Supplementary Information for

A Simple and Efficient Fluorescent Sensor for Histidine

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I. General data

ESI-MS and HRMS spectral data were recorded on a Finnigan LCQ^{DECA} and a Bruker Daltonics Bio TOF mass spectrometer, respectively. Fluorescence emission spectra were obtained using FluoroMax-4 Spectrofluorophotometer (HORIBA Jobin Yvon) at 298 K. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. All the solvents were dried according to the standard methods prior to use. All of the solvents were either HPLC or spectroscopic grade in the optical spectroscopic studies.

II. Experimental procedures and characterizations

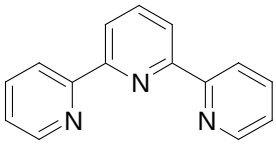
Preparation and Characterization of the Tpy-Cu^{2+} complex.

A mixture of Tpy (233 mg, 1.0 mmol) and $CuCl_2{\cdot}2H_2O$ (205 mg, 1.2 mmol) was stirred in H_2O (30 mL) at room temperature for 30 min until the solution became clear. Then the solution was concentrated to about 5 mL and which produced green precipitate. The green solid was collected by filtration and washed with CH_3OH (5 mL). After dried under vacuum, $TpyCuCl_2$ was obtained. The HR-MS (ES+) spectrum of $TpyCuCl_2$ is included.

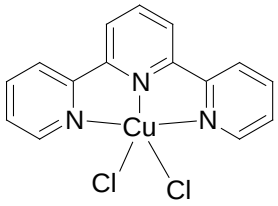
CV Measurement

Electrochemical measurements were performed with a CHI 660C instruments. All the measurements were carried out in a one-compartment cell under a nitrogen atmosphere at 25 ^{o}C equipped with Pt working electrode, a platinum counter electrode, and a $Hg(l)/Hg_2Cl_2(s)$ reference electrode. The supported electrolyte was a 0.1 M NaCl solution. The host solution was mixed with different equivalent of amino acids or imidazole at room temperature in a volumetric flask and diluted to the desired concentration. The resulting solutions were allowed to stand at room temperature for 2 h before measurement. The electrodes were soaked in 2N HNO_3 for 15 min and then washed with distilled water before each measurement.

III. Supplementary Fluorescence Spectra

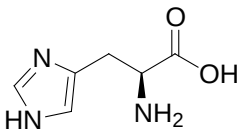


Tpy

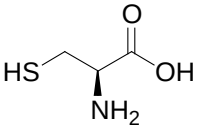


Tpy-Cu

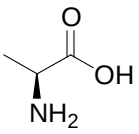
Amino acids and other species:



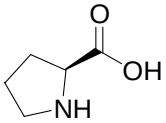
Histidine



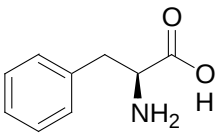
Cysteine



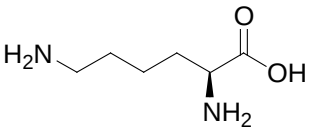
Alanine



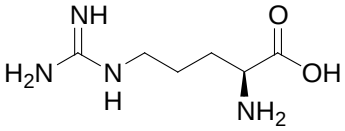
Proline



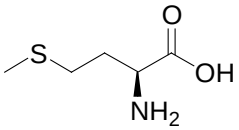
Phenylalanine



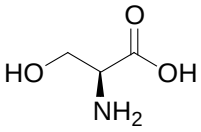
Lysine



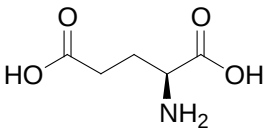
Arginine



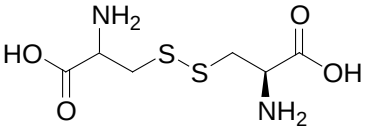
Methionine



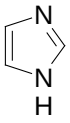
Serine



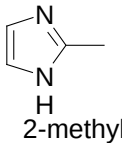
Glu



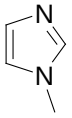
Cystine



1*H*-imidazole



2-methyl-1*H*-imidazole



1-methyl-1*H*-imidazole

Figure S1. Fluorescence spectra of Tpy and the isolated $TpyCuCl_2$ (2.0×10^{-5} M in 25 mM hepes buffer solution, pH = 7.35) (λ_{exc}=298nm, slits: 5 nm/5 nm).

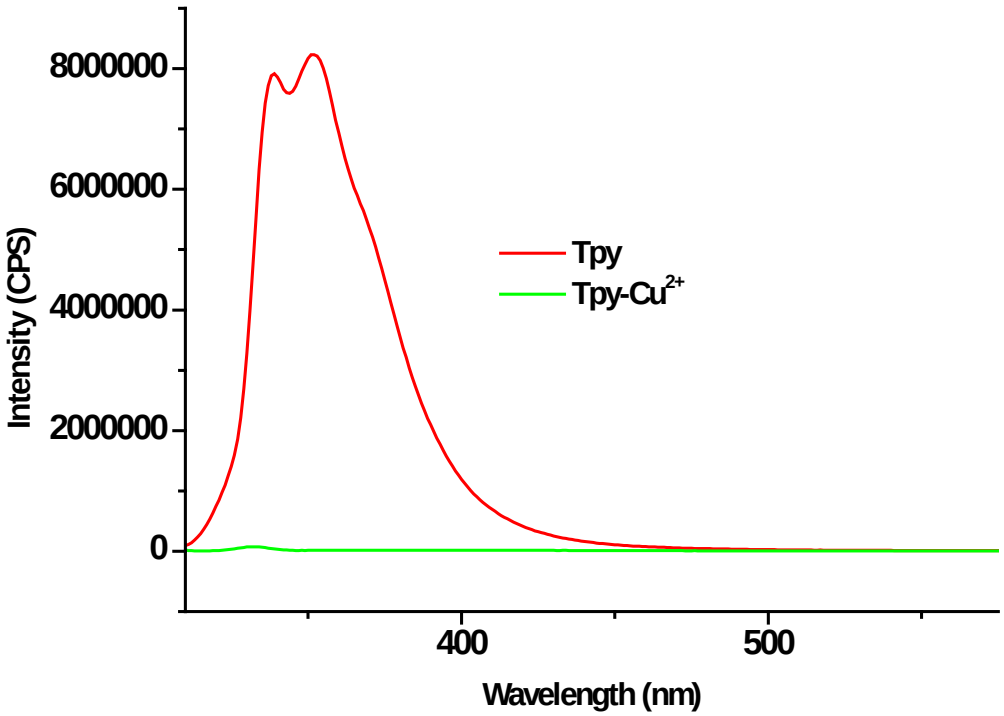


Figure S2. Fluorescence responses of $TpyCu^{2+}$ (2.0×10^{-5} M in 25 mM hepes buffer solution, pH = 7.35) toward L-cysteine (0, 0.5. 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.5, 12.0, 12.5 and 13.0 equiv) (λ_{exc} = 298 nm, slits: 5 nm/5 nm).

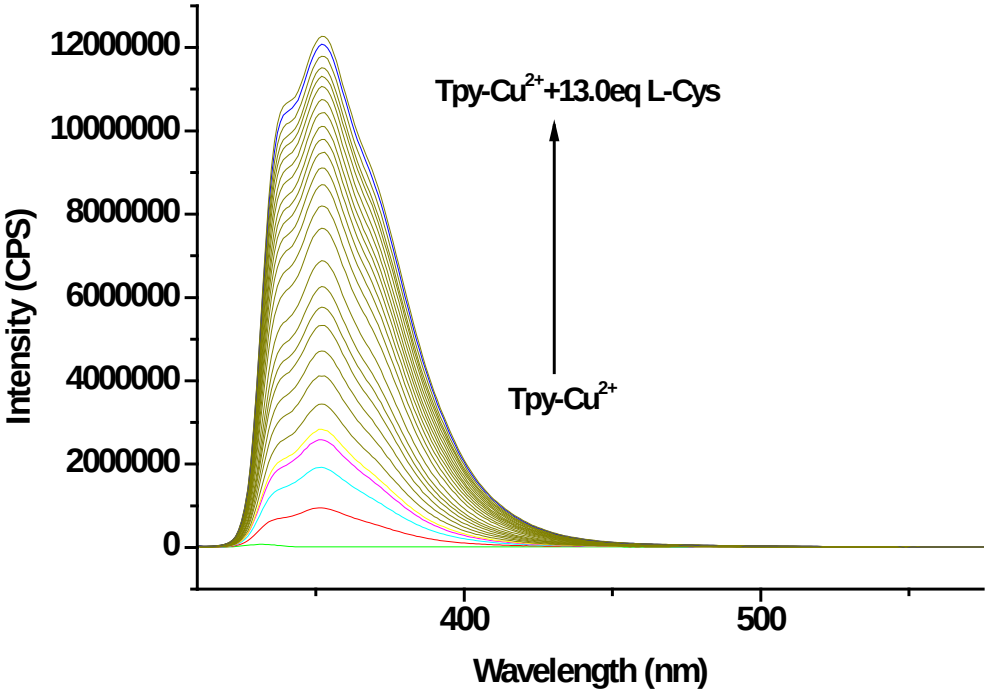


Figure S3. Fluorescence responses of $TpyCu^{2+}$ (2.0×10^{-5} M in 25 mM hepes buffer solution, pH = 7.35) toward L-cystine (0, 0.5. 1.0, 2.0, 3.0, 4.0 and 5.0 equiv) (λ_{exc} = 298 nm, slits: 5 nm/5 nm).

