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# Turn-on Luminescent Probe for Cysteine/Homocysteine Based on a Ruthenium(II) Complex

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A unique ruthenium(II) complex, tris(4-methyl-2,2′-bipyridyl-4′-carboxaldehyde)Ru(II) hexafluorophosphate [Ru(CHO-bpy)$_3$]($PF_6$)$_2$, has been designed and synthesized as a highly sensitive and selective luminescence probe for the recognition and detection of cysteine (Cys) and homocysteine (Hcy). The almost non-luminescent probe can rapidly react with Cys and Hcy to yield the corresponding thiazolidine and thiazinane derivatives, accompanied by the remarkable luminescence enhancement and a large blue-shift of the maximum emission wavelength from 720 to 635 nm. The dose-dependent luminescence enhancement of the probe shows a good linearity in the Cys/Hcy concentration range of 15 to 180 $\mu$M with the detection limits of 1.41 $\mu$M and 1.19 $\mu$M for Cys and Hcy, respectively. Furthermore, the luminescence response of the probe is highly specific to Cys/Hcy only even in the presence of various amino acids, protein, and DNA. The results of this work not only demonstrate the efficacy and advantages of the Ru(II) complex-based luminescence probe for the sensitive and selective detection of Cys/Hcy but also provide a useful strategy for the rational design of Ru(II) complex-based luminescence probes for various biological molecules.

## Introduction

The thiol-containing amino acid including cysteine (Cys), homocysteine (Hcy), and glutathione (GSH) plays crucial roles in physiological matrixes.[1] In organisms, Cys and Hcy are essential biological molecules required for the growth of cells and tissues, and abnormal levels of Cys and Hcy are implicated in a variety of diseases. The deficiency of Cys is involved in slow growth, hair depigmentation, edema, lethargy, liver damage, muscle and fat loss, skin lesions, and weakness.[2] Additionally, it is now known that there is a relationship between the increased Hcy level and certain types of cardiovascular risk, and Alzheimer's disease, and dietary, environmental and genetic causes (common polymorphic variation) have been implicated as a mechanism of raised Hcy level.[1a,3] Therefore, the rapid, sensitive, and selective detections of Cys and Hcy are of great importance for investigating their functions in cells and disease diagnosis.

To date, a number of analytical methods for Cys and Hcy have been developed in conjunction with high-performance liquid chromatography (HPLC),[4] capillary electrophoresis (CE),[4,5] electrochemical,[4,6] UV−vis spectroscopy,[7] Fourier transform infrared (FTIR) spectroscopy,[8] mass spectrometry,[9] and fluorescence spectroscopy.[10] Of these methods, the fluorometric assay method using a specific fluorescence

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probe is considered to be one of most promising methods because of its high sensitivity and selectivity. The well-known cyclization reactions of $\beta$-/$\gamma$-aminoalkylthiols (containing both SH and $NH_2$ groups) with organic aldehydes, which form the thiazolidine and thiazinane derivatives, have been widely used to design fluorescent probes for the detection of total concentration of Cys/Hcy and molecular imaging.[11−13] However, most of the reported Cys/Hcy fluorescence probes were designed by using organic fluorescence dyes. Some characteristics of these probes are undesirable, such as lower photostability, high background fluorescence, long response time, and the UV excitation and emission wavelengths. The small Stokes shift of an organic fluorescence probe is also unfavorable since it can cause self-quenching and measurement errors by excitation and scattered lights.[14] The use of luminescent transition-metal complexes as luminescence probes has recently attracted considerable interest, especially those of Ru(II), Re(I), and Ir(III),[15] and several metal complex-based luminescent probes for the detection of Cys/Hcy have been reported.[11a,15e,16] As luminescent probes, Ru(II) complexes have several very desirable features including the intense visible absorption and emission, large Stokes shift, high photo-, thermal and chemical stabilities, and very low cytotoxicity.[17] Owing to these outstanding photochemical and photophysical properties, a variety of sensitive and selective luminescence probes based on Ru(II) complexes, especially the Ru(II)-bipyridine complex, have been developed for the recognition and detection of anions,[18] metal cations,[19] and molecular oxygen[20] in recent years.

