2.5 shows that no apparent change in the fluorescence intensity was observed upon treatment with 0.25 mL CO₂ gas after 180 s. And the time-dependent spectral change reveals that after bubbling 0.5 and 1.0 mL CO₂ gas into the detecting solution, the reaction will be completed within 120 s. These results indicate that the response of 9 to CO₂ is fast. In addition, the detection limit was calculated to be as low as 2.4 × 10⁻⁶ M (Figure 3.2.6). Because of the good
sensitivity and high selectivity coupled with the fast spectral response, 9 can be considered as a suitable candidate for the real application of CO₂ detection.

**Figure 3.2.6** Changes in the fluorescence intensity of a solution of 9 in aqueous solution (50 μM) with various quantities of CO₂ gas.

Furthermore, to emphasize the practical application of 9, a porous film was fabricated as an efficient CO₂ gas sensor. 9 along with sodium carboxymethyl cellulose was dissolved in water to form a rubber matrix, which is highly permeable to CO₂. Concentration of 9 in sodium carboxymethyl cellulose was calculated in weight percent. We fabricated the film with a concentration of 1.0% of 9. The porous film has a clearly visible response to CO₂ gas, which is illustrated in Figure 3.2.7a. The left part shows a photographic image of the film, which is purple black under the daylight and non-emissive after excitation by the UV light (365 nm). The purple black color of the 9 was faded to colorless when CO₂ gas was purged. In the meantime, the
obvious bright blue fluorescence was observed under the UV light after the porous film was exposed to CO₂ gas. These observations are in accordance with the sensing results in aqueous solution.

![Photographic images and PL spectra](image)

**Figure 3.2.7** (a) Photographic images of the porous film under the daylight and UV light. (b) The PL spectra of porous film before (black line) and after (red line) the treatment of CO₂ gas.

### 3.2.5 Detection mechanism of CO₂ gas

In order to confirm the sensing process of CO₂ gas by 9, ^1^H NMR spectroscopic experiments were performed in DMSO-\(d_6\). From Figure 3.2.8, we can see that the resonance signals of protons of 9 and TPE-OH in the ^1^H NMR spectra are quite different. The notable change in chemical shifts was observed after the subsequent exposure of 9 to CO₂ gas, which induced almost complete recovery of the chemical shifts of protons on the phenyl ring, restoring them to the positions observed for TPE-OH. However, the proton signals on the phenol group did not appear after
bubbling CO$_2$ into the solution. The $^1$H NMR spectral change upon the addition of HCO$_3^-$ into TPE-OH solution indicates that the absence of the proton signals on phenol group might be due to the interaction between HCO$_3^-$ and phenol group. These results further support the proposal above that the negatively charged 9 was transformed to neutral TPE-OH after bubbling CO$_2$ gas into the solution, thus leading to the remarkable changes in color and photoluminescence.

![Figure 3.2.8](image)

**Figure 3.2.8** Partial $^1$H NMR spectra of TPE-OH, 9, 9 with CO$_2$ and TPE-OH with HCO$_3^-$ in DMSO-$d_6$. 

85
3.2.6 Cytotoxicity

![Graph showing cell viability values vs concentration of TPE-ONa](image)

**Figure 3.2.9** Cell viability values (%) assessed using an MTT proliferation test versus incubation concentrations of 9. HeLa cells were cultured in the presence of 0–25 μM 9 at 37 °C for 24 h.

The excellent CO₂ sensing results obtained in the aqueous solution encouraged us to explore the biological applications of 9. Firstly, the cytotoxicity towards HeLa cells was evaluated by a standard MTT (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay. The results are illustrated in Figure 3.2.9. After the treatment of the live HeLa cells with different concentrations of 9 for 24 h, the cellular viabilities were estimated to be approximately 92% at 25 μM, suggesting the low cytotoxicity of 9. Thus, it has potential to serve as a probe for biological applications.
3.2.7 Biological application

![Fluorescence images of 9 in HeLa cells.](image)

**Figure 3.2.10** Fluorescence images of 9 in HeLa cells. (a, b) HeLa cells incubated with 10 μM 9 for 2 h at 37 °C and normal atmospheric condition (0.038% CO₂). (c, d) HeLa cells further incubated for 1 h at 37 °C and 5% CO₂. (e) Average fluorescence intensities of 9 treated with 0.038% CO₂ and further treated with 5% CO₂.

The confocal fluorescence microscopy experiment was carried out to explore the capability of 9 as a specific bioprobe for monitoring the variation of external CO₂ concentration of living cells. Before washing with Roswell Park Memorial Institute 1640 (RPMI 1640) for three times, the live HeLa cells were incubated with 10 μM of 9 for 2 h at 37 °C and normal atmospheric condition (0.038% CO₂). As shown in Figure 3.2.10, only a very weak intracellular fluorescence signal could be observed. Then, the percentage of CO₂ in the incubation chamber was increased from 0.038% to 5% for a further 1h. After the variation, the remarkable intracellular fluorescence signal was subsequently observed (Figure 3.2.10). The average intracellular intensity analysis suggests that the emission intensity was enhanced by approximately 4-folds. 
after the concentration of CO\textsubscript{2} increased from 0.038\% to 5\%. These observations suggest that the increase of external CO\textsubscript{2} concentration causes the enhancement of fluorescence emission intensity, revealing the capability of 9 for measuring the external CO\textsubscript{2} concentration changes of living cells.

3.2.8 Conclusions

In summary, we have developed a water-soluble fluorescent probe based on tetraphenylethene derivative for CO\textsubscript{2} detection. After bubbling CO\textsubscript{2} into the detection solution, remarkable color change and fluorescence enhancement could be observed. The response of 9 to CO\textsubscript{2} in aqueous solution is fast and the detection limit is about 2.4 \times 10^{-6} \text{ M}. To emphasize the practical application of 9, a porous film was successfully fabricated by mixing the dye with sodium carboxymethyl cellulose in water, which can serve as an efficient CO\textsubscript{2} gas sensor. More importantly, 9 exhibits low cytotoxicity towards live cells and has the ability to monitor the external CO\textsubscript{2} concentration changes of living cells.
References


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[22] H. F. Shi, H. B. Sun, H. R. Yang, S. J. Liu, G Jenkins, W. Feng, F. Y. Li, Q. Zhao,


Chapter 4 An Emerging Application of Soft Salts in Ratiometric and Lifetime Imaging of Living Systems

4.1 Introduction

Fluorescence bioimaging based on fluorescent probes provides a powerful approach for visualizing morphological details in biological systems with subcellular resolution.\(^1\) However, most traditional fluorescent probes often suffer from the interference due to autofluorescence and scattered light, which increases the background and reduces the signal-to-noise ratio (SNR). Recently, an emerging technique, namely, photoluminescence lifetime imaging microscopy (PLIM), offers an effective way of eliminating unwanted background interference based on the lifetime difference between the probe and interference signal. In addition, lifetime as the detecting signal is independent of excitation laser intensity, target molecule concentration and photobleaching, and it is very beneficial for imaging applications. Phosphorescent transition-metal complexes (PTMCs) typically exhibiting long emission lifetime, evident Stokes shifts and high photostability,\(^2\text{-}^4\) are ideal candidates for biological applications, especially for lifetime imaging applications,\(^5\text{-}^7\) although examples of PTMCs for monitoring intracellular biomolecules by PLIM are quite rare.

Despite the advantages of PTMCs-based probes, most previously reported probes were based on single emission intensity changes. The diversities in cell morphology
within different districts might influence the quality and quantity of emission signals, which can result in substantial misinterpretations when dynamic changes of intracellular biomolecules are investigated. Therefore, accurate and quantitative measurements of the actual concentrations of intracellular biomolecules or the relative changes of concentrations in a living cell are difficult. Ratiometric measurement is normally used to address this issue. It can permit simultaneous recording of two separate wavelengths instead of measuring single emission intensity changes and thus offer built-in correction for environmental effects, leading to a more favorable system for imaging living cells and tissues. However, most ratiometric probes developed recently for the imaging of biological molecules are based on organic dyes or nanoparticles. It is still a challenge to design PTMCs-based ratiometric probes due to their complicated excited-state properties.

Luminescent ion pairs, which consist of two photoactive coordination complexes with opposite charges, are called “soft salts” due to the soft nature of the ions. Recently, Thompson and his co-workers have studied the photophysical properties of soft salts in detail and successfully applied them in organic light-emitting diodes. However, studies on soft salts have not received too much attention yet. Considering that two emission wavelengths from a soft salt can be easily separated by chemical modification of cyclometalated ligands of the two ionic complexes, soft salt will be a good and versatile platform for the design of phosphorescent ratiometric probes. To date, however, the applications of soft salts in chemical sensing and biological systems are still the unexploited areas.
Here we present the first example of a soft salt based ratiometric probe for imaging and measuring pH variations in living cells. Intracellular pH is a crucial parameter associated with cellular behaviors and pathological conditions, such as cell proliferation, apoptosis,\textsuperscript{[17]} drug resistance,\textsuperscript{[18]} enzymatic activity,\textsuperscript{[19]} and ion transport.\textsuperscript{[20]} Abnormal cellular pH value is an indicator of inappropriate cellular functions, which are associated with many common diseases, for example, stroke, cancer, Alzheimer’s disease and so on. It is thus vital to monitor pH alterations in biological cells and tissues to understand the physiological and pathological processes.\textsuperscript{[21,22]}

Figure 4.1 schematically describes the design concept. We selected the cationic complex C\textsubscript{1} with pendant pyridyl moieties as the pH-sensitive phosphor and the anionic complex A\textsubscript{1} as the pH-insensitive phosphor. These two luminophores were connected by electrostatic interaction to form the soft salt complex 10. When 10 is excited at 380 nm, the excitation photons are mainly absorbed by the A\textsubscript{1} moiety, and the energy is partially transferred from A\textsubscript{1} to C\textsubscript{1} moiety. Complex 10 is expected to give two emission bands, namely pH-insensitive blue and pH-sensitive red phosphorescence emissions. Thus, the ratio of the phosphorescence intensities can respond to different pH values (2.03–7.94). Moreover, 10 exhibits two well-resolved emission peaks separated by about 150 nm (from 475 to 625 nm), which avoids the mutual interference of two emission bands and allows for the high-resolution and sensitive ratiometric response of pH variations. Hence, complex 10 could act as an ideal ratiometric probe to monitor the pH variations in biological cells. Furthermore,
to utilize the long phosphorescence lifetime of complex 10, PLIM experiment was
carried out to monitor intracellular pH alterations.

Figure 4.1 Design concept of a ratiometric pH probe and chemical structures of
complexes A1, C1 and 10.

4.2 Synthesis

The cyclometalated iridium(III) chloro-bridged dimer [Ir(C^N)2Cl]2 (C^N =
2-(2,4-difluorophenyl)pyridine (dfppy) or 2-phenylpyridine (ppy)) and the
2,2′:4,4″:4′,4″′-quaterpyridyl (qpy) ligand were synthesized according to the
literature methods.[23,24] The cationic iridium(III) complex [Ir(ppy)2qpy]^+Cl^- (C1) was
prepared by refluxing biscyclometalated iridium(III) dichloro-bridged dimer in the
presence of an excess of qpy ligand.[25] The anionic iridium(III) complex
[Ir(dfppy)2(CN)2] Bu4N+ (A1) was synthesized from [Ir(dfppy)2Cl]2 and
tetrabutylammonium cyanide (10 equiv) in dichloromethane at 50 °C for 4 h. By mixing two oppositely charged iridium(III) complexes A1 and C1 (1 : 1 molar ratio) in the mixture of CH₃CN-H₂O (1 : 1, v : v) at room temperature, the soft salt (10) was obtained through the metathesis reaction. The obtained complexes were characterized by ¹H and ¹³C NMR spectroscopy, MALDI-TOF spectrometry and elemental analysis.

