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PAPER

Synthesis of a new highly sensitive near-infrared fluorescent Iridium(III) probe and its application for the highly selective detection of glutathione

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A new near-infrared fluorescent probe **1** based on the IR 780 skeleton was designed, synthesized and structurally characterized for the development of a chemosensor. UV-vis absorption and fluorescence spectroscopic studies show that probe **1** exhibits a high selectivity and sensitivity towards Iridium (III) in an EtOH/H_2O (1:4, v/v, pH 7.4, HEPES buffer, 1 mM) solution by forming a 1:1 complexation with Iridium (III). Experimental results further demonstrate that probe **1**-Iridium(III) complex also has an excellent application for the highly selective detection of glutathione.

Introduction

Glutathione (GSH) is a peptide that is synthesized naturally in the human pathologies and is widely distributed in various organs of the body. GSH helps to maintain the normal function of the immune system[1] and shows anti-oxidation and integration detoxification activity.[2] The main physiological role of GSH is the ability to rid the body of free radicals by acting as an important antioxidant, protecting the thiol groups of many proteins and enzyme molecules.[3] To date, many investigations have shown that some diseases are more closely related to the levels of GSH, including hepatic, cardiac and renal failure,[4] leucocyte loss,[5] Parkinson's disease,[6] and cancer.[7] Therefore, accessing the level of GSH in a biological system is helpful in the early diagnosis of certain diseases, and designing a new probe to detect GSH with high selectivity, sensitivity and simplicity is extremely necessary for diagnosing those diseases.

Over the past several years, some new fluorescent probes (including fluoresceins, rhodamine, BODIPY, stilbene, naphthalimideand phthalocyanine dyes) have been designed to distinguish thiol from other amino acids in living systems.[8] For example, phthalocyanine dyes with a wide spectrum, a molar extinction coefficient, sensitivity, non-fluorescent background fluorescence quantum yield are gradually replacing the traditional fluorescent marker dyes to become a new generation of marker dyes.[9] Moreover, the indocyanine green(ICG) of the phthalocyanine dyes has been certified by the FDA.[10] In the relevant papers, some probes composed of phthalocyanine dyes have also been developed for identification of GSH.[11] In these papers, GSH is detected to achieve the change in fluorescence by the method of bond cleavage, but most of researchers suffer from the problem which has low thiol selectivity for GSH, [12] due to GSH, Cys and Hcy often interfered with each other during the process of detection.[13-14] Because the emission wavelength of phthalocyanine dyes is so long (650-900 nm), it is quite difficult to quench the fluorescence by the photo introduced electron transfer (PET) approach.[15] In addition, some metal ions are well known to play an important role in quenching the fluorescence of the probe.[16] Several research groups have prepared a series of fluorescence probes that can be used for fluorescence turn-on sensors to detect cell receptors.[17] In those articles we can find that those 'on-off' chemical sensor achieved the purpose of changing fluourescence by complexation of metal with probe.

Therefore, a design probe of phthalocyanine is a good method to detect GSH efficiently by metal complexation. In this paper, we designed a new composite probe containing metal ions to achieve the target of identification of GSH. The (3,5-bis(2,5-dioxocyclopent-3-en-1-yl)benzoyl chloride, containing the IR780 skeleton, was used to design a novel near-infrared fluorescent probe. After the Michael addition reaction of GSH with a group from dimaleimide, the GSH was selectively distinguished with the aid of the metal ions.[18]The results showed that the Iridium(III) was the best metal ion suitable for quenching the fluorescence of probe **1** in EtOH/H_2O (1:4, v/v, pH 7.40, HEPES buffer, 1 mM) solution. The Iridium(III) exhibited a high sensitivity and strong complexation capacity. At the same time, the composite Iridium(III) probe exhibited a high selectivity and sensitivity toward GSH.

Results and discussion

Synthesis of probe 1

Target **probe 1** was synthesized by multi-step reactions in accordance with the route as shown in **Scheme 1**. Reaction of piperazine with IR-

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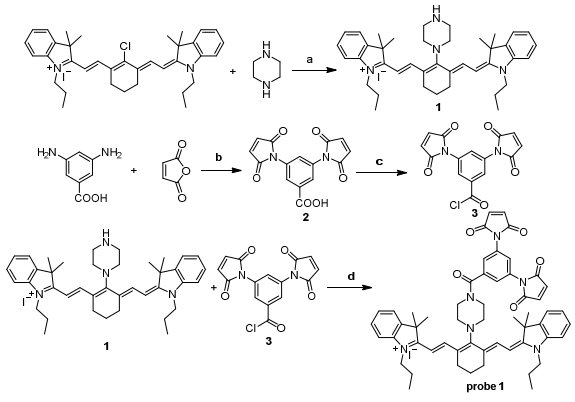
780 iodine under the condition of nucleophilic substitution reaction afforded compound **1** according to the reported procedure.[12] Subsequently, for synthesis of reaction unit of GSH-selective, we employed 3,5-diaminobenzoic acid and maleic anhydrideas starting materials to prepare compound **2**, which was then under the condition of acylation reaction to produce the key intermediate **3**.[19] Finally, Treatment of compound **1** with compound **3** in the presence of Et_3N efficiently provided probe **1** in 80% yield. The chemical structures from compound **1** to probe **1** were all confirmed by ^{1}H-NMR, ^{13}C-NMR and HRMS (Fig. S1-7, ESI†).

Effect of pH values

The pH-dependence of probe **1** (10 μM) was first evaluated in an optimized EtOH/H_2O (1:4, v/v) solution (Fig. S8, ESI†), exhibiting a suitable pH range for probe **1** in biological applications. The fluorescence intensities of probe **1** were recorded at 30 min after the addition of **probe 1** into solutions of different pH values (the pH values included 2.7, 3.4, 4.5, 5.6, 6.3, 7.0, 7.3, 7.5, 8.3, 9.4) at $\lambda_{ex/em}$=764/800 nm. As shown in **Fig. 1**, the results demonstrated that the fluorescence of probe **1** have a good stability in the pH range of 2.7-9.4 and the fluorescence intensity of probe **1-Ir^{3+}** complex can be influenced in the pH range of 5.6-9.4. Hence, the pH range of 5.6-9.4 was a reasonable value for detection of the fluorescence performance of probe **1**. Based on the above range of reasonable pH values, further fluorescence and UV–vis studies were detected in a EtOH/H_2O (1:4, v/v, pH, 7.4, HEPES buffer, 1 mM) solution.