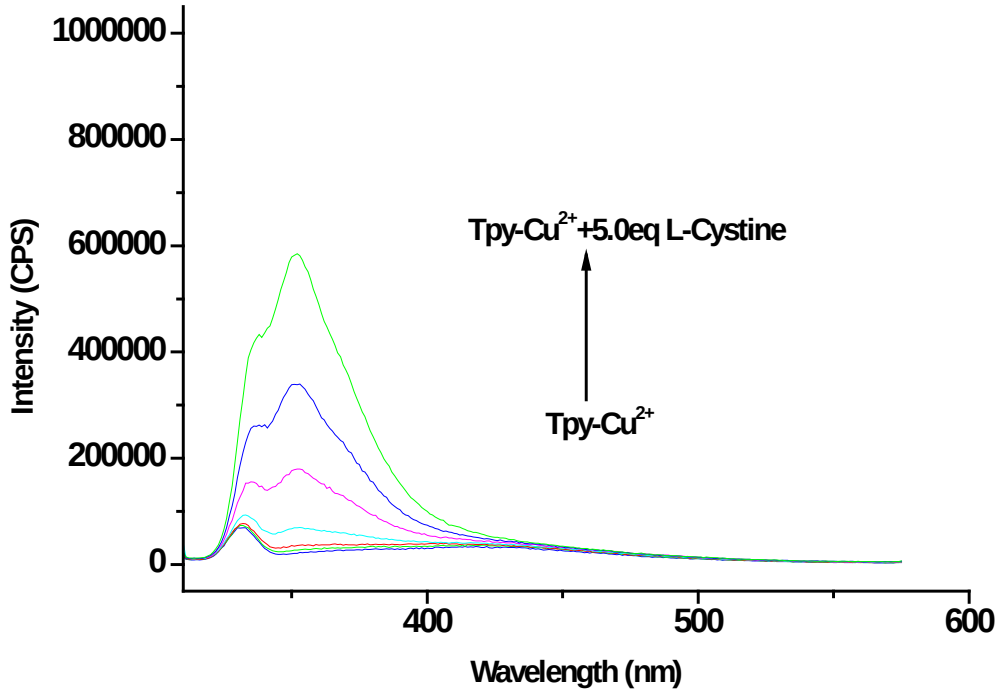
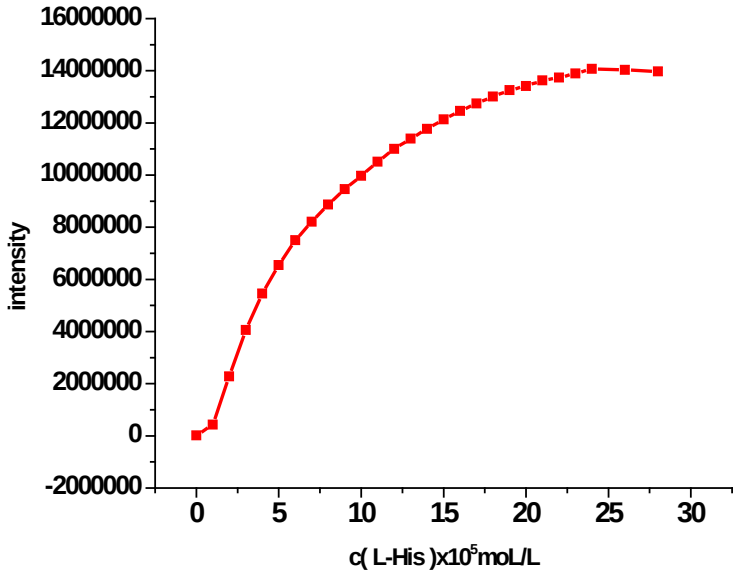
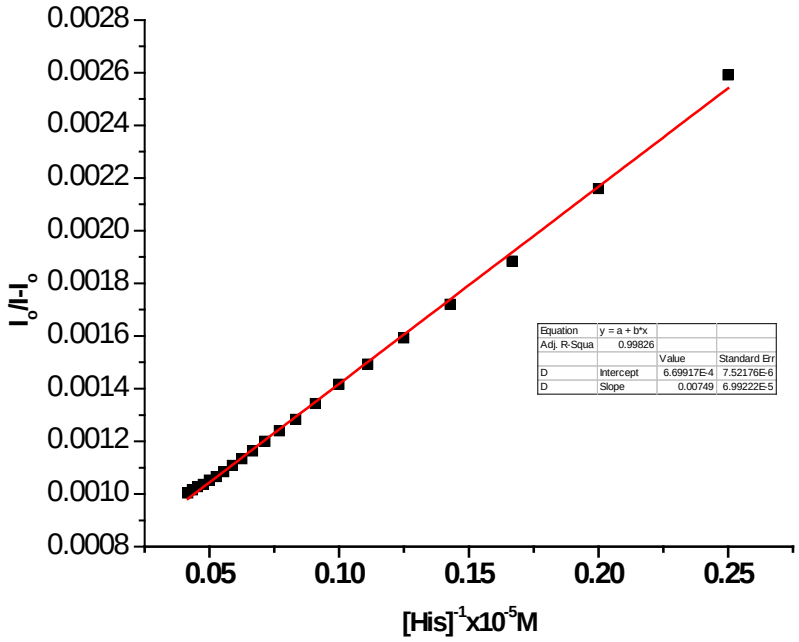


Figure S4. Fluorescence responses of $TpyCu^{2+}$ (2.0×10^{-5} M in 25 mM hepes buffer solution, pH = 7.35) toward L-histidine at λ = 352 nm (λ_{exc} = 298 nm, slits: 5 nm/5 nm).



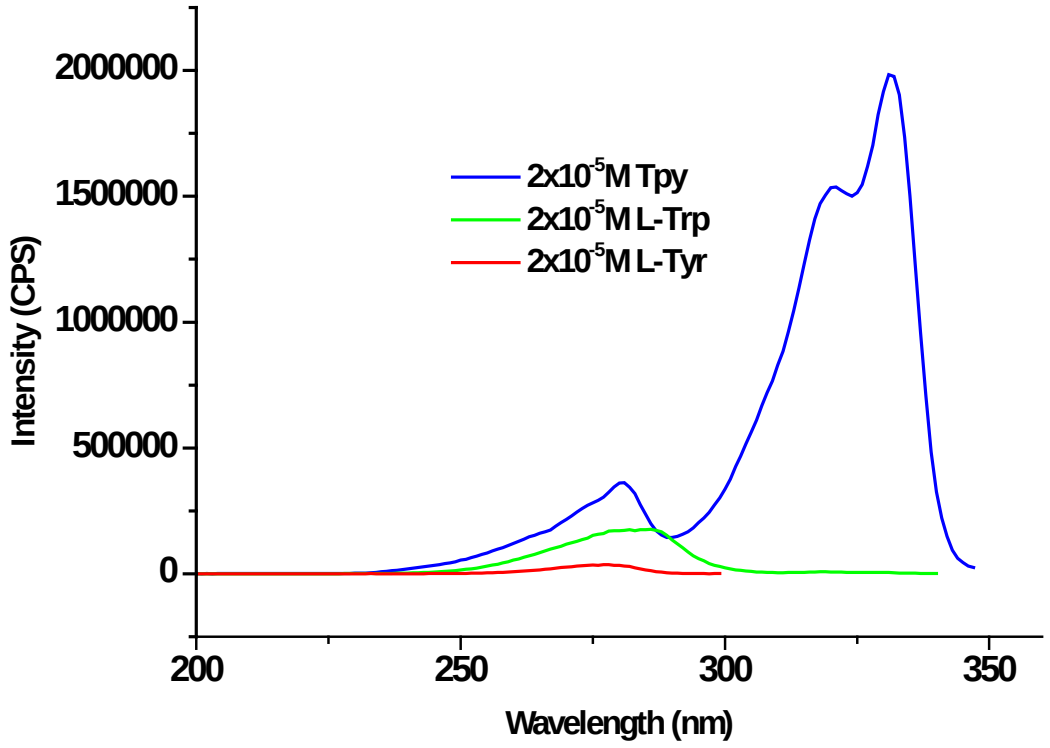
We calculated the binding constant of $TpyCu^{2+}$ with L-histidine by using the linear Benesi-Hilderand expression:

$$\frac{I_0}{I - I_0} = \frac{b}{a - b}\left\{\frac{1}{K[M]} + 1\right\}$$



1/K = 0.00749 x 10^{-5}/6.66917x10^{-4}
The binding constant of $TpyCu^{2+}$ with histidine is 8.944 x 10^3

Figure S5. Fluorescence excitation spectra of Tpy, L-Trp, L-Tyr at the same concentration (25 mM HEPES, pH 7.35).



