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Time-resolved photoluminescence spectroscopy for the detection of cysteine and other thiol containing amino acids in complex strongly autofluorescent media†

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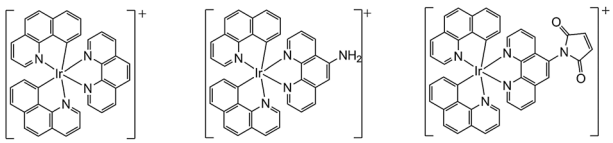
A thiol probe based on an iridium complex with long-lived photoluminescence was synthesized, which can be used for the detection of thiols even in the presence of strong background fluorescence. This system provides an easy and fast methodology for detecting thiol containing amino acids, which has potential applications in clinical diagnostics.

Small-molecule thiols are ubiquitous in biological systems for maintaining redox homeostasis.[1,2] Abnormal amounts of thiols in biological fluids have been associated with cardiovascular diseases,[3] Alzheimer's disease,[4,5] idiopathic pulmonary fibrosis[6] and acquired immune deficiency syndrome,[7] among others. Therefore, the development of effective and sensitive probes that would allow the detection of thiol-containing molecules is pivotal for improving diagnosis methods for these diseases.

Although a variety of thiol probes have been reported using electrochemical,[8–12] colorimetric[13–16] and photoluminescence methods,[1,2,17–27] it is noticeable that all these probes were designed to function in background free environments. Also, many of these probes function only in buffer media containing a considerable amount of an organic solvent.[14,19,23,24,26,27] In this manuscript, a photoluminescent iridium(III) complex modified with a maleimide group is reported as light switching thiol probe in aqueous solution. One important advantage of metal complexes is their long-lived photoluminescence lifetime, which allows detecting analytes even in the presence of short-lived background fluorescence by using time-gating techniques.[28,29]

During previous research, we found that the photoluminescence of iridium(III) complexes such as $[Ir(bq)_2(phen)]^+$ (bq = benzo[*h*]quinoline; phen = 1,10-phenanthroline) is strongly sensitive to modification of the phen ligand. For example $[Ir(bq)_2(phen\text{-}NH_2)]^+$ (phen-NH_2 = 1,10-phenanthroline-5-amino) is non-photoluminescent (Scheme 1). Based on this interesting phenomenon, $[Ir(bq)_2(phen\text{-}M)]^+$ (**1**, phen-M = 1,10-phenanthroline-5-maleimide) was synthesized (for synthesis see ESI†), which also displays no photoluminescence in aqueous solution. However, when **1** is in the presence of small thiol-containing amino acids such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), it presents up to a 60-fold increase in photoluminescence (see Fig. 1). In order to evaluate the selectivity of **1**, other natural amino acids that do not contain thiol groups, including L-alanine (Ala), L-arginine (Arg), L-asparagine (Asp), L-histidine (His), L-leucine (Leu), L-lysine (Lys), L-phenylalanine (Phe), L-serine (Ser), L-threonine (Thr), L-tryptophan (Try), L-tyrosine (Tyr), L-valine (Val), L-cystine (oxidized cysteine molecule), and two proteins, bovine serum albumin (BSA) and lysozyme, were separately added to **1**. The photoluminescence intensity of **1** in the presence of different amino acids and proteins was explored. As can be seen from Fig. 1, only thiol-containing amino acids produced a light-switching response from **1**.



Scheme 1 Chemical structure of $[Ir(bq)_2(phen)]^+$, $[Ir(bq)_2(phen\text{-}NH_2)]^+$ and $[Ir(bq)_2(phen\text{-}M)]^+$ (**1**).

