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-Cu2+ complex.

A mixture of Tpy (233 mg, 1.0 mmol) and CuCl2·2H2O (205 mg, 1.2 mmol) was stirred in H2O (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 CH3OH (5 mL). After dried under vacuum, TpyCuCl2 was obtained. The HR-MS (ES+) spectrum of TpyCuCl2 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 °C equipped with Pt working electrode, a platinum counter electrode, and a Hg(l)/Hg2Cl2(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 HNO3 for 15 min and then washed with distilled water before each measurement.
III. Supplementary Fluorescence Spectra

Amino acids and other species:

- Histidine
- Cysteine
- Alanine
- Proline
- Phenylalanine
- Lysine
- Arginine
- Methionine
- Serine
- Glu
- Cystine
- 1H-imidazole
- 2-methyl-1H-imidazole
- 1-methyl-1H-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) ($\lambda_{exc}$=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) ($\lambda_{\text{exc}}$ = 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) ($\lambda_{\text{exc}}$ = 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:

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

$$\frac{1}{K} = 0.00749 \times 10^{-5}/6.66917\times10^{-4}$$

The binding constant of TpyCu$^{2+}$ with histidine is $8.944 \times 10^3$.
**Figure S5.** Fluorescence excitation spectra of Tpy, L-Trp, L-Tyr at the same concentration (25 mM HEPES, pH 7.35).

<table>
<thead>
<tr>
<th></th>
<th>Maximum emission wavelength</th>
<th>The wavelength range that can be used for excitation</th>
<th>Maximum excitation wavelength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tpy</td>
<td>352nm</td>
<td>239nm-345nm</td>
<td>331nm</td>
</tr>
<tr>
<td>L-Trp</td>
<td>357nm</td>
<td>240nm-311.4nm</td>
<td>278nm</td>
</tr>
<tr>
<td>L-Tyr</td>
<td>304nm</td>
<td>250nm-293nm</td>
<td>286nm</td>
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**Figure S6.** Fluorescence response of Tpy-CuCl₂ (2.0×10⁻⁵M in 25mM hepes buffer solution, pH=7.35) toward L-His, L-Trp and L-Tyr (λᵢₑₓ= 320 nm, slits: 5nm/5nm)

**Figure S7.** Fluorescence response of Tpy-Cu (2.0×10⁻⁵M 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 H₂O) (λᵢₑₓ= 320 nm, slits: 5nm/5nm)
Figure S8. Fluorescence Responses of Tpy-CuCl₂ (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-CuCl₂ (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-CuCl₂ (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 (2.0×10⁻⁵ 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)]

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

<table>
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<tr>
<th></th>
<th>(\lambda_{exc}) (slits: 5/5 nm)</th>
<th>F (Integral fluorescence intensity)</th>
<th>A (Absorbance at 298nm)</th>
<th>Integration range</th>
<th>Y (Fluorescence quantum yield)</th>
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<tr>
<td>quinine bisulfate</td>
<td>298 nm</td>
<td>637673285</td>
<td>0.015</td>
<td>370-580nm</td>
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<tr>
<td>Tpy</td>
<td>298 nm</td>
<td>144062710</td>
<td>0.045</td>
<td>325-500nm</td>
<td>0.041</td>
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<tr>
<td>Tpy-Cu^{2+}+12eqHis</td>
<td>298 nm</td>
<td>68793865</td>
<td>0.033</td>
<td>325-500nm</td>
<td>0.027</td>
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IV. Distinguish Histidine from Cysteine by Using the Fluorescence Response of TpyCuCl₂

Oxidation of Cysteine to Cystine: To a solution of L-Cysteine (7.3 mg, 0.06 mmol) in 10 mL H₂O was added NaI (0.09 mg, 1 mol %) and 50% H₂O₂ (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 TpyCuCl₂ (2.0×10⁻⁵ M) at λₐₘᵋ = 352 nm to L-Cysteine upon treatment with H₂O₂ and NaI in aqueous buffered solution (25 mM HEPES, p = 7.35) (λᵫᵧc = 298 nm, slits: 5 nm/5 nm).
**Figure S13.** Fluorescence response of TpyCuCl₂ (2.0×10⁻⁵ M) at λₘᵋ = 352 nm to L-Cysteine and L-Histidine upon treatment with H₂O₂ and NaI in aqueous buffered solution (25 mM HEPES, pH 7.35) (λₑₓᶜₑ = 298 nm, slits: 5 nm/5 nm).

![Graph showing fluorescence response](image)

**Figure S14.** Fluorescence spectra of TpyCuCl₂ (2.0×10⁻⁵ M) to L-Cysteine and L-Histidine upon treatment with H₂O₂ and NaI in aqueous solution (25 mM HEPES buffered, pH 7.35) (λₑₓᶜₑ = 298 nm, slits: 5 nm/5 nm).

![Graph showing fluorescence spectra](image)
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$_2$Cl$_2$(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 $\ell$-histidine, 1 eq $\ell$-alanine and 1 eq imidazole. Reference electrode: saturated Hg(l)/Hg$_2$Cl$_2$(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 $\ell$-histidine. Reference electrode = saturated Hg(l)/Hg$_2$Cl$_2$(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$_2$Cl$_2$(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+}.

![UV Titration of Tpy](image1)

**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.

![UV spectra of TpyCu^{2+}](image2)
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$_6$H$_{10}$N$_3$O$_2$\(^+\)
  - Exact Mass: 156.0768
- Chemical Formula: C$_{12}$H$_{19}$N$_6$O$_4$\(^+\)
  - Exact Mass: 311.1462
- Chemical Formula: C$_{15}$H$_{13}$ClCuN$_3$O
  - Exact Mass: 349.0143
- Chemical Formula: C$_{12}$H$_{18}$CuN$_6$O$_4$\(^+\)
  - Exact Mass: 373.0686
- Chemical Formula: C$_{21}$H$_{22}$ClCuN$_6$O$_3$\(^+\)
  - 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$_{11}$NaN$_3$\(^+\)
  - 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 TpyCuCl₂: L-histidine = 1:10
Figure S24. Following peaks were observed in the mass spectrum of the mixture of TpyCuCl2:L-histidine=1:1

- Chemical Formula: $\text{C}_6\text{H}_{10}\text{N}_3\text{O}_2^+$
  - Exact Mass: 156.0768

- Chemical Formula: $\text{C}_{12}\text{H}_{19}\text{N}_6\text{O}_4^+$
  - Exact Mass: 311.1462

- Chemical Formula: $\text{C}_{15}\text{H}_{13}\text{ClCuN}_3\text{O}^{2+}$
  - Exact Mass: 349.0143

- Chemical Formula: $\text{C}_{12}\text{H}_{18}\text{CuN}_6\text{O}_4^+$
  - Exact Mass: 373.0686

- Chemical Formula: $\text{C}_{12}\text{H}_{17}\text{CuKN}_6\text{O}_4^{2+}$
  - Exact Mass: 411.0244

- Chemical Formula: $\text{C}_{21}\text{H}_{22}\text{ClCuN}_6\text{O}_3^{2+}$
  - Exact Mass: 504.0838

- Chemical Formula: $\text{C}_{21}\text{H}_{21}\text{ClCuKN}_6\text{O}_3^{2+}$
  - Exact Mass: 542.0397
Figure S25. The mass spectrum of the mixture of TpyCuCl$_2$·L-histidine=1:1