	Maximum emission wavelength	The wavelength range that can be used for excitation	Maximum excitation wavelength
Tpy	352nm	239nm-345nm	331nm
L-Trp	357nm	240nm-311.4nm	278nm
L-Tyr	304nm	250nm-293nm	286nm

Figure S6. Fluorescence response of Tpy-$CuCl_2$ (2.0×10^{-5}M in 25mM hepes buffer solution, pH=7.35) toward L-His, L-Trp and L-Tyr (λ_{exc}= 320 nm, slits: 5nm/5nm)

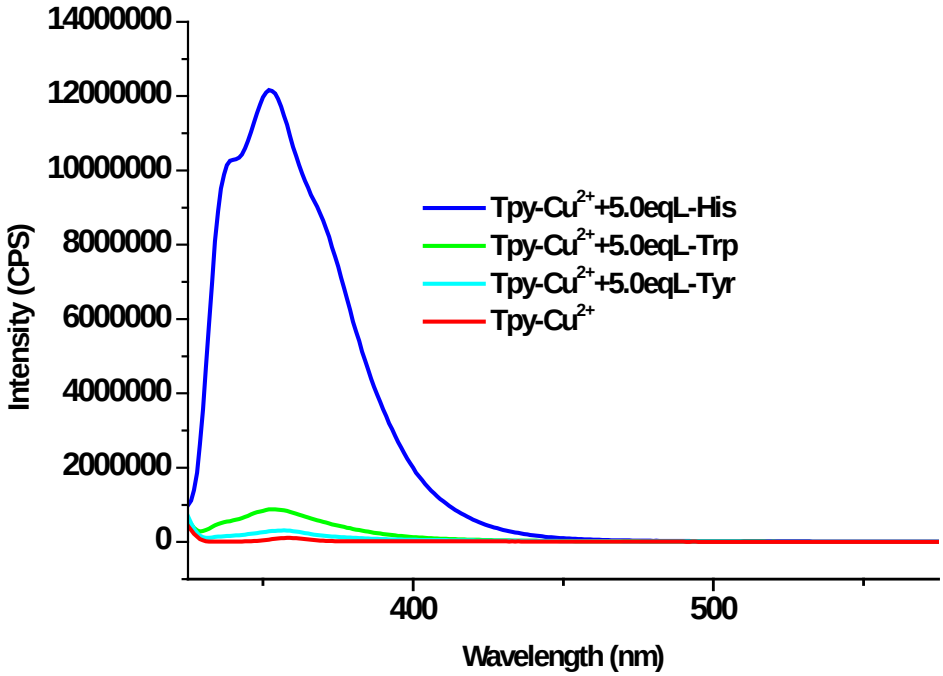


Figure S7. Fluorescence reponse of Tpy-Cu (2.0×10^{-5}M in 25 mM hepes buffer solution, pH=7.35) towards L-His, L-Trp and L-Tyr at 352.4 nm (1.0×10^{-4} M in H_2O) (λ_{exc}= 320 nm, slits: 5nm/5nm)

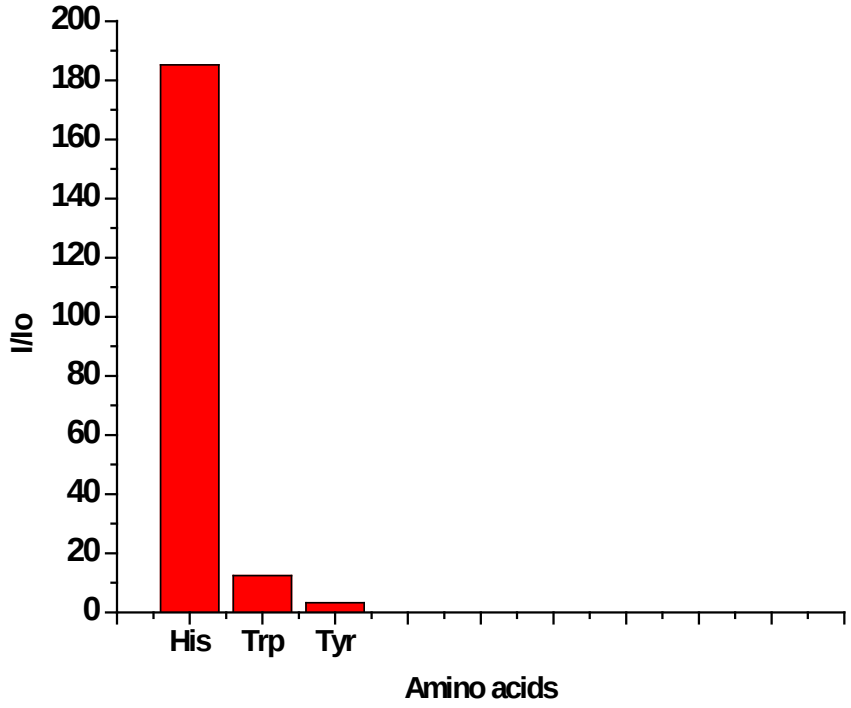


Figure S8. Fluorescence Responses of Tpy-$CuCl_2$ (2.0×10^{-5} M in hepes buffer solution, pH =7.35) toward L-Histidine (0, 0.5. 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.5, 12.0 ando 13.0 equiv) (λ_{exc}=320nm, slits: 5nm/5nm)

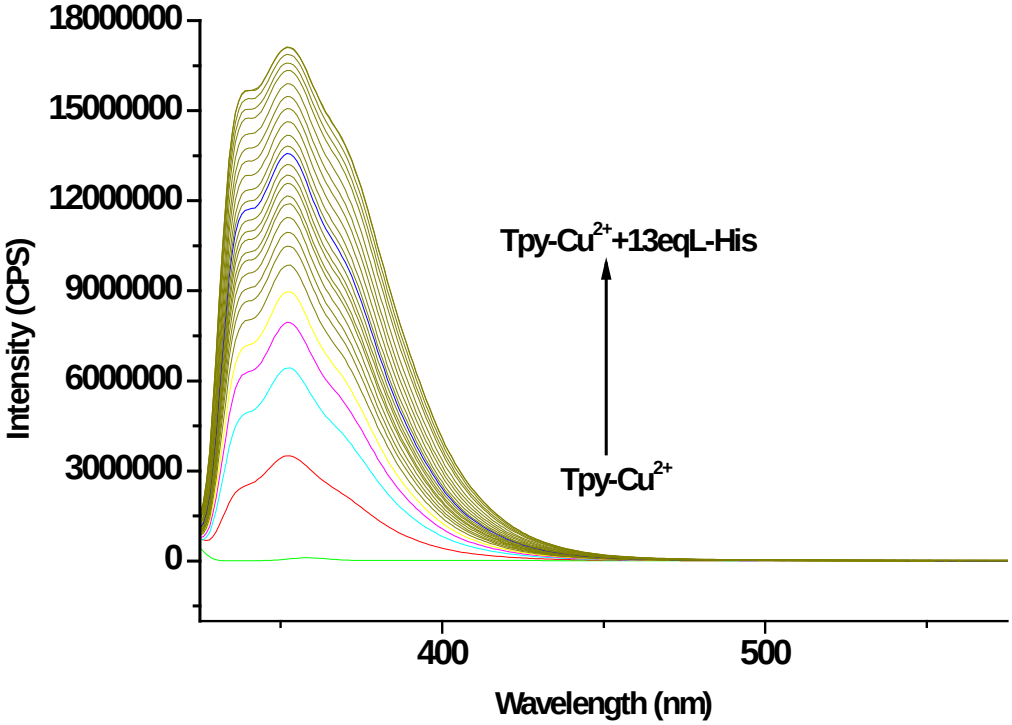


Figure S9. Fluorescence Responses of Tpy-$CuCl_2$ (2.0×10^{-5} M in hepes buffer solution, pH=7.35) toward L-Trp (0, 1.0, 2.0, 3.0, 4.0 and 5.0 equiv) (λ_{exc}=320nm, slits: 5nm/5nm).