Response time of probe 1 for Iridium(III)

To evaluate the sensitivity of probe **1** toward Ir^{3+}, the response time of probe **1** with Iridium(III) was detected in HEPES (1 mM, pH = 7.4) containing 20% EtOH at $\lambda_{ex/em}$ =764/800 nm. The concentration of probe **1** and Iridium(III) was 10 μm and 50 μm. As shown in **Fig. 2,** the fluorescence intensity of probe **1** at 764 nm decreased rapidly after Ir^{3+} (50 μM) was added into probe **1** (10 μM) solution, and stabilized after 20 min after the addition of Iridium(III). These results indicated that probe **1** was a sensitive sensor for Ir^{3+} in a HEPES (1mM, pH = 7.4) solution containing 20% EtOH. Therefore, the reaction time of 20 min was used in subsequent experiments



Scheme 1. Reagents and conditions: (a) CH_3CN, N_2, 82 °C, 4 h, 90%; (b) (i) $CHCl_3$, reflux 20 h, (ii) Ac_2O, NaOAc, 100 °C, 2 h, 69% for two steps; CH_2Cl_2/Et_3N, 75−82%. (c) $SOCl_2$, reflux, 10 h, 90%; (d) Et_3N, DCM, N_2, rt, 5 h, 60%.

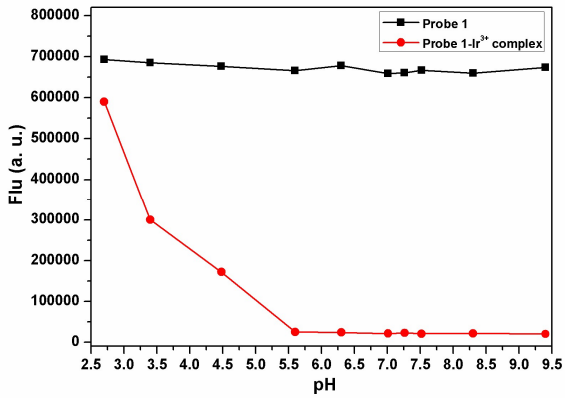


Fig. 1. The spectra of probe **1** (10 μM) in solutions of different pH values containing 20% EtOH. Fluorescence spectra (λ_{ex} = 764 nm, λ_{em} = 800 nm, slit: 5/5 nm). Each spectrum was recorded 30 min after the addition of probe **1**.

to ensure enough time for metal ions to complex with the probe **1.**

Selectivity studies for metal ions using UV-vis spectra:

The selectivity of probe **1** (10 μM) in a HEPES (1mM, pH = 7.4) containing 20% EtOH was examined with various metal ions (including Na^+, K^+, Ca^{2+}, Cu^+, Mg^{2+}, Fe^{2+}, Fe^{3+}, Cu^{2+}, Al^{3+}, Sn^{2+}, Ag^+, Hg^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Cr^{3+}, Pb^{2+}, Ir^{3+}, Rh^{3+}, Mn^{2+}, Li^+, and Ba^{2+}, at 50 μM).As shown in **Fig. 3** after Iridium(III) ions complexed with the probe **1**, the maximum UV absorption wavelength (λ_{max} =691) had a slight blue shift, and the absorption intensity (ε_{max}= 0.531) was significantly weaker than all the other metal ions.The results indicate that probe **1** exhibits excellent selectivity for Iridium(III) in a EtOH/H_2O (4:1, v/v, pH 7.40, HEPES buffer, 1mM) solution.

To gain better insight into the effect of the relationship between the probe **1** and Iridium(III), the probe **1** (10μM) was titrated with

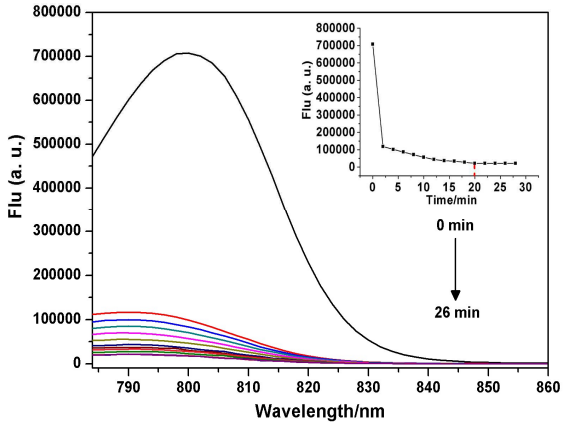


Fig. 2. The spectra of probe **1** (10 μM) with Iridium(III) (50 μM) in a HEPES (1mM, pH = 7.4) solution containing 20% EtOH. Fluorescent spectra (λ_{ex} = 764 nm, λ_{em} = 800 nm, slit: 5/5 nm). The spectra were recorded at 2 min intervals after the addition of Iridium(III). Inset: trend of fluorescence intensity, with probe **1** recorded at different times.

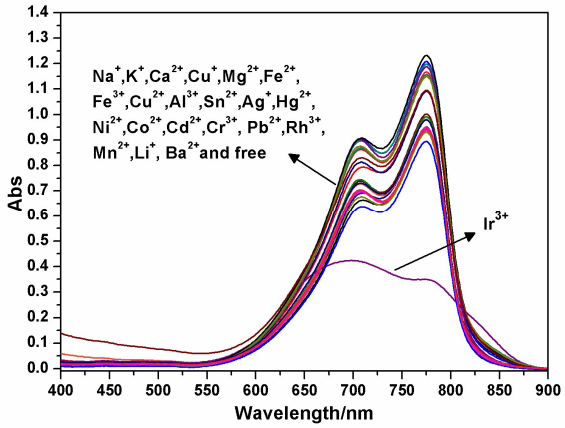


Fig. 3. The spectra of probe **1** (10 μM) with different metal ions (50 μM) in HEPES (1mM, pH = 7.4) containing 20% EtOH. Each spectrum was recorded 20 min after the addition of the metal ions.

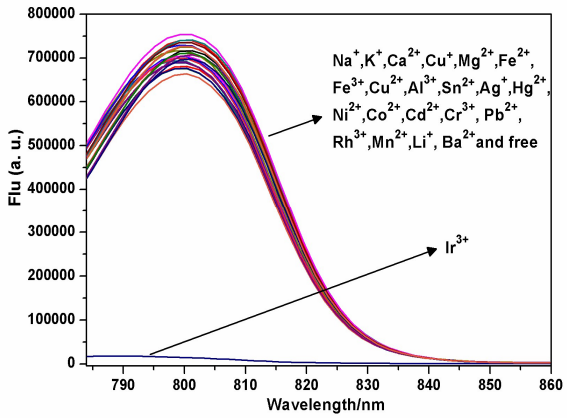


Fig. 5. The spectra of probe **1** (10 μM) with different metal ions (50 μM) in HEPES (1mM, pH = 7.4) containing 20% EtOH. Fluorescent spectra (λ_{ex} = 764 nm, λ_{em} = 800 nm, slit: 5/5 nm). Each spectrum was recorded 20 min after the addition of the metal ions.

Iridium(III) in a HEPES (1mM, pH = 7.4) solution containing 20% EtOH and analyzed using UV–vis absorbance spectra. As shown in **Fig. 4**. with the increase in the concentration of Iridium(III), UV absorption of the probe **1** decreased continuously at 775 nm. When the concentration of Iridium(III) was increased by a factor of 3, the UV absorbance intensity of probe **1** reached its lowest value.