The quantitative recognition of probe **1** by thiol-containing amino acids was assayed using Cys, Hcy, and GSH. As shown in the Fig. 1 inset, sequential titration of Cys (0–11 μM) into a solution of **1** (10 μM) leads to an outstanding enhancement of the photoluminescence emission at 590 nm. The linear increase in the photoluminescence signal and the saturation of the photoluminescence signal at 10 μM indicate that the reaction of **1** and Cys is stoichiometric, which was further confirmed by ^{1}H NMR titration and ESI-MS (Fig. S1 and S2, ESI†). Similar experimental results were observed when Hcy and GSH were assayed respectively (Fig. S4 and S6, ESI†). The sensitivity of probe **1** was calculated as the minimum amount of thiol-containing amino acid necessary to achieve a three-fold increase in its photoluminescence. The determined detection limits were 14.3, 16.4 and 18.6 nM for Hcy, Cys and GSH

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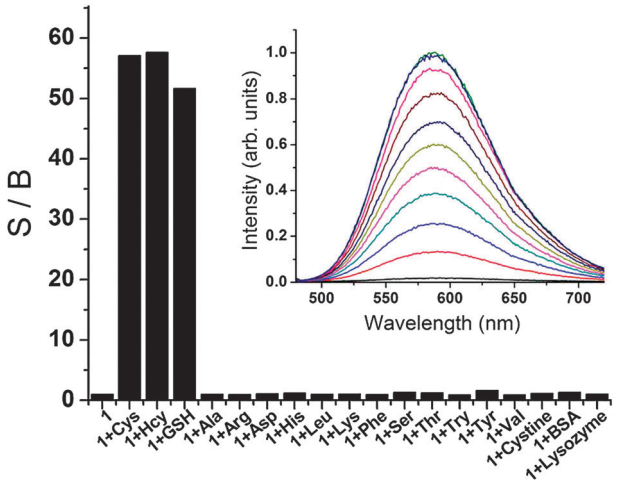

Fig. 1 Relative photoluminescence intensity of 10 μM probe **1** at 590 nm before and after addition of 10 μM of various amino acids, GSH, cysteine and proteins (PBS 6.7 mM, pH 7.2, 1% DMF; incubation at room temperature for 1 minute after mixing). S/B ratio is the ratio of the probe photoluminescence after and before the addition of the amino acid. **1** + Cys, **1** + Hcy, and **1** + GSH samples were run in triplicate, and their S/B ratios presented standard deviations of less than 3%. Inset: photoluminescence spectra of **1** with different concentrations of Cys.

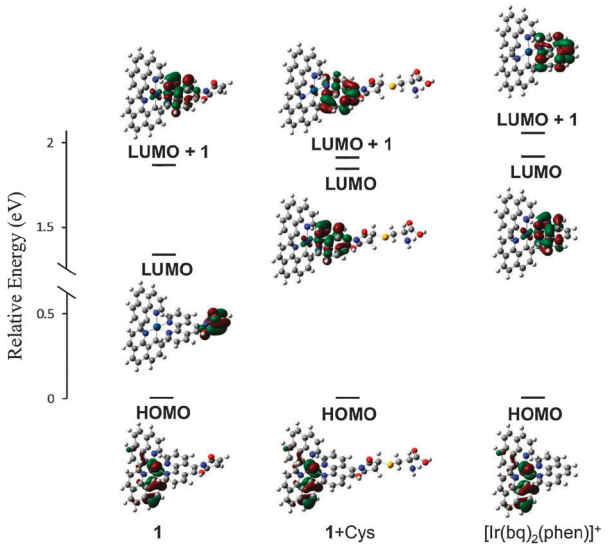

Fig. 2 Molecular orbital diagrams of **1**, **1** + Cys and $[Ir(bq)_2(phen)]^+$. The energy of the HOMOs was set to 0 eV for easy comparison (isovalue = 0.04).

respectively (Table S2, ESI†). It is important to note that **1** does not present a response towards L-cystine (cysteine oxidized dimer), which is in contrast to the low detection limit for L-cysteine and indicates a strong selectivity towards the reduced form of the thiol-containing amino acids. Furthermore, the reaction of **1** with lysozyme (8 Cys) and BSA (35 Cys) did not show any increase in photoluminescence. This can be explained by the fact that all Cys in lysozyme and 34 out of 35 Cys in BSA are participating in disulfide bonds. Interestingly, while this free Cys in BSA has shown to bind maleimide-containing molecules[30] it seems to be unreactive towards **1**, which suggest that the bulky and inflexible structure of **1** requires a widely exposed thiol group for the reaction to occur. To confirm this, we ran the reaction at 60 °C, which would help the BSA to explore more conformations than at room temperature. We found that the photoluminescence increased by 30 fold after 5 minutes, while the control experiment with **1** in buffer at 60 °C just showed marginal photoluminescence.