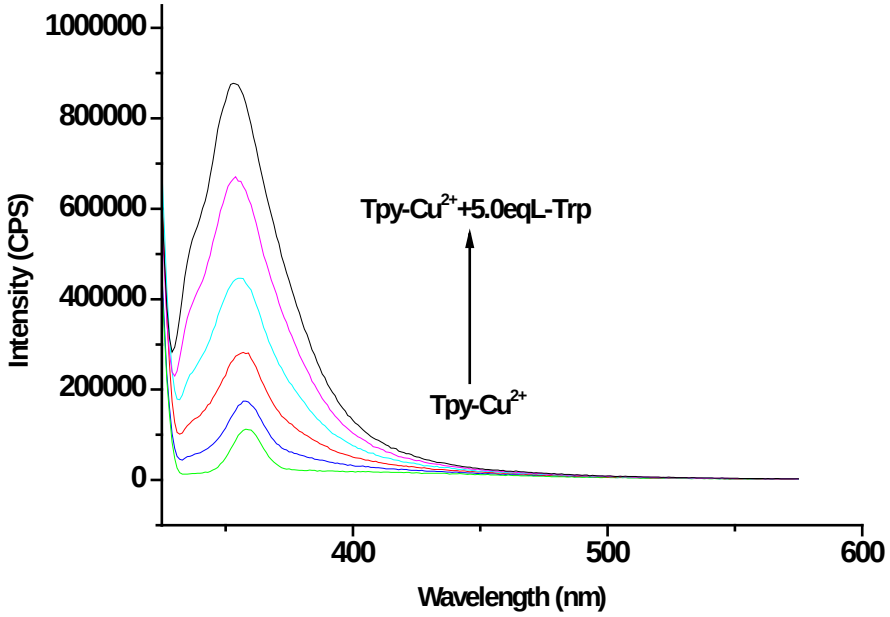


Figure S10. Fluorescence Responses of Tpy-$CuCl_2$ (2.0×10^{-5} M in hepes buffer solution, pH=7.35) toward L-Tyr (0, 1.0, 2.0, 3.0, 4.0 and 5.0 equiv) (λ_{exc}=320nm, slits: 5nm/5nm)

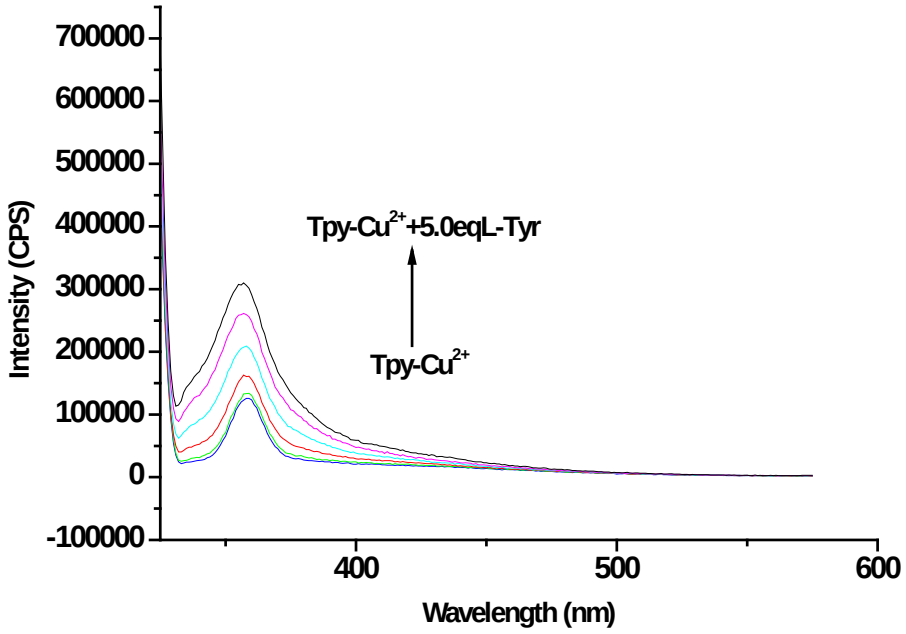


Figure S11. Fluorescence of L-Trp in hepes buffer solution (25 mM, pH=7.35) at 0, C ($2.0x10^{-5}$ M), 2C, 3C, 4C and 5C (λ_{exc}=320nm, slits: 5nm/5nm)

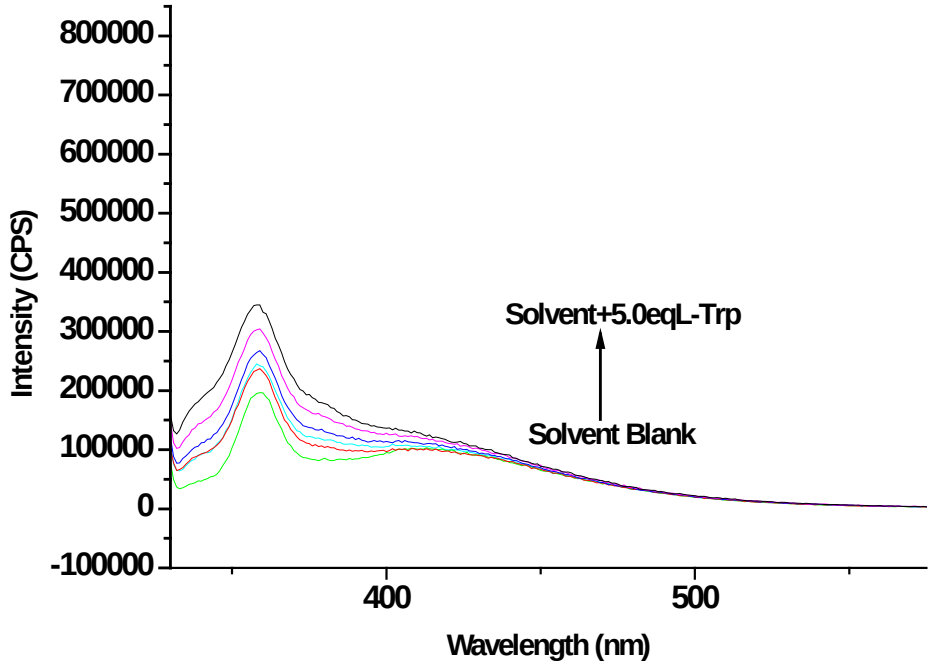


Table S1. Fluorescence quantum yields of Tpy ($5x10^{-6}$M) and Tpy-Cu^{2+}+12eqL-His ($5x10^{-6}$M) [Fluorescent reference material: quinine bisulfate ($5x10^{-6}$M)]

$$Y_u = Y_s \cdot \frac{F_u}{F_s} \cdot \frac{A_s}{A_u}$$

	λexc (slits: 5/5 nm)	**F** (Integral fluorescence intensity)	**A** (Absorbance at 298nm)	Integration range	**Y (Fluorescence quantum yield)**
quinine bisulfate	**298 nm**	637673285	0.015	370-580nm	0.55
Tpy	**298 nm**	144062710	0.045	325-500nm	**0.041**
Tpy-Cu^{2+}+12eqHis	**298 nm**	68793865	0.033	325-500nm	**0.027**

IV. Distinguish Histidine from Cysteine by Using the Fluorescence Response of $TpyCuCl_2$

Oxidation of Cysteine to Cystine: To a solution of L-Cysteine (7.3 mg,0.06 mmol) in 10 mL H_2O was added NaI (0.09 mg, 1 mol %) and 50% H_2O_2(2.91 μL,0.06 mmol). Stirring the mixture at room temperature for 0.5 h gave L-cystine precipitate.

Figure S12. Fluorescence responses of $TpyCuCl_2$ (2.0×10^{-5} M) at λ_{emi} = 352 nm to L-Cysteine upon treatment with H_2O_2 and NaI in aqueous buffered solution (25 mM HEPES, p =7.35) (λexc = 298 nm, slits: 5 nm/5 nm).