Selectivity studies for metal ions by fluorescence spectra:

To achieve further in-depth analysis of the selectivity of probe **1** for metal ions, the fluorescence intensity of probe **1** was also further investigated by fluorescence spectra with various metal ions in a HEPES (1 mM, pH = 7.4) solution containing 20%EtOH at $\lambda_{ex/em}$ =764/800 nm under the same conditions. As shown in **Fig. 5**, When Ir^{3+} (50 μM) was introduced to the solution of probe **1**, a remarkable fluorescence quenching was observed. All other metal ions showed no obvious fluorescence quench under the same conditions. This result indicated that the probe **1** had better selectivity for Iridium(III) due to the quenching of its fluorescence. Therefore, the experiments of fluorescence titration

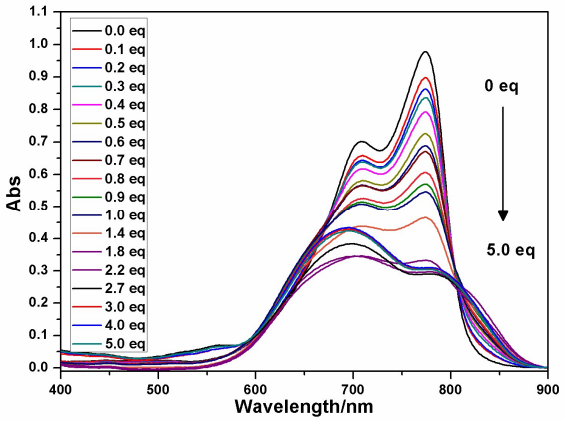


Fig. 4. The UV–vis absorbance spectra of probe **1** (10 μM) for different concentrations of Iridium(III) in HEPES (1mM, pH = 7.40) containing 20% EtOH. Each spectrum was recorded 20 min after the addition of Iridium(III).

further demonstrated that probe **1** functions as a highly selective and sensitive fluorescent chemosensor for Iridium(III)

Fluorescence titration of Probe 1 with Ir^{3+}:

To gain further insight into the binding of probe **1** (10 μM) with Ir^{3+}, Ir^{3+} titration against probe **1** in an EtOH/H_2O (4:1, v/v, pH 7.4, HEPES buffer, 1mM, $\lambda_{ex/em}$= 764/800 nm) was monitored by using fluorescence spectra. As shown in **Fig. 6**, when no Ir^{3+} ions were added to the solutions of probe **1**, the free probe **1** (10 μM) exhibited very strong fluorescence (λex =764 nm) at 800 nm. However, upon the addition of the Ir^{3+} (0-5eq.), the titration of Ir^{3+} ions with probe **1** led to a significant decrease in the emission intensity at 800 nm.The fluorescence spectraalso had been slightly blue shifted from 800 nm to 790 nm with the increase concentrations of Iridium(III). The emission intensity reached its lowest value after the addition of 5 equivalent of Ir^{3+}.

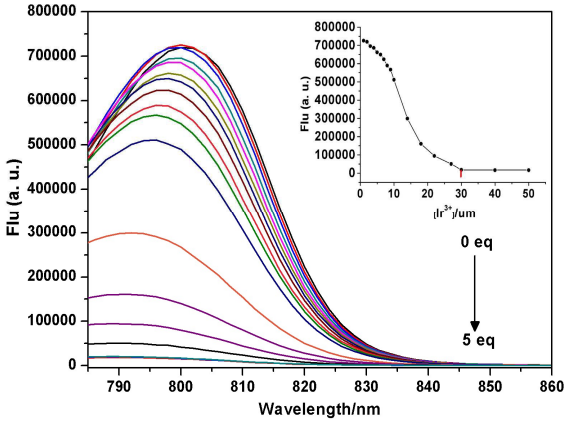


Fig. 6. The spectra of probe **1** (10 uM) for different concentrations of Iridium(III) in HEPES (1mM, pH = 7.4) containing 20% EtOH. Fluorescent spectra: (λ_{ex} = 764 nm, λ_{em} = 800 nm, slit: 5/5 nm). Each spectrum was recorded 20 min after the addition of Iridium(III). Inset: plots of fluorescence intensity where probe **1** was recorded with different concentrations of Iridium(III).

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Next, the complexation ratio of probe **1** with Iridium(III) was also investigated in the EtOH/H_2O mixed solution (1:4, v/v, pH 7.4, HEPES buffer, 1mM, $\lambda_{ex/em}$ =764/800 nm) by a Job's plot. The molar concentration of Iridium(III) ranged from 0 to 1 in a solution of [Ir^{3+}] + [probe **1**]; the total concentration of probe 1 with Iridium(III) was 40 μM. The results revealed that when the mole fraction of Iridium(III) was 0.5, the fluorescence intensity of probe **1** reached the maximum value (**Fig. 7**), indicating that the ratio by which probe **1** complexed with Iridium(III) is 1:1.

Detection limit of probe1 with Iridium(III):

The detection limit of probe **1** for Iridium(III) was further calculated based on an experiment with the fluorescence titration. The fluorescence spectrum of probe **1** was collected 10 times. Each spectrum was recorded at 10 min intervals at $\lambda_{ex/em}$=764/800 nm. As shown in **Fig. 8**, the Iridium(III) concentration changed over the range of 2-7 μM. A good linear regression equation was obtained as y = -20507x+76135 (R = 0.9940). The detection limit of Iridium(III) was calculated as 2.0 × 10^{-8} M by (K = 3), showing a high detection sensitivity for Iridium(III).[20]

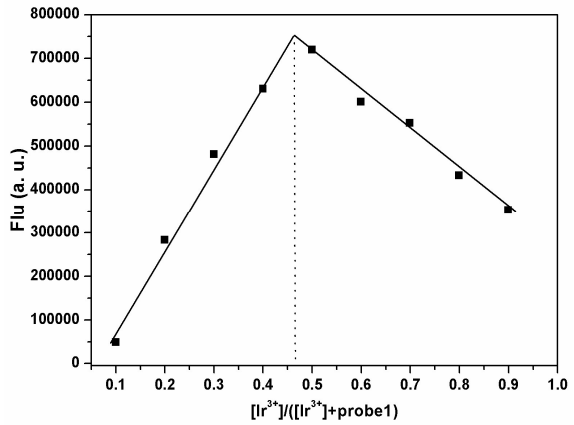

Fig. 7. Job's plot of probe **1** in an EtOH/H_2O solution (1:4, v/v, pH 7.4, HEPES buffer, 1mM) with a total concentration of [probe **1**] + Ir^{3+} = 40 μM. Fluorescent spectra (λ_{ex} = 764 nm, λ_{em} = 800 nm,).

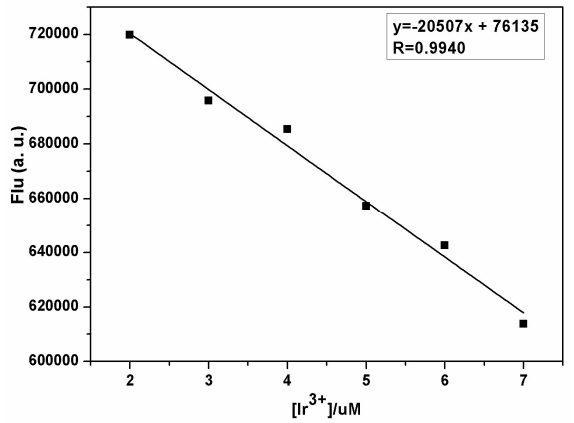

Fig. 8. Detection limit of probe **1** with Iridium(III). The spectra of probe 1 (10 uM) with different concentrations of Iridium(III) in HEPES (1mM, pH = 7.4) containing 20% EtOH. Fluorescent spectra (λ_{ex} = 764 nm, λ_{em} = 800 nm, slit: 5/5 nm).