In order to explore the nature of the sensing mechanism of **1** to thiols, DFT calculations of the frontier orbitals of **1** and its adduct with Cys were performed for the HOMO, LUMO and LUMO + 1 (Fig. 2). The LUMO of $[Ir(bq)_2(phen)]^+$ was found to possess phen(π*) character as expected from these kinds of metal complexes.[31,32] In contrast to this, the LUMO of **1** is located at the maleimide group and at a considerably lower energy than that of $[Ir(bq)_2(phen)]^+$. On the other hand, the LUMO of **1**-Cys is phen(π*) and resembles that of $[Ir(bq)_2(phen)]^+$. Given that both $[Ir(bq)_2(phen)]^+$ and **1**-Cys are photoluminescent, it can be concluded that the population of this phen(π*) excited state results in a radiative deactivation to the ground state. The lack of photoluminescence from **1** is consistent with the population of the maleimide molecular orbitals in the excited state, which are lower in energy than the LUMO of $[Ir(bq)_2(phen)]^+$ and **1**-Cys, and decay to the ground state by non-radiative pathways. This is in agreement with the photophysical behavior of other maleimide containing molecules.[17]

Up to this point we have demonstrated that **1** is an effective probe for thiol-containing amino acids in aqueous solution with low detection limits. This probe, however, presents the additional advantage of having a relatively long photoluminescence lifetime (for **1**-Cys the average lifetime is 99.2 ns, see Table S1, ESI†), which could be used to detect thiol-containing amino acids in media with high autofluorescence background. A high autofluorescence background will obscure the photoluminescence of the probe, preventing the detection of the analyte. To demonstrate the ability of this probe to overcome strong autofluorescent background, and to selectively detect thiol containing molecules in a complex environment we used Dulbecco's Modified Eagle (DME) as a medium for our experiments. The DME medium has an emission at around 595 nm, which overlaps completely with that of **1**, degrading the large S/B ratio of the probe to basically nothing (see Fig. 3a). Furthermore, this medium, which is popular for cell cultures, contains a complex mixture of inorganic salts, amino acids, vitamins and other molecules such as glucose and pyruvic acid. Moreover, it lacks L-cysteine, but contains L-cystine (the oxidized form of cysteine), which is unreactive to **1**.

Fig. 3b shows the photoluminescence time-decay transients for **1** in DME medium before and after the addition of Cys. The photoluminescence decay before the addition of Cys (red curve, Fig. 3b) basically shows the autofluorescence decay of the DME medium, since **1** presents virtually no photoluminescence. However, when Cys is added (blue curve, Fig. 3b) a long-lived photoluminescence arises which is due to the presence of **1**-Cys. It is evident from Fig. 3b that the short-lived fluorescence of the DME medium decays much faster than the relatively long-lived photoluminescence of **1**-Cys.