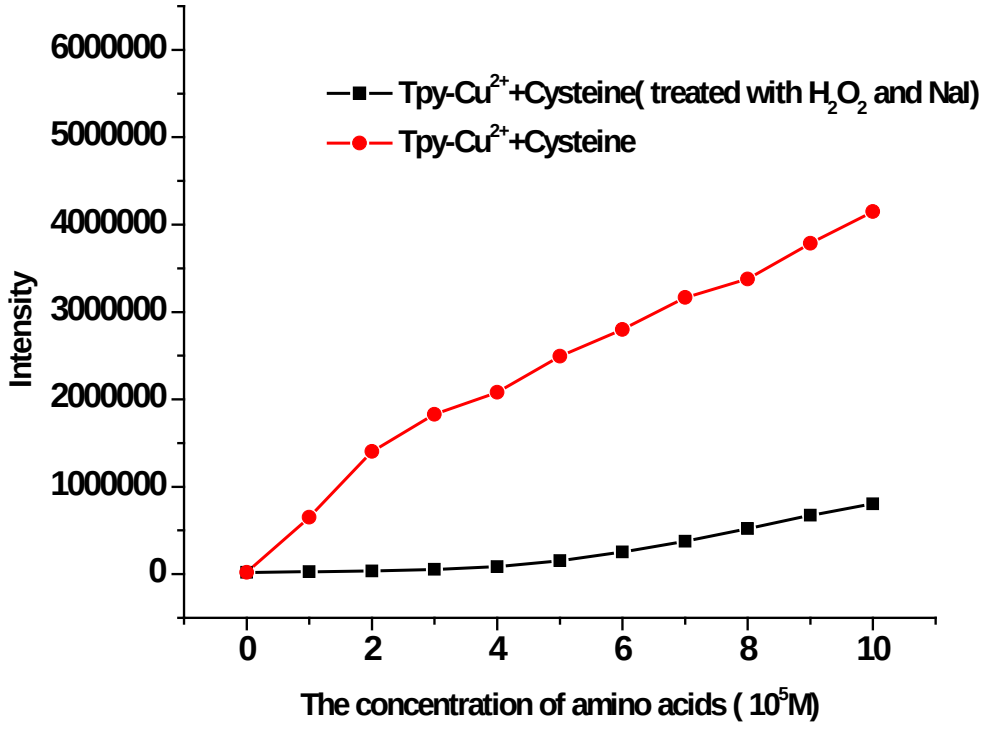


Figure S13. Fluorescence response of $TpyCuCl_2$ (2.0×10^{-5} M) at λ_{emi} = 352 nm to L-Cysteine and L-Histidine upon treatment with H_2O_2 and NaI in aqueous buffered solution (25 mM HEPES, pH 7.35) (λexc = 298 nm, slits: 5 nm/5 nm).

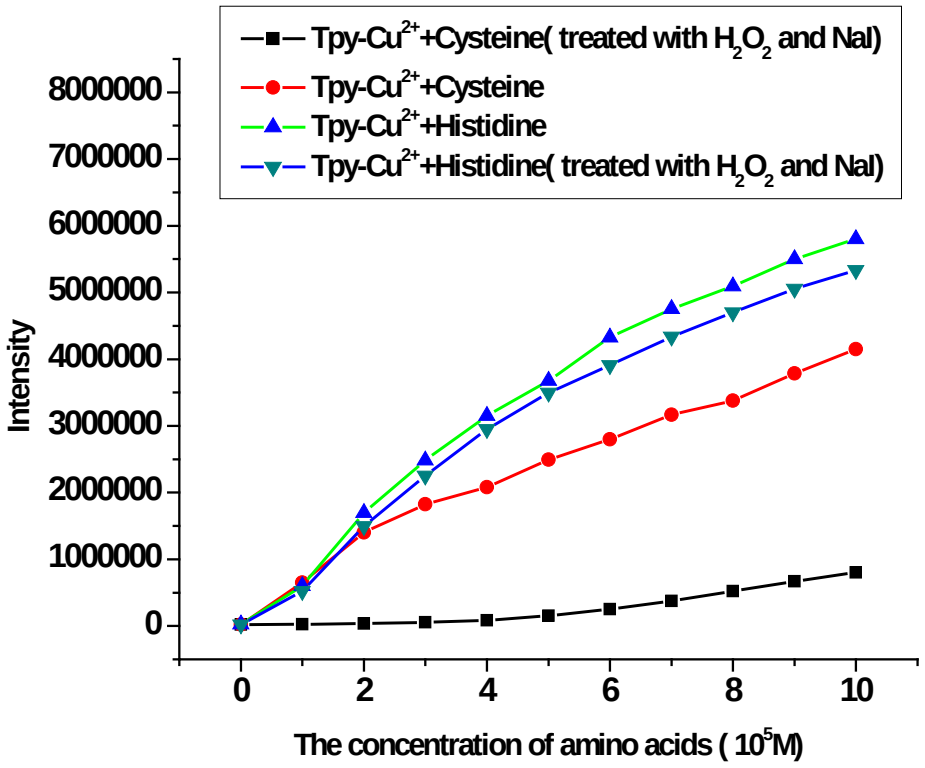
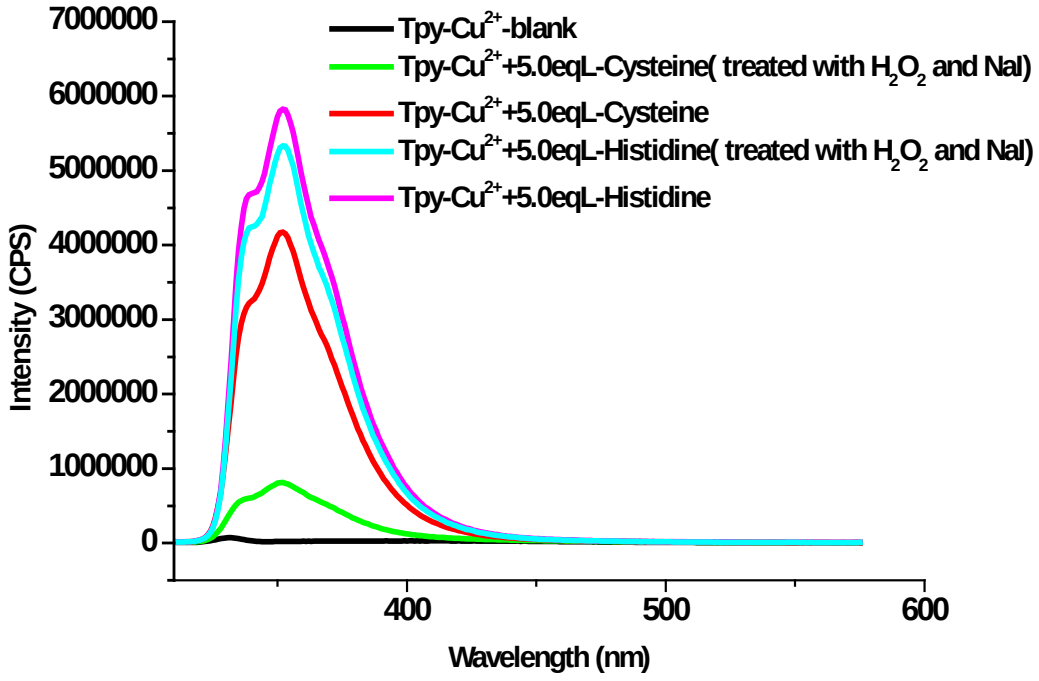


Figure S14. Fluorescence spectra of $TpyCuCl_2$ (2.0×10^{-5} M) to L-Cysteine and L-Histidine upon treatment with H_2O_2 and NaI in aqueous solution (25 mM HEPES buffered, pH 7.35) (λexc = 298 nm, slits: 5 nm/5 nm).



V. Supplementary CV Plots

Figure S15. CV titration profile of $TpyCu^{2+}$ (1 mM) with various amount of imidazole. Reference electrode: saturated $Hg(l)/Hg_2Cl_2(s)$; supporting electrolyte : 0.1 M NaCl solution; scan rate = 50 mV S^{-1}.