In this work, a unique Ru(II) complex-based luminescent probe, $[Ru(CHO\text{-}bpy)_3](PF_6)_2$ (CHO-bpy: 4-methyl-2,2′-bipyridyl-4′-carboxaldehyde), was designed and synthesized for the selective recognition and sensitive detection of Cys/Hcy. This probe is based on a Ru(II) complex with three aldehyde-substituted bipyridine ligands, since the strong electron-withdrawing aldehyde group in the ligand can effectively quench the metal-to-ligand charge-transfer (MLCT) luminescence of the Ru(II) complex. The new Ru(II) complex is almost non-luminescent, and can specifically react with Cys and Hcy to form highly luminescent thiazolidine and thiazinane derivatives, $[Ru(Cys\text{-}bpy)_3]^{2+}$ and $[Ru(Hcy\text{-}bpy)_3]^{2+}$, accompanied by a large blue-shift of the maximum emission wavelength from 720 to 635 nm, which enables the probe to be used for the sensitive luminescence detection of Cys or Hcy. Furthermore, a strong luminescence response was found upon reaction of $[Ru(CHO\text{-}byp)_3]^{2+}$ with Cys/Hcy only, but not with other amino acids, protein, and DNA, indicating an extremely high specificity for Cys/Hcy. To the best of our knowledge, it is the first Ru(II) complex-based luminescence probe for the selective recognition and detection of Cys/Hcy. Scheme 1 shows the structures of $[Ru(CHO\text{-}bpy)_3]$, $[Ru(Cys\text{-}bpy)_3]^{2+}$, and $[Ru(Hcy\text{-}bpy)_3]^{2+}$, and the luminescence response reaction of $[Ru(CHO\text{-}bpy)_3]$ toward Cys/Hcy.

## Experimental Section

**Materials and Physical Measurements.** 4-Methyl-2,2′-bipyridyl-4′-carboxaldehyde (CHO-bpy) was synthesized by using a literature method.[21] Unless otherwise stated, all chemical materials were purchased from commercial sources and used without further purification.

The NMR spectra were recorded on a Bruker Avance spectrometer (400 MHz). ESI-MS spectra were measured on a HP1100LC/MSD MS spectrometer. Absorption spectra were measured on a Perkin-Elmer Lambda 35 UV−vis spectrometer. Elemental analysis was carried out on a Vario-EL analyzer. Luminescence spectra were measured on a Perkin-Elmer LS 50B luminescence spectrometer with excitation and emission slits of 10 nm.

**Synthesis of $[Ru(CHO\text{-}bpy)_3](PF_6)_2$.** Under an argon atmosphere, 79.3 mg of CHO-bpy (0.4 mmol) in 15 mL of EtOH was added to a solution of $RuCl_3$ (20.7 mg, 0.1 mmol) in 20 mL of 3:1 $EtOH/H_2O$. The reaction mixture was refluxed for 24 h, and then cooled to room temperature. After the solvent was evaporated, the residue was dissolved in a small amount of water, and then the aqueous solution was washed with $CH_2Cl_2$. A saturated

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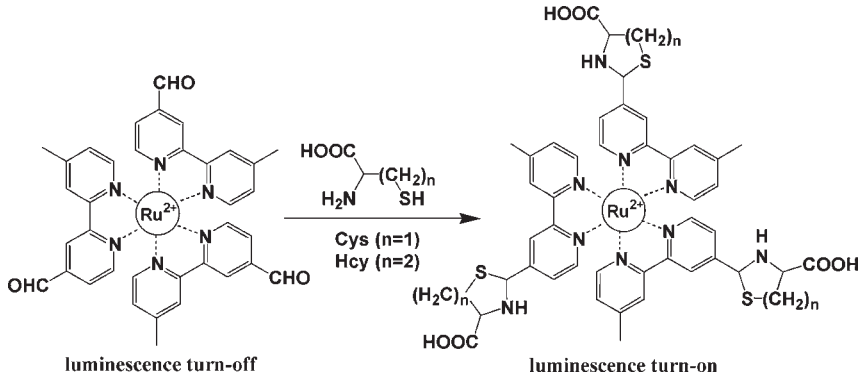
**Scheme 1.** Luminescence Response Reaction of $[Ru(CHO\text{-}bpy)_3]^{2+}$ Towards Cys/Hcy




solution of $NH_4PF_6$ was added to yield red precipitate of the product, which was further purified by neutral aluminum oxide column chromatography with $CH_2Cl_2$/MeOH (10/1, v/v) as eluent. The target Ru(II) complex was isolated as a red solid (62.1 mg, 63% yield). $^1$H NMR (400 MHz, $CD_3CN$): $\delta$ = 10.15 (s, 3H, CHO), 8.84 (s, 3H, H3′), 8.55 (s, 3H, H3), 7.96 (m, 3H, H6′), 7.74 (m, 3H, H6), 7.52 (m, 3H, H3′), 7.30 (m, 3H, H3), 2.57 (s, 9H, $CH_3$). $^{13}$C NMR (100 MHz, $CD_3CN$): $\delta$ = 20.24, 122.20, 125.53, 125.90, 129.05, 142.11, 150.77, 151.33, 153.42, 155.53, 158.58, 190.53. ESI-MS ($m/z$): 348.1 ($[M\text{-}2PF_6]^{2+}$). Elemental analysis calcd for $C_{36}H_{30}F_{12}N_6O_3P_2Ru$ (%): C 44.24, H 3.17, N 8.27; found: C 43.87, H 3.07, N 8.53.