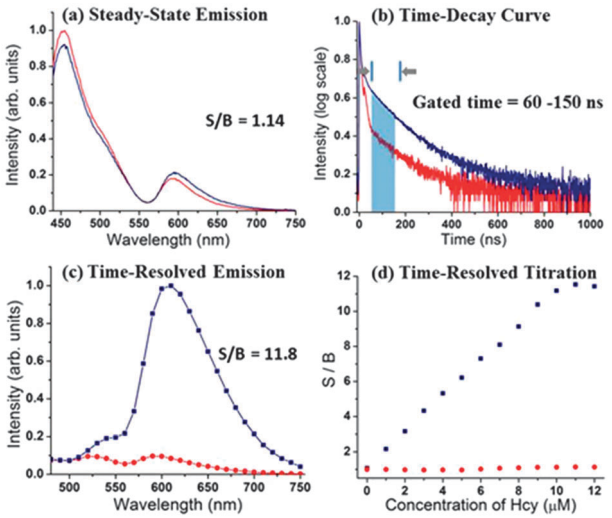

Fig. 3 (a) Steady-state emission of 10 μM solution of **1** in Dulbecco's Modified Eagle medium (1% DMF) with (blue line) and without (red line) addition of 10 μM cysteine; (b) time-decay transients of the same solution before (red line) and after (blue line) addition of 10 μM cysteine (λ_{exc} = 370 nm, λ_{em} = 590 nm); (c) time-resolved emission of the same solution with time-gating from 60 to 150 ns after laser excitation; (d) titration of cysteine to 10 μM solution of **1** in Dulbecco's Modified Eagle medium (1% DMF) using a steady-state fluorometer (red points) and a time-resolved fluorometer with time-gating from 60 to 150 ns (blue points).

Time-resolved photoluminescence spectroscopy allows selecting a time-window after the fluorescence background has decayed but where still the photoluminescence of **1**-Cys is strong. This time window was selected to be from 60–150 ns and was determined using a recently published method by our group.[28] Fig. 3c shows the time-resolved emission spectra (TRES) of **1** with and without Cys in the presence of strong autofluorescent background from DME media, and time-gating from 60–150 ns. As expected, most of the autofluorescent background has been eliminated allowing the selective observation of the photoluminescence of the long-lived probe. An S/B ratio of 11.8 can be obtained using time-gating, which is in contrast to the poor S/B ratio shown in Fig. 3a. Furthermore, Fig. 3d shows the titration of **1** with Cys in autofluorescent DME medium using steady-state and time-resolved photoluminescence spectroscopy. This figure convincingly shows that by using steady-state methods no thiol can be detected, which is a consequence of the strong fluorescence of DME media obscuring the assay. In contrast to this, using time-resolved photoluminescence spectroscopy and time-gating from 60–150 ns result in the unambiguous detection of Cys. Similarly, thiol detection experiments with the addition of the fluorophore sulforhodamine in PBS buffer solution can be found in the ESI,† and lead to the same results (Fig. S3–S7, ESI†). These results provide concrete evidence of the advantages of this probe for the detection of thiol-containing amino acids in complex environments with strong autofluorescence.

In summary, a thiol probe based on an iridium complex with long-lived photoluminescence was synthesized, which can be used for the detection of thiols even in the presence of strong background fluorescence. This system provides an easy and fast methodology for detecting thiol containing amino acids, which has potential applications in clinical diagnostics.