Calculations for the binding association constant:

The association constant for probe **1** with Iridium(III) ion was calculated using a Benesi–Hildebrand plot.[21] The result is shown in **Fig. 9**. A good linear regression equation was obtained as y = -2*10^{-11}x+1*10^{-5} based on the fluorescence titration curves of probe **1** with Iridium(III) ion.Thus, the result of K_a (the binding association constant for Iridium(III)was 5.0×10^5 M^{-1} in a HEPES (1mM, pH = 7.4) solution containing 20% EtOH at $\lambda_{ex/em}$= 764/800 nm, indicating that Iridium(III) with probe have a strong binding capacity.

Fluorescence spectroscopic studies of probe 1and probe 1-Ir^{3+} complex with various amino acid:

We have concluded that probe **1** could strongly and selectively bind with Ir^{3+} to form a probe **1**–Ir^{3+} complex, causing a considerable change in the spectral properties of probe **1**. We further studied the effects of different amino acids on the effect of probe **1**–Ir^{3+} complex. First, we performed the UV-vis absorption (**Fig. 10A**) and fluorescence spectroscopic (**Fig. 10B**) experiments of the probe **1**(10 uM) in the presence of different amino acids such as Ala, Arg, Gly, Glu, Lys, Met, Pro, Cys, GSH, Thr, Trp, Val, and Hcy (20 uM)in a HEPES (1mM, pH = 7.4) solution containing 20% EtOH. Every spectrum was recorded 60 min after the addition of the amino acids (20 μM) into probe **1**(10 μM).[22-23] Both UV-vis absorption and Fluorescence spectroscopic results showed that all amino acids failed to produce any discernible spectral change under the same conditions.

Next, to better understand the property of probe **1**– Ir^{3+} complex on sensing of amino acids in a HEPES (1mM, pH = 7.4) solution containing 20% EtOH. We further performed UV-vis absorption and fluorescence titration experiments of probe **1**– Ir^{3+} complex with various amino acids. Every spectrum was recorded after the addition of Iridium (III) (30 μM) into probe **1** (10 μM) for 30 min followed by further addition of the amino acids (20 μM) for another 30 min. As shown in **Fig. 11,** After the addition of GSH(20μM, 2 eq.) to the solution, a significant absorption enhancement at 775 nm was observed (**Fig. 11A**). No obvious response was observed at 775 nm after other amino acids were added under the same conditions. The sensoring behavior of probe **1**– Ir^{3+} complex towards different amino acids was also investigated by fluorescence spectroscopy to further evaluate the selectivity of probe **1**– Ir^{3+} complex towards GSH. The change in fluorescence intensity upon addition

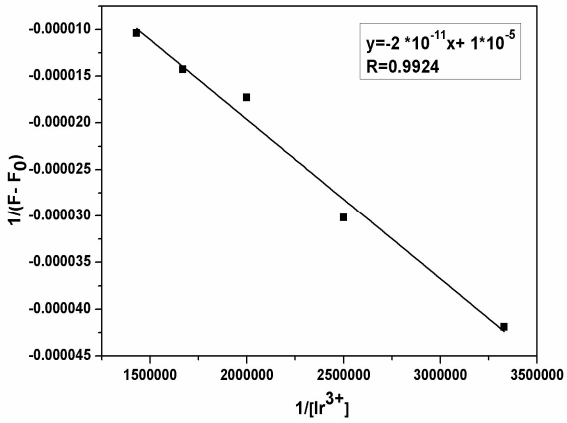

Fig. 9. The binding association constant of probe **1** with Iridium(III) was based on a Benesi–Hildebrand plot, fluorescent spectra (λ_{ex} = 764 nm, λ_{em} = 800 nm, slit: 5/5 nm)

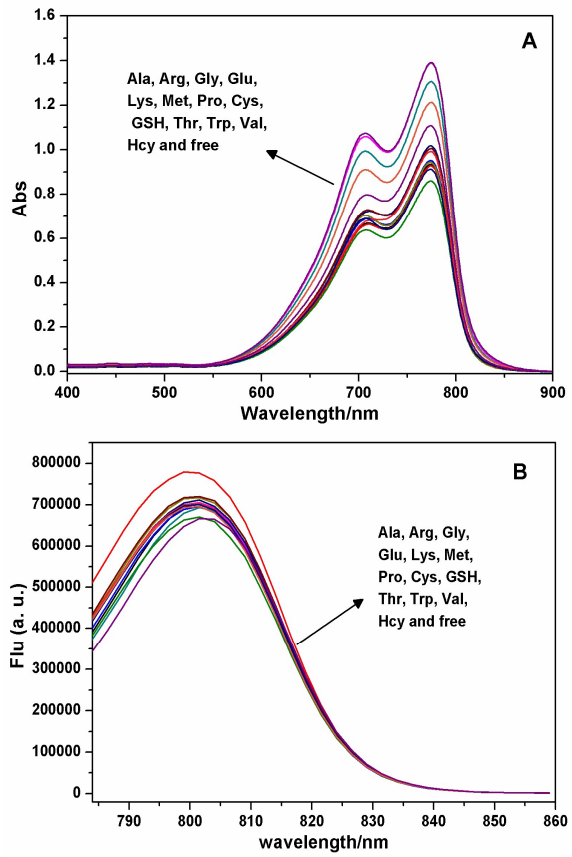

Fig. 10. The spectra of probe **1** (10 uM) relative to different amino acids (20 uM) in HEPES (1mM, pH = 7.4) containing 20% EtOH: (A) UV/Vis absorption spectra and (B) Fluorescent spectra (λex = 764 nm, λem = 800 nm, slit: 5/5 nm)**.** Fluorescent spectra (λex = 764 nm, λem = 800 nm, slit: 5/5 nm). Each spectrum was recorded 60 min after the addition of amino acids.