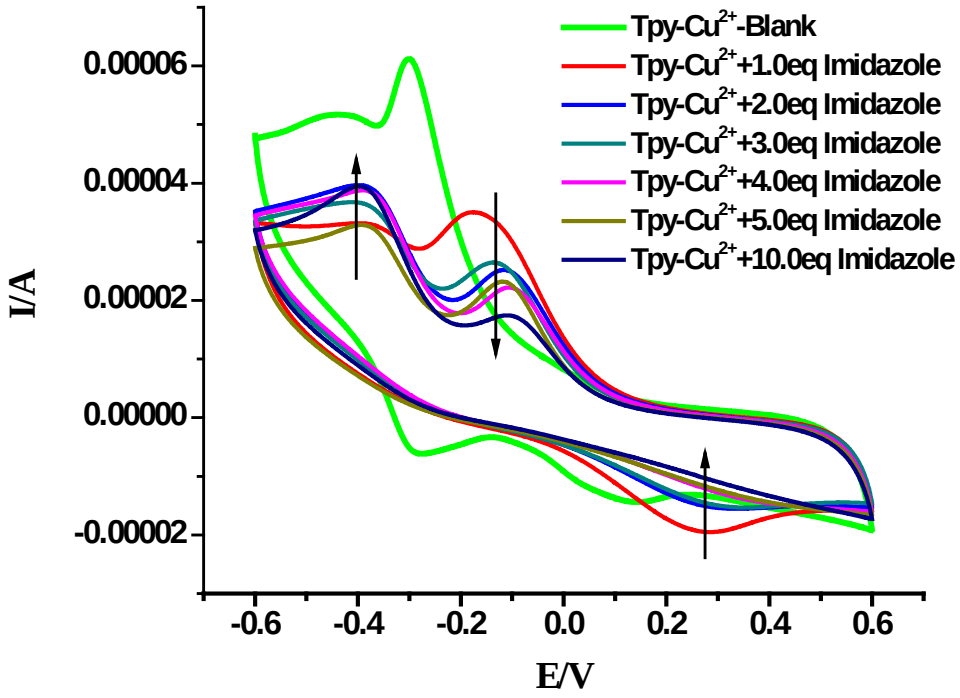


Figure S16. Comparison of the CV titration profile of $TpyCu^{2+}$ (1 mM) with 1 eq L-histidine, 1 eq L-alanine and 1 eq imidazole. Reference electrode: saturated $Hg(l)/Hg_2Cl_2(s)$; supporting electrolyte: 0.1M NaCl solution; scan rate = 50 mV S^{-1}.

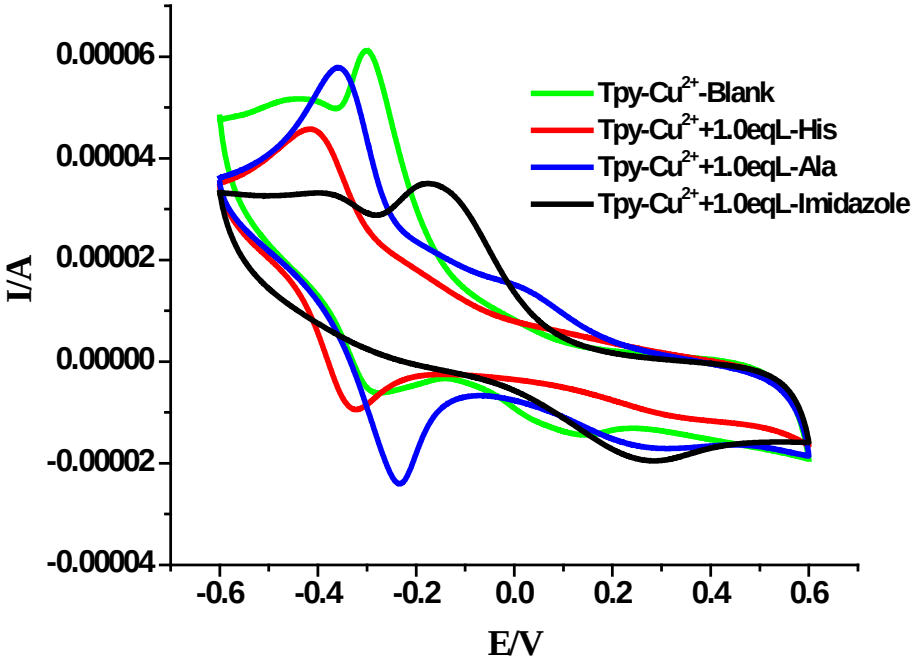


Figure S17. The CV titration profile of Cu^{2+} (1 mM) with 0.5, 1.0, 2.0 and 3.0 equiv L-histidine. Reference electrode = saturated $Hg(l)/Hg_2Cl_2(s)$; supporting electrolyte: 0.1M NaCl solution; scan rate = 50 mV S^{-1}.

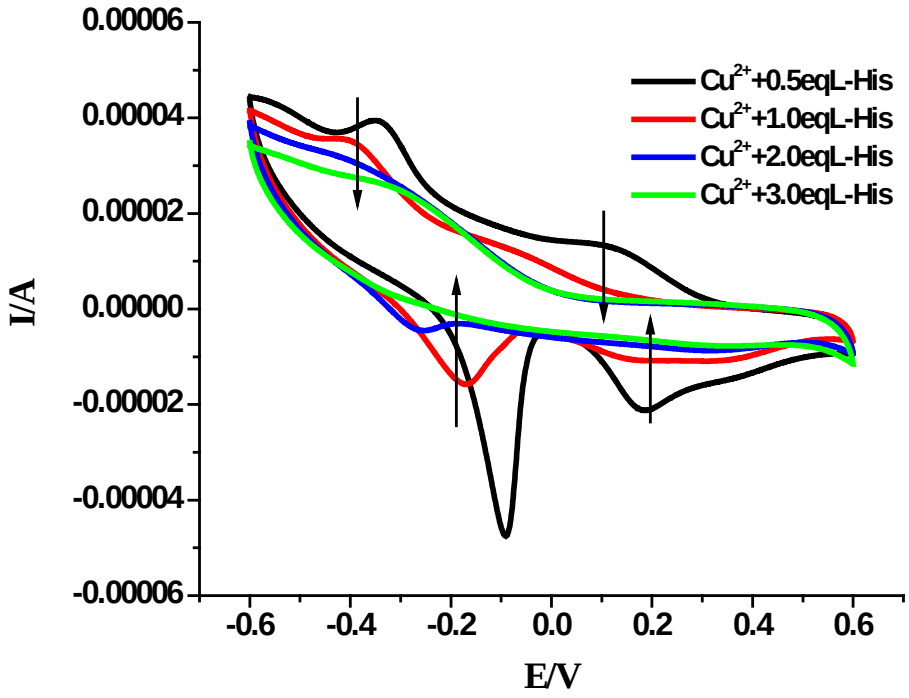
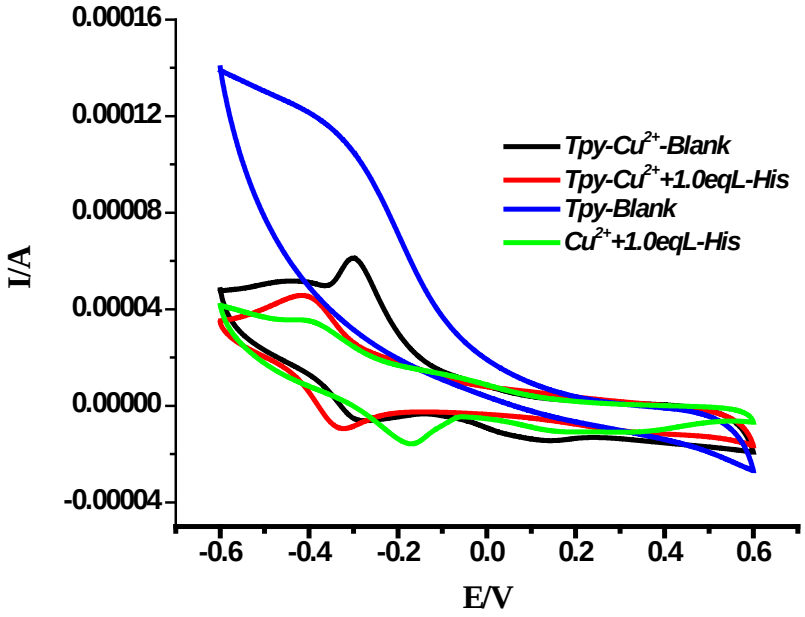


Figure S18. Comparison of the CV titration profile of $TpyCu^{2+}$ (1 mM)、$TpyCu^{2+}$-1.0 eq L-histidine (1 mM)、Tpy (1 mM with 5%THF to increase its solubility)、Cu^{2+}-1.0 eq L-histidine (1 mM). Reference electrode = saturated $Hg(l)/Hg_2Cl_2(s)$; supporting electrolyte: 0.1 M NaCl solution; scan rate = 50 mV S^{-1}



VI. Supplementary UV Spectra

Figure S19. UV Titration of Tpy (2.0×10^{-5}M in 25 mM hepes buffer solution, pH = 7.35) with $CuCl_2.2H_2O$ to generate $TpyCu^{2+}$.

