

Colorimetric Sensing of α -Amino Acids and its Application for the “Label-Free” Detection of Protease

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Experimental Section.

Materials and Instrumentations

Compound **1** was obtained following literature procedures.¹ Trypsin was obtained from Sigma, and other proteins were purchased from Beijing Xinjingke Biotechnology Co., Ltd. All other reagents were of analytical reagent grade and used without further purification. Doubly distilled water was used for all experiments. UV-visible spectra were obtained using a Shimadzu UV-2550 spectrometer, and the pH values were determined by using a DELTA 320 PH dollar.

UV absorption changes of **1**+ Cu²⁺ with different amino acids.

A solution of **1** (10 μ M) was prepared in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/CH₃CN. The solution of amino acids (1 \times 10⁻² mol/L) was prepared in distilled water. A solution of **1** (3.0 mL) was placed in a quartz cell (10.0 mm width) and the UV absorption spectrum was recorded. After the addition of the copper ion solution (1 \times 10⁻³ mol/L, 21 μ L), the solution of one of amino acids (1 \times 10⁻² mol/L) was introduced in portions and the UV absorption changes were recorded at room temperature each time.

Trypsin Assay.

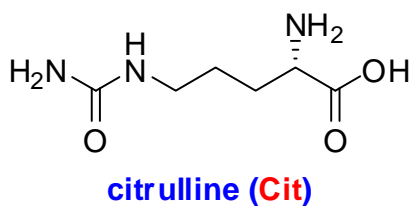
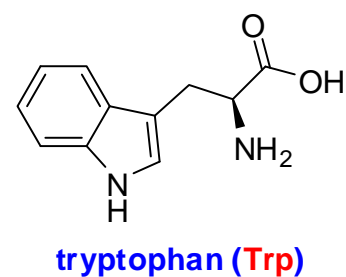
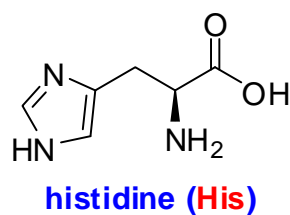
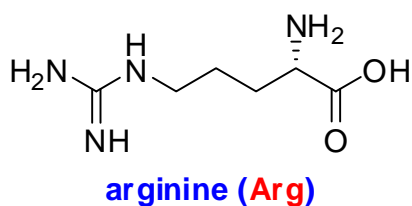
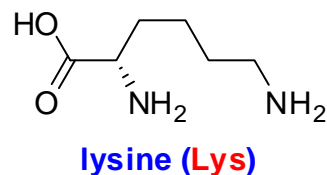
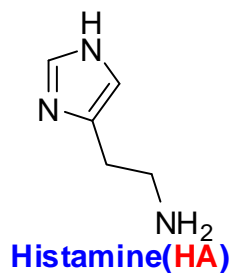
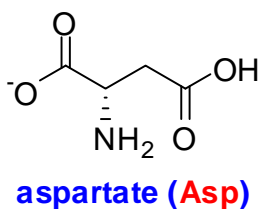
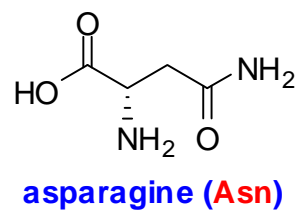
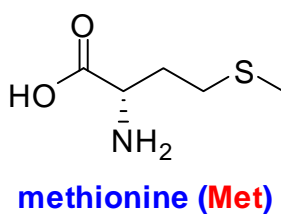
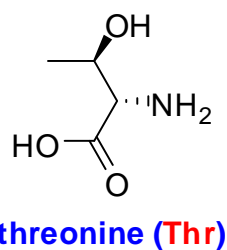
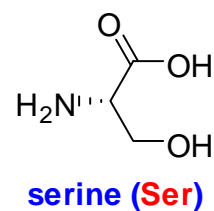
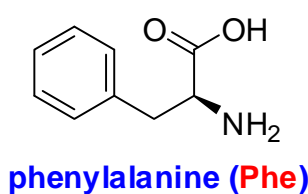
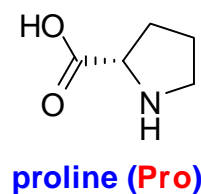
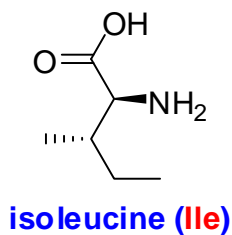
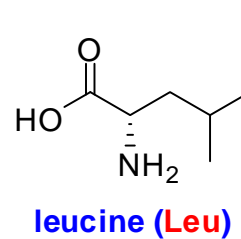
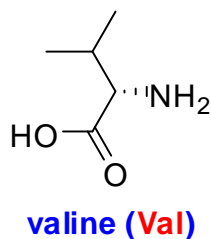
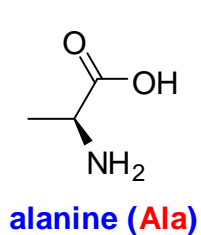
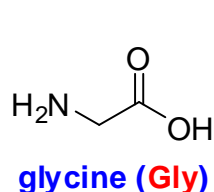
A solution of **1** (10 μ M) was prepared in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/CH₃CN. A solution of **1** (3.0 mL) was placed in a quartz cell ([**1**] = 1.0 \times 10⁻⁵ mol/L, [Cu²⁺] = 7.0 \times 10⁻⁶ mol/L), [BSA] = 10 μ g/mL). Then 0, 1.0, 2.0, 3.0 and 5.0 μ g/mL of trypsin were added into the quartz cell, respectively. The UV absorption spectrum were recorded at 2 min

intervals over 0~40 min. The plots of the relative absorption difference at 555 nm versus the trypsin incubating time were obtained.

Reference.

1. Y. Xiang, A. Tong, P. Jin, Y. Ju, *Org. Lett.* **2006**, 8, 2863-2866.

Chart S1. The structure of general α -amino acid.



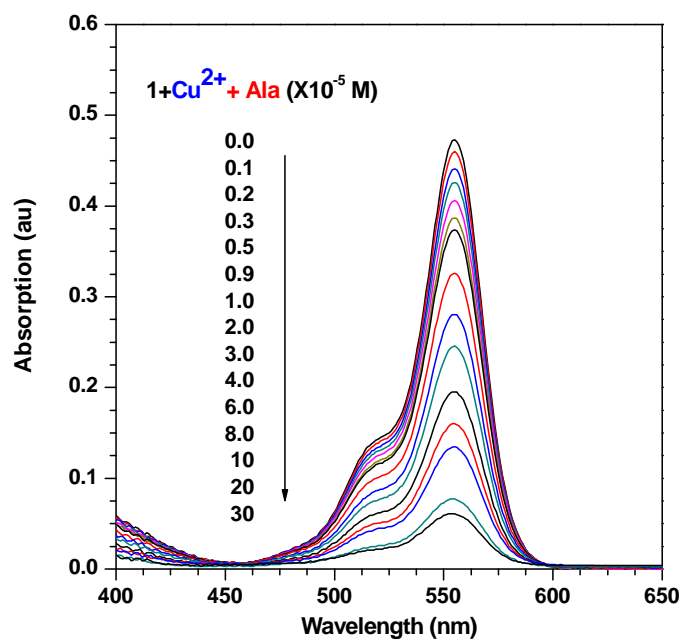


Figure S1a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1** + Cu^{2+} with increasing amounts of **Ala**. The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

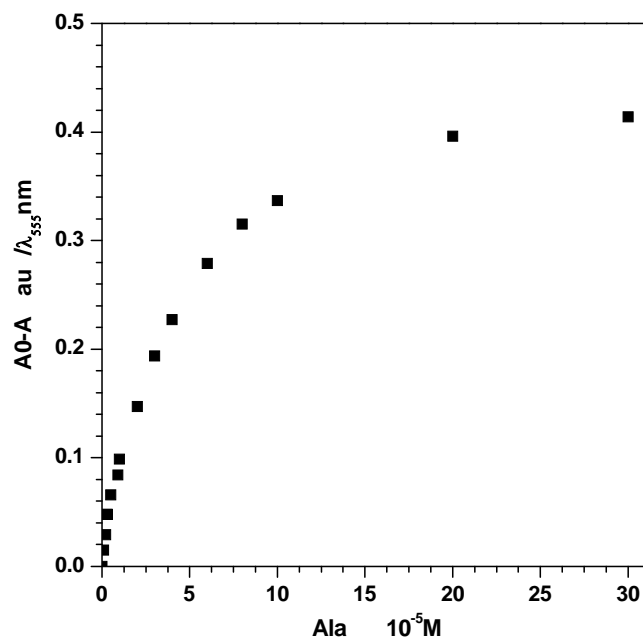


Figure S1b. Absorption difference at $\lambda = 555$ nm versus the concentration of **Ala** in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