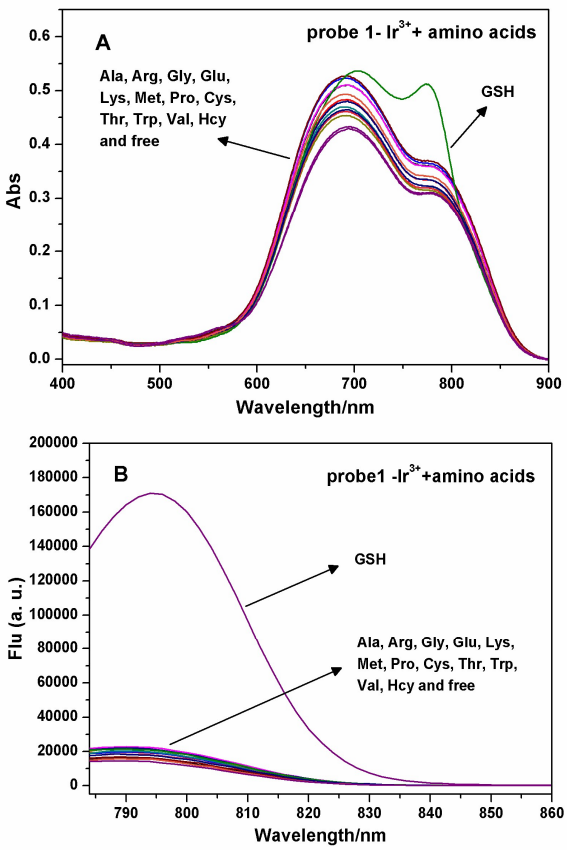

Fig. 11. The spectra of the probe **1** – Ir^{3+} ensemble(probe **1** 10 μM, Iridium(III) 30 μM) with different amino acids (20 μM) in HEPES (1mM, pH = 7.4) containing 20% EtOH. (A) UV/Vis absorption spectra and (B) Fluorescence spectra (λ_{ex} = 764 nm, λ_{em} = 800 nm, slit: 5/5 nm). Probe **1** complexed with Iridium (III) for 20 minutes. Then, each spectrum was recorded 60 min after the addition of amino acids.

of various amino acids under the same conditions was recorded. As shown in **Fig. 11B**, the fluorescence spectra of probe **1**– Ir^{3+} complex exhibit a weak fluorescence at 800 nm (λ_{ex} = 764 nm). When GSH (20μM, 2 equiv) was added to the solution containing probe **1**– Ir^{3+} complex, a strong emission band centered at 580 nm appeared, and a greater than 8-fold fluorescence enhancement was observed. Compared with probe **1**, 24.29 per fluorescence intensity was recovered upon the addition of GSH to probe **1** – Ir^{3+} complex. However, no significant variation in the fluorescence spectra was observed with any other amino acids under identical conditions. Therefore, both the UV-vis absoprtion and the fluorescence titration experiments demonstrated that probe **1**– Ir^{3+} complexfunctions as a highly sensitive and selective fluorescent chemosensor for GSH.

Competitive selectivity of probe 1-Ir^{3+}complex for GSH with other amino acids and anions:

To further evaluate the selectivity of the **probe 1**– Ir^{3+}complex for GSH, the competitive selectivity of the **probe 1**– Ir^{3+} complex for GSH under the same conditions was also examined in the presence of other amino acids. As shown in **Fig. 12**, no significant variation in the emission of the probe **1**– Ir^{3+} GSH complex was observed when comparing the results with or without other amino acids. These results clearly indicated that the detectionof GSH is not interfered with by other amino acids and that probe **1**– Ir^{3+} complex could be used as a selective GSH fluorescent chemosensor.

Additionally, the competitive selectivity of the probe **1**– Ir^{3+} complex for GSH under the identical conditions was also examined in the presence of other anions. As shown in **Fig. 13**, the emission of the probe **1**– Ir^{3+} GSH complex shows no significant change when introducing various anions including: F^-, Cl^-, Br^-, I^-, HCO_3^-, SO_4^{2-}, $H_2PO_4^-$, S_2^{2-}, HPO_4^{2-}, PO_4^{3-}, NO_3^- and CO_3^{2-}. These results further demonstrate that the detection of GSH is not remarkable interfered by other anions and thus the probe **1**– Ir^{3+} complex could be used as a highly selective GSH fluorescent chemosensor.

Mechanism studies:

NMR Spectral analysis of probe **1** with Iridium(III): to determine the reaction mechanism of probe **1** with Iridium (III), the ^{1}H-NMR spectral analysis of probe **1** was studied between the absence and presence of Iridium(III) (Fig. 14). Obviously, the probe **1** (5 mg) can be quite uniformly dispersed in the CD_3OD: D_2O (2:1, v/v, 500 μL) (I). Then Iridium(III)(1 eq) was added into the CD_3OD:D_2O (2:1, v/v) containing 5

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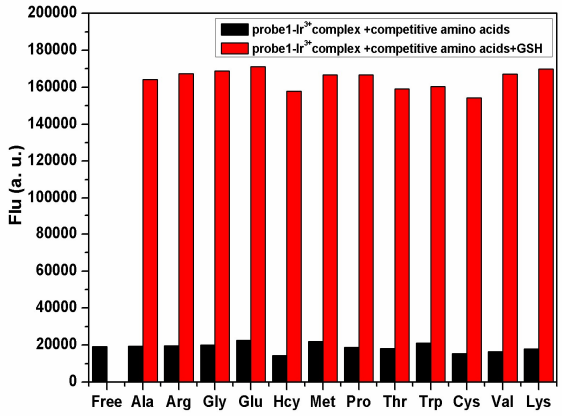

Fig. 12. The competitive selectivity of probe **1** – Ir^{3+} for GSH with other amino acids was examined with various amino acids (including Ala, Arg, Gly, Glu, Lys, Met, Pro, Cys, GSH, Thr, Trp, Val, and Hcy, 20 μM). The selectivity of these amino acids was detected in HEPES (1mM, pH = 7.4) containing 20% EtOH at $\lambda_{ex/em}$=764/800 nm. Every spectrum was recorded at 20 min after the addition of Iridium(III) (30 μM) and 60 min after the addition of the amino acids.

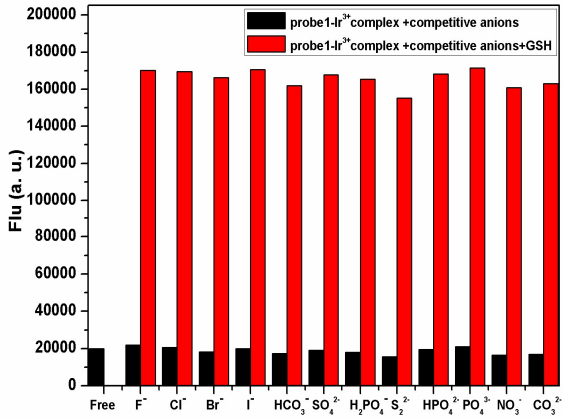

Fig. 13. The competitive selectivity of probe **1** – Ir^{3+} for GSH was examined with various anions (including: F^-, Cl^-, Br^-, I^-, HCO_3^-, SO_4^{2-}, $H_2PO_4^-$, S_2^{2-}, HPO_4^{2-}, PO_4^{3-}, NO_3^- and CO_3^{2-}, 20 μM). The selectivity of these anions was detected in HEPES (1mM, pH = 7.4) containing 20% EtOH at $\lambda_{ex/em}$=764/800 nm. Every spectrum was recorded at 20 min after the addition of Iridium(III) (30 μM) and 60 min after the addition of the amino acids.

mg probe **1** (II), suggesting that the chemical shift of the resulting product was identical to the free probe **1**, except its intensity of the resulting product was significantly reduced upon addition of Iridium(III). Next, when Iridium(III)(2 eq) was added, the NMR's relative intensity of the final product had been almost completely disappeared upon introducing Iridium(III), and the final product of probe **1** with Iridium(III) can rapidly precipitated. The above results means that complexation of probe **1** ± with Iridium(III) can generated between the probe **1** and the Iridium(III), meanwhile, since Iridium(III) complexed with probe **1**, it also led to reduce the solubility of the probe **1** and further precipitate from solvent system.