4.3 Photophysical properties

The photophysical data of A1, C1 and 10 are summarized in Table 4.1. Spectroscopic studies of A1 and C1 in acetonitrile are shown in Figure 4.2. The emission peak of anionic complex A1 located at around 451 and 475 nm displays vibronic progressions, which is the result of a triplet ligand-centered (³LC) transition on the cyclometalated ligands.[26] The cationic complex C1 shows a broad and featureless spectrum with the emission maximum at 625 nm. Therefore, C1 is expected to emit from metal-to-ligand charge-transfer (MLCT) state.[27] The photoluminescent (PL) spectrum of 10 exhibits concentration dependence, which is shown in Figure 4.2b. The ratio of emission peaks of A1 and C1 varies significantly depending on the solution concentration, suggesting that the degree of energy transfer between the two complexes is different. At 380 nm excitation, the emission is mainly from the anionic complex A1 at the relatively low concentration of 10⁻⁶ M, which might be due to the fact that the quantum efficiency of A1 is much higher than that of C1. The blue emission decreases as the concentration of solution increases, with the cationic
complex C1 acting as a quencher of A1, and the emission of A1 is barely observed at a concentration of $10^{-3}$ M and above.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Solvent</th>
<th>$\lambda_{\text{abs}}, \text{nm}$ (log$\varepsilon$)</th>
<th>$\lambda_{\text{PL}}, \text{nm}$</th>
<th>$\tau_{\text{ms}}$</th>
<th>$\Phi_{\text{em}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>CH$_3$CN</td>
<td>251 (4.92) 293 (4.58) 364 (4.05)</td>
<td>451, 475</td>
<td>1329</td>
<td>0.62</td>
</tr>
<tr>
<td>C1</td>
<td>CH$_3$CN</td>
<td>249 (4.70) 291 (4.43) 386 (3.78)</td>
<td>633</td>
<td>413</td>
<td>0.18</td>
</tr>
<tr>
<td>10</td>
<td>CH$_3$CN</td>
<td>249 (4.92) 289 (4.56) 364 (4.01)</td>
<td>453, 480, 633</td>
<td>819/398</td>
<td>0.22</td>
</tr>
</tbody>
</table>

The quenching study was carried out to investigate the energy transfer between two ionic components in 10. As shown in Figure 4.2c, with the addition of an increasing amount of cationic complex C1 into an acetonitrile solution of the anionic complex A1 ($10^{-5}$ M), the red emission increased gradually at the expense of the blue emission from A1, indicating that the energy transfer/quenching process is very efficient between two ionic complexes. The quenching rate constant ($K_q$) can be extracted by dividing the slope of the fitted straight line by $I_0$ (the fluorescence intensity with no quencher present) (Figure 4.2d). The calculation yielded a $K_q$ value of $5.49 \times 10^{10}$ M$^{-1}$ s$^{-1}$. As shown in Figure 4.2a, the overlap between the absorption of C1 and the emission of A1 is poor, so the electron exchange (Dexter) energy transfer might be responsible for the energy transfer in this system.$^{[20]}$
Figure 4.2 (a) Normalized absorption and photoluminescence spectra of A1 and C1 in acetonitrile solution. (b) Photoluminescence spectra of 10 at different concentrations in acetonitrile solution. (c) Photoluminescence spectra of anionic complex A1 (10⁻⁵ M) in acetonitrile solution with various amounts of cationic complex C1 (0–1.0 × 10⁻⁵ M). (d) Stern-Volmer plot of the quenching study between C1 and A1 ([Q] is the concentration of quencher).

4.4 Ratiometric response to pH variations

The phosphorescence emission spectra of A1, C1 and 10 were examined in acetonitrile/phosphate buffer solution (1 : 9, v : v) at various pH values (2.03–7.94). There are no obvious spectral variations for A1 solutions of different pH values (Figure 4.3a). For C1, the emission intensity at 625 nm decreases dramatically with
the decrease of pH value, which can serve as an on-off single intensity based pH probe (Figure 4.3b). Protonation makes pyridine ring in the ancillary ligand of C1 a stronger electron acceptor, which can cause quenching of the phosphorescence.

**Figure 4.3** (a) Changes in the phosphorescence emission spectra of A1 (2.0 × 10^{-5} M) in the pH range of 2.03–7.94 in CH_{3}CN/buffer (1 : 9, v : v). (b) Changes in the phosphorescence emission spectra of C1 (2.0 × 10^{-5} M) in the pH range of 2.03–7.94 in CH_{3}CN/buffer (1 : 9, v : v). (c) Changes in the phosphorescence emission spectra of 10 (2.0 × 10^{-5} M) in the pH range of 2.03–7.94 in CH_{3}CN/buffer (1 : 9, v : v). (d) Plot of I_{625 nm}/I_{451 nm} versus pH values. I_{625 nm} and I_{451 nm} indicate the phosphorescence intensity at 625 nm and 451 nm, respectively.

To realize a ratiometric probe, a soft salt 10 constituted of A1 and C1 by electrostatic interaction has been developed. The phosphorescence spectral changes of
**10** at different pH values are displayed in Figure 4.3c. Increasing pH values results in higher phosphorescent intensity of **C1** at 625 nm ($I_{625\text{ nm}}$), while the emission intensity of **A1** at 451 nm ($I_{451\text{ nm}}$) remains unchanged. Such a change of phosphorescence emission color from blue to red with increasing pH value can be easily observed by naked eyes (Figure 4.3c). The relative ratio of phosphorescence intensities ($I_{625\text{ nm}}/I_{451\text{ nm}}$) increased by 16-fold (from 0.18 to 2.86) over the pH range of 2.03–7.94 (Figure 4.3d), which covers most physiological pH values. In addition, the phosphorescence response of **10** to pH value displays an excellent reversibility (Figure 4.4).

![Figure 4.4 pH reversibility study of 10 between pH 2 and 8.](image)

We investigated the interference on the pH measurement by biological molecules, and the phosphorescence spectral responses of **10** in the presence of oxidative-stress-associated redox chemicals (such as cysteine (Cys), homocysteine (Hcy), glutathione (GSH) and H$_2$O$_2$) and essential metal ions (such as K$^+$, Na$^+$, Zn$^{2+}$, Cu$^{2+}$, Ca$^{2+}$, Mn$^{2+}$, Mg$^{2+}$, Fe$^{2+}$ and Fe$^{3+}$) were measured. There are no detectable
spectroscopic changes observed (Figure 4.5), which indicates that 10 could act as a phosphorescent probe for the detection of intracellular pH alterations without any interference. In addition, the stability of 10 in acetonitrile/phosphate buffer solution (1:9, v:v) at 37 °C was investigated. Figure 4.6 shows that the relative ratio of phosphorescence intensities ($I_{625\text{ nm}}/I_{451\text{ nm}}$) barely changed at 37 °C even for 2 h, suggesting the good stability of 10.

Figure 4.5 Phosphorescence spectra of 10 (2.0 × 10^{-5} M) in the presence of 1.0 × 10^{-4} M of oxidative-stress-associated redox chemicals and metal ions (Cys, Hcy, GSH, H_2O_2, K^+, Na^+, Zn^{2+}, Cu^{2+}, Ca^{2+}, Mn^{2+}, Mg^{2+}, Fe^{2+} and Fe^{3+}) in CH_3CN/buffer (1 : 9, v : v).
Figure 4.6 Relative ratio of phosphorescence intensity ($I_{625\,nm}/I_{451\,nm}$) changes of 10 in the pH range of 2-8 at 37 °C.

4.5 Cytotoxicity

Figure 4.7 Cell viability values (%) assessed using an MTT test versus incubation concentrations of 10. HepG-2 cells were cultured in the presence of 0–200 μM 10 at 37 °C for 24 h.
The cytotoxicity towards HepG-2 cells was evaluated by a standard MTT (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay. The results are illustrated in Figure 4.7. After the treatment of the living HepG-2 cells with different concentrations of 10 for 24 h, the cellular viabilities were estimated to be approximately 85% at 200 μM, apparently indicating the good biocompatibility and low cytotoxicity of 10.

4.6 Ratiometric imaging

![Figure 4.8](image)

**Figure 4.8** (a, b) Confocal luminescence, (c) overlay images and (d) bright-field images of living HepG-2 cells. HepG-2 cells incubated with 10 μM 10 for 1 h at 37 °C.

Practical application of complexes C1, A1 and 10 in luminescence imaging of living HepG-2 cells was investigated using a confocal fluorescence microscopy. After incubation with 10 μM of C1 (A1 or 10) for 1 h at 37 °C, notable intracellular
luminescence was observed in HepG-2 cells (Figure 4.9 and Figure 4.10). The overlay of confocal luminescence and bright-field images demonstrated that luminescence was evident in the cytoplasm region.

Figure 4.9 Real-time monitoring of live cell staining with A1 and C1, respectively.

Figure 4.10 Real-time monitoring of live cell staining with 10.

To determine the kinetics of complex internalization, time-lapse imaging was carried out to monitor the progression of A1, C1 and 10 in HepG-2 cells via a Live Cell Workstation. Confocal images were obtained after 15 min, 30 min, 45 min, and 1
h, respectively. As shown in Figure 4.9, only very weak luminescence was observed in the cells in first 45 min for A1, indicating the slow cellular uptake rate of A1. In contrast, for C1, notable luminescence was detected in the cells within a short period of time (15 min). These findings suggest that the cellular uptake rates are different for A1 and C1, which might be due to the different ionic nature of complexes. However, when 10 was treated with HepG-2 cells, both the blue and red luminescence was observed in the cells at 15 min (Figure 4.10), implying that the cellular uptake rates for the each counterpart of the soft salt are similar.

![Figure 4.11](image)

**Figure 4.11** (a) Living HepG-2 cells co-stained with 10 μM A1 and C1 for 1 h at 37 °C, and (b) living HepG-2 cells incubated with 10 under the same conditions.

In addition, the overlapping rate was calculated to demonstrate that two counterparts of the soft salt remained intact in the cells. From Figure 4.11 we can see that the overlapping rate between blue and red channels was calculated to be 70.3% when living cells were co-stained with A1 and C1. However, after the treatment of 10
with living cells, the overlapping rate of two channels was 94.6%. These observations suggest that the cationic and anionic parts of 10 should be kept intact rather than fell apart in the cells.

<table>
<thead>
<tr>
<th>pH</th>
<th>3.98</th>
<th>5.02</th>
<th>6.08</th>
<th>7.01</th>
<th>8.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td><img src="image1" alt="Image A1 pH 3.98" /></td>
<td><img src="image2" alt="Image A1 pH 5.02" /></td>
<td><img src="image3" alt="Image A1 pH 6.08" /></td>
<td><img src="image4" alt="Image A1 pH 7.01" /></td>
<td><img src="image5" alt="Image A1 pH 8.01" /></td>
</tr>
<tr>
<td>C1</td>
<td><img src="image6" alt="Image C1 pH 3.98" /></td>
<td><img src="image7" alt="Image C1 pH 5.02" /></td>
<td><img src="image8" alt="Image C1 pH 6.08" /></td>
<td><img src="image9" alt="Image C1 pH 7.01" /></td>
<td><img src="image10" alt="Image C1 pH 8.01" /></td>
</tr>
</tbody>
</table>

**Figure 4.12** Phosphorescence images of A1 and C1 in HepG-2 cells clamped at pH 3.98, 5.02, 6.08, 7.01 and 8.0, respectively.

![Graph A1 C1 pH](image11)

**Figure 4.13** Average intracellular emission intensity of A1 and C1 at different pH values.
Figure 4.14 (a) Phosphorescence images of 10 in HepG-2 cells clamped at pH 3.98, 5.02, 6.08, 7.01 and 8.01, respectively. The excitation wavelength was 405 nm and the images of the first row (blue channel) and second row (red channel) were collected in the ranges of 430–480 nm and 600–700 nm, respectively. Overlay images (third row) and ratio images were obtained from the red and blue channels (fourth row). (b) Phosphorescence emission spectra of the HepG-2 cells at pH 3.98 and 8.01.

Then, these complexes were used for monitoring intracellular pH changes. The HepG-2 cells were cultured with 10 μL nigericin (10 ng/mL) for 10 min to
homogenize intracellular pH value first. Remarkable intracellular luminescence enhancement was observed with the increase in pH value for C1 (Figures 4.12 and 4.13). In contrast, no obvious luminescence intensity change can be detected in HepG-2 cells for A1 (Figures 4.12 and 4.13). We subsequently exploited the ratiometric probe 10 to examine the cellular pH value changes in living cells. As shown in Figure 4.14, the luminescence from the red channel (600–700 nm) in cells increases with increasing pH value, whereas that from the blue channel (430–480 nm) hardly alters. These imaging results have further been demonstrated by the measured phosphorescence emission spectra of HepG-2 cells at pH 3.98 and 8.01 (Figure 4.14), which show the significant variation in the red channel and small change in the blue channel. The obtained intracellular phosphorescence emission spectra are also similar to those measured in solution. Thus, the variations in the ratio of blue to red intensity reveal the ability of 10 to measure a pH-dependent signal linearly over the pH range of 4–8 ($R^2 = 0.9854$).

### 4.7 Lifetime imaging

<table>
<thead>
<tr>
<th>Table 4.2 Emission lifetime of 10 at different pH values</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 3.99</td>
</tr>
<tr>
<td>$\tau_{451 \text{ nm}}$ (ns)</td>
</tr>
<tr>
<td>$\tau_{625 \text{ nm}}$ (ns)</td>
</tr>
</tbody>
</table>
Figure 4.15 Phosphorescence intensity images collected at different time ranges (0-700 ns).

<table>
<thead>
<tr>
<th>pH</th>
<th>3.98</th>
<th>5.02</th>
<th>6.08</th>
<th>7.01</th>
<th>8.01</th>
</tr>
</thead>
</table>

Figure 4.16 Phosphorescence lifetime images of 10 in living HepG-2 cells at different pH values. HepG-2 cells were incubated for 1 h at 37 °C.