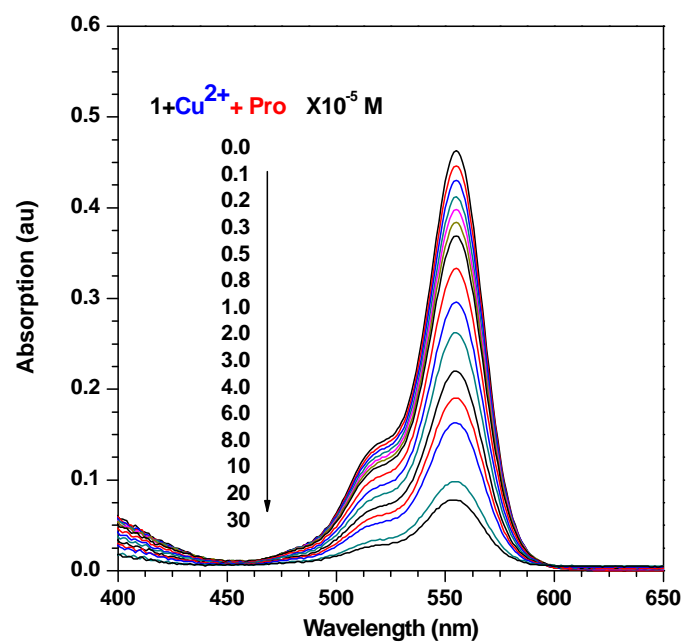


Figure S2a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1** + Cu^{2+} with increasing amounts of **Pro**. The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

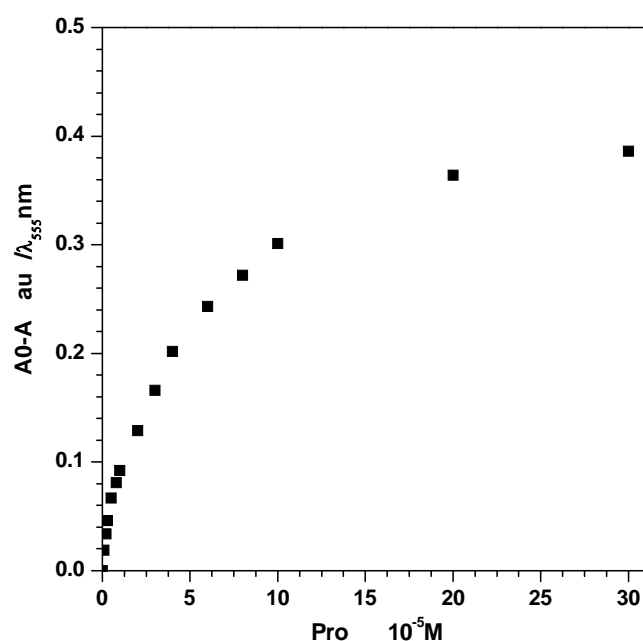


Figure S2b. Absorption difference at $\lambda = 555$ nm versus the concentration of **Pro** in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

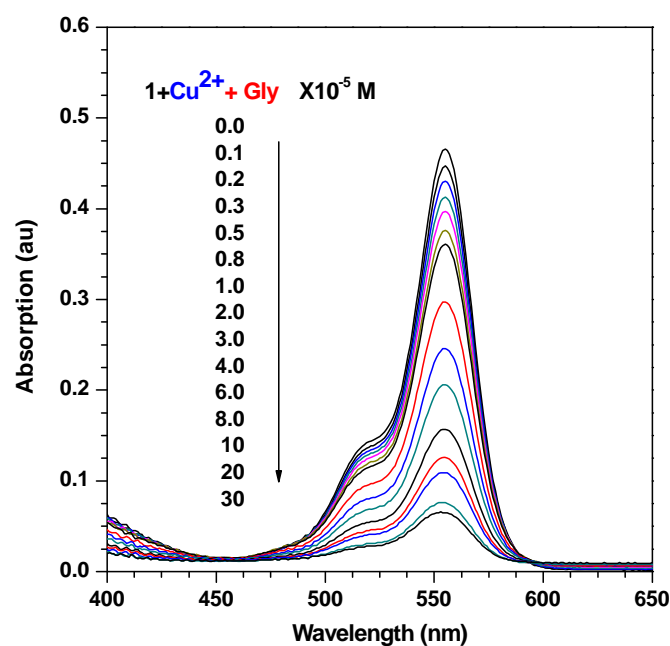


Figure S3a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1** + Cu^{2+} with increasing amounts of **Gly**. The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

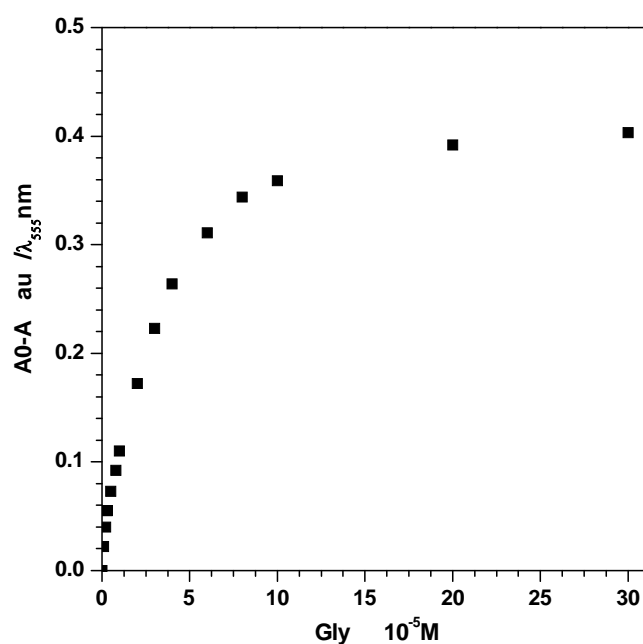


Figure S3b. Absorption difference at $\lambda = 555$ nm versus the concentration of **Gly** in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

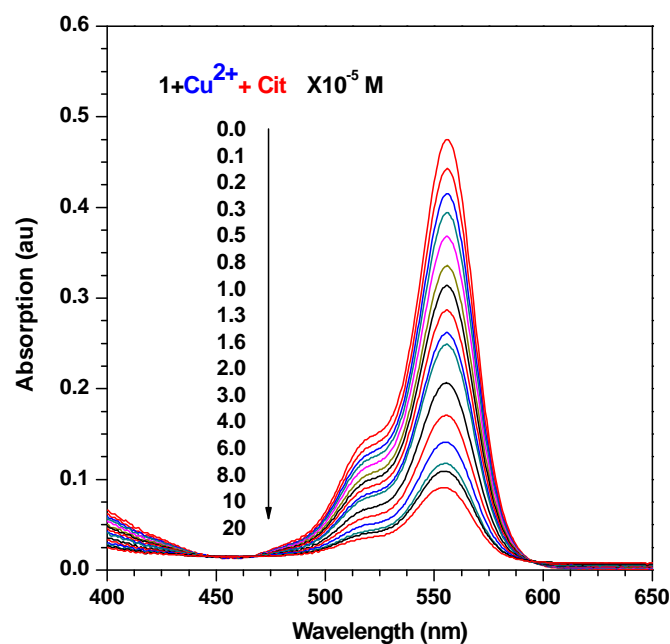


Figure S4a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1**+ Cu^{2+} with increasing amounts of **Cit**. The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