Reaction mechanism of probe **1** with GSH: from the above experimental results with UV–vis and fluorescence spectral data, the reaction mechanism for probe **1** (10 μM) with GSH (20 μM) was studied in 3 mL HEPES (1mM, pH = 7.4) containing 20% EtOH at room temperature by MS. The MS were recorded after the addition of GSH for 60 min. The intense peak at *m/z* 1497.6211 was present in the HRMS spectrum (Fig. 15A).

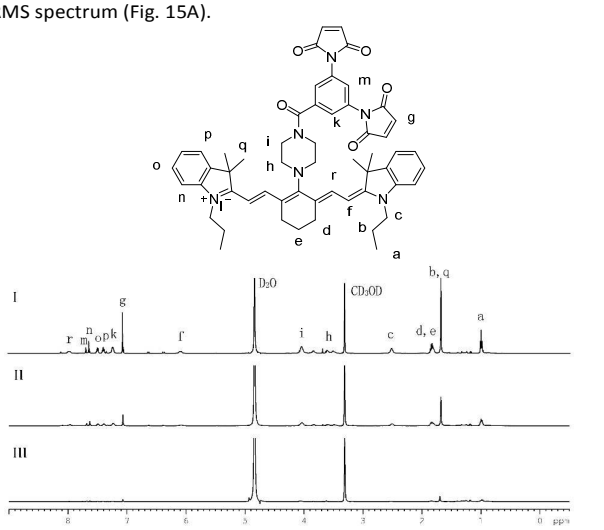

Fig. 14. The ^{1}H NMR (500 MHz) spectra of probe **1** (I), probe **1** + Iridium(III)(1 eq) (II), and probe **1** + Iridium(III)(2 eq)(III) in the CD_3OD:D_2O (2:1, v/v).

In contrast to that probe **1**'s the original molecular weight of 883.4566(Fig. 15B), revealing that the two molecules GSH just reacted with probe **1**. That is to say, the probe **1** reaction occurred by the Michael addition reaction with GSH (2 eq).

To further explain the interaction relationship between probe **1** with Iridium trihloride and GSH, the energy-optimized structures of probe **1**, Iridium trihloride and GSH were obtained by DFT calculations with the B3LYP method using a site of Gaussian 09 programs. As shown in Fig. 16, Spatial distributions showed that the conjugated structure of IR780 was changed after the chlorine atom of IR780 were replaced by nitrogen atom of the piperazine(Fig. 16a). After Iridium(III) complexed with probe **1**, the conjugate structure of the probe **1** was obviously destroyed by Iridium(III) (Fig. 16b). Then, with the addition of GSH, the conjugate structure of IR780 was restored duo to move up the Iridium(III) (Fig. 16c). As a consequence, the orbital energies of the HOMO and LUMO of probe **1**, probe **1** – Ir^{3+}, probe **1** – Ir^{3+}-GSH were generated by DFT calculations too (Fig 17). Indicating that the LUMO of probe **1** was distributed on one of the two maleimide group moiety, the HOMO was spread over the group of IR780(Fig 17a). However, after the probe **1** complexed with Iridium(III) (Fig 17b), the π electrons on the LUMO of probe **1** was distributed on the right of the group of IR780 moiety, the HOMO was spread over the two maleimide group moieties. Subsequently, adding the GSH(Fig 17c), the electron cloud of new product also occurred significant changes, the π electrons on the LUMO of probe **1** was distributed on the IR780 group, benzene and two molecules of GSH, and the HOMO was spread over the IR780 group and the right of GSH. The energy gaps between the LUMO and HOMO in the probe **1**, probe **1** – Ir^{3+}, probe **1** – Ir^{3+}-GSH were calculated to be 54.15, 39.97 and 4.36 kcal/mol respectively. The results show that the binding of GSH to probe **1** – Ir^{3+} reduced the

energy gap of HOMO – LUMO of the new product and stabilized the system.

Base on the above points, we try to deduce the following inference about the mechanism: probe **1** – Ir^{3+} ensemble shows a high selectivity for GSH. The probe **1** fluorescence is quenched owing to leading to the destruction the conjugate structure of the probe **1** by Iridium(III) and its poor

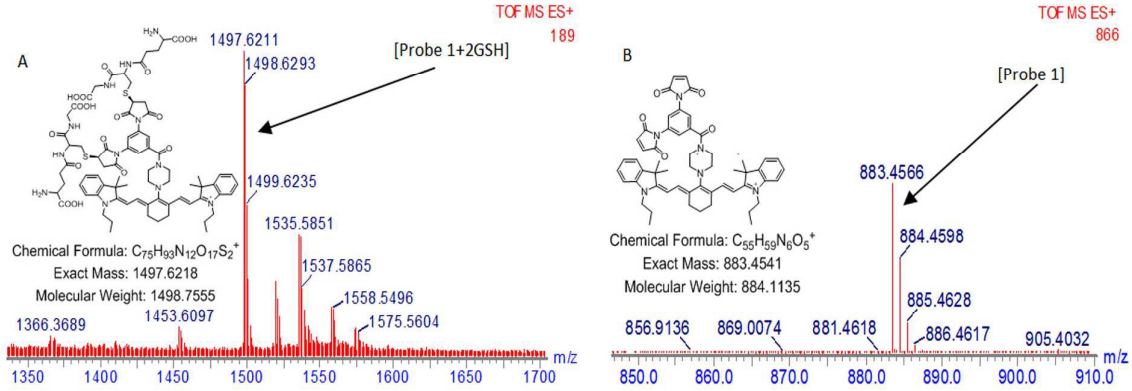

Fig. 15. HRMS spectrum of probe **1** with GSH (A) and probe **1** (B).

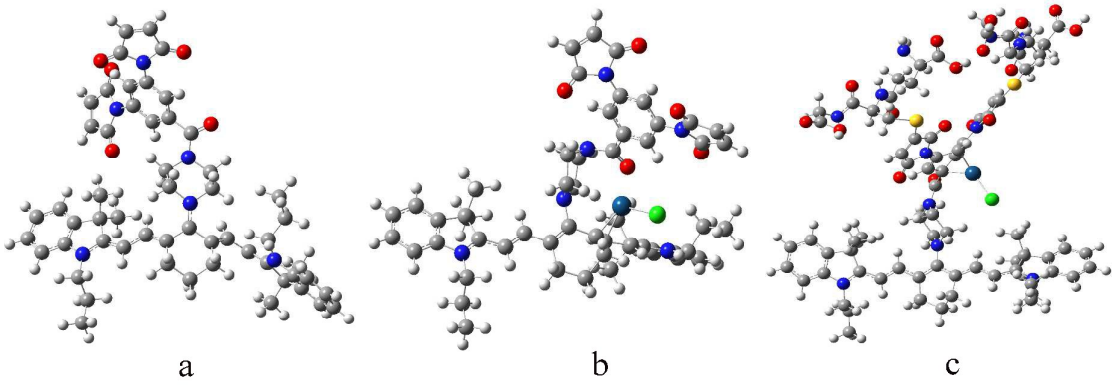

Fig. 16. Energy-minimized structures of probe **1** (a), probe **1** – Ir^{3+}(b) and probe **1** - Ir^{3+}-GSH(c)

solubility in the EtOH/H_2O (1:4, v/v, pH 7.4, HEPES buffer, 1mM) solution after the complexation of probe **1** with Iridium (III). However, with the addition of GSH, the conjugate structure of IR780 was restored owing to shifting the Iridium (III). In addition, GSH is a hydrophilic compound, it is easy to improve the solubility of the complexation of Iridium (III) with probe **1**, when GSH reacts with probe 1. Based on the above mechanism, probe **1** illustrate a excellent selectivity for GSH in presence of Iridium(III).