To utilize the long phosphorescence lifetime of complex 10, a PLIM experiment was carried out for living HepG-2 cells. We expected that PLIM can separate the long-lived phosphorescence signal from other contributions to the total photoluminescence. The emission lifetimes of 10 were first measured in CH₃CN-buffer (1 : 9, v : v) of different pH values (Table 4.2). The lifetime from 625 nm is increased with increasing pH value (from 73.2 to 327.5 ns), but that from 451 nm stays unchanged (around 700.0 ns), which is similar to the variation trend of the emission intensity. Then, the PLIM experiment was performed after the living
HepG-2 cells were incubated with 10 μM 10 at 37 °C for 1 h. As shown in Figure 4.16, the average photoluminescence lifetime (τ_{avg}) of 10 experienced an obvious increase with the decrease of pH value. The τ_{avg} of 10 was determined to be 178 ns by PLIM when the intracellular pH value was 8.01. As the intracellular pH value decreased from 8.01 to 3.98, the τ_{avg} of 10 were measured to be 209 ns, 247 ns, 271 ns and 312 ns, respectively. The PLIM experiment collected photons randomly from both blue and red emission, and only red emission was affected by the change of pH value. Therefore, increasing photons from blue emission are collected as the pH decreases due to the reduction of the red emission intensity. And the fact that τ_{625 nm} is decreasing as the pH decreases (Table 4.2) indicates that the increase in average photoluminescence lifetime from pH 8 (τ_{avg} = 178 ns) to pH 4 (τ_{avg} = 312 ns) is reasonable. Thus, the result has demonstrated that 10 has the ability to detect the intracellular pH alterations by the photoluminescence lifetime, which highlights the capability of removing background fluorescence.

4.8 Quantitative measurement of intracellular pH fluctuations

The ratio channel, obtained based on the above two distinguishable emission channels, shows a characteristic pH-dependent signal, demonstrating the ability of 10 to examine a pH-dependent signal linearly over the pH range of 3.98 to 8.01 (Figure 4.17). According to this calibration curve, the averaged intracellular pH value of intact HepG-2 cells was measured to be 6.80 ± 0.20 (Figure 4.17). Furthermore, the effects of different redox substances on intracellular pH fluctuations were investigated based
on the calibration curve. As shown in Figure 4.17, the pH value for H$_2$O$_2$ treated cells was determined to be 7.20 ± 0.15, which indicates that H$_2$O$_2$ makes the HepG-2 cells more basic. This observation is in good agreement with the previous report that the oxidative stress (such as H$_2$O$_2$) can cause the inactivation of lysosomal V-ATPase, consequently resulting in increasing the pH value of the lysosomes.[28] Then, NEM (N-ethylmaleimide, a GSH inhibitor) or NAC (N-acetylcysteine, a GSH precursor) was applied to HepG-2 cells to control the intracellular GSH level. The intracellular pH value was measured to be 7.10 ± 0.12 after decreasing the concentration of GSH by NEM (Figure 4.17). Possible reasons for the basification of cells are as follows: (i) an oxidative cellular environment caused by the decrease of GSH induces lysosomal inactivation; (ii) the function of the Na$^+$/H$^+$ antiporter may be affected by the decrease of GSH level.[29] Interestingly, the generation of GSH by NAC decreases the intracellular pH to 4.80 ± 0.16 (Figure 4.17). We believe that a reductive cellular environment caused by the high concentration of GSH induces the activation of lysosomal V-ATPase, which is a possible explanation for this acidification.[30] Ratiometric images directly reveal the intracellular pH changes caused by the oxidative stress. To take advantage of ratiometric measurement, accurate and quantitative determinations of actual intracellular pH value and its relative changes can be well achieved. Moreover, when the lifetime serves as a signal, these intracellular pH variations could also be detected by PLIM. Figure 4.17c shows the phosphorescence lifetime images of intact cells, H$_2$O$_2$ treated, NEM treated and NAC treated cells, and their $\tau_{avg}$ were determined to be 217 ns, 206 ns, 201 ns and 263 ns,
respectively. This result highlights that the detection of intracellular pH alterations by lifetime signal can avoid the interferences from autofluorescence, scattered light as well as other technical artifacts.

Figure 4.17 (a) Intracellular pH calibration curve of 10 in HepG-2 cells. (b) Ratiometric images and (c) phosphorescence lifetime images of 10 (10 μM). Intact cells, H₂O₂ (100 μM) treated, NEM (100 μM) treated and NAC (100 μM) treated cells were incubated for 1 h at 37 °C.

4.9 Conclusions

In conclusion, we have developed a novel soft salt based phosphorescent probe. This type of probe consists of two oppositely charged ionic complexes with two distinguishable emission colors, which makes it a perfect candidate as a ratiometric probe. The emission color of 10 changes from blue to red with increasing pH value. 10 is cell-permeable and exhibits low cytotoxicity, and it has been successfully applied for ratiometric pH imaging with the use of confocal microscopy.
demonstrating its great potential for intracellular environment monitoring. Furthermore, phosphorescence lifetime imaging experiments can detect intracellular pH variations by photoluminescence lifetime measurements, which allowed for eliminating background fluorescence and selecting long-lived phosphorescence images. Quantitative measurement of intracellular pH fluctuations caused by oxidative stress has been successfully carried out for 10 based on the pH-dependent calibration curve. To our knowledge, this work represents the first example of a soft salt based probe for chemical sensing and biological applications. We expect that this work can provide valuable information for the future rational design of phosphorescent ratiometric and lifetime probes.
References


Chapter 5 Color Tuning of Zinc(II) Bis-terpyridine Complexes via the Variation of Counterions and Their Applications in White Light Generation, Electrochromic and Information Protection

5.1 Introduction

Zn(II) complexes represent an emerging class of templates for the development of novel photoelectronic materials, chemical sensing as well as bioimaging systems in the recent decades.\textsuperscript{[1-4]} Because Zn(II) complexes are highly promising with respect to low cost, thermodynamically stable bond, large two-photon absorption (TPA) cross section and high photoluminescence quantum efficiency.\textsuperscript{[1-4]} To realize the full color display, fluorescence color tuning has been studied in the past few decades. To date, tremendous progress has been made in fluorescence color tuning of Zn(II) complexes by structural modification in the skeletal structure as well as electron-donating or -withdrawing substituents of the ligand. Therefore, it would be significant to develop other approaches for fluorescence color tuning.

Electrochromic materials have been intensively studied because these materials can be potentially applied in various applications such as sensors, reflective-type displays, memory devices, and smart mirrors, etc.\textsuperscript{[5-18]} In most cases, electrochromic materials are based on the change in absorption properties.\textsuperscript{[7-11]} In fact, luminescence is intrinsically more sensitive than absorption as a sensing technique. However, the
electrochromic luminescent materials are still rare and in view of their advantages including easy tuning of luminescence color, short response time and relatively high contrast, \cite{14-18} it is desirable to develop novel type of electrochromic luminescent materials. Current literature shows that information recording and storage device was fabricated by utilizing the phosphorescent cationic iridium complexes. In addition, information security protection technology has attracted much interest since it can be applied to the production of secret media, such as banknotes, identity cards, trademark tags and so on, and to prevent forgery, tampering or counterfeiting. To date, several strategies have been exploited for data security protection, including mass spectra, Raman spectra and phosphorescence lifetime. Therefore, the development of novel electrochromic luminescent materials and new approach to achieve the information security protection is highly desirable.

In this chapter, we demonstrate the full potential of a color tuning methodology for cationic Zn(II) bis-terpyridine complexes by the variation of counterions and suggest its most probable mechanism. A series of cationic bis-terpyridine Zn(II) complexes (11a-11d) exhibits the emission colors from green yellow to orange red (549 nm to 622 nm) in CH$_2$Cl$_2$ solution controlled by different counterions (CH$_3$COO$^-$, BF$_4^-$, ClO$_4^-$ and PF$_6^-$). It is believed that the differences in basicity with various counterions are responsible for the changes in photophysical properties of these Zn(II) complexes. In addition, it is found that the counterions play crucial roles in regulating the lowest-energy excited state of these Zn(II) complexes. On the basis of these findings, a white light emission has been generated by doping 0.6% complex 11d into
poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (PEG-PPG-PEG). In addition, the emission colors of these complexes were changed under an applied voltage, and a quasi-solid data recording and storage device has been successfully fabricated to show the distinguishable luminescence color alteration from orange to sky blue at a low voltage. Furthermore, by utilizing the advantageous relatively large TPA cross section of these Zn(II) complexes, for the first time, data encryption and decryption were achieved by the TPA excitation method.

![Chemical structures of complexes 11a-11d.](image)

**Figure 5.1** Chemical structures of complexes 11a-11d.

### 5.2 Synthesis

The synthesis of 4-([2,2':6',2''-terpyridin]-4'-yl)-N,N-diphenylaniline (tpypa) was carried out as published previously.\cite{cite} The Zn(II) bis-terpyridine complexes of [Zn(tpypa)2]^{2+}X^- (11a-11d, X = CH_3COO, BF_4, ClO_4, PF_6) were obtained by refluxing ligand tpypa with zinc(II) acetate (0.5 equiv) in methanol, followed by anion exchange with NaBF_4, or NaClO_4, or NaPF_6. The obtained complexes were
characterized by $^1$H and $^{13}$C NMR spectroscopy, MALDI-TOF spectrometry and elemental analysis. Single crystals of 11b-11d were obtained by slow diffusion of hexane to its dichloromethane solution (Figure 5.2). Basic crystallographic data are summarized in Table 5.1 and selected bond distances and angles for the complexes are given in Table 5.2. As confirmed by X-ray crystallography, the zinc ion can be considered to have a distorted octahedral coordination geometry. Interestingly, the bond lengths between zinc ion and terpyridine ligand are different with various counterions. For example, in 11d, the bond lengths of Zn-N are 2.165, 2.056, 2.151, 2.172, 2.054, 2.155, respectively, which is shorter than that of 11b (2.182, 2.071, 2.191, 2.204, 2.070 and 2.199) and 11c (2.202, 2.106, 2.178, 2.203, 2.107 and 2.187) (Table 5.2).

### Table 5.1 Summary of X-ray crystallographic data for complexes 11b, 11c and 11d

<table>
<thead>
<tr>
<th>Complexes</th>
<th>11b</th>
<th>11c</th>
<th>11d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical Formula</td>
<td>$C_{67.5}H_{51}Cl_3B_2F_8N_8$</td>
<td>$C_{68}H_{52}Cl_4N_8O_8$</td>
<td>$C_{66}H_{48}F_{12}N_8P_2Z$</td>
</tr>
<tr>
<td>Zn</td>
<td>Zn</td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>Formula Weight</td>
<td>1319.50</td>
<td>1387.24</td>
<td>1308.43</td>
</tr>
<tr>
<td>Crystal System</td>
<td>Triclinic</td>
<td>Triclinic</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space Group</td>
<td>$P\bar{1}$</td>
<td>$P\bar{1}$</td>
<td>$P21/c$</td>
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<tr>
<td>$a$ (Å)</td>
<td>9.194(4)</td>
<td>9.341(3)</td>
<td>10.684(2)</td>
</tr>
<tr>
<td>$b$ (Å)</td>
<td>17.447(8)</td>
<td>17.194(7)</td>
<td>16.579(3)</td>
</tr>
<tr>
<td></td>
<td>19.681(9)</td>
<td>20.094(8)</td>
<td>36.027(7)</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>$c$ (Å)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha$ (°)</td>
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<td>98.23(2)</td>
<td>90</td>
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<tr>
<td>$\beta$ (°)</td>
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<td>91.11</td>
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<tr>
<td>$\gamma$ (°)</td>
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<td>90</td>
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<tr>
<td>$V$ (Å$^3$)</td>
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<td>3131(2)</td>
<td>6380</td>
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<tr>
<td>$Z$ value</td>
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<td>2</td>
<td>4</td>
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<tr>
<td>$D$ (calcd) (g cm$^{-3}$)</td>
<td>1.444</td>
<td>1.471</td>
<td>1.362</td>
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<td>Temperature (K)</td>
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<td>173(2)</td>
<td>173(2)</td>
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<tr>
<td>$\mu$ (mm$^{-1}$)</td>
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<td>3.430</td>
<td>1.716</td>
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<tr>
<td>$F$ (000)</td>
<td>1350</td>
<td>1424</td>
<td>2672</td>
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<tr>
<td>$R_1$, $wR_2$[$I &gt; 2\sigma(I)$]</td>
<td>0.0854, 0.2234</td>
<td>0.1401, 0.3711</td>
<td>0.0558, 0.1360</td>
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<td>$R$ indices (all data)</td>
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<td>0.1826, 0.4042</td>
<td>0.0710, 0.1435</td>
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<td>Goodness-of-fit on $F^2$</td>
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<td>1.738</td>
<td>1.042</td>
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</table>
Figure 5.2 Single crystal structures of 11b-11c.
Table 5.2 Selected bond lengths [Å] in single crystal structures of 11b, 11c and 11d