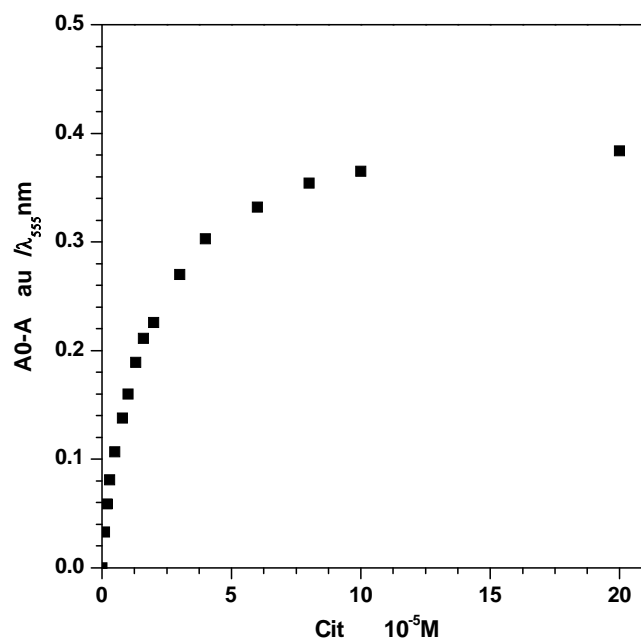


Figure S4b. Absorption difference at $\lambda = 555$ nm versus the concentration of **Cit** in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

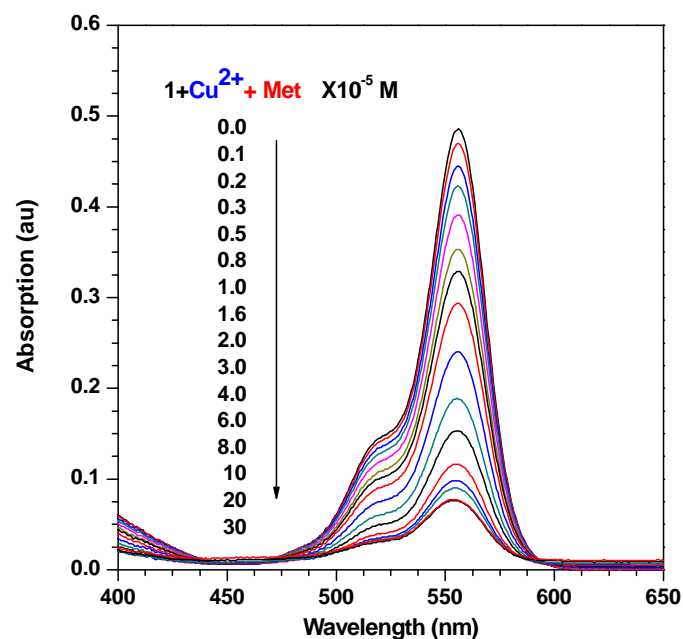


Figure S5a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1** + Cu^{2+} with increasing amounts of **Met**. The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

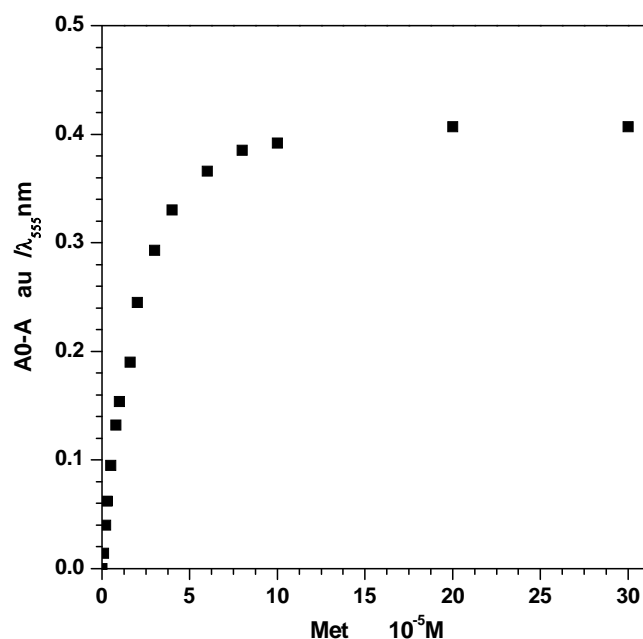


Figure S5b. Absorption difference at $\lambda = 555$ nm versus the concentration of **Met** in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

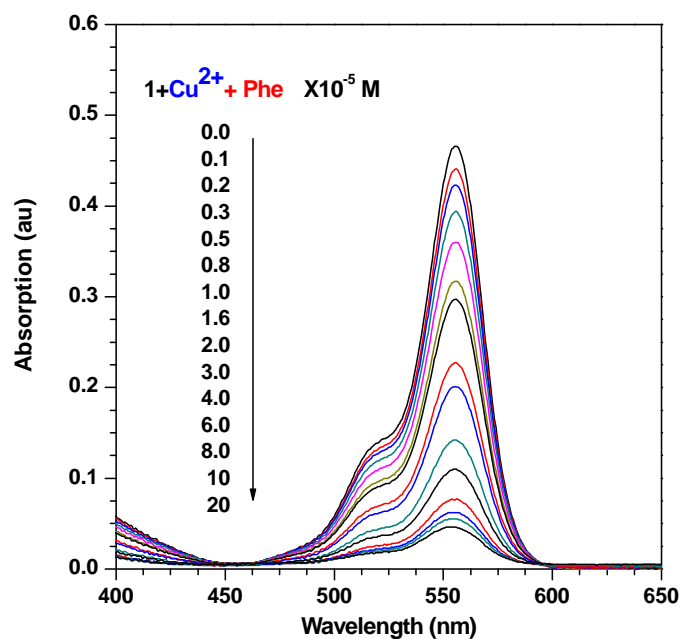


Figure S6a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1**+ Cu^{2+} with increasing amounts of **Phe**. The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

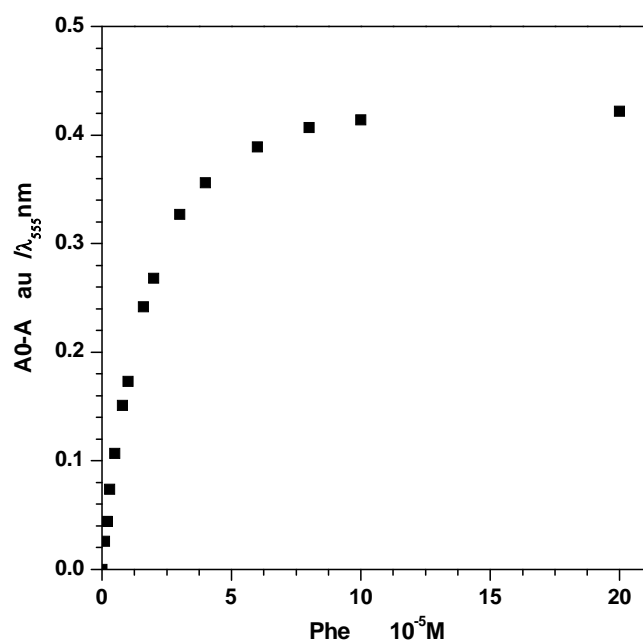


Figure S6b. Absorption difference at $\lambda = 555$ nm versus the concentration of **Phe** in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

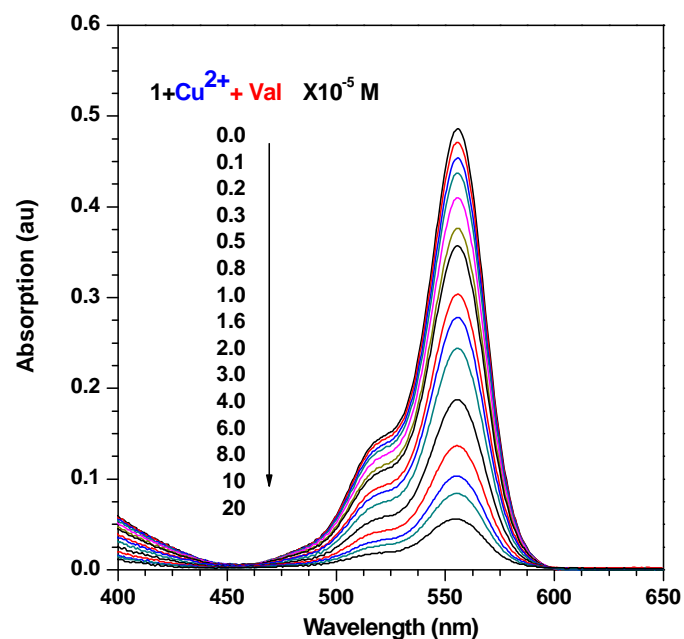


Figure S7a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1**+ Cu^{2+} with increasing amounts of **Val**. The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