**Confirmation of the Products of $[Ru(CHO\text{-}bpy)_3](PF_6)_2$ Reacted with Cys and Hcy.** To a solution of 98.6 mg of $[Ru(CHO\text{-}bpy)_3](PF_6)_2$ (0.1 mmol) in 10 mL of $CH_3CN$ was added dropwise 5 mL of aqueous solution of Cys or Hcy (3 M) with vigorous stirring. After the solution was stirred at 37 °C for 2 h, the electrospray ionization (ESI) mass spectrum of the solution was measured. $[Ru(Cys\text{-}bpy)_3](PF_6)_2$: $m/z$ (%), 503.4 (100) $[M - 2PF_6]^{2+}$; $[Ru(Hcy\text{-}bpy)_3](PF_6)_2$: $m/z$ (%), 524.7 (100) $[M - 2PF_6]^{2+}$.

**Luminescence Response of $[Ru(CHO\text{-}bpy)_3]^{2+}$ toward Cys/Hcy.** The luminescence response of $[Ru(CHO\text{-}bpy)_3]^{2+}$ toward Cys/Hcy was measured in a DMSO-HEPES buffer (50 mM, pH 7.0, 9:1 v/v) at room temperature. After different concentrations of Cys or Hcy were added to a solution of $[Ru(CHO\text{-}bpy)_3]^{2+}$ (10 $\mu$M), respectively, the solutions were stirred for 15 min, and then subjected to the luminescence measurements.

**Reactions of $[Ru(CHO\text{-}bpy)_3]^{2+}$ with Different Amino Acids, Protein, DNA, and Hydroxy/Mercapto Ethylamines.** All the reactions were carried out in DMSO-HEPES buffer (50 mM, pH 7.0, 9:1 v/v) with the same concentrations of $[Ru(CHO\text{-}bpy)_3]^{2+}$ (10 $\mu$M) and amino acids (1.2 mM), bovine serum albumin (BSA, 1.2 mM), calf thymus DNA (1.2 $\mu$g/mL), ethanol amine (1.2 mM), and cysteamine (1.2 mM) for 15 min at room temperature.

## Results and Discussion

**Design, Synthesis, and Photophysical Properties of the Ru(II) Complex Probe.** Tris(bipyridine)Ru(II) complex is a well-known luminescent Ru(II) complex, and has shown a variety of utilities for the luminescent sensings of anions, metal cations, and molecular oxygen.[18−20] However, the luminescent Ru(II)-bipyridine complexes that can be directly applied to probe the biomolecules have rarely been reported.[22]

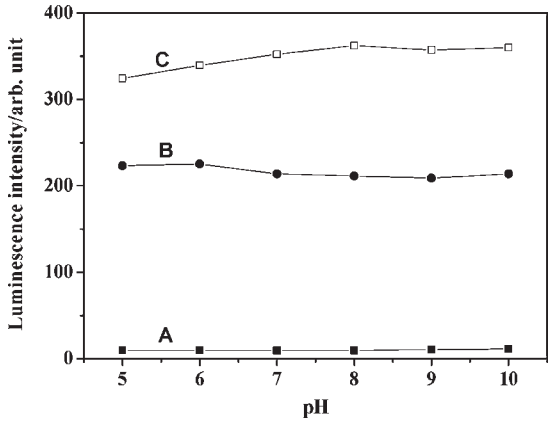
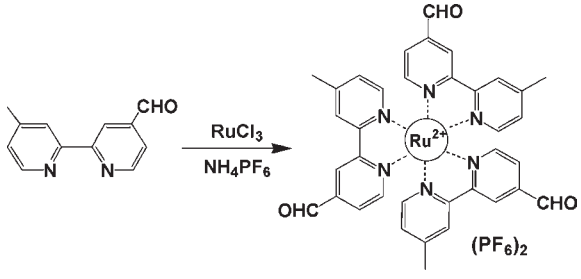



**Figure 1.** Effects of pH on the luminescence intensities of $[Ru(CHO\text{-}bpy)_3]^{2+}$ (10 $\mu$M, A), $[Ru(Cys\text{-}bpy)_3]^{2+}$ (10 $\mu$M, B), and $[Ru(Hcy\text{-}bpy)_3]^{2+}$ (10 $\mu$M, C) in the DMSO-HEPES buffers (50 mM) with different pHs.