<table>
<thead>
<tr>
<th></th>
<th>Bond Length [Å]</th>
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<tbody>
<tr>
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</tr>
<tr>
<td>Zn(1)-N(6)</td>
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</tr>
<tr>
<td>Zn(1)-N(5)</td>
<td>2.204(4)</td>
</tr>
<tr>
<td>11c</td>
<td></td>
</tr>
<tr>
<td>Zn(1)-N(2)</td>
<td>2.106(6)</td>
</tr>
<tr>
<td>Zn(1)-N(6)</td>
<td>2.107(5)</td>
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<tr>
<td>Zn(1)-N(3)</td>
<td>2.178(6)</td>
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<tr>
<td>Zn(1)-N(7)</td>
<td>2.187(8)</td>
</tr>
<tr>
<td>Zn(1)-N(1)</td>
<td>2.202(7)</td>
</tr>
<tr>
<td>Zn(1)-N(5)</td>
<td>2.203(8)</td>
</tr>
<tr>
<td>11d</td>
<td></td>
</tr>
<tr>
<td>Zn(1)-N(6)</td>
<td>2.054(4)</td>
</tr>
<tr>
<td>Zn(1)-N(2)</td>
<td>2.056(4)</td>
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<tr>
<td>Zn(1)-N(3)</td>
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<tr>
<td>Zn(1)-N(7)</td>
<td>2.155(5)</td>
</tr>
<tr>
<td>Zn(1)-N(1)</td>
<td>2.165(4)</td>
</tr>
<tr>
<td>Zn(1)-N(5)</td>
<td>2.172(4)</td>
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5.3 Fluorescence color tuning by counterions

The rationalization of photophysical properties of 11a-11d requires the determination of the energy and the nature of the highest-occupied and the lowest-unoccupied molecular orbitals (HOMO and LUMO). Time-dependent density functional theory (TDDFT) calculation was performed on these Zn(II) complexes to explore the nature of their excited states. Figure 5.3 illustrates that the HOMO is located primarily on the triphenylamine group, while the LUMO and LUMO+4 mainly reside on the terpyridine (Tpy) moiety. For them, the $S_1$ originates from HOMO → LUMO (68%) and HOMO → LUMO+4 (13%) transitions. These calculation results demonstrate
that intraligand (IL) and intraligand charge transfer (ILCT) transition states participate in the excited states of these Zn(II) complexes.

![HOMO and LUMO distributions of Zn(II) complexes.](image)

**Figure 5.3** HOMO and LUMO distributions of Zn(II) complexes.

The photophysical data of 11a-11d are listed in Table 5.3, and the UV-visible absorption and photoluminescence (PL) spectra of all complexes (10 μM) in CH₂Cl₂ are illustrated in Figure 5.4. As shown in Figure 5.4a, the spectra of 11a-11d feature an intense band at around 300 nm, which can be assigned to the ligand-centered (LC) π-π* transition. The strong absorption bands in the region from 350 to 500 nm can be assigned to intraligand charge transfer (ILCT) transition, since MLCT has to be excluded for Zn(II) complexes because of their d¹⁰ electron configuration.²²,²³
<table>
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<tr>
<th>Complexes</th>
<th>Solvent</th>
<th>$\lambda_{\text{abs}}, \text{nm}$</th>
<th>$\lambda_{\text{PL}}, \text{nm}$</th>
<th>$\tau, \text{ns}$</th>
<th>$\Phi_{\text{em}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>11a</td>
<td>CH$_2$Cl$_2$</td>
<td>284, 302, 407</td>
<td>549</td>
<td>1.85</td>
<td>0.26</td>
</tr>
<tr>
<td>11b</td>
<td>CH$_2$Cl$_2$</td>
<td>284, 321, 334, 457</td>
<td>576</td>
<td>1.67</td>
<td>0.19</td>
</tr>
<tr>
<td>11c</td>
<td>CH$_2$Cl$_2$</td>
<td>284, 321, 333, 457</td>
<td>594</td>
<td>2.31</td>
<td>0.16</td>
</tr>
<tr>
<td>11d</td>
<td>CH$_2$Cl$_2$</td>
<td>284, 319, 332, 458</td>
<td>622</td>
<td>2.12</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Generally, fluorescence color tuning is achieved through the change of the HOMO-LUMO energy gap. The emission bathochromic shift is realized by the stabilization of the LUMO energy level and destabilization of the HOMO energy level, while the blue shift is obtained in the opposite way. For 11a-11d, the emission colors in CH$_2$Cl$_2$ are gradually red shifted from green yellow to orange red (549 nm–622 nm) in the order CH$_3$COO$^-$ → BF$_4^-$ → ClO$_4^-$ → PF$_6^-$ (Figures 5.4b and 5.4c). It is believed that the different basicity of counterions is responsible for the changes of photophysical properties. The ionic charge introduced by Zn$^{2+}$ when it is coordinated to Tpya ligand makes the Tpy unit a stronger electron-withdrawing group, which is beneficial to stabilizing the LUMO energy level, while the HOMO energy level is barely affected. It is known that the net charge quantity of zinc center of the complex varies as a function of the basicity of the counterions. In the case of PF$_6^-$ as the counterions, the zinc center may have more net charge due to the weak basicity of PF$_6^-$. In contrast, when CH$_3$COO$^-$ of strong basicity is used as counterions, the zinc centers...
center may have less net charge. Accordingly, with the increase of basicity of the counterions, the electron-withdrawing ability of Tpy unit decreases. Therefore, as illustrated in Figure 5.4d, the emission exhibits a blue shift due to the destabilization of LUMO energy level as induced by the increase of basicity of counterions (CH$_3$COO$^-$ > BF$_4^-$ > ClO$_4^-$ > PF$_6^-$). These demonstrations indicate that by controlling the basicity of counterions it could be an effective way for fluorescence color tuning of Zn(II) complexes.

![Figure 5.4](image)

Figure 5.4 (a) Absorption spectra and (b) room-temperature normalized photoluminescence spectra of complexes 11a–11d in CH$_2$Cl$_2$. (c) Photos of the emission of 11a–11d in CH$_2$Cl$_2$. (d) Schematic illustration of the influence of counterions on the LUMO energy level of Zn(II) complexes.
In addition, it is believed that counterions provide a stabilizing force for the ILCT excited state of Zn(II) complexes. To confirm that the counterions can influence the photophysical properties of Zn(II) complexes, the UV-visible absorption and PL spectra of 11d were recorded in different solvents. As shown in Figure 5.5a, the low energy bands originated from ILCT transition are greatly affected by the polarity of solvents. The intense ILCT absorption band is dramatically decreased with the increasing polarity of the solvents, and only very weak ILCT band can be observed in DMSO and DMF. Besides, the emission band shows a blue shift with the increasing solvent polarity as well. Complex 11d displays an orange red fluorescence emission at 622 nm in CH$_2$Cl$_2$ solution, and a green fluorescence emission at 540 nm was observed in the more polar CHCl$_3$ solution. As the polarity of the solvent increases, the emission peak is further shifted to around 495 nm in CH$_3$CN, DMSO and DMF. In less polar solvent, the electrostatic interaction between counterions and Zn(II) complex is believed to be stronger due to the poorer coordinating capacity of the solvent, which is beneficial to stabilizing the ILCT excited state. When the polarity increases, the electrostatic interaction between counterions and Zn(II) complex decreases because the counterions are driven out by the polar solvent molecules. Such change may result in the destabilization of ILCT excited state of Zn(II) complex (Figure 5.5). Accordingly, the high polarity solvent leads to a switch in the nature of the lowest-energy excited state from ILCT to IL character of Zn(II) complex. To trace the detailed change of solvent-dependent emission, the PL spectrum of 11d was recorded in CH$_2$Cl$_2$ with increasing solvent polarity by increasing the DMF portion.
It was observed obviously that the long emission (622 nm) originating from ILCT excited state was gradually quenched, but a new emission (518 nm) originating from IL excited state was emerged. This observation confirmed that the switch of excited state from ILCT to LC can be achieved by attenuating the electrostatic interaction between counterions and complex.

**Figure 5.5** (a) Room-temperature normalized photoluminescence spectra of complex 11d in CH₂Cl₂, CHCl₃, CH₃CN, DMSO and DMF. (b) PL spectra of complex 11d in CH₂Cl₂–DMF mixtures (10 μM) with different DMF fractions. (c) PL spectra of complex 11d in CH₂Cl₂–DMF mixtures (10 μM) with different DMF fractions. (d) Photos of the emission of 11d in CH₂Cl₂, CHCl₃, CH₃CN, DMSO and DMF.
Counterions stabilize the ILCT excited state
When counterions are separated, the ILCT excited state is destabilized
Further destabilization of ILCT excited state

5.4 Tuning for White-Light Emission

Getting the insight into the way of controlling the lowest-energy excited state of these Zn(II) complexes can provide us with an opportunity to possibly achieve white light generation. Obviously, by doping these Zn(II) complexes into polymer film with an appropriate polarity and a suitable concentration, the electrostatic interaction between counterions and Zn(II) complex can be well regulated. In other words, the fluorescence emission from IL and ILCT excited state can be tuned to emit at the same time. Hence, it could potentially generate white-light emission by a single emitter, a property that is of great importance for the lighting industry.[23-26]

PEG-PPG-PEG was selected as the matrix due to its appropriate polarity and

Figure 5.6 Schematic illustration of the change of the lowest-energy excited state of Zn(II) complex induced by counterions.
non-fluorescent properties. Indeed, simply by doping 11d into PEG-PPG-PEG (11d contents at 0.2%, 0.4%, 0.6% and 3.0% of the polymer weight, respectively), the fluorescence emission colors of blue, gray, white and yellow were observed. The PL spectra of 11d doped polymer are shown in Figure 5.7a. In general, the CIE coordinates and other aspects of the emission color quality can be easily tuned by adjusting the concentration of the 11d dopant in the polymer host. For instance, when 11d content was 0.2%, the emission is from IL excited state, because the weak electrostatic interaction between counterions and Zn(II) complex leads to the destabilization of ILCT excited state. Thus, a strong blue fluorescence in the overall emission feature (CIE (0.18, 0.16)) was observed. The electrostatic interaction was enhanced when the 11d content was raised to 0.4%, leading to an increase in the stabilization of ILCT excited state. Consequently, the emission from ILCT excited state can be seen in the PL spectrum and the polymer film emits gray light at the weight percentage of 0.4%. A distinct white-light emission was observed by doping 0.6% 11d into PEG-PPG-PEG, and the corresponding CIE coordinates are (0.29, 0.34), which is close to the pure white light. When the weight percentage was increased to 3.0%, only ILCT emission band was displayed in the PL spectrum since the strong electrostatic interaction stabilizes the ILCT excited state.
Figure 5.7 (a) Normalized photoluminescence spectra of complex 11d at increasing concentrations (from 0.2% to 3.0%) in polymer films. (b) The photograph of complex 11d in polyether films with different concentrations. (c) CIE-1931 chromaticity diagram and the positions for the emissions of complex 11d in polyether films with different concentrations.

5.5 Electrochromic fluorescence

Additionally, the discovery of controlling the lowest-energy excited state of these Zn(II) complexes by adjusting electrostatic interaction between counterions and Zn(II) ion encourages us to investigate the electrochromic applications of these Zn(II) complexes. The experiment of fluorescence emission color change manipulated by electric field was then carried out. Two tin (Sn) electrodes were immersed in a CH₂Cl₂ solution of 11d with a distance of 20 mm between them (Figure 5.8a). In the absence of an applied voltage, an intense photoluminescence peak centered at 622 nm
can be discerned (Figure 5.8b) and the solution emits a bright orange red fluorescence under UV light (Figure 5.8a). Upon the application of a voltage of 15 V, the photoluminescence near the cathode shows a drastic blue shift (Figure 5.8b) and the fluorescence color of the solution is changed to sky blue. It is noteworthy that the applied electric field does not cause the alteration of fluorescence color near the anode, indicating that the photoluminescence shifting may originate from the migration of PF$_6^-$.

One can see from the $^1$H NMR and MS spectral data near both of the two electrodes is that the structure of 11d remained intact under our experimental conditions (Figures 5.9 and 5.10). On the other hand, the concentration of the PF$_6^-$ increased by 35% near the anode and decreased by 27% near the cathode based on the integrals in the $^{19}$F NMR spectra (Figure 5.11). It is believed that this non-homogeneous distribution of PF$_6^-$ led to a dilution of electrostatic interaction between PF$_6^-$ and 11d near the cathode. Consequently, the ILCT excited state of 11d near the cathode was destabilized, and the lowest-energy excited state was altered to the IL excited state. Thus, a fluorescence color change of the solution from orange to sky blue was observed (Figure 5.11). The same phenomenon was witnessed for 11b and 11d. For 11a, because the electrostatic interaction between complex and CH$_3$COO$^-$ is more covalent in nature, the applied voltage under our experiment condition cannot drive the CH$_3$COO$^-$ out of Zn(II) complex. Thereby, the similar observation of electric induced fluorescence color change was absent for 11a. These results further demonstrate that adjusting the electrostatic interaction of counterions
with Zn(II) complexes can control the lowest-energy excited state of Zn(II) complexes.