Conclusions

In summary, a new near-infrared composite probe (probe **1** complexed with Iridium(III)) was prepared for the determination of GSH based on the Michael addition reaction and metal complexation. Chemical structure of the probe **1** was characterized by ^{1}H-NMR, ^{13}C-NMR and HRMS. The results of performance testing (UV–vis and fluorescence response) show that the chemosensor of probe **1** had excellent selectivity and sensitivity for Iridium(III) in EtOH/H_2O (1:4, v/v, pH 7.4, HEPES buffer, 1mM) solution. Their fluorescence intensities were basically stable in the pH range of 5.6- 9.4. The 1:1 complexation mode was achieved based on a Job's plot. The Ka was calculated to be $5.0\times10^5 M^{-1}$. Moreover probe **1**-Ir^{3+} complex had excellent selectivity and sensitivity for GSH in aEtOH/H_2O (1:4, v/v, pH 7.4, HEPES buffer, 1mM) solution. Fluorescence of probe **1** was not only destruction of the conjugate structure of the probe **1**, but also precipitation from the solvent after the complexation of probe **1** with Iridium(III), once the hydrophilic GSH reacted with the probe **1**, probe **1** again tended to hydrophilic and resumed its partial fluorescent.

Experimental

All chemicals were purchased from J&K Scientific (Shanghai, China) and used without further purification. Acetonitrile was HPLC grade, other solvents were of analytical grade, and double-distilled water was used throughout all experiments.

The salts used in stock aqueous solutions of ions were NaCl, KCl, $CaCl_2$, CuCl, $MgCl_2 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $AlCl_3$, $SnCl_2 \cdot 2H_2O$, $AgNO_3$, $HgCl_2$, $NiCl_2 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$, $CdCl_2 \cdot 5H_2O$, $CrCl_3 \cdot 6H_2O$, $PbCl_2$, $IrCl_3 \cdot H_2O$, $RhCl_3 \cdot H_2O$, $MnCl_2 \cdot 4H_2O$, Li_2CO_3, $Ba(NO_3)_2$, KF $\cdot 2H_2O$, KBr, KI, $Na_2S \cdot 6H_2O$, $NaHCO_3$, $NaSO_4$, KH_2PO_4, K_2HPO_4, $K_3PO_4 \cdot 2H_2O$, $NaNO_3$, and K_2CO_3. Column chromatography was performed on Haiyang silica gel (type: 200–300 mesh ZCX-2). TLC was performed on Haiyang silica gel F254 plate.

Paper

The ^{1}H- (500 MHz) and ^{13}C-NMR (126 MHz) spectra were recorded on a Bruker Avance 500 spectrometer with $CDCl_3$ and DMSO-d_6 as solvents and tetramethylsilane (TMS) as an internal standard. High-resolution mass spectra (HRMS) were recorded on a double-focusing high-resolution instrument (Autospec; Micromass Inc.) under electron ionization conditions. All pH measurements were measured by PHS-25C Precision pH/mV Meter (Aolilong, Hangzhou, China) at room temperature (approximately 298 K). UV-vis spectra were recorded on a UV-3600 spectrophotometer (Shimadzu, Japan). Fluorescence

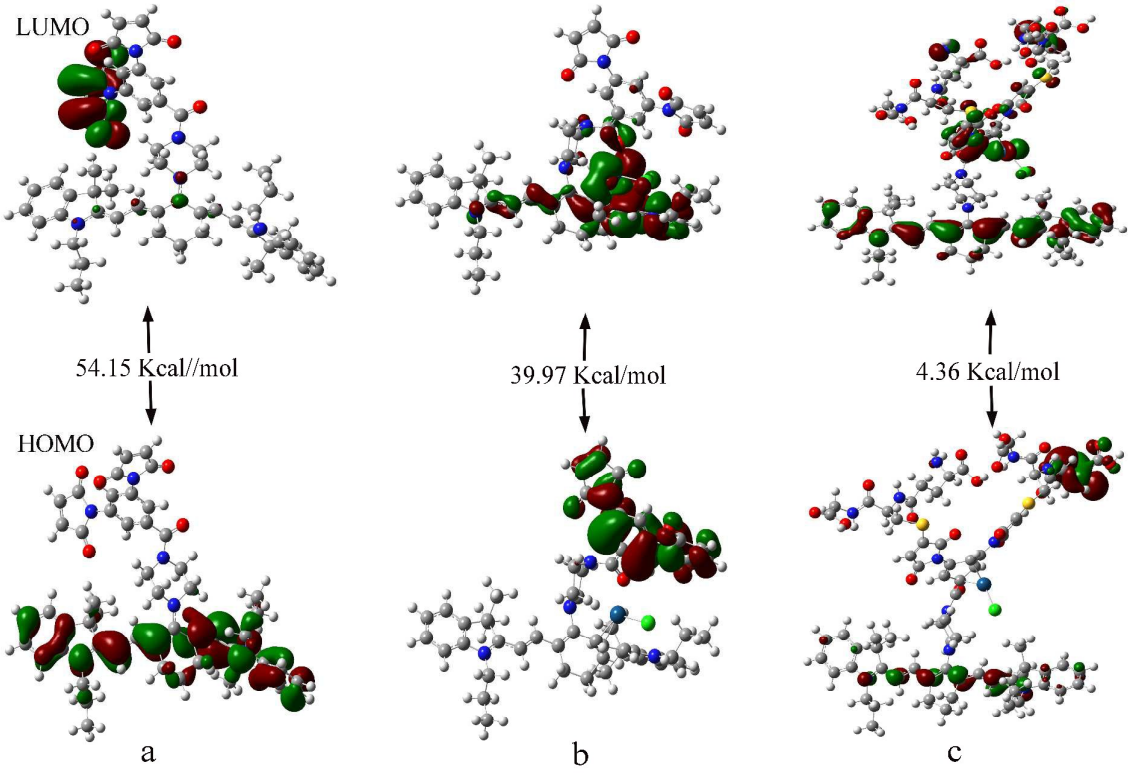

Fig. 17. HOMO and LUMO orbitals of probe **1** (a), probe **1** – Ir^{3+}(b) and probe **1** – Ir^{3+}-GSH(c)

measurements were recorded on an Edinburgh FLS920 fluorescence spectrophotometer (Livingston, UK).