**Scheme 2.** Synthesis Procedure of $[Ru(CHO\text{-}bpy)_3](PF_6)_2$




In this work, a Ru(II)-bipyridine complex-based luminescent probe specific for Cys/Hcy was designed by using an aldehyde-substituted bipyridine, CHO-bpy, as the ligand. The aldehyde group in the Ru(II) complex was employed as a specific reactive moiety for trapping Cys/Hcy because it is not only a well-known response scaffold specific for Cys/Hcy but also a strong electron-withdrawing group that can effectively quench the luminescence of the Ru(II) complex.[11a,23] Therefore, the complex itself is almost non-luminescent. After reacting with Cys/Hcy to form the corresponding thiazolidine/thiazinane derivatives, because of the disappearance of electron-withdrawing effect of the aldehyde group, the luminescence of the Ru(II) complex is turned on; thus, the Ru(II) complex becomes highly luminescent (Scheme 1).

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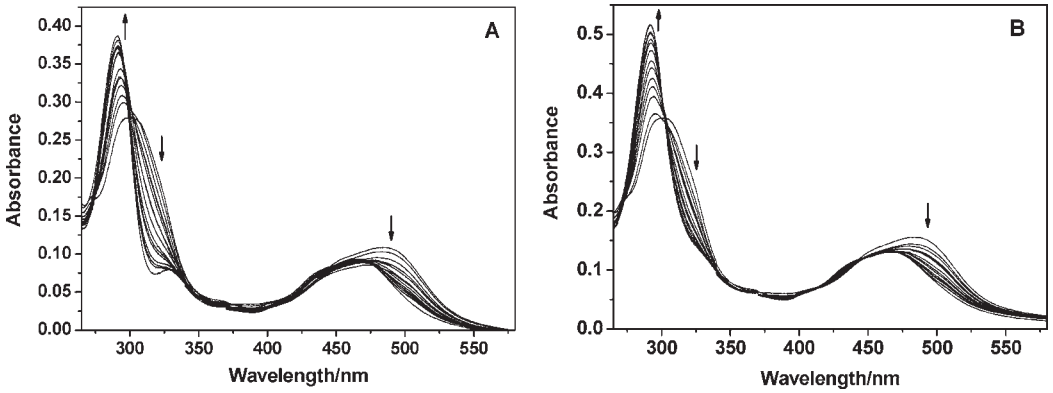


**Figure 2.** UV−vis absorption spectra of $[Ru(CHO\text{-}bpy)_3]^{2+}$ (10 $\mu$M) in the presence of different concentrations of Cys (A) and Hcy (B). The concentrations of Cys and Hcy are 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, and 1200 $\mu$M, respectively.

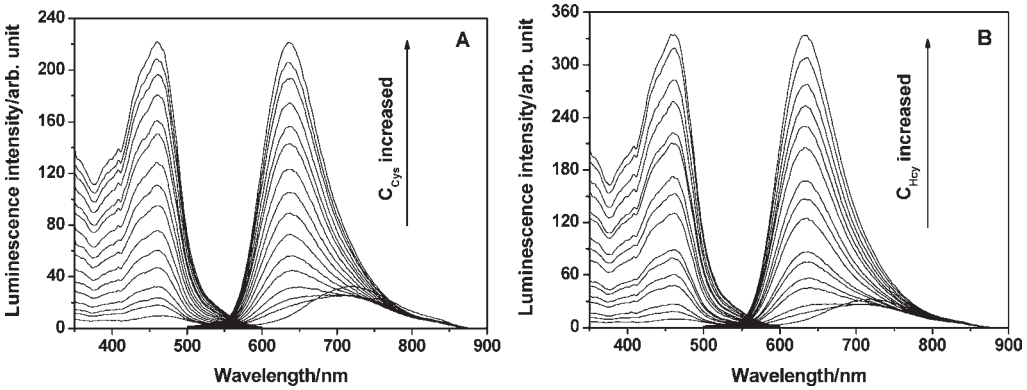


**Figure 3.** Excitation and emission spectra of $[Ru(CHO\text{-}bpy)_3]^{2+}$ (10 $\mu$M) in the presence of different concentrations of Cys (A) and Hcy (B). The concentrations of Cys and Hcy are 50, 100, 150, 200, 250, 300, 400, 500, 600, 750, 900, 1200, 1500, 2100, and 3000 $\mu$M, respectively.