![Figure 5.8](image)

**Figure 5.8** (a) The photographs of electrochromic fluorescence of 11d (20 μM) in CH$_3$CN. (b) The emission spectra of 11d before and after applying a voltage in CH$_3$CN.

![Figure 5.9](image)

**Figure 5.9** $^1$H NMR spectra of 11d in CD$_3$CN during the electrochromic fluorescence experiment.
Figure 5.10 Mass spectra of 11d in CD$_3$CN during the electrochromic fluorescence experiment.

Figure 5.11 Concentration variation of PF$_6^-$ based on the $^{19}$F NMR spectra during the electrochromic fluorescence experiment.
5.6 Information recording device based on electrochromic fluorescence

![Image of quasi-solid film under UV lamp]

**Figure 5.12** Quasi-solid film under a UV lamp.

On the basis of this electrochromic fluorescence behavior, we successfully demonstrated the use of our material as an optical recording medium. The characters “HK” were written on the surface of a quasi-solid film with a needle electrode pen (Figure 5.12). The quasi-solid film was fabricated by mixing 11d with electrochemically and thermally stable ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate (BMIM\(^{+}\)PF\(_6^{-}\)), and SiO\(_2\) nanoparticles. The complexes, counterions and the ionic liquid within the SiO\(_2\) can freely migrate due to the presence of inorganic network channels. The information recording device was simply constructed by coating quasi-solid film on an indium tin oxide (ITO) electrode plate. When the movable needle electrode with a voltage of 4 V contacted the surface of quasi-solid film, the electrically-induced separation of PF\(_6^{-}\) and 11d can cause the switching of the ILCT to IL excited state. Thus, a sky blue luminescence spot immediately appeared at the contact point of the film. With the movement of the
needle electrode, the trace can be recorded on the surface of device as a distinguishable sky blue fluorescence. Therefore, the electrically-manipulated information recording was achieved by utilizing electric field to regulate the lowest-energy excited state of 11d.

5.7 Information encryption and decryption via two-photon excitation imaging

Information encryption and decryption plays a significant role in our daily life and have attracted widespread scientific and technological interest. In this part, a new approach, which takes advantage of the difference in two-photon absorption (TPA) cross section between 11d and common organic dyes, was developed to achieve this goal. We first measured the TPA cross section of 11d and some common organic dyes. As shown in Figure 5.13b, the TPA cross section of 11d was measured to be 298 GM at 950 nm in CH₂Cl₂ solution, which is much larger than these common organic dyes (Fluorescein, Rhodamine 6G, Coumarin 540 and BODIPY) (Table 5.4). Figure 5.13c is the schematic illustration of the process of information encoding and decoding. First, an organic dye with strong emission but small TPA cross section was used to encrypt the fluorescence of 11d (Figure 5.13b). Next, the TPA excitation technique was employed to identify the difference in TPA cross section between 11d and common organic dyes, and thus decrypt the information. In this study, a green-emitting fluorescent dye BODIPY was chosen and doped into the quasi-solid film to provide the interference fluorescence background. When a character “W” was
written on the device containing both BODIPY and 11d, the information was encrypted and thus unreadable via common fluorescent imaging microscope (FIM) (Figure 5.13b). Since the TPA cross section of 11d (298 GM) is much larger than that of the BODIPY dye (7.49 GM) at 950 nm, the encrypted information was successfully decrypted by the TPA excitation. As shown in Figure 5.13b, a clear readable “W” was shown under the two-photon fluorescent imaging microscope (TPFIM). These results demonstrate that the information encryption can be achieved by combining a strongly emissive and small the TPA cross section organic dye with 11d, and the information decryption can be achieved by utilizing the TPA excitation technique. Therefore, this demonstration opens a new opportunity in the data security protection.

**Figure 5.11** (a) TPA cross sections of complex 11d and different common organic dyes (Fluorescein, Rhodamine 6G, Coumarin 540 and BODIPY). (b) Fluorescence
microscopy image and fluorescence two-photon excitation microscopy image for complex 11d with BODIPY.

Table 5.4 TPA cross sections of complex 11d and different common organic dyes (Fluorescein, Rhodamine 6G, Coumarin 540 and BODIPY)

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<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Fluorescein</th>
<th>Rhodamine 6G</th>
<th>Coumarin 540</th>
<th>BODIPY</th>
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<tr>
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</table>
5.8 Conclusion

In summary, a novel approach of fluorescence color tuning for Zn(II) bis-terpyridine complexes by the variation of counterions (CH\(_3\)COO\(^-\), BF\(_4\)\(^-\), ClO\(_4\)\(^-\), and PF\(_6\)\(^-\)) has been demonstrated. A series of cationic bis-terpyridine Zn(II) complexes (11a-11d) with different counterions is found to exhibit the emission color from green yellow to orange red (549 nm to 622 nm) in CH\(_2\)Cl\(_2\) solution. It is believed that the different basicity of counterions is responsible for the changes of photophysical properties. In addition, it is found that the counterions play crucial roles in regulating the lowest-energy excited state of these Zn(II) complexes. On the basis of these discoveries, the CIE coordinates and the emission colors can be simply tuned by adjusting the concentration of 11d in the PEG-PPG-PEG. Under an electric field of about 15 V applied onto the electrodes, the emission colors of the solutions of 11b-11d near the cathode have changed their original fluorescence colors to sky blue. Based on this interesting electrochromic fluorescence property of 11d, a quasi-solid information recording device has been successfully designed and constructed. Furthermore, data encryption has been realized by combining 11d with BODIPY, and
information decoding process has been accomplished by employing the TPA excitation technique for the first time, in which the large TPA cross section of 1d is differentiated from small TPA cross section of common organic dyes.

References


Chapter 6 Summary and Future Prospects

The development of novel photofunctional molecular materials for the applications in chemical sensing, bioimaging and electrochromic has been the focus in this thesis.

In chapter 2, we have synthesized a series of water-soluble phosphorescent cationic iridium(III) solvato complexes (1-7) as multicolor cellular probes for imaging in living cells (as shown in Scheme 6.1). All of these complexes can be dissolved in PBS. The emission of complexes can be tuned from green to red by changing the chemical structure of cyclometalating ligands. All complexes exhibit low cytotoxicity to living cells and exhibit cell membrane permeability and specific staining of cytoplasm. They enter the cells by the mechanism of energy-independent passive diffusion mechanisms. More importantly, complex 7 can act as a two-photon phosphorescent cellular probe, and fluorescence lifetime imaging microscopy is successfully applied for bioimaging in the presence of short-lived background fluorescence.

We developed two excellent optical probes for CO₂ detection in Chapter 3 (as shown in Scheme 6.2). The first one for the CO₂ detection is a phosphorescent probe based on an iridium(III) complex with 2-phenylimidazo-[4,5-f][1,10]phenanthroline. After bubbling CO₂ into the detection solution, the quenched phosphorescence by the addition of CH₃COO⁻ can be recovered. Photobleaching experiment demonstrates that this phosphorescent CO₂ probe shows higher photostability than some of the reported organic probes. More importantly, the time-resolved PL experiment demonstrates that this probe can be used to detect CO₂ in the presence of strong background
fluorescence, which improves the sensitivity and signal-to-noise ratio of the sensor in complicated media. The second one is a water-soluble fluorescent probe based on tetraphenylethene derivative. After bubbling $\text{CO}_2$ into the detection solution, remarkable color change and fluorescence enhancement could be observed. The response of this probe to $\text{CO}_2$ in aqueous solution is fast and the detection limit is about $2.4 \times 10^{-6}$ M. To emphasize the practical application of this probe, a porous film was successfully fabricated by mixing the dye with sodium carboxymethyl cellulose in water, which can serve as an efficient $\text{CO}_2$ gas sensor. More importantly, this probe exhibits low cytotoxicity towards live cells and has the ability to monitor the external $\text{CO}_2$ concentration changes of living cells.

Chapter 4 focused on the development of novel soft salt based phosphorescent probe (Scheme 6.3). This type of probe consists of two oppositely charged ionic complexes with two distinguishable emission colors, which makes it a perfect candidate as a ratiometric probe. The emission color of \textit{10} changes from blue to red with increasing pH value. \textit{10} is cell-permeable and exhibits low cytotoxicity, and it has been successfully applied for ratiometric pH imaging with the use of confocal microscopy, demonstrating its great potential for intracellular environment monitoring. Furthermore, phosphorescence lifetime imaging experiments can detect intracellular pH variations by photoluminescence lifetime measurements, which allowed for eliminating background fluorescence and selecting long-lived phosphorescence images. Quantitative measurement of intracellular pH fluctuations caused by oxidative stress has been successfully carried out for \textit{10} based on the pH-dependent
calibration curve.

A series of cationic Zn(II) complexes has been designed and synthesized in chapter 5 (Scheme 6.4). The photophysical properties of these Zn(II) complexes are affected by the counterions. By altering the counterions, the emission peak can be changed from 549 nm to 622 nm. Interestingly, the CIE coordinates and the emission colors can be simply tuned by adjusting the concentration of 11d in the polyether. Under an electric field of about 15 V applied onto the electrodes, the emission color of the solution of 11b-11d near the cathode changed its original emission color to sky blue. Based on this interesting electrochromic fluorescence of 11d, a quasi-solid information recording device has been successfully designed. Furthermore, data encryption has been realized by combining 1d with BODIPY, and information decoding has been accomplished, for the first time, by employing TPA excitation techniques, in which the large TPA cross section of 11d is differentiated from small TPA cross section of common organic dyes.
Scheme 6.1 Chemical structures of water-soluble bioprobes in Chapter 2.

Scheme 6.2 Chemical structures of CO₂ probes in Chapter 3.
Scheme 6.3 Chemical structure of soft salt based pH probe in Chapter 4.

Scheme 6.4 Chemical structures of electrochromic materials in Chapter 5.
Chapter 7 Experimental Details

7.1 General

7.1.1 Measurements and instruments

Commercially available chemical reagents were used without further purification. The solvents were carefully dried and distilled from appropriate drying agents prior to use. NMR spectra were taken on a Bruker Ultrashield 400 MHz FT-NMR spectrometer. Mass spectra were obtained on a Bruker Autoflex matrix assisted laser desorption/ionization time of flight mass spectrometer (MALDI-TOF MS). X-Ray diffraction data were collected at 293 K using graphite-monochromated Mo–Kα radiation (λ = 0.71073 Å) on a Bruker APEX DUO diffractometer. The collected frames were processed with the software SAINT[1] and an absorption correction (SADABS)[2] was applied to the collected reflections. The structure was solved by the Direct methods (SHELXTL)[3] in conjunction with standard difference Fourier techniques and subsequently refined by full-matrix least-squares analyses on F². UV-visible absorption spectra were recorded using an HP-8453 spectrophotometer. Photoluminescence spectra were measured on an Edinburgh LFS920 fluorescence spectrophotometer. The quantum efficiencies of complexes were measured in solutions at room temperature with an aerated aqueous solution of [Ru(bpy)₃]Cl₂ as an external standard (Φ = 0.028).[4] Time-resolved emission spectra (TRES) and
emission lifetimes were obtained through a time-correlated single photon counting (TCSPC) technique using an Edinburgh FL 920 instrument with a laser (365 nm) as the excitation source. Time-gated acquisition of photoluminescence spectra was carried out by employing the TRES technique. The two-photon imaging setup was integrated with an Olympus IX81 laser scanning confocal microscope. The fluorescence signal was detected using the confocal microscope and correlative calculation of the data was performed by professional software which was provided by PicoQuant Company. A 405 nm picosecond pulsed diode laser (<1 μW) with 2.5 MHz repetition rate was used for excitation. The device with the written “W” was put on the platform, and a 2 mm × 2 mm sample area consisting of 200× pixels was scanned with an acquisition rate of 2 ms/pixel.

7.1.2 Cell culture

The Hela cell line and HepG-2 cell line were supplied by the Institute of Biochemistry and Cell Biology, SIBS, CAS (China). The Hela cells were grown in RPMI 1640 (Roswell Park Memorial Institute’s Medium) supplemented with 10% FBS (Fetal Bovine Serum) at 37 °C and 5% CO₂. Cells (5×10⁶/L) were plated on 18 mm glass coverslips and allowed to adhere for 24 h.
7.1.3 Cytotoxicity assay

The cytotoxicity of the complexes toward the Hela cells or HepG-2 cells has been measured by the methyl thiazolyl tetrazolium (MTT) assay. Before incubated at 37 °C under 5% CO₂ atmosphere for 24 h, Hela cells in log phase were seeded into a 96-well cell-culture plate at 1×10⁴ / well. The complexes 1, 6 and 7, (100μL/well) at concentrations of 400, 200, 100, 40, 20, 10 μM, or compound 9 at concentrations of 25, 10, 5 μM, or complex 10 at concentrations of 200, 100, 50, 25 μM were added to the wells of the treatment group, and MTT containing 0.2% DMSO (100 μL/well) to the negative control group. The cells incubated at 37 °C under 5% CO₂ atmosphere for 24 h. 20 μL MTT solution (5 mg/mL) was added to each well of the 96-well assay plate, and the solution was incubated for another 3 h under the same condition. A Tecan Infinite M200 monochromator based multifunction microplate reader was used for measuring the OD570 (Absorbance value) of each well referenced at 690 nm. The following formula was used to calculate the viability of cell growth:

\[
\text{viability (\%)} = \frac{\text{mean of absorbance value of treatment group}}{\text{mean absorbance value of control}} \times 100.
\]

7.1.4 Cell imaging

The complexes 1-7 were dissolved in PBS to yield 20 μM solutions. Before washing with PBS, the Hela cells were incubated solely with the solution of 1 (or 2-7) for 30 min at 37 °C or at 4 °C. Then, the experiments were carried out on an Olympus FV1000 laser scanning confocal microscope and a 60x oil-immersion objective lens.
A semiconductor laser was served as excitation of the Hela cells incubated with 1 (or 2-7) at 405 nm. Emission was collected at 480-580, 500-600, 570-670, 500-600, 530-630, 600-700 and 600-700 nm for the Hela cells incubated solely with 1 (or 2-7), respectively.