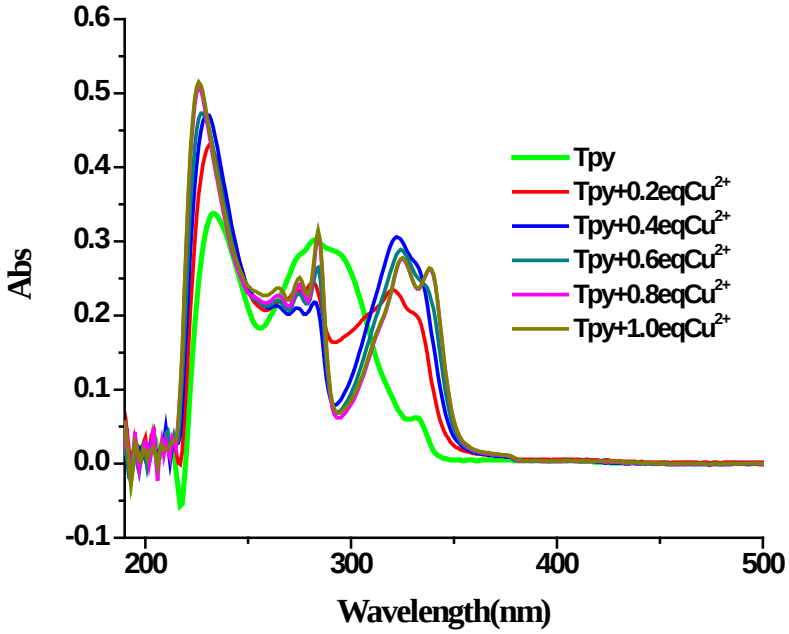
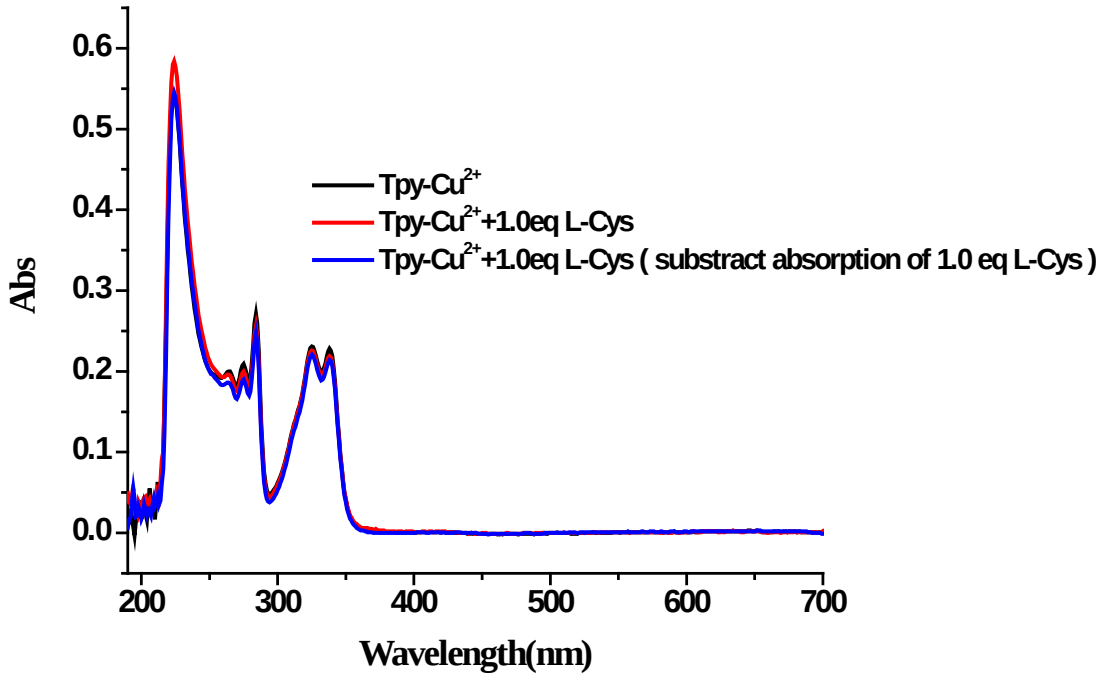
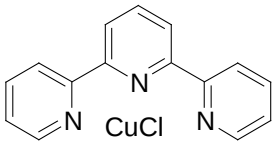


Figure S20. The UV spectra of $TpyCu^{2+}$ (2.0×10^{-5} M in 25 mM hepes buffer solution, pH=7.35) with/without 1.0 equiv L-cysteine.

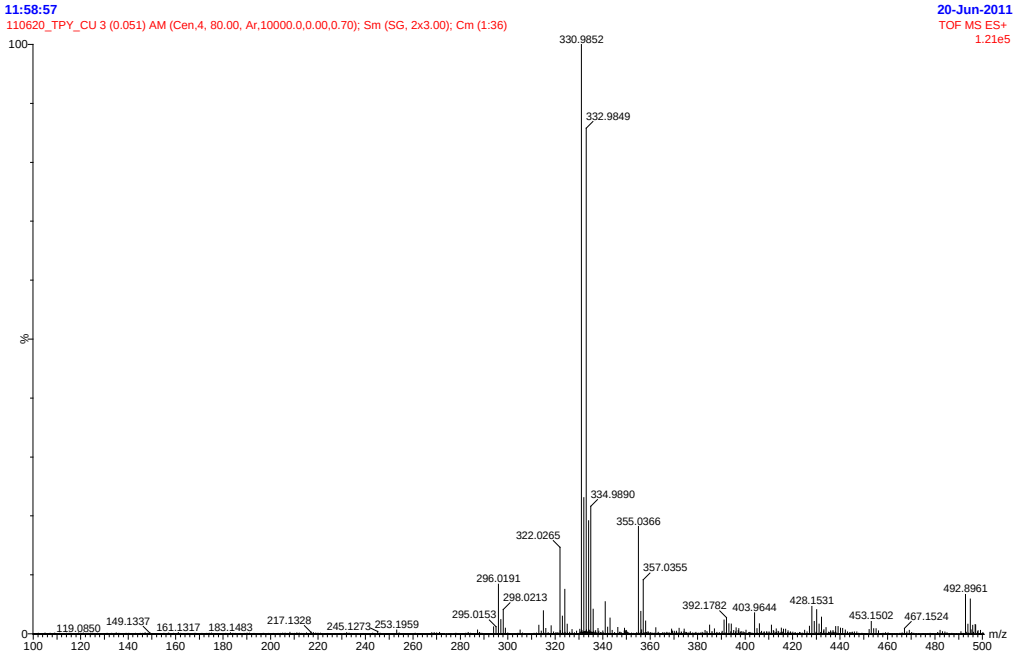


VII. High Resolution Mass Spectrum (ES+) of $TpyCuCl_2$

Figure S21. High Resolution Mass Spectrum (ES+) of $TpyCuCl_2$

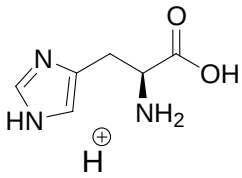


Chemical Formula: $C_{15}H_{11}ClCuN_3$
Exact Mass: 330.9938

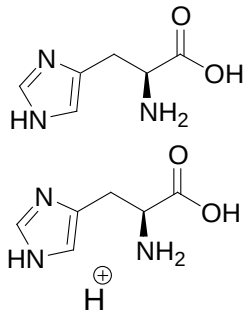


VIII. Mass Spectroscopic Analysis of the Mixture of $TpyCu^{2+}$ with L-Histidine.

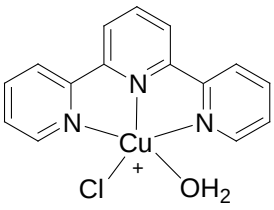
Figure S22. For the mixture of $TpyCuCl_2$:L-histidine = 1:10 in pure water, the following ions were observed in the mass spectrum:



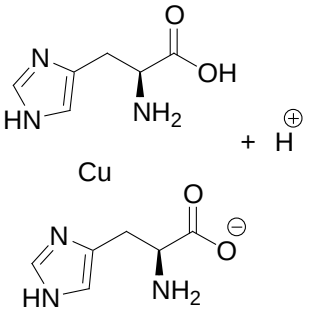
Chemical Formula: $C_6H_{10}N_3O_2^+$
Exact Mass: 156.0768