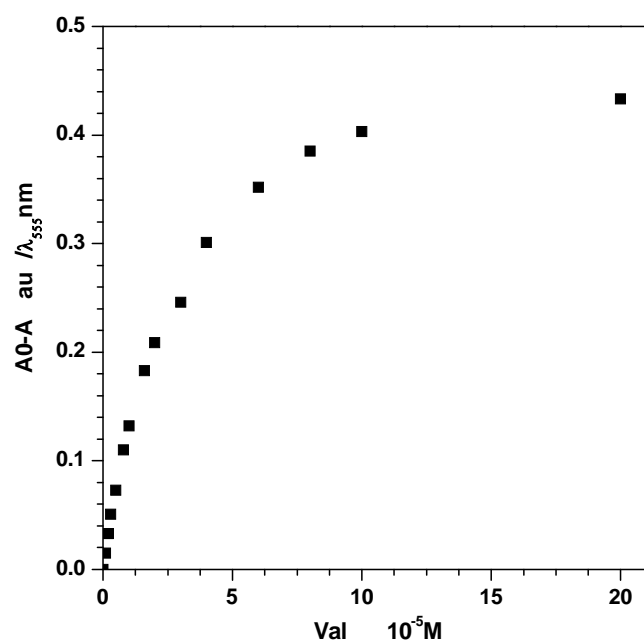


Figure S7b. Absorption difference at $\lambda = 555$ nm versus the concentration of **Val** in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

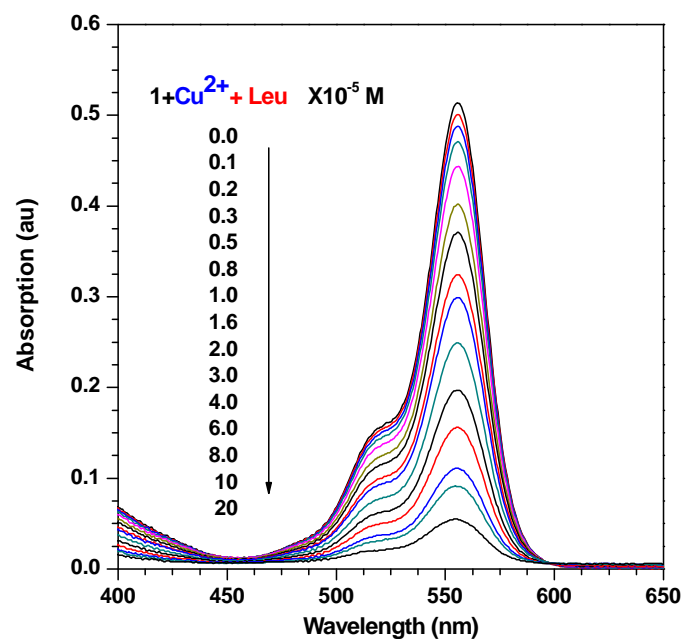


Figure S8a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1**+ Cu^{2+} with increasing amounts of **Leu**. The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

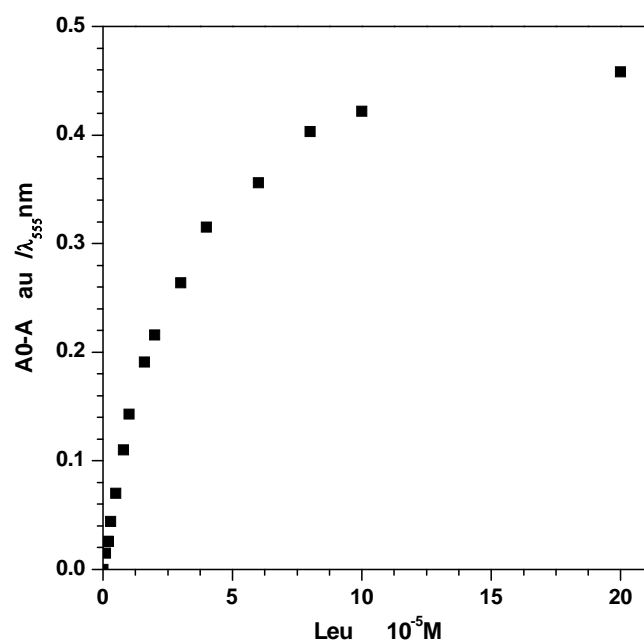


Figure S8b. Absorption difference at $\lambda = 555$ nm versus the concentration of **Leu** in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

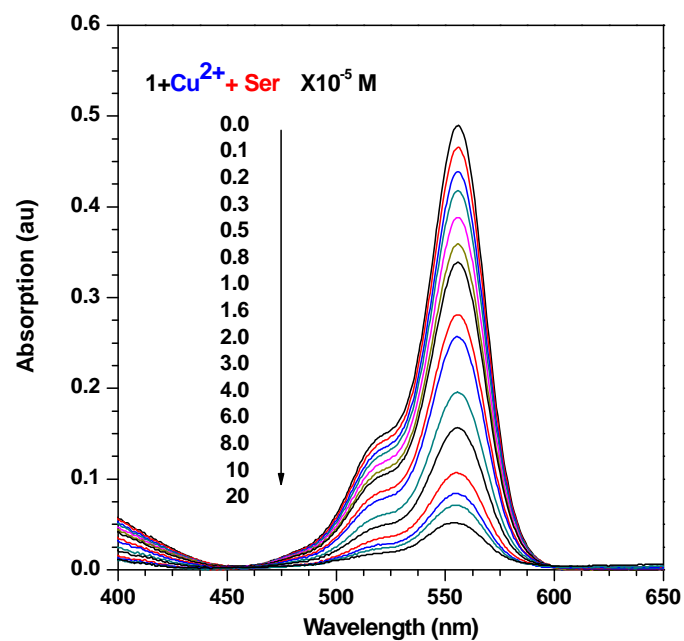


Figure S9a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1**+ Cu^{2+} with increasing amounts of **Ser**. The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

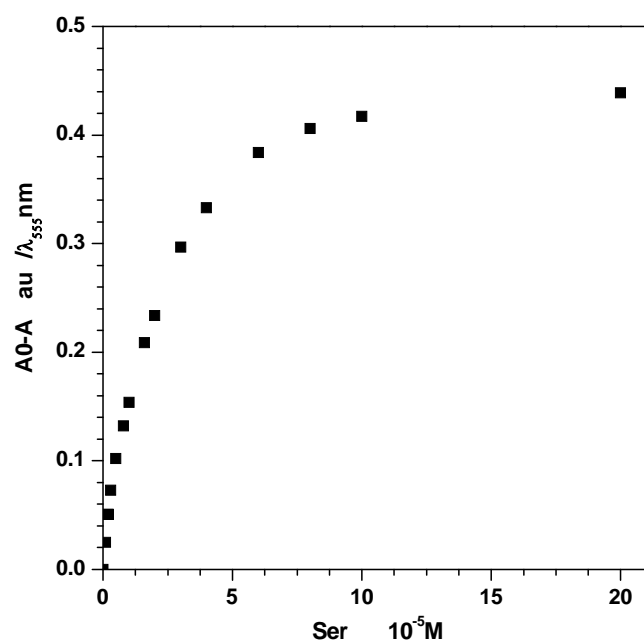


Figure S9b. Absorption difference at $\lambda = 555$ nm versus the concentration of **Ser** in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

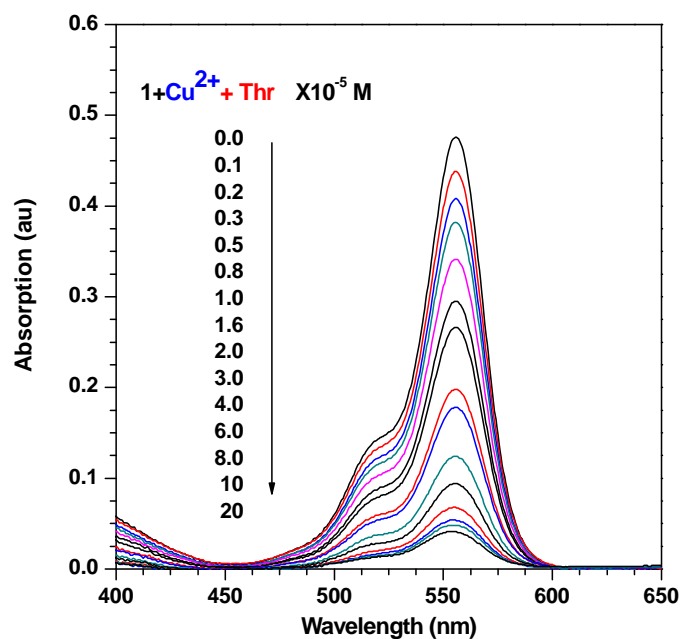


Figure S10a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1** + Cu^{2+} with increasing amounts of **Thr**. The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