Complexes C1, A1 and 10 were dissolved in DMSO/RPMI 1640 (v : v, 1 : 99) to yield 10 μM solutions. Before washing with PBS, the HepG-2 cells were incubated solely with the solution of C1 (or A1 and 10) for 1 h at 37 °C. Then, the experiments were carried out on an Olympus FV1000 laser scanning confocal microscope and a 60x oil-immersion objective lens. A semiconductor laser served as the excitation source of the Hep-2 cells incubated with C1 (or A1 and 10) at 405 nm.

### 7.1.5 Two-photon living cell imaging

Two photon confocal luminescence imaging was carried out on an Olympus IX81 laser scanning confocal microscope equipped with a 40 immersion objective lens. A semiconductor laser was served as excitation of the Hela cells incubated with 3, 6 and 7 at 800 nm. The G & R optical spectroscope was used to collect two-photon emission, of which G and R contains 495–540 nm and 575–630 nm bands respectively. Complexes 3, 6 and 7 were dissolved in PBS to yield 20 μM solutions. The Hela cells were incubated with the solution of 3, 6 and 7 for 30 min at 37 °C.
7.1.6 Lifetime imaging

The FLIM image setup is integrated with Olympus IX81 laser scanning confocal microscope. The fluorescence signal was detected by the system of the confocal microscope and correlative calculation of the data was carried out by professional software which was provided by Pico Quant Company. The light from the pulse diode laser head (Pico Quant, PDL 800-D) with excitation wavelength of 405 nm and frequency of 0.5 MHz was focused onto the sample with a 40x/NA 0.95 objective lens for single-photon excitation. The emitted fluorescence signal was collected at 600-700 nm.

7.1.7 Intracellular pH calibration

The HepG-2 cells were incubated at 37 °C for 30 min in a high K⁺ buffer (30 mM NaCl, 120 mM KCl, 1 mM CaCl₂, 0.5 mM MgSO₄, 1 mM NaH₂PO₄, 5 mM glucose, 20 mM HEPES) with various pH values (3.98-8.01) in the presence of 10 μL nigericin (10 ng/mL). HepG-2 cells were incubated with C1 (or A1 and 10) for 1 h at 37 °C. The phosphorescence and lifetime images were then measured, and the pH calibration curve was constructed with Olympus FV1000 confocal microscope.
7.2 Materials

7.2.1 Synthetic procedure of the compounds in chapter 2

**Synthesis of L1**

Pd(PPh₃)₄ (100 mg) was added to a mixture of 3-boronic acid-N-phenylcarbazole (0.926 g, 3.25 mmole) and 2-bromopyridine (0.289 g, 1.56 mmole) in THF (50 mL) and 2 M aqueous Na₂CO₃ (5 mL) under an inert atmosphere of nitrogen. The reaction was heated to 90 °C for 2 days. After being cooled to room temperature, water was added and the solution mixture was extracted with ethyl acetate. The combined organic layer was dried by MgSO₄ and was filtered and concentrated under reduced pressure. The residue was purified through column chromatography over silica gel eluting with ethyl acetate and hexane to give the product. Yield 69%. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.80 (s, 1H, Ar), 8.72-8.73 (d, 1H, Ar), 8.18-8.20 (d, 1H, Ar), 8.02-8.05 (d, 1H, Ar), 7.82-7.84 (d, 1H, Ar), 7.73-7.77 (t, 1H, Ar), 7.51-7.60 (m, 3H, Ar), 7.43-7.48 (m, 3H, Ar), 7.39-7.41 (d, 2H, Ar), 7.28-7.32 (m, 1H, Ar), 7.19-7.21 (d, 1H, Ar). MS (MALDI-TOF) [m/z]: 320.7.

**Synthesis of L2**

This compound was prepared following the same procedures as described above of L1 but 2-chloro-5-(trifluoromethyl)pyridine was used. Yield 74%. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.88 (s, 1H, Ar), 8.78 (s, 1H, Ar), 8.15-8.13 (d, 1H, Ar), 7.98-7.94 (d, 1H, Ar), 7.82-7.80 (d, 2H, Ar), 7.52-7.24 (m, 9H, Ar). MS (MALDI-TOF) [m/z]: 387.9.
Synthesis of \textbf{L3}

This compound was prepared following the same procedures as described above of \textbf{L1} but 1-chloro-isoquinoline was used. Yield 58\%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ (ppm) 8.66-8.64 (d, 1H, Ar), 8.50 (s, 1H, Ar), 8.26-8.15 (dd, 2H, Ar), 7.90-7.87 (d, 1H, Ar), 7.79-7.44 (m, 12 H, Ar), 7.29-7.23 (m, 1H, Ar). MS (MALDI-TOF) [\textit{m/z}]: 370.4.

Synthesis of \textbf{L4}

This compound was prepared following the same procedures as described above of \textbf{L1} but 9, 9-diethyl 2-boronic acid fluorene and 2-bromopyridine was used. Yield 69\%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ (ppm) 8.74-8.76 (d, 1H, Ar), 8.03-8.05 (d, 1H, Ar), 7.97-8.00 (d, 1H, Ar), 7.77-7.85 (m, 4H, Ar), 7.35-7.40 (m, 3H, Ar), 7.24-7.28 (t, 1H, Ar), 2.05-2.21 (m, 4H, CH$_2$), 0.35-0.39 (t, 6H, CH$_3$). MS (MALDI-TOF) [\textit{m/z}]: 299.5.

Synthesis of \textbf{L5}

This compound was prepared following the same procedures as described above of \textbf{L1} but 9, 9-diethyl 2-boronic acid fluorene and 2-chloro-5-(trifluoromethyl)pyridine was used. Yield 79\%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ (ppm) 8.97-8.99 (d, 1H, Ar), 8.08-8.10 (d, 1H, Ar), 8.01-8.04 (dd, 1H, Ar), 7.97-8.00 (dd, 4H, Ar), 7.91-7.93 (m, 1H, Ar), 7.75-7.79 (m, 1H, Ar), 7.31-7.38 (m, 3H, Ar), 2.05-2.20 (m, 4H, CH$_2$), 0.34-0.38 (t, 6H, CH$_3$). MS (MALDI-TOF) [\textit{m/z}]: 367.7.
Synthesis of **L6**

This compound was prepared following the same procedures as described above of **L1** but 9, 9-diethyl 2-boronic acid fluorene and 1-chloroisoquinoline was used. Yield 56%. $^1$H NMR (400 MHz, CDCl$_3$): δ (ppm) 8.64-8.66 (d, 1H, Ar), 8.12-8.14 (d, 1H, Ar), 7.86-7.91 (dd, 2H, Ar), 7.77-7.80 (m, 1H, Ar), 7.66-7.72 (m, 4H, Ar), 7.53-7.57 (t, 1H, Ar), 7.35-7.40 (m, 3H, Ar), 2.04-2.13 (m, 4H, CH$_2$), 0.40-0.43 (t, 6H, CH$_3$). MS (MALDI-TOF) [m/z]: 349.5.

**Synthesis of L7**

Compound **9**' (220 mg, 0.59 mmol), 3-formyl-N-hexylcarbazole (165 mg, 0.59 mmol) and t-BuOK (322 mg, 3 mmol) were mixed in dry THF (50 mL) at 0 °C under an inert atmosphere of nitrogen. Then, the reaction mixture was warmed to room temperature for 12 h. The mixture was dispersed in 100 mL ethanol. The residual solid was filtered and recrystallized from dichloromethane/ethanol, giving pale yellow crystals. Yield 78%. $^1$H NMR (400 MHz, CDCl$_3$): δ (ppm) 8.94 (s, 1H, Ar), 8.26 (s, 1H, Ar), 8.12-8.14 (d, 1H, Ar), 8.05-8.08 (d, 2H, Ar), 7.95-7.98 (d, 1H, Ar), 7.85-7.87 (d, 1H, Ar), 7.67-7.71 (t, 3H, Ar), 7.39-7.48 (m, 4H, Ar), 7.24-7.28 (d, 1H, CH=CH), 7.18-7.22 (d, 1H, CH=CH), 4.28-4.32 (t, 2H, CH$_2$), 1.84-1.92 (m, 2H, CH$_2$), 1.26-1.36 (m, 6H, CH$_2$), 0.85-0.90 (t, 3H, CH$_3$). MS (MALDI-TOF) [m/z]: 498.6.

**General procedures of synthesis of iridium complexes 1-7**

All the complexes were synthesized by reacting IrCl$_3$·3H$_2$O with 2.5 equivalents of
the corresponding cyclometalating ligand in a mixture of 2-ethoxyethanol and water (3:1, v/v). The mixture was refluxed for 24 hours and then cooled to room temperature. Precipitates were gradually formed during the reaction. The precipitate was collected by filtration and washed with hexane followed by ethanol. The solvent was then pumped dry completely to give the crude iridium(III) dimer for subsequent reaction, without further purification, and this crude dimer was refluxed with AgOTf in the mixture of EtOH/H₂O (v : v = 9 : 1) overnight. After the mixture was cooled to room temperature, the solution was filtered and the precipitate was washed three times (2 mL) with solvent ligand H₂O. The filtrate and washings were combined and reduced by evaporation to a volume of 1 mL. This solution was cooled to 0 °C, and 25 mL ether was slowly added while stirring. The precipitate which formed was collected by filtration and washed with ether and hexane, and then dried under vacuum for 24 h to obtain the products.

Yield 65%. ¹H NMR (400 MHz, MeOD): δ (ppm) 9.72-9.74 (d, 1H, Ar), 9.42-9.44 (d, 1H, Ar), 8.67-8.71 (d, 2H, Ar), 8.33-8.39 (dd, 2H, Ar), 8.10-8.12 (d, 6H, Ar), 8.00-8.03 (t, 2H, Ar), 7.68-7.72 (t, 2H, Ar), 7.51-7.57 (m, 7H, Ar), 7.44-7.47 (m, 3H, Ar), 7.39-7.41 (d, 2H, Ar), 7.20-7.24 (d, 2H, Ar). ¹³C NMR (100 MHz, CDCl₃), δ (ppm): 158.51, 143.07, 141.90, 139.50, 136.55, 135.93, 131.43, 130.98, 130.46, 130.24, 128.45, 128.11, 127.51, 127.17, 126.19, 124.37, 123.58, 123.17, 122.58, 121.39, 121.14, 120.60, 111.18, 110.42. MS (MALDI-TOF) [m/z]: 831.3 [1−2H₂O]⁺.
2 Yield 71%. $^1$H NMR (400 MHz, MeOD): δ (ppm) 9.03 (s, 2H, Ar), 8.54-8.57 (d, 2H, Ar), 8.39-8.41 (d, 2H, Ar), 8.14-8.20 (dd, 4H, Ar), 8.02-8.06 (t, 2H, Ar), 7.64-7.68 (t, 2H, Ar), 7.68-7.72 (t, 2H, Ar), 7.43-7.55 (m, 6H, Ar), 7.30-7.38 (m, 2H, Ar), 7.13-7.22 (m, 4H, Ar). $^{13}$C NMR (100 MHz, CDCl$_3$), δ (ppm): 173.37, 152.23, 147.09, 146.45, 146.11, 143.08, 141.49, 139.52, 137.59, 129.19, 128.00, 125.63, 124.25, 124.05, 123.39, 122.14, 120.88, 119.99. MS (MALDI-TOF) [m/e]: 967.5 [2–2H$_2$O]$^+$. 