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Notes and references

1 X. Chen, Y. Zhou, X. Peng and J. Yoon, *Chem. Soc. Rev.*, 2010, **39**, 2120.
2 D. Kand, A. M. Kalle, S. J. Varma and P. Talukdar, *Chem. Commun.*, 2012, **48**, 2722.
3 H. Refsum, P. M. Ueland, O. Nygård and S. E. Vollset, *Annu. Rev. Med.*, 1998, **49**, 31.
4 S. Seshadri, A. Beiser, J. Selhub, P. F. Jacques, I. H. Rosenberg, R. B. D'Agostino, P. W. F. Wilson and P. A. Wolf, *N. Engl. J. Med.*, 2002, **346**, 476.
5 C. Cecchi, S. Latorraca, S. Sorbi, T. Iantomasi, F. Favilli, M. T. Vincenzini and G. Liguri, *Neurosci. Lett.*, 1999, **275**, 152.
6 A. C. White, V. J. Thannickal and B. L. Fanburg, *J. Nutr. Biochem.*, 1994, **5**, 218.
7 W. Dröge, *Pharmacology*, 1993, **46**, 61.
8 T. Inoue and J. R. Kirchhoff, *Anal. Chem.*, 2000, **72**, 5755.
9 F. Ricci, F. Arduini, A. Amine, D. Moscone and G. Palleschi, *J. Electroanal. Chem.*, 2004, **563**, 229.
10 T. Inoue and J. R. Kirchhoff, *Anal. Chem.*, 2002, **74**, 1349.
11 S. Fei, J. Chen, S. Yao, G. Deng, D. He and Y. Kuang, *Anal. Biochem.*, 2005, **339**, 29.
12 W. Wang, L. Li, S. Liu, C. Ma and S. Zhang, *J. Am. Chem. Soc.*, 2008, **130**, 10846.
13 W. Wang, O. Rusin, X. Xu, K. K. Kim, J. O. Escobedo, S. O. Fakayode, K. A. Fletcher, M. Lowry, C. M. Schowalter, C. M. Lawrence, F. R. Fronczek, I. M. Warner and R. M. Strongin, *J. Am. Chem. Soc.*, 2005, **127**, 15949.
14 J. V. Ros-Lis, B. García, D. Jiménez, R. Martínez-Máñez, F. Sancenón, J. Soto, F. Gonzalvo and M. C. Valldecabres, *J. Am. Chem. Soc.*, 2004, **126**, 4064.
15 D. Zhang, *Inorg. Chem. Commun.*, 2009, **12**, 1255.
16 F.-J. Huo, Y.-Q. Sun, J. Su, J.-B. Chao, H.-J. Zhi and C.-X. Yin, *Org. Lett.*, 2009, **11**, 4918.
17 T. Matsumoto, Y. Urano, T. Shoda, H. Kojima and T. Nagano, *Org. Lett.*, 2007, **9**, 3375.
18 J. E. T. Corrie, *J. Chem. Soc., Perkin Trans. 1*, 1994, 2975.
19 J. Guy, K. Caron, S. Dufresne, S. W. Michnick, W. G. Skene and J. W. Keillor, *J. Am. Chem. Soc.*, 2007, **129**, 11969.
20 L. Yi, H. Li, L. Sun, L. Liu, C. Zhang and Z. Xi, *Angew. Chem., Int. Ed.*, 2009, **48**, 4034.
21 W. Lin, L. Yuan, Z. Cao, Y. Feng and L. Long, *Chem.–Eur. J.*, 2009, **15**, 5096.
22 T. J. Tolbert and C.-H. Wong, *Angew. Chem., Int. Ed.*, 2002, **41**, 2171.
23 H. Chen, Q. Zhao, Y. Wu, F. Li, H. Yang, T. Yi and C. Huang, *Inorg. Chem.*, 2007, **46**, 11075.
24 M. Zhang, M. Yu, F. Li, M. Zhu, M. Li, Y. Gao, L. Li, Z. Liu, J. Zhang, D. Zhang, T. Yi and C. Huang, *J. Am. Chem. Soc.*, 2007, **129**, 10322.
25 H. Maeda, H. Matsuno, M. Ushida, K. Katayama, K. Saeki and N. Itoh, *Angew. Chem., Int. Ed.*, 2005, **44**, 2922.
26 H.-Y. Shiu, M.-K. Wong and C.-M. Che, *Chem. Commun.*, 2011, **47**, 4367.
27 N. Zhao, Y.-H. Wu, L.-X. Shi, Q.-P. Lin and Z.-N. Chen, *Dalton Trans.*, 2010, **39**, 8288.
28 K. Huang and A. A. Martí, *Anal. Chem*, 2012, **84**, 8075.
29 N. P. Cook, V. Torres, D. Jain and A. A. Martí, *J. Am. Chem. Soc.*, 2011, **133**, 11121.
30 M. Oblak, A. Prezelj, S. Pecar and T. Solmajer, *Z. Naturforsch., C*, 2004, **59**, 880.
31 Q. Zhao, S. Liu, M. Shi, F. Li, H. Jing, T. Yi and C. Huang, *Organometallics*, 2007, **26**, 5922.
32 C. Dragonetti, L. Falciola, P. Mussini, S. Righetto, D. Roberto, R. Ugo, A. Valore, F. De Angelis, S. Fantacci, A. Sgamellotti, M. Ramon and M. Muccini, *Inorg. Chem.*, 2007, **46**, 8533.