The new complex $[Ru(CHO\text{-}bpy)_3]^{2+}$ was easily synthesized with a one-step reaction by refluxing 4 equiv of CHO-bpy with $RuCl_3$ in $EtOH/H_2O$ (Scheme 2). After the complex was isolated by the addition of $NH_4PF_6$ and further purified by a chromatography method, the pure complex $[Ru(CHO\text{-}bpy)_3](PF_6)_2$ was obtained with a high yield. The composition of the complex was confirmed by the NMR, ESI-MS, and elementary analysis. In addition, the products of $[Ru(CHO\text{-}bpy)_3]^{2+}$ reacted with Cys and Hcy, $[Ru(Cys\text{-}bpy)_3]^{2+}$, and $[Ru(Hcy\text{-}bpy)_3]^{2+}$ were also confirmed by the ESI-MS analysis.

To evaluate the effects of pH on the reaction between $[Ru(CHO\text{-}bpy)_3]^{2+}$ and Cys/Hcy, the luminescence intensities of $[Ru(CHO\text{-}bpy)_3]^{2+}$ before and after the addition of Cys/Hcy were measured at different pHs ranging from 5.0 to 10.0. As shown in Figure 1, the luminescence intensity of $[Ru(CHO\text{-}bpy)_3]^{2+}$ was weak and stable with the pH changes. After the addition of Cys/Hcy, the luminescence intensity was significantly increased at all pHs. The luminescence intensity of $[Ru(Cys\text{-}bpy)_3]^{2+}$ was slightly decreased at pH > 7.0, and that of $[Ru(Hcy\text{-}bpy)_3]^{2+}$ was slightly increased at pH > 7.0. However, the luminescence intensity changes of the three complexes with the change of pH are small. This result indicates that $[Ru(CHO\text{-}bpy)_3]^{2+}$ can work well as a luminescent probe for the detection of Cys/Hcy in weakly acidic, neutral, and weakly basic buffers.

Figure 2 shows the UV−vis absorption spectra of $[Ru(CHO\text{-}bpy)_3]^{2+}$ (10 $\mu$M) in the presence of different concentrations of Cys/Hcy in the DMSO-HEPES buffer. The UV−vis spectrum of $[Ru(CHO\text{-}bpy)_3]^{2+}$ exhibits two strong absorption bands at 300 and 485 nm, respectively. The high-energy absorption at 300 nm is attributed to the spin-allowed ligand localized $\pi-\pi^*$ transition, while the low-energy absorption at 485 nm can be assigned to the absorption of the metal-to-ligand charge transfer (MLCT) transition. In the presence of Cys/Hcy, the two absorption bands of the complex were shifted to 290 and 465 nm, respectively, accompanied by the solution's color change from orange to yellow. This result indicates that the formations of the corresponding thiazolidine and thiazinane derivatives after $[Ru(CHO\text{-}bpy)_3]^{2+}$ is reacted with Cys and Hcy can result in the degradation of the strong electron-withdrawing effects derived from the aldehyde groups of the Ru(II) complex.

Figure 3 shows the excitation and emission spectra of $[Ru(CHO\text{-}bpy)_3]^{2+}$ (10 $\mu$M) in the presence of different concentrations of Cys/Hcy in the DMSO-HEPES buffer. In comparison with the emission spectrum of $[Ru(bpy)_3]^{2+}$ ($\lambda_{max}$ = 612 nm),[24] the emission spectrum of $[Ru(CHO\text{-}bpy)_3]^{2+}$ shows a very weak and remarkably red-shifted MLCT emission band centered at 720 nm because of the strong electron-withdrawing effect of the three aldehyde groups. However, in the presence of Cys/Hcy, the MLCT-based emission band of the Ru(II) complex displays a large blue-shift from 720 to 635 nm, accompanied by the significant increase in the luminescence intensity (Figure 3). This result also demonstrates that the formations of

(24) Juris, A.; Balzani, V. *Coord. Chem. Rev.* **1988**, *84*, 85–277.

thiazolidines in the Ru(II) complex can effectively reduce the electron-withdrawing effect of the ligand, resulting in the remarkable luminescence enhancement and a large blue-shift of the maximum emission wavelength of the complex.[11a,b,d]