3 Yield 83%. $^1$H NMR (400 MHz, MeOD): δ (ppm) 8.51-8.52 (d, 2H, Ar), 8.47 (s, 2H, Ar), 8.19-8.23 (t, 4H, Ar), 8.02-8.04 (d, 2H, Ar), 7.84-7.85 (d, 2H, Ar), 7.79-7.81 (d, 2H, Ar), 7.63-7.74 (m, 10H, Ar), 7.55-7.59 (t, 4H, Ar), 7.42-7.47 (m, 4H, Ar), 7.29-7.33 (t, 2H, Ar). $^{13}$C NMR (100 MHz, CDCl$_3$), δ (ppm): 151.64, 149.44, 142.72, 142.15, 141.53, 140.02, 137.52, 137.07, 136.32, 135.81, 129.97, 129.09, 127.66, 127.11, 126.66, 126.28, 125.11, 124.42, 123.91, 121.43, 120.56, 120.31, 119.87, 119.15, 115.89, 115.82, 109.99. MS (MALDI-TOF) [m/z]: 931.4 [3–2H$_2$O]$^+$. 

4 Yield 76%. $^1$H NMR (400 MHz, MeOD): δ (ppm) 8.99-9.00 (d, 2H, Ar), 8.28-8.30 (d, 2H, Ar), 8.12-8.16 (t, 2H, Ar), 7.97-8.01 (t, 1H, Ar), 7.93-7.95 (d, 1H, Ar), 7.73 (s, 2H, Ar), 7.56-7.60 (t, 2H, Ar), 7.39-7.46 (m, 2H, Ar), 7.16-7.25 (m, 4H, Ar), 6.46-6.48 (d, 2H, Ar), 1.87-2.11 (m, 8H, CH$_2$), 0.22-0.26 (t, 12H, CH$_3$). $^{13}$C NMR (100 MHz, CDCl$_3$), δ (ppm): 168.85, 152.22, 150.89, 144.23, 142.98, 142.24, 141.22, 138.08, 135.98, 127.30, 126.62, 126.44, 125.91, 122.95, 122.68, 121.67, 121.56, 119.71, 118.66, 117.99, 55.16, 32.86, 32.63, 8.65. MS (MALDI-TOF) [m/z]: 789.3
[4–2H₂O]⁺.

5 Yield 63%. ¹H NMR (400 MHz, MeOD): δ (ppm) 9.23 (d, 2H, Ar), 8.50-8.52 (d, 2H, Ar), 8.42-8.45 (d, 2H, Ar), 7.87 (s, 2H, Ar), 7.21-7.26 (m, 8H, Ar), 6.44 (s, 2H, Ar), 1.91-2.08 (m, 8H, CH₂), 0.21-0.29 (t, 12H, CH₃). ¹³C NMR (100 MHz, CDCl₃), δ (ppm): 173.37, 152.31, 147.09, 146.41, 146.17, 143.01, 141.49, 139.52, 137.56, 129.29, 128.00, 124.25, 124.05, 123.39, 122.09, 120.88, 64.32, 56.85, 33.95, 33.38, 8.47. MS (MALDI-TOF) [m/z]: 925.3 [5–2H₂O]⁺.

6 Yield 72%. ¹H NMR (400 MHz, MeOD): δ (ppm) 8.96-8.98 (d, 1H, Ar), 8.88-8.90 (d, 1H, Ar), 8.49-8.50 (d, 2H, Ar), 8.26-8.28 (d, 1H, Ar), 8.13 (s, 1H, Ar), 8.01-8.07 (d, 2H, Ar), 7.94-7.99 (m, 2H, Ar), 7.78-7.86 (m, 6H, Ar), 7.61-7.65 (m, 2H, Ar), 7.38-7.43 (m, 2H, Ar), 7.16-7.24 (m, 2H, Ar), 7.00-7.06 (t, 1H, Ar), 6.86-6.88 (d, 1H, Ar), 1.91-2.03 (m, 8H, CH₂), 0.34-0.38 (t, 12H, CH₃). ¹³C NMR (100 MHz, CDCl₃), δ (ppm): 207.59, 169.50, 161.52, 161.20, 151.17, 150.39, 149.97, 144.48, 144.26, 142.42, 142.06, 137.00, 130.08, 129.01, 127.71, 127.40, 127.22, 127.04, 126.11, 124.55, 123.01, 119.88, 119.53, 56.32, 55.32, 32.72, 30.11, 8.64. MS (MALDI-TOF) [m/z]: 889.3 [6–2H₂O]⁺.

7 Yield 52%. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 10.09 (s, 2H, Ar), 9.28 (s, 2H, Ar), 8.62 (s, 2H, Ar), 8.57-8.59 (d, 4H, Ar), 8.29-8.32 (d, 2H, Ar), 8.14-8.16 (d, 2H, Ar), 8.01-8.06 (m, 6H, Ar), 7.36-7.59 (m, 8H, Ar), 7.18-7.24 (m, 4H, CH=CH), 4.28-4.32
(t, 4H, CH₂), 1.82-1.90 (m, 4H, CH₂), 1.26-1.36 (m, 12H, CH₂), 0.86-0.92 (t, 6H, CH₃). ¹³C NMR (100 MHz, CDCl₃), δ (ppm): 150.31, 146.60, 144.05, 140.88, 139.21, 137.65, 135.39, 132.90, 129.63, 129.57, 128.47, 126.70, 125.96, 125.53, 124.58, 124.15, 123.18, 122.72, 121.58, 120.67, 120.25, 119.06, 118.44, 115.21, 113.13, 111.80, 109.28, 108.95, 106.94, 103.17, 99.88, 43.20, 31.34, 28.91, 26.77, 22.51, 13.98. MS (MALDI-TOF) [m/z]: 1187.5 [72H₂O]+.

7.2.2 Synthetic procedure of the compounds in chapter 3

Synthesis of Complex 8

The ligand 1-phenylisoquinoline was synthesized according to our previous report. The cyclometalated iridium(III) chloro-bridged dimer [Ir(piq)₂Cl]₂ was prepared according to the literature method. A solution of [Ir(piq)₂Cl]₂ (0.079 mmol) and 2-phenylimidazo-[4,5-f][1,10]phenanthroline (0.158 mmol) in CH₂Cl₂/MeOH (15 mL, 2:1 v/v) was heated to reflux. After 4 h, the red solution was cooled to room temperature, and then a 10-fold excess of potassium hexafluorophosphate was added. The suspension was stirred for 1 h and then was filtered to remove the insoluble inorganic salts. The solution was evaporated to dryness under reduced pressure. The crude product was applied to a silica gel column and eluted with CH₂Cl₂/acetone (10:1, v/v) to afford a red solid in 69% yield. ¹H NMR (400 MHz, CD₃CN), δ (ppm): 9.12 (t, 4H, J = 17.2 Hz, phenanthroline), 8.47 (d, 2H, J = 8.0 Hz. isoquinoline), 8.33 (d, 2H, J = 7.2 Hz, phenyl), 8.19 (d, 2H, J = 4.0 Hz, isoquinoline), 7.95 (d, 2H, J = 7.2
Hz, isoquinoline), 7.83–7.91 (m, 6H, isoquinoline), 7.69 (t, 3H, J = 12.0 Hz, phenyl), 7.41 (d, 2H, J = 4.0 Hz, isoquinoline), 7.29 (d, 2H, J = 4.0 Hz, isoquinoline), 7.24 (t, 2H, J = 14.0 Hz, isoquinoline), 6.99 (t, 2H, J = 16.0 Hz, isoquinoline), 6.46 (d, 2H, J = 8.0 Hz, isoquinoline). $^{13}$C NMR (100 MHz, CD$_3$CN), δ (ppm): 168.6, 153.7, 153.0, 149.1, 145.8, 144.6, 141.0, 137.1, 132.2, 132.1, 131.9, 130.8, 130.5, 129.4, 129.2, 128.9, 127.5, 126.8, 126.6, 126.2, 122.3, 121.8 (aromatic). MALDI-TOF MS [m/z]: 899.32 [M–PF$_6$]$^+$. Anal. calcd for C$_{49}$H$_{33}$N$_6$PF$_6$Ir: C, 56.43; H, 3.19; N, 8.06. Found: C, 56.65; H, 3.44; N, 7.90%.

Synthesis of TPE-OCH$_3$

Bis(4-methoxyphenyl)methanone (4.84 g, 20 mmol) and zinc powder (2.60 g, 40 mmol) were placed in a round bottomed flask under nitrogen. Dry THF (100 mL) was added and TiCl$_4$ (2.2 mL, 20 mmol) was added dropwise to the solution, and then the mixture was refluxed overnight. After cooling to room temperature, the solution was washed with water, and the mixture was extracted with CH$_2$Cl$_2$. Subsequently, the organic layer was collected and evaporated. The resulting crude mixture was subjected to a silica gel column using ethyl acetate/hexane (1/5, v/v) as the eluent to give TPE-OCH$_3$ in 74% yield. $^1$H NMR (400 MHz, CDCl$_3$): δ 6.97 (d, J = 8.60 Hz, 8H, phenyl group), 6.67 (d, J = 8.71 Hz, 8H, phenyl group), 3.77 (s, 12H, methyl group). MALDI-TOF MS [m/z]: 453.2 [M+H]$^+$. 

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**Synthesis of TPE-OH**

A 5 mL CH₂Cl₂ solution of BBr₃ (53 mmol) was added dropwise to a solution of TPE-OCH₃ (3.0 g, 6.63 mmol) in 20 mL dry CH₂Cl₂ while keeping at 0 °C. After the addition was completed, the reaction mixture was allowed to stir at room temperature overnight. The reaction mixture was hydrolyzed by dropwise addition of 20 mL H₂O. After the filtration, the precipitate was washed with H₂O and diethyl ether to give TPE-OH in 88% yield. ¹H NMR (400 MHz, DMSO-d₆): δ 9.24 (s, 4H, phenolic group), 6.70 (d, J = 8.51 Hz, 8H, phenyl group), 6.47 (d, J = 8.55 Hz, 8H, phenyl group). ¹³C NMR (100 MHz, DMSO-d₆): δ 160.0, 142.3, 139.9, 136.8, 119.3 ppm. MALDI-TOF MS [m/z]: 397.2 [M+H]⁺. Elemental analysis (calcd, found for C₂₆H₂₀O₄): C (78.77, 78.84), H (5.09, 5.14), O (16.14, 16.02).

**Synthesis of 9**

A solution of TPE-OH (396 mg, 1 mmol) and CH₃ONa (216 mg, 4 mmol) in CH₃OH (10 mL) was heated to reflux. After 2 h, the purple black solution was cooled to room temperature, and then the solution was evaporated to dryness under reduced pressure to give a purple black solid in 97% yield. ¹H NMR (400 MHz, DMSO-d₆): δ 7.19 (d, J = 9.2 Hz, 8H, phenyl group), 6.11 (d, J = 8.6 Hz, 8H, phenyl group). ¹³C NMR (100 MHz, DMSO-d₆): δ 167.4, 137.7, 132.3, 123.1, 118.0 ppm. Elemental analysis (calcd, found for C₂₆H₁₆O₄Na₄): C (64.47, 64.55), H (3.33, 3.42), O (13.21, 13.09).
7.2.3 Synthetic procedure of the compounds in chapter 4

Synthesis of 2,2′:4,4″:4′,4‴-quaterpyridyl (apy)

4,4′-Bipyridine (2 g) and 10% palladium on carbon (400 mg) in DMF were heated at 180 °C for 48 h under an inert atmosphere of nitrogen. After being cooled to room temperature, the mixture was filtered and then DMF was removed under reduced pressure. The residual solid was recrystallized from acetone to give white crystals. Yield 19%. $^1$H NMR (400 MHz, CDCl$_3$): δ (ppm) 8.81 (d, $J= 8$ Hz, 2H), 8.76 (d, $J= 8$ Hz, 6H), 7.67 (dd, $J= 4$ Hz, 4H), 7.67 (d, $J= 8$ Hz, 2H). MS (MALDI-TOF) [m/z]: 310.4 [$M^+$].

Synthesis of C1

A mixture of iridium bis(2-phenylpyridine) dichloro-bridged dimer (34.5 mg, 0.032 mmol) and 2,2′:4,4″:4′,4‴-quaterpyridyl (25 mg, 0.08 mmol) was dissolved in a mixture of dichloromethane and methanol (2 : 1, v : v) and the mixture was refluxed for 16 h. The solution was concentrated and washed with hexane to afford the crude product. Then, the product was recrystallized by vapor diffusion of diethyl ether into acetonitrile. Yield 65%. $^1$H NMR (400 MHz, acetonitrile-$d_3$): δ (ppm) 8.97 (s, 2H), 8.82 (d, $J= 8$ Hz, 4H), 8.10 (d, $J= 4$ Hz, 4H), 7.87-8.81 (m, 10H), 7.70 (d, $J= 4$ Hz, 2H), 7.09 (dd, $J= 16$ Hz, 4H), 6.98 (t, $J= 16$ Hz, 2H), 6.33 (d, $J= 8$ Hz, 2H). $^{13}$C NMR (100 MHz, acetone-$d_6$), δ (ppm): 168.67, 157.71, 152.21, 151.90, 151.23, 150.28, 149.41, 144.96, 143.86, 139.74, 132.50, 131.40, 127.37, 125.95, 124.61,
123.98, 123.58, 122.48, 120.96. MS (MALDI-TOF) [m/z]: 831.3 [M–Cl]^+. Elemental analysis (calcd, found for C_{40}H_{48}F_{4}IrN_{5}): C (55.41, 55.74), H (5.58, 5.93), N (8.08, 8.24).