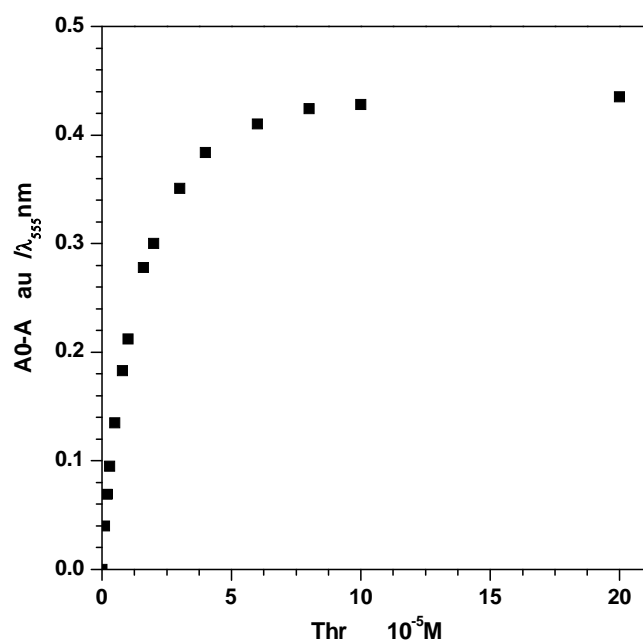


Figure S10b. Absorption difference at $\lambda = 555$ nm versus the concentration of **Thr** in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

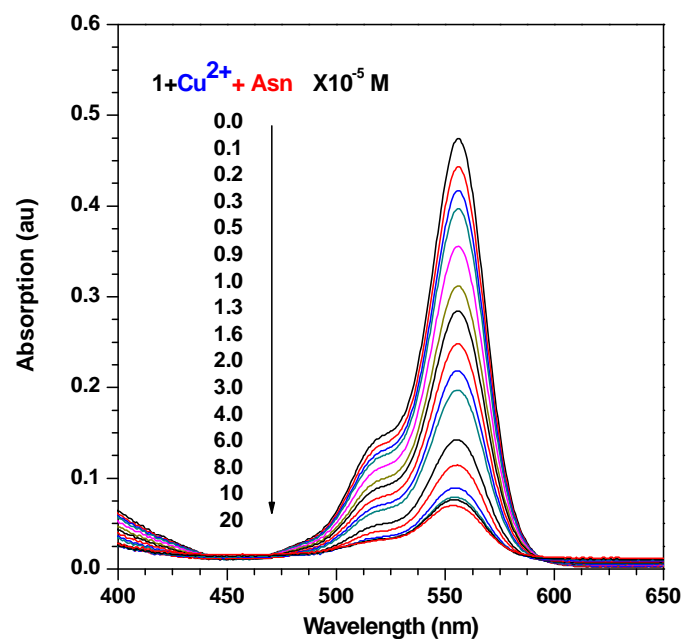


Figure S11a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1** + Cu^{2+} with increasing amounts of **Asn**. The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

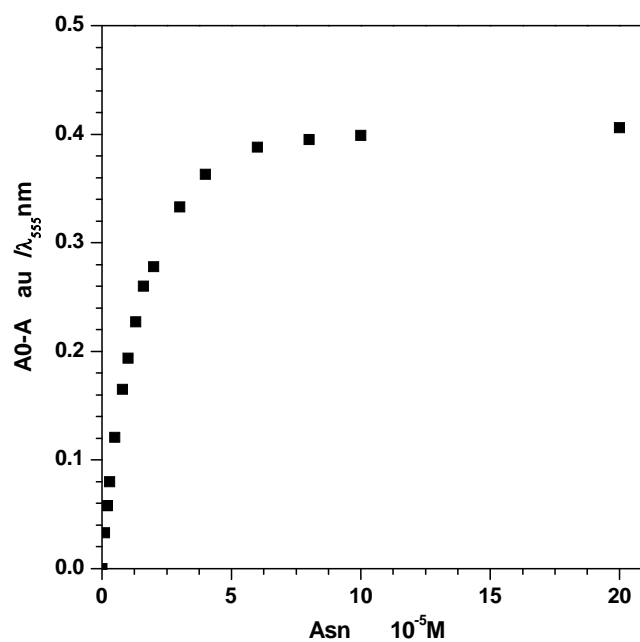


Figure S11b. Absorption difference at $\lambda = 555$ nm versus the concentration of **Asn** in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

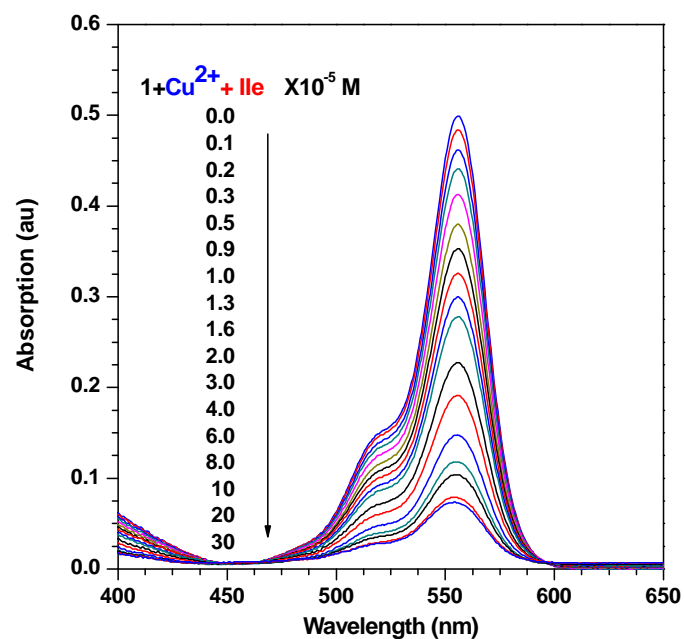


Figure S12a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1**+ Cu^{2+} with increasing amounts of **IIe**. The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

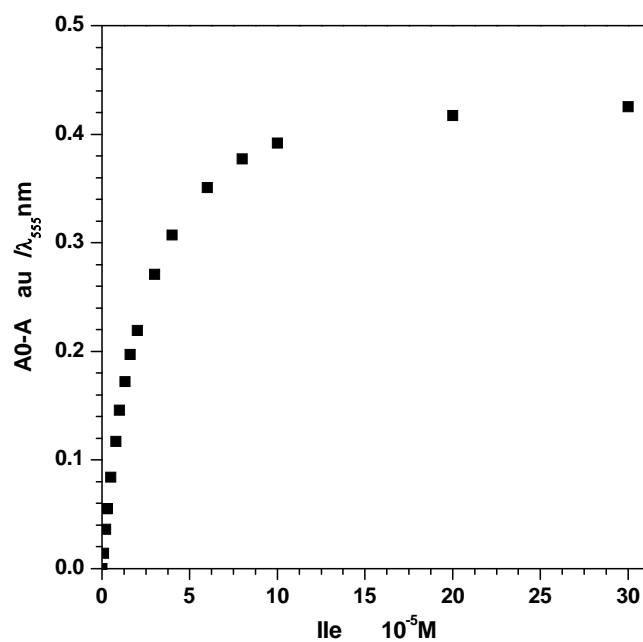


Figure S12b. Absorption difference at $\lambda = 555$ nm versus the concentration of **IIe** in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