**Luminescence Response of $[Ru(CHO\text{-}bpy)_3]^{2+}$ toward Cys/Hcy.** The time course of the luminescence response of $[Ru(CHO\text{-}bpy)_3]^{2+}$ to the addition of Cys/Hcy in the DMSO-HEPES buffer was investigated by monitoring the luminescence intensity change at 635 nm with excitation at 460 nm. As shown in Figure 4, the pure $[Ru(CHO\text{-}bpy)_3]^{2+}$ shows a very weak and stable luminescence intensity. Upon addition of Cys or Hcy, the luminescence intensity is rapidly increased and reaches to the maximum value within a few seconds, and then keeps at a steady level under the continuous excitation. When another Cys or Hcy is added, the luminescence intensity is rapidly increased again and reaches to another maximum value. This result demonstrates that the reaction between $[Ru(CHO\text{-}bpy)_3]^{2+}$ and Cys/Hcy is very fast, and the reaction product is highly stable. The faster reaction of $[Ru(CHO\text{-}bpy)_3]^{2+}$ with Cys/Hcy than other single aldehyde group containing fluorescence probes[10a,11f] can be considered to be attributed to the presence of the three aldehyde groups in the Ru(II) complex. The reaction of the three aldehyde groups in $[Ru(CHO\text{-}bpy)_3]^{2+}$ with Cys/Hcy was further confirmed by the Job's plotting analysis. As shown in Figure 5, the reaction between $[Ru(CHO\text{-}bpy)_3]^{2+}$ and Cys/Hcy shows a 1:3 stoichiometry, which indicates that all of the three aldehyde groups in the Ru(II) complex can react with Cys/Hcy.

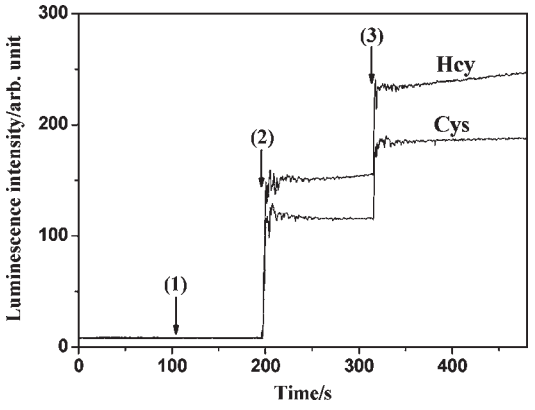


**Figure 4.** Time course of the luminescence response of $[Ru(CHO\text{-}bpy)_3]^{2+}$ (10 $\mu$M) to the addition of Cys/Hcy. The (1) shows the luminescence intensity before the addition of Cys/Hcy, and (2) and (3) show twice the additions of Cys and Hcy (0.5 mM).

To investigate the utility of $[Ru(CHO\text{-}bpy)_3]^{2+}$ as a luminescent probe for the quantitative detection of Cys/Hcy, the luminescence intensities of $[Ru(CHO\text{-}bpy)_3]^{2+}$ (10 $\mu$M) in the presence of different concentrations of Cys/Hcy in the DMSO-HEPES buffer were measured at room temperature. As shown in Figure 6A, with the increase of the Cys concentration, the luminescence intensity of the solution at 635 nm is gradually increased. The dose-dependent luminescence enhancement shows a good linearity that can be expressed as $I = 0.659C_{Cys} + 9.68$ ($r = 0.998$) in the Cys concentration range of 15 to 180 $\mu$M. The detection limit for Cys, calculated as the concentration corresponding to three standard deviations of the background signal, is 1.41 $\mu$M. Similarly, the dose-dependent luminescence enhancement of $[Ru(CHO\text{-}bpy)_3]^{2+}$ in the presence of different concentrations of Hcy also shows a good linearity that can be expressed as $I = 0.878C_{Hcy} + 9.22$ ($r = 0.999$) with a detection limit of 1.19 $\mu$M (Figure 6B). The above results demonstrate that $[Ru(CHO\text{-}bpy)_3]^{2+}$ can be used as a luminescent probe for the highly sensitive detections of Cys and Hcy, respectively.

**Reactions of $[Ru(CHO\text{-}bpy)_3]^{2+}$ with Different Amino Acids, Protein, DNA, and Hydroxy/Mercapto Ethylamines.** To evaluate the recognition specificity of $[Ru(CHO\text{-}bpy)_3]^{2+}$ toward Cys/Hcy, the reactions of $[Ru(CHO\text{-}bpy)_3]^{2+}$ (10 $\mu$M) with various amino acids, protein, DNA, ethanol amine ($NH_2$-EtOH), and cysteamine ($NH_2$-EtSH) in the DMSO-HEPES buffer were examined at room temperature. As shown in Figure 7, $[Ru(CHO\text{-}bpy)_3]^{2+}$ did not show remarkable luminescent responses upon the additions of diverse amino acids, protein, DNA, and amino alcohol including glycine (Gly), leucine (Leu), aspartic acid (Asp), proline (Pro), arginine (Arg), lysine (Lys), tyrosine (Tyr), valine (Val), glutathione (GSH), threonine (Thr), serine (Ser), alanine (Ala), histidine (His), methinonine (Met), tryptophan (Trp), BSA, calf thymus DNA, and $NH_2$-EtOH, whereas the luminescence intensity of $[Ru(CHO\text{-}bpy)_3]^{2+}$ was significantly enhanced upon the addition of $\beta$-/$\gamma$-aminoalkylthiols, Cys, Hcy, and $NH_2$-EtSH. The bottom picture of Figure 7 shows the luminescence color