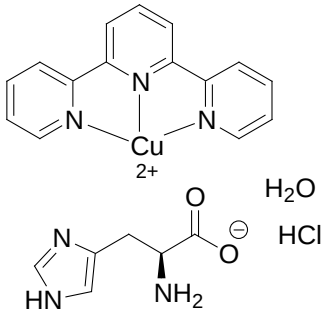
Chemical Formula: $C_{12}H_{19}N_6O_4^+$
Exact Mass: 311.1462



Chemical Formula: $C_{15}H_{13}ClCuN_3O$
Exact Mass: 349.0143

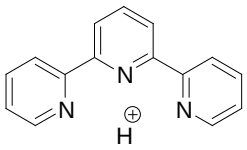


Chemical Formula: $C_{12}H_{18}CuN_6O_4$
Exact Mass: 373.0686

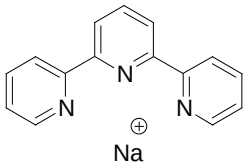


Chemical Formula: $C_{21}H_{22}ClCuN_6O_3^+$
Exact Mass: 504.0838

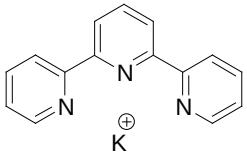
The following ions were not found, indicating no free terpyridine.



Chemical Formula: $C_{15}H_{12}N_3^+$
Exact Mass: 234.1026



Chemical Formula: $C_{15}H_{11}N_3Na^+$
Exact Mass: 256.0845



Chemical Formula: $C_{15}H_{11}KN_3^+$
Exact Mass: 272.0585

Figure S23. The mass spectrum of the mixture of $TpyCuCl_2$: L-histidine = 1:10

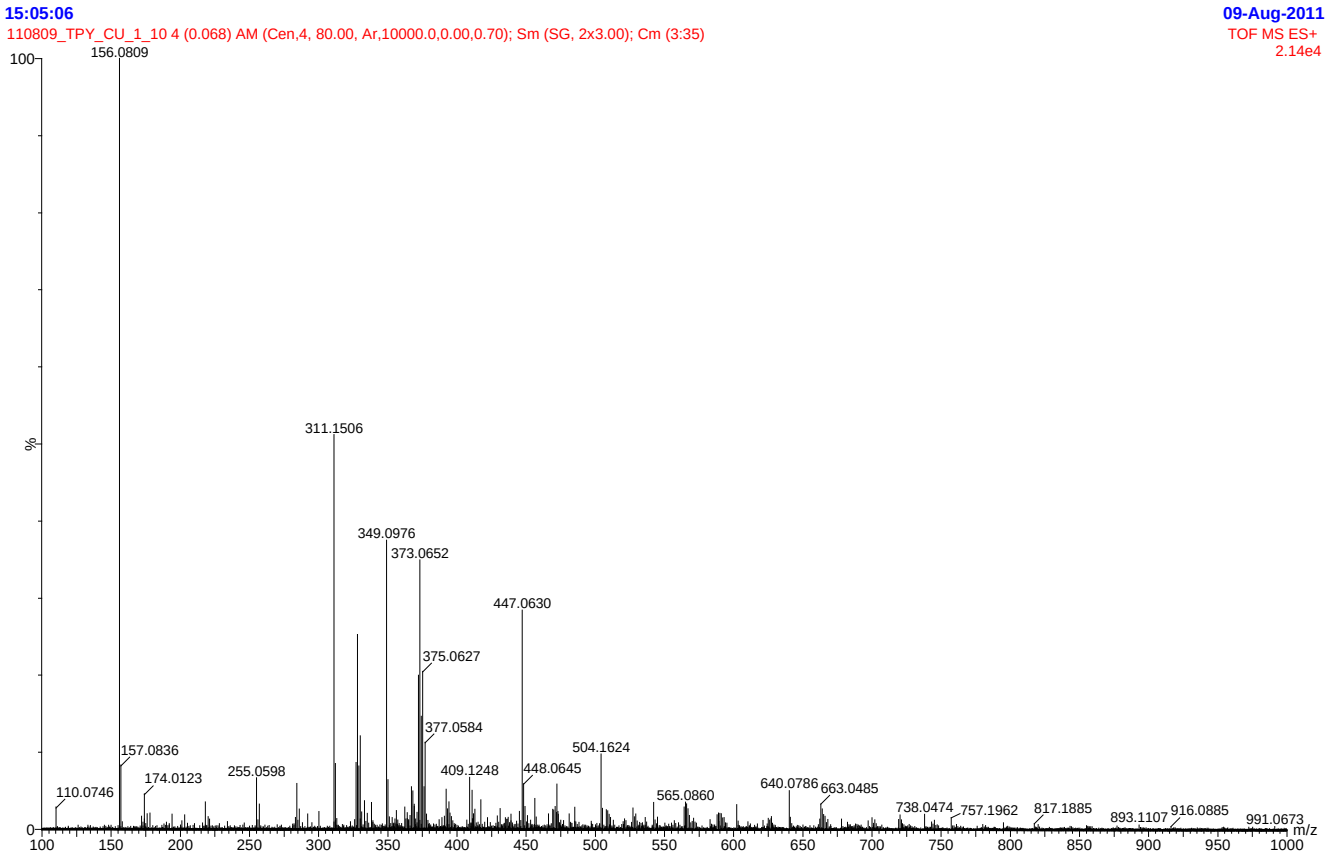
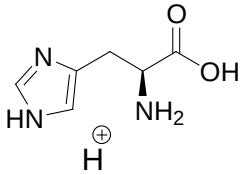
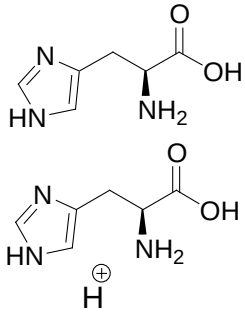


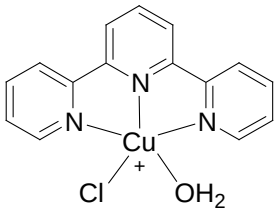
Figure S24. Following peaks were observed in the mass spectrum of the mixture of $TpyCuCl_2$:L-histidine=1:1



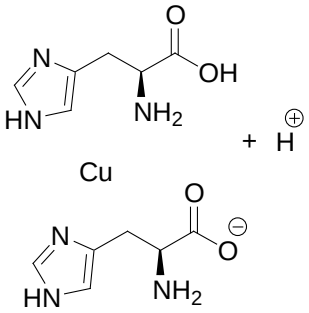
Chemical Formula: $C_6H_{10}N_3O_2^+$
Exact Mass: 156.0768



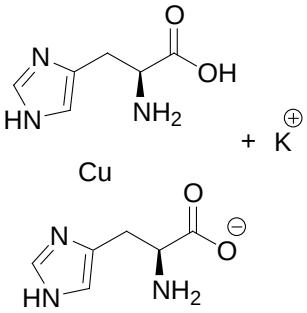
Chemical Formula: $C_{12}H_{19}N_6O_4^+$
Exact Mass: 311.1462



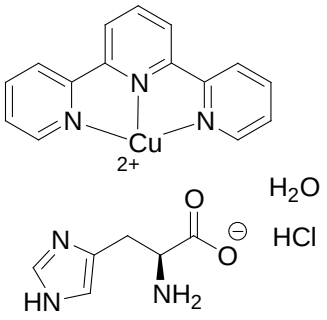
Chemical Formula: $C_{15}H_{13}ClCuN_3O$
Exact Mass: 349.0143



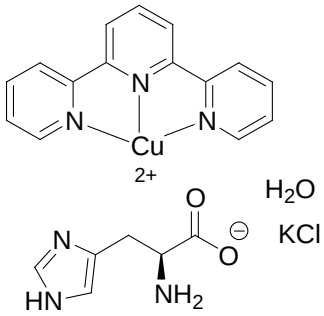
Chemical Formula: $C_{12}H_{18}CuN_6O_4$
Exact Mass: 373.0686



Chemical Formula: $C_{12}H_{17}CuKN_6O_4$
Exact Mass: 411.0244



Chemical Formula: $C_{21}H_{22}ClCuN_6O_3^+$
Exact Mass: 504.0838



Chemical Formula: $C_{21}H_{21}ClCuKN_6O_3^+$
Exact Mass: 542.0397

Figure S25. The mass spectrum of the mixture of $TpyCuCl_2$:L-histidine=1:1