Synthesis of A1

Iridium bis(2-(2,4-difluorophenyl)pyridine) dichloro-bridged dimer (146 mg, 0.12 mmol) was combined with tetrabutylammonium cyanide (360 mg, 1.2 mmol) in dichloromethane at 50 °C for 4 h. After removing dichloromethane under reduced pressure, the product was purified by aluminum oxide (chromatography) with dichloromethane and methanol (10 : 1, v : v) as the eluent. Yield 78%. ^1H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 9.53 (d, J = 8 Hz, 2H), 8.20 (d, J = 8 Hz, 2H), 8.02 (t, J = 16 Hz, 2H), 7.44 (t, J = 12 Hz, 2H), 6.64-6.59 (m, 12 H), 5.52 (d, J = 8 Hz, 2H), 3.33-3.17 (m, 8H), 1.61-1.53 (m, 8H), 1.34–1.25 (m, 8H), 0.92 (t, J = 16 Hz, 12H). ^13C NMR (100 MHz, DMSO-d$_6$): δ (ppm) 170.2, 163.42, 154.97, 145.83, 139.76, 137.44, 132.17, 129.64, 124.72, 123.36, 121.62, 120.11, 59.49, 31.15, 23.52, 19.67, 13.95. MS (MALDI-TOF) [m/z]: 625.3 [M–Bu$_4$N]$^+$. Elemental analysis (calcd, found for C$_{42}$H$_{30}$ClIrN$_6$): C (59.60, 59.95), H (3.57, 3.89), N (9.93, 10.11).

Synthesis of 10

[Ir(dfppy)$_2$(CN)$_2$]Bu$_4$N$^+$ (25 mg, 0.035 mmol) and [Ir(ppy)$_2$qpy]$^+Cl$ (25 mg, 0.04 mmol) were added to CH$_3$CN (10 mL). The reaction mixture was stirred for 2 h at
room temperature and then extracted with CH₂Cl₂. The combined organic solution was concentrated by rotary evaporation. The resulting solid was washed with diethyl ether to afford 10 as a red solid. Yield 85%. ¹H NMR (400 MHz, Acetonitrile-d₃): δ (ppm) 9.51 (d, J = 4 Hz, 2H), 8.93 (s, 2H), 8.71 (d, J = 4 Hz, 4H), 8.15 (d, J = 4 Hz, 2H), 8.02 (d, J = 8 Hz, 4H), 7.81–7.74 (m, 12H), 7.62 (d, J = 4 Hz, 2H), 7.17 (t, J = 12 Hz, 2H), 7.02–6.95 (m, 4H), 6.89 (t, J = 12 Hz, 2H), 6.33 (t, J = 16 Hz, 2H), 6.25 (d, J = 8 Hz, 2H), 5.55 (d, J = 8 Hz, 2H). ¹³C NMR (100 MHz, DMSO-d₆): δ (ppm) 167.22, 164.30, 156.61, 156.36, 154.18, 150.94, 149.68, 147.96, 146.30, 144.88, 144.30, 142.94, 139.37, 138.04, 131.52, 130.80, 128.59, 128.27, 126.89, 125.64, 124.51, 123.86, 123.54, 123.02, 122.82, 122.52, 122.30, 121.88, 120.60, 118.50, 112.30, 97.03, 55.38. MS (MALDI-TOF) [m/z]: 831.3 [M⁺], 625.3 [M⁺]. Elemental analysis (calcd, found for C₆₆H₄₂F₄Ir₂N₁₀): C (55.22, 55.58), H (2.95, 3.38), N (9.76, 9.69).

7.2.3 Synthetic procedure of the compounds in chapter 5

Synthesis of 4-(diphenylamino)benzaldehyde

POCl₃ (2 mL) was dropped slowly into DMF (6 mL) at 0 °C and stirred for 30 min at room temperature. To the above solution was added a CH₂Cl₂ solution of triphenylamine (3.8 g, 15.4 mmol). After the mixture was refluxed overnight, the solution was poured into ice water and then extracted by CH₂Cl₂. Product was obtained by recrystallization in ethanol (95% yield). ¹H NMR (400 MHz, CDCl₃) δ =
9.79 (s, 1H), 7.67 (d, J = 8.9, 2H), 7.18–7.15 (m, 6H), 7.36–7.30 (t, J = 7.8, 4H), 7.00 (d, J = 8.9, 2H).

Synthesis of 4-[(2,2′:6′,2″-terpyridin]-4′-yl]-N,N-diphenylaniline

To a stirred mixture of 4-(diphenylamino)benzaldehyde (1 g, 3.41 mmol) and 2-acetylpyridine (1.73 g, 14.31 mmol) in 15 mL EtOH, followed KOH powder (0.57 g, 14.1 mmol) and ammonia (5 mL) were added. After the dark pink solution had been stirred at 25 °C for 12 h, the precipitate was isolated by filtration and washed with EtOH. Purification was accomplished readily by recrystallization from ethanol (65% yield). 1H NMR (400 MHz, CDCl3) δ = 8.73–8.71 (m, J = 8.8 Hz, 4H), 8.68–8.66 (d, J = 8.0 Hz, 2H), 7.90–7.86 (t, J = 15.6 Hz, 2H), 7.81–7.78 (d, J = 8.4, 2H), 7.37–7.34 (t, J = 12 Hz, 2H), 7.32–7.28 (t, J = 16 Hz, 4H), 7.19–7.15 (t, J = 15.2 Hz, 6H), 7.09–7.05 (m, J = 14.8 Hz, 2H).

Synthesis of Zn(II) complexes

General procedure for the synthesis of Zn(II) bis-terpyridine complexes [Zn(L)2]2+(X)2 : A solution of Zn(CH3COO)2·2H2O (0.5 mmol) in absolute methanol (30 mL) was added to a solution of the terpyridine ligand (1 mmol) in absolute methanol (100 mL). After refluxing for 12 h, the mixture was evaporated and the residue washed successively with methanol (3 x 20 mL), water (2 x 20 mL), and diethyl ether (2 x 20 mL). Further purification of the crude product was achieved by
recrystallization from acetonitrile/diethyl ether. Complexes 11b-11d were synthesized by anion exchange with NaBF₄, or NaClO₄, or KPF₆ in CH₃CN solution.

11a ¹H NMR (400 MHz, CDCl₃) δ = 9.04–9.03 (d, J = 8.8 Hz, 4H), 8.28 (s, 4H), 8.23–8.21 (d, J = 8.0 Hz, 4H), 8.03–7.99 (t, J = 15.6 Hz, 4H), 7.61–7.57 (m, 8H), 7.36–7.32 (t, J = 12 Hz, 8H), 7.20–7.18 (m, 12H), 7.16–7.13 (t, J = 15.2 Hz, 4H), 1.89 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ = 178.04, 139.21, 129.64, 128.06, 126.36, 125.17, 124.34, 122.91, 122.14, 121.83, 120.42, 118.19. MS (MALDI-TOF) [m/z]: 1016.83 [M–2CH₃COO]²⁺. Elemental analysis (calcd, found for C₇₀H₅₄N₈O₄Zn): C (73.90, 74.14), H (4.75, 4.93), N (9.85, 9.54).

11b ¹H NMR (400 MHz, CD₃CN) δ = 8.89 (s, 4H), 8.70–8.68 (d, J = 8.4 Hz, 4H), 8.16–8.12 (t, J = 14.4 Hz, 4H), 8.10–8.08 (d, J = 8.8 Hz, 4H), 7.81–7.80 (d, J = 4.8 Hz, 4H), 7.45–7.41 (t, J = 15.6 Hz, 8H), 7.39–7.36 (t, J = 12.4 Hz, 4H), 7.25–7.21 (m, J = 19.6 Hz, 16H). ¹³C NMR (100 MHz, CD₃CN) δ = 155.03, 150.71, 149.30, 147.69, 147.58, 146.33, 140.78, 129.51, 128.71, 127.15, 129.99, 125.60, 124.56, 122.72, 120.59, 119.76. MS (MALDI-TOF) [m/z]: 1016.27 [M–2BF₄]²⁺. Elemental analysis (calcd, found for C₆₆H₄₈B₂F₈N₈Zn): C (66.43, 66.71), H (4.03, 4.35), N (9.39, 9.24).

11c ¹H NMR (400 MHz, CD₃CN) δ = 8.89 (s, 4H), 8.70–8.68 (d, J = 8.0 Hz, 4H), 8.15–8.08 (m, J = 28 Hz, 8H), 7.81–7.80 (d, J = 4.8 Hz, 4H), 7.44–7.40 (t, J = 15.6 Hz, 8H), 7.38–7.35 (t, J = 12.4 Hz, 4H), 7.24–7.19 (m, J = 20.8 Hz, 16H). ¹³C NMR
(100 MHz, CD$_3$CN) $\delta =$ 155.08, 150.73, 149.33, 147.77, 147.59, 146.36, 140.81, 129.54, 128.72, 127.17, 127.02, 125.61, 124.56, 122.73, 120.60, 119.76. MS (MALDI-TOF) [$m/z$]: 1016.33 [M–2ClO$_4^-$]$^{2+}$. Elemental analysis (calcd, found for C$_{66}$H$_{48}$Cl$_2$N$_8$O$_8$Zn): C (65.05, 65.24), H (3.94, 3.93), N (9.20, 9.24).

11d $^1$H NMR (400 MHz, CD$_3$CN) $\delta =$ 8.90 (s, 4H), 8.70–8.68 (d, $J =$ 8.0 Hz, 4H), 8.17–8.13 (t, $J =$ 16.8 Hz, 4H), 8.11–8.09 (d, $J =$ 8.8 Hz, 4H), 7.83–7.82 (d, $J =$ 4.4 Hz, 4H), 7.46–7.42 (t, $J =$ 16 Hz, 8H), 7.40–7.37 (t, $J =$ 12.4 Hz, 4H), 7.26–7.22 (m, $J =$ 19.2 Hz, 16H). $^{13}$C NMR (100 MHz, CD$_3$CN) $\delta =$ 155.15, 150.81, 149.38, 147.79, 147.62, 146.39, 140.85, 129.56, 128.74, 127.22, 127.21, 125.66, 124.58, 122.74, 120.61, 119.77. MS (MALDI-TOF) [$m/z$]: 1016.65 [M–2PF$_6^-$]$^{2+}$. Elemental analysis (calcd, found for C$_{66}$H$_{48}$F$_{12}$N$_8$P$_2$Zn): C (60.53, 60.74), H (3.67, 3.93), N (8.56, 8.24).

Reference

List of Publications


• **Yun Ma**, Shujuan Liu, Huiran Yang, Yongquan Wu, Huibin Sun, Qiang Zhao, Fuyou Li, Wei Huang, A water-soluble phosphorescent polymer for time-resolved assay and bioimaging of cysteine/homocysteine, *Journal of Materials Chemistry B*, 2013, 1, 319-329

• **Yun Ma**, Shujuan Liu, Huiran Yang, Yongquan Wu, Chengjiang Yang, Xiangmei Liu, Qiang Zhao, Fuyou Li, Wei Huang, Water-soluble phosphorescent iridium(III) complexes as multicolor probes for imaging of homocysteine and cysteine in living cells, *Journal of Materials Chemistry*, 2011, 21, 18974-18982 (selected as back cover)

• Shujuan Liu, Huibin Sun, **Yun Ma**, Shanghui Ye, Qiang Zhao, Shi Sun, Xin Mou, Wei Huang, Rational Design of Metallophosphors with Tunable Aggregation-Induced Phosphorescent Emission and Their Promising Applications in Time-Resolved Luminescence Assay and Targeted Fluorescence Imaging of Cancer Cells, *Journal of Materials Chemistry*, 2012, 22, 22167-2173.

• Xiangmei Liu, Na Xi, Shujuan Liu, Yun Ma, Huiran Yang, Yangfei Lin, Haoran Li, Junhui He, Fuyou Li, Qiang Zhao, Wei Huang. Highly selective phosphorescent nanoprobes for sensing and bioimaging of homocysteine and cysteine, *Journal of Materials Chemistry*, **2012**, *22*, 7894-7901.

• Shujuan Liu, Weili Qiao, Guoyi Cao, Yang Chen, Yun Ma, Yanqing Huang, Xiangmei Liu, Wenjuan Xu, Qiang Zhao, Wei Huang. Smart Poly( N-isopropylacrylamide) Containing Iridium(III) Complexes as Water-Soluble Phosphorescent Probe for Sensing and Bioimaging of Homocysteine and Cysteine, *Macromolecular Rapid Communications*, **2013**, *34*, 81-86.
CURRICULUM VITAE

Academic qualifications of the thesis author, Mr. MA Yun:

- Received the degree of Bachelor of Macromolecular Materials and Engineering from Qingdao University, June 2008.

- Received the degree of Master of Advanced Materials from Nanjing University of Posts and Telecommunications, April 2012.

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