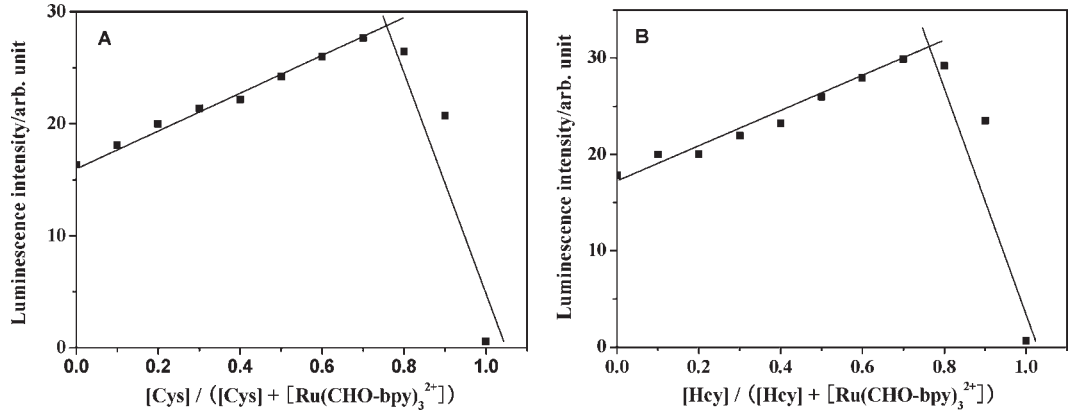


**Figure 5.** Job's plots of the reactions between $[Ru(CHO\text{-}bpy)_3]^{2+}$ and Cys (A)/Hcy (B) in the DMSO-HEPES buffer (50 mM). The total concentrations of $[Ru(CHO\text{-}bpy)_3]^{2+}$ and Cys/Hcy were kept at 100 $\mu$M.

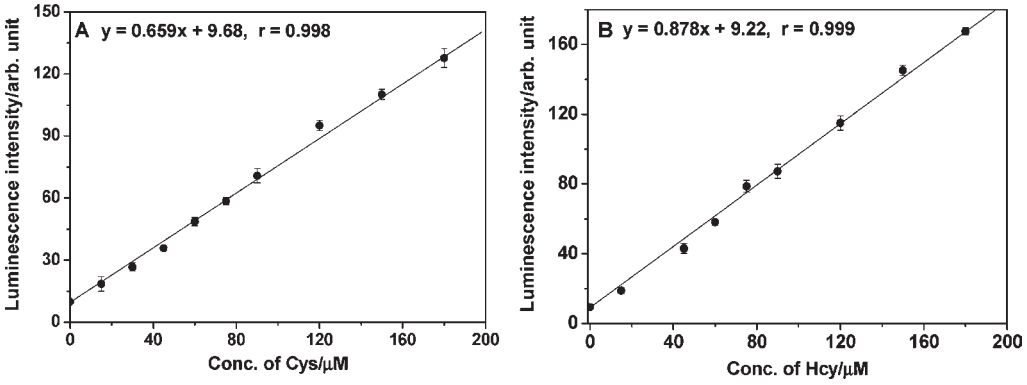


**Figure 6.** Calibration curves for the luminescence detections of Cys (A) and Hcy (B) using $[Ru(CHO\text{-}bpy)_3]^{2+}$ (10 $\mu$M) as a probe.