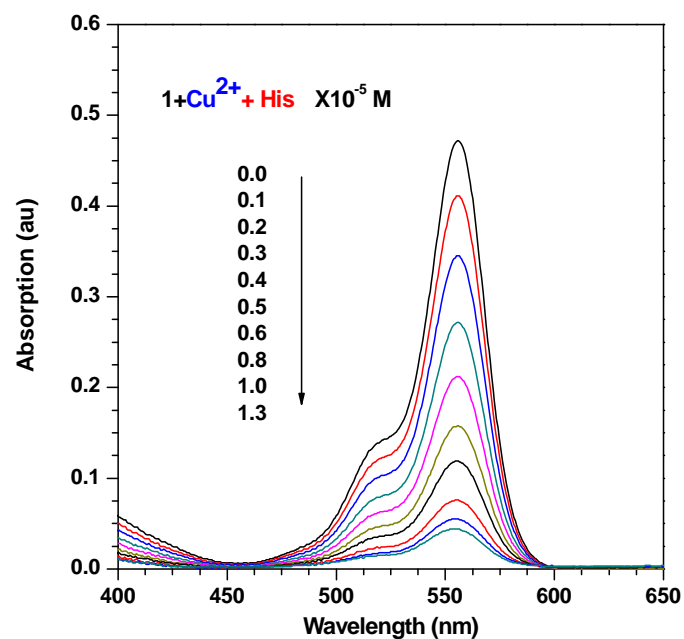


Figure S13a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1** + Cu^{2+} with increasing amounts of **His**. The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

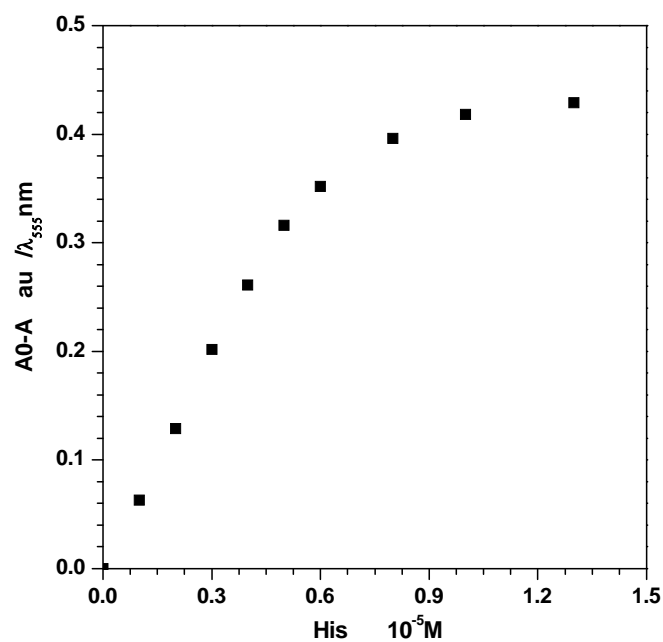


Figure S13b. Absorption difference at $\lambda = 555$ nm versus the concentration of **His** in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

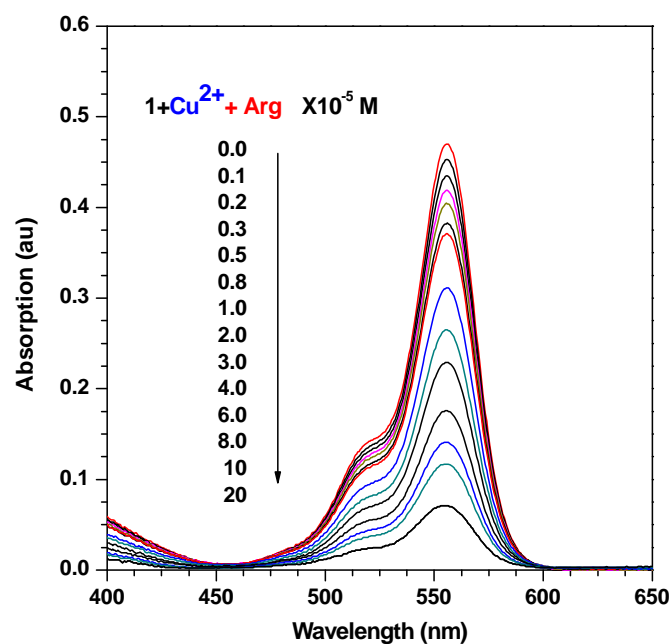


Figure S14a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1** + Cu^{2+} with increasing amounts of **Arg**. The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

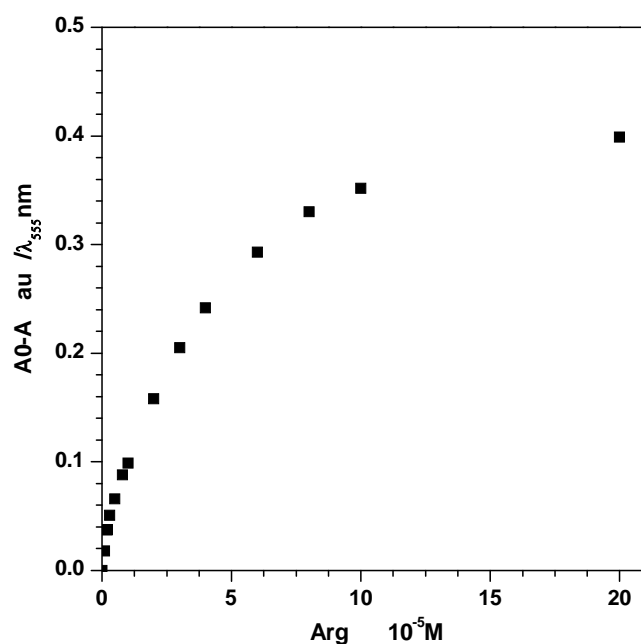


Figure S14b. Absorption difference at $\lambda = 555$ nm versus the concentration of **Arg** in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

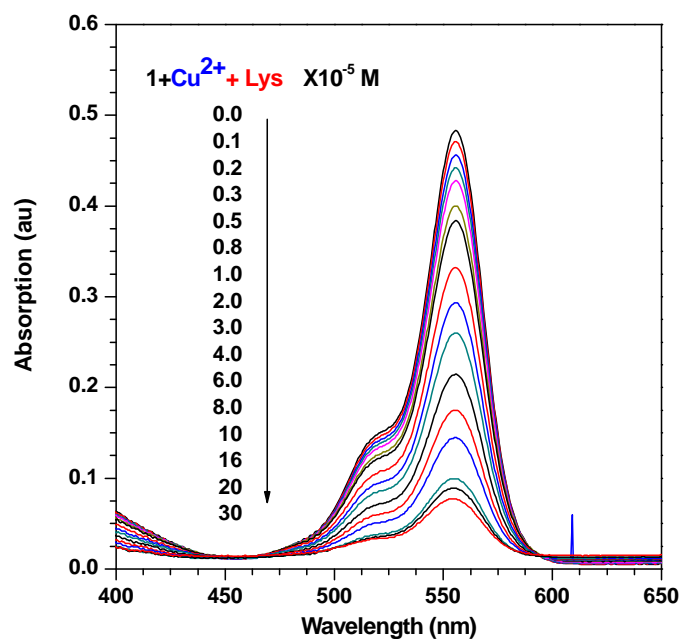


Figure S15a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1**+ Cu^{2+} with increasing amounts of **Lys**. The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

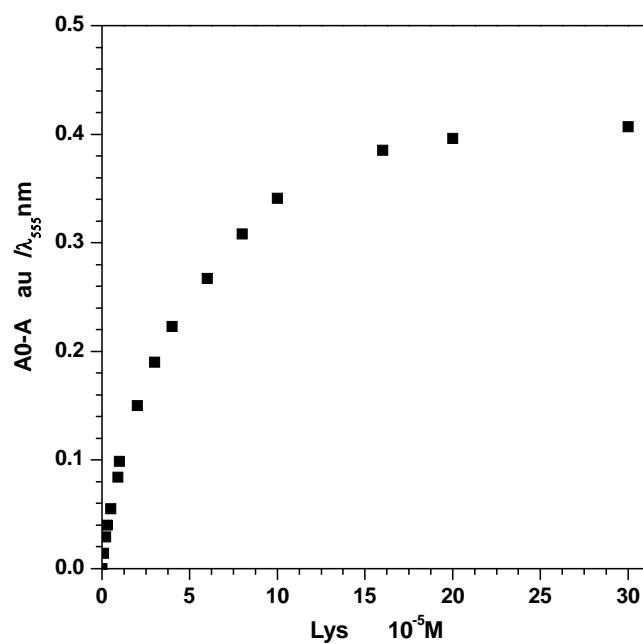


Figure S15b. Absorption difference at $\lambda = 555$ nm versus the concentration of **Lys** in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

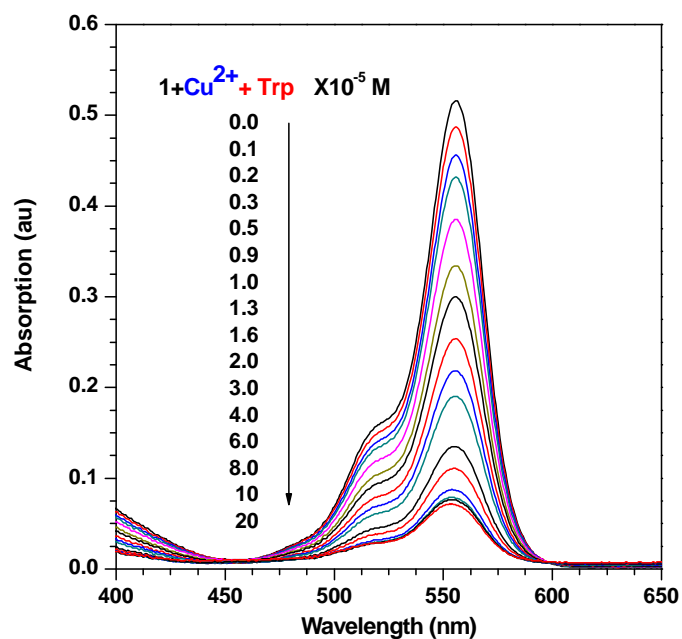


Figure S16a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1**+ Cu^{2+} with increasing amounts of **Trp**. The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

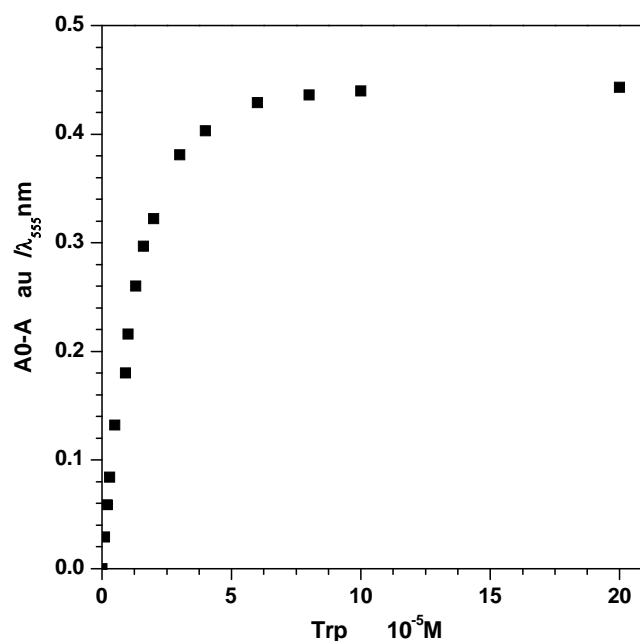


Figure S16b. Absorption difference at $\lambda = 555$ nm versus the concentration of **Trp** in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

Chart S2. The structure of the three isomers of aminobutyric acids.

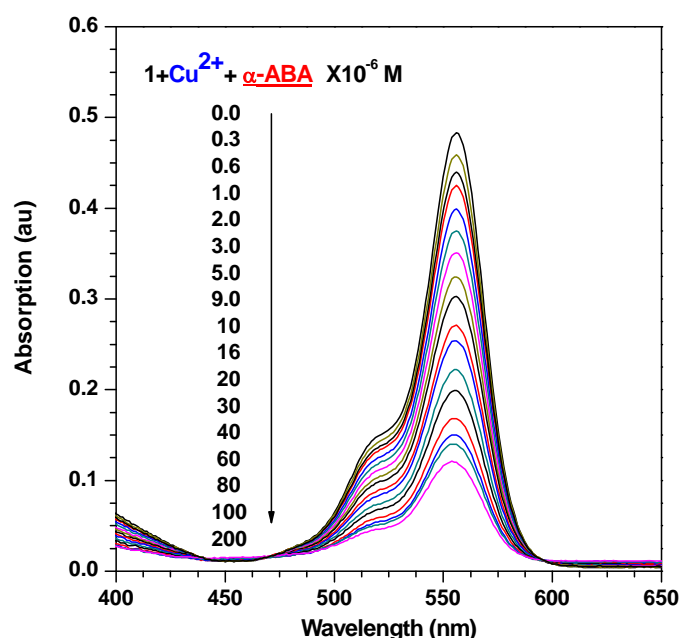
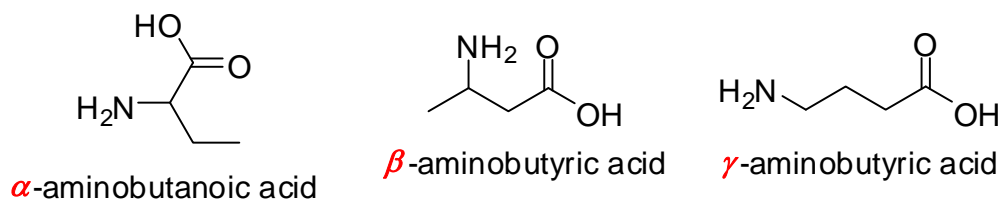


Figure S17a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1** + Cu^{2+} with increasing amounts of α -amino-butyric acid (α -ABA). The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

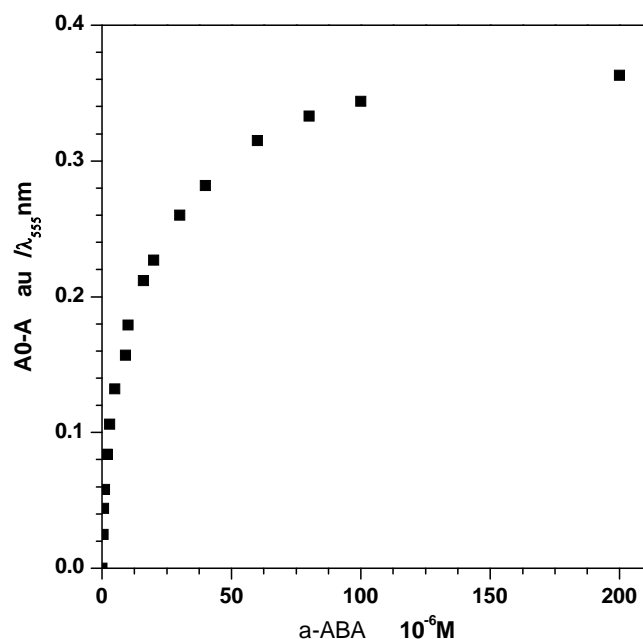


Figure S17b. Absorption difference at $\lambda = 555$ nm versus the concentration of α -amino-butyric acid (α -ABA) in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

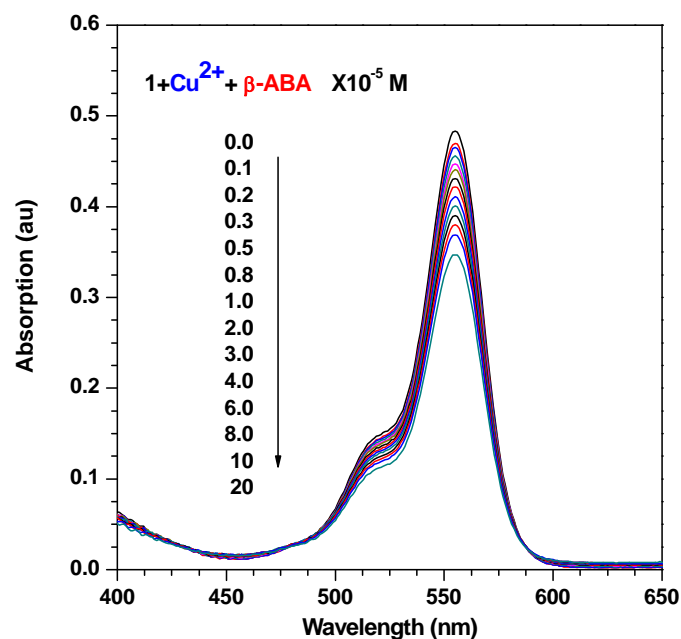


Figure S18a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1** + Cu^{2+} with increasing amounts of β -amino-butyric acid (β -ABA). The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

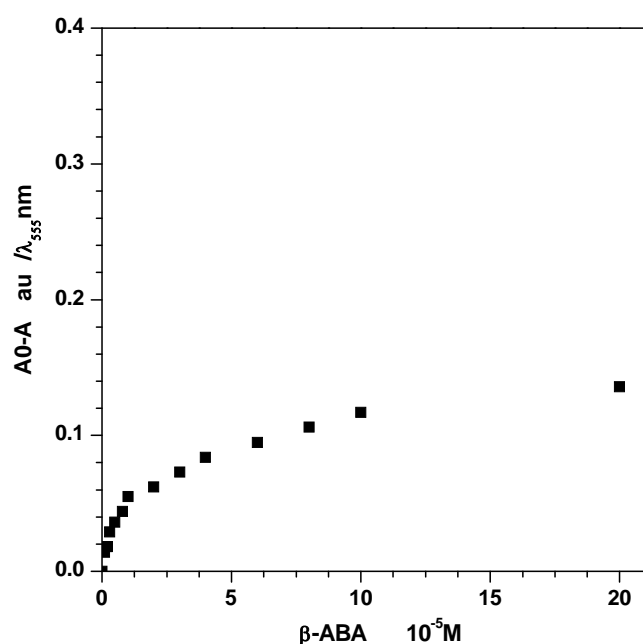


Figure S18b. Absorption difference at $\lambda = 555$ nm versus the concentration of β -amino-butyric acid (β -ABA) in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

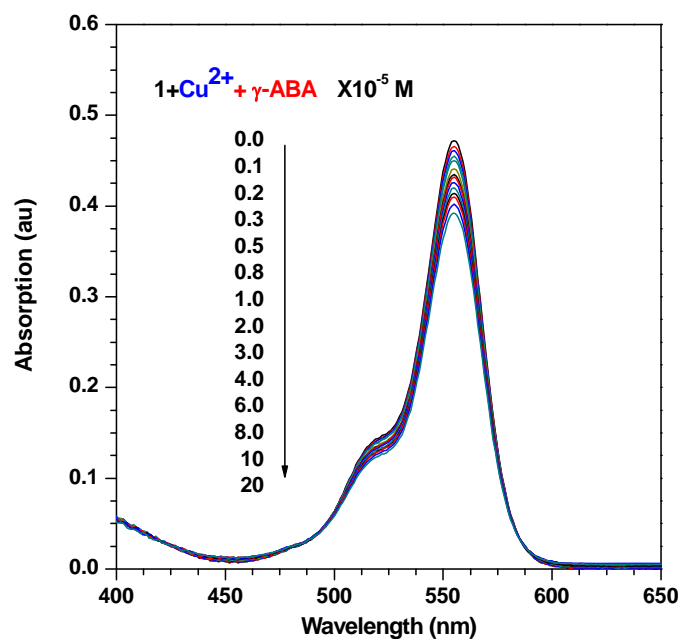


Figure S19a. UV-vis spectra of **1** (1.0×10^{-5} mol/L) after the addition of Cu^{2+} , and titration of **1** + Cu^{2+} with increasing amounts of γ -amino-butyric acid (γ -ABA). The concentration of Cu^{2+} was 7.0×10^{-6} mol/L.

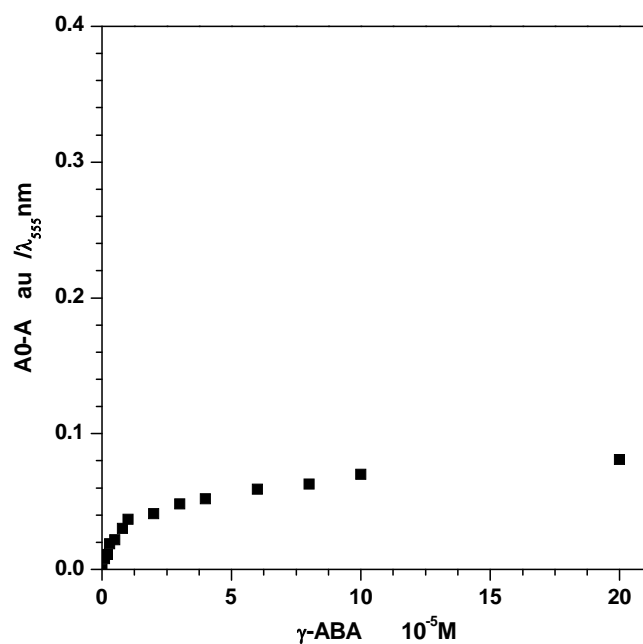


Figure S19b. Absorption difference at $\lambda = 555$ nm versus the concentration of γ -amino-butyric acid (γ -ABA) in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN with the concentrations of **1** and Cu^{2+} at 1.0×10^{-5} and 7.0×10^{-6} mol/L, respectively.

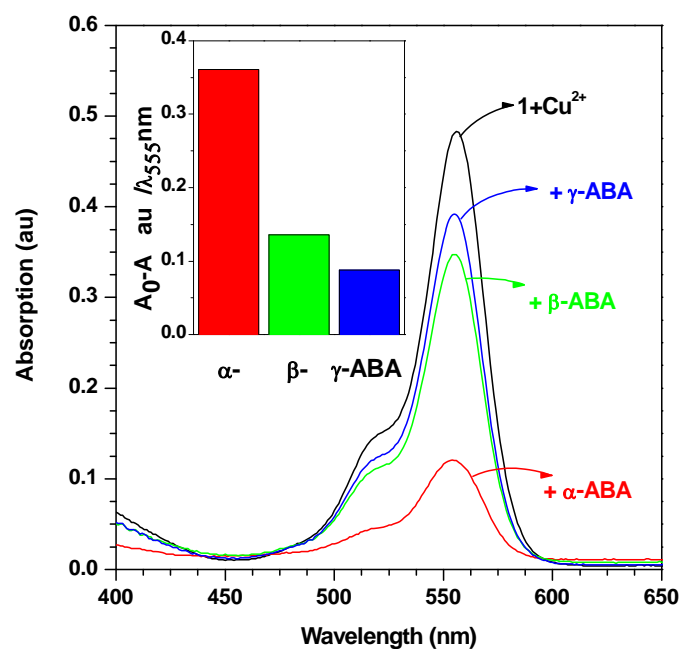


Figure S20. UV-vis spectra of $1+\text{Cu}^{2+}$, before and after the addition of α -, β - and γ -ABA (2×10^{-4} M), respectively. Inset: UV-vis response profiles of $1+\text{Cu}^{2+}$ upon the addition of ABAs. The concentration of **1** was 1.0×10^{-5} mol/L, while that of Cu^{2+} 7.0×10^{-6} mol/L.

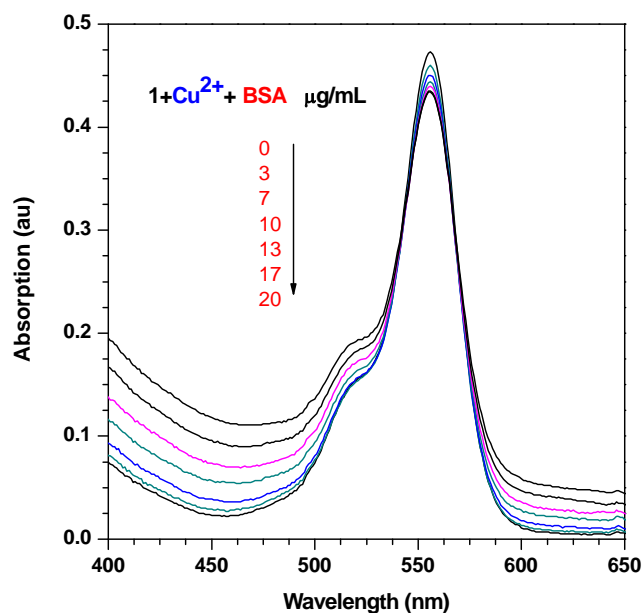


Figure S21. UV-vis titration of $1+\text{Cu}^{2+}$ with increasing amounts of BSA. The concentration of **1** was 1.0×10^{-5} mol/L, while that of Cu^{2+} 7.0×10^{-6} mol/L.