Stock solution preparation for spectral detection

The stock solutions (50 uM) of the metal ions of Cd^{2+}, Co^{2+}, Cr^{3+}, Ir^{3+}, Ag^{+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Na^{+}, Al^{3+}, Ba^{2+}, Ca^{2+},Cu^{+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Sn^{2+},K^{+}, Li^{+} and Zn^{2+}, and amino acids of Ala (alanine), Arg (arginine), Gly (glycine), Glu (glutamine), Lys (lysine), Met (methionine), Pro (proline), Cys (cysteine), GSH, Thr (threonine), Trp (tryptophane), Val (valine), and Hcy (homocysteine) were prepared in EtOH/H_2O (1:4, v/v, pH 7.40). The working solution of probe **1** (10 μM) was prepared in EtOH/H_2O (1:4, v/v, pH 7.4). Detection solutions of probe **1** were freshly prepared for spectroscopic measurements.

UV–vis and fluorescence spectral studies

All experiments were performed in EtOH/H_2O solution (1:4, v/v, pH 7.4, HEPES buffer, 1mM). In the selectivity experiments, the test samples were prepared by adding an appropriate amount of the cation stock or amino acid solution (50 μM) to a 3 mL solution of the probe **1** (10 μM). In the titration experiment, a 10 μM solution of the probe **1** was prepared at room temperature with an appropriate amount of the ion stock solution or GSH solution, which was added to the quartz optical cell using a micropipette. Spectral data were recorded 30 min after the addition of the amino acids or metal ions. In the fluorescence measurements (slit: 5/5 nm), excitation was provided at 764 nm and emission was 800 nm, collected from 784 to 860 nm.

Synthesis of compound 1[12]

Under a N_2 atmosphere, piperazine (34.5 mg, 0.4 mm) was added to a 5ml solution of IR-780 iodine(67 mg, 0.1 mmol) in anhydrous acetonitrile. After stirring for 4 h at 82□, the solvent was removed *in vacuo*.The resulting residue was purified by column chromatography (silica gel; DCM/MeOH, 100/2, v/v) to give compound **1** (68 mg, 90%) as a blue solid. ^{1}H-NMR (500 MHz, $CDCl_3$, 298 K, TMS) δ (ppm): 7.69 (d, J = 13.0 Hz, 2H), 7.29−7.36 (m, 4H), 7.15 (s, 2H), 6.97 (d, J = 6.0 Hz, 2H), 5.79 (d, J = 12.0 Hz, 2H), 3.97(d, 8H), 3.32 (s,4H), 2.50 (s, 4H), 1.87 (s,6H), 1.74 (s, 12H), 1.08 (s, 6H). ^{13}C-NMR (126 MHz, $CDCl_3$, 298 K, TMS) δ (ppm): 172.7, 169.5, 142.8, 141.2, 140.5, 128.3, 123.7, 123.4, 122.3, 109.4, 96.2, 55.2, 48.4, 46.8, 45.3, 29.6, 25.1, 21.7, 20.4, 11.7.

Synthesis of compound 2[19]

Maleic anhydride (193 mg,1.97 mmol) was added to a 6 mL solution of 3,5-diaminobenzoic acid (100 mg, 0.66 mmol) in $CHCl_3$. After refluxing for 20 h, the residue of yellow precipitate was filtered, and sodium acetate (17.6 mg, 0.26 mmol) and acetic anhydride (5 mL) were added. The reacting mixture was heated for 2 h at 100 ℃. The resulting reaction mixturewas poured on ice, and cold water (15 mL) was added. The mixture was stirred vigorously for an additionhour.. The precipitate was filtered and washed with cold water (50 mL) to afford compound **2** (144.38 mg, 69%) as a beige solid.^{1}H-NMR (500 MHz, DMSO-*d6*, 298 K, TMS) δ (ppm): 7.97 (s, 2H), 7.64 (s, 1H), 7.22 (s,4H). ^{13}C-NMR (126 MHz, DMSO-*d6*, 298 K, TMS) δ (ppm): 170.0, 166.4, 135.3, 132.8, 132.5, 129.0, 126.8.

Synthesis of compound 3[19]

Compound **2** (200 mg, 0.65 mmol) was added to thionyl chloride (10 mL). The mixture was reflux at 85 ℃until solid particles disappeared completely. The excess $SOCl_2$ was evaporated in vacuo, then the resulting solid was dried under vacuum to afford compound **3** (191.49 mg, 90%) as a beige solid that was used without further purification to add directly into the next reaction.

Synthesis of probe 1

Compound 3 (29 mg, 0.75 mmol) and Et_3N (12.14 mg, 0.11 mmol) in anhydrous DCM (2 mL)were added dropwise to 3ml anhydrous DCM solution containing compound **1** (60 mg, 0.08 mmolat 0 ℃. The reaction mixture was allowed to stir for an additional 5 h under ~~an~~ a N_2 atmosphere at room temperature. After completion of the reaction, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (silica gel; DCM/MeOH, 100/1, v/v) to give probe **1** (56.53 mg, 80%) as a classical blue solid.^{1}H-NMR (500 MHz, $CDCl_3$, 298 K, TMS) δ (ppm): 7.86 (d, *J* = 13.7 Hz, 2H), 7.73 (t, *J* = 1.9 Hz, 1H), 7.67 (d, *J* = 2.0 Hz, 2H), 7.407 – 7.32 (m, 4H), 7.20 (t, *J* = 7.5, 0.9 Hz, 2H), 7.08 (d, *J* = 8.1 Hz, 2H), 6.93 (s, 4H), 6.01 (d, *J* = 13.7 Hz, 2H), 4.03 (t, *J* = 7.3 Hz, 4H), 3.65 (s, 4H), 2.56 (t, *J* = 6.4 Hz, 4H), 1.89 (p, *J* = 7.7 Hz, 6H), 1.73 (s, 16H), 1.07 (t, *J* = 7.40 Hz, 6H).^{13}C-NMR (126 MHz, $CDCl_3$, 298 K, TMS) δ (ppm): 170.2, 169.7, 168.7, 168.6, 142.6, 141.6, 140.5, 135.9, 134.5, 132.5, 128.6, 126.8, 124.3, 123.4, 123.2, 122.1, 110.1, 98.5, 54.0, 48.6, 45.8, 28.8, 25.4, 21.5, 20.6, 11.7. HRMS $(M^-I^-)^+$ found, 883.4566; calcd for $C_{55}H_{59}N_6O_5^+$, 883.4521.

Acknowledgments

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Synthesis of a new highly sensitive near-infrared fluorescent Iridium(III) probe and its application for the highly selective detection of glutathione

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A new near-infrared fluorescent probe **1** based on the IR 780 skeleton was designed, synthesized and structurally characterized for the development of a chemosensor. Further research demonstrate that probe **1**-Iridium(III) complex has an excellent application for the highly selective detection of glutathione.