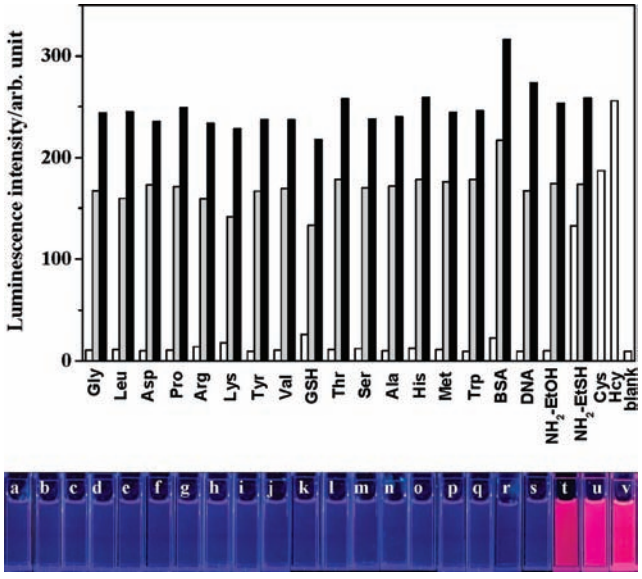


**Figure 7.** Luminescence intensities (635 nm) of the products of $[Ru(CHO\text{-}bpy)_3]^{2+}$ (10 $\mu$M) reacted with different amino acids (1.2 mM), BSA (1.2 mM), DNA (1.2 $\mu$g/mL), ethanol amine (1.2 mM), and cysteamine (1.2 mM) (white bars). The gray and black bars show the luminescence intensities (635 nm) of the products of $[Ru(CHO\text{-}bpy)_3]^{2+}$ reacted with different amino acids, BSA, DNA, ethanol amine, and cysteamine in the presence of Cys (1.2 mM) and Hcy (1.2 mM), respectively. The bottom picture is the luminescence color photographs of the products of $[Ru(CHO\text{-}bpy)_3]^{2+}$ reacted with different molecules (a, blank; b, Gly; c, Leu; d, Asp; e, Pro; f, Arg; g, Lys; h, Tyr; i, Val; j, GSH; k, Thr; l, Ser; m, Ala; n, His; o, Met; p, Trp; q, BSA; r, calf thymus DNA; s, ethanol amine; t, cysteamine; u, Cys; v, Hcy) under a 365 nm UV lamp.

photographs of $[Ru(CHO\text{-}bpy)_3]^{2+}$ (10 $\mu$M) in the presence of different amino acids (1.2 mM), BSA (1.2 mM), DNA (1.2 $\mu$g/mL), and $NH_2$-EtOH/$NH_2$-EtSH (1.2 mM) under a 365 nm UV lamp, which indicates that the enhanced luminescence of $[Ru(CHO\text{-}bpy)_3]^{2+}$ in the presence of three $\beta$-/$\gamma$-aminoalkylthiols can be clearly perceived even by the naked eyes. In addition, to evaluate the utility of $[Ru(CHO\text{-}bpy)_3]^{2+}$ as a luminescent probe for the detection of Cys/Hcy in complicated biological samples, a $[Ru(CHO\text{-}bpy)_3]^{2+}$ (10 $\mu$M) solution was also treated with Cys and Hcy (1.2 mM) in the presence of different amino acids (1.2 mM), BSA (1.2 mM), calf thymus DNA (1.2 $\mu$g/mL), and $NH_2$-EtOH/$NH_2$-EtSH (1.2 mM), respectively. As shown in Figure 7, all of the chosen amino acids, DNA, and hydroxy/mercapto ethylamines have virtually no significant influence on the luminescence detection of Cys and Hcy (in the presence of Cys/Hcy, the reaction of $[Ru(CHO\text{-}bpy)_3]^{2+}$ with $NH_2$-EtSH seems to be inhibited). It was found that, in the presence of BSA, the luminescence intensities of the products of $[Ru(CHO\text{-}bpy)_3]^{2+}$ reacted with Cys and Hcy were 16% and 23% increased, respectively. This phenomenon might be caused by the interaction (e.g., the hydrophobic interaction) between the Ru(II) complex and BSA molecules. However, the effect of protein can be rectified by using the protein-containing buffer as the solvent for the standard solution preparation.

## Conclusion

In this work, a novel Ru(II) complex-based luminescence probe for the specific recognition and detection of Cys and Hcy has been successfully developed by introducing three aldehyde groups into a tris(bipyridine)Ru(II) complex. The new probe can specifically and rapidly react with Cys/Hcy to form the corresponding thiazolidine and thiazinane derivatives, resulting in the remarkable enhancement of the MLCT emission and a large blue-shift of the maximum emission wavelength. Compared to the previously reported fluorescence probes for the detection of Cys/Hcy, the new Ru(II) complex-based luminescence probe has several distinct advantages including short response time, visible-light excitation and emission wavelengths with a larger Stokes shift, a remarkable change of the maximum emission wavelength, and high selectivity and sensitivity. The successful development of this probe provides a new strategy for the rational design of Ru(II) complex-based luminescence probes for various biological molecules, which could be expected to extend the applications of luminescent Ru(II) complexes in biological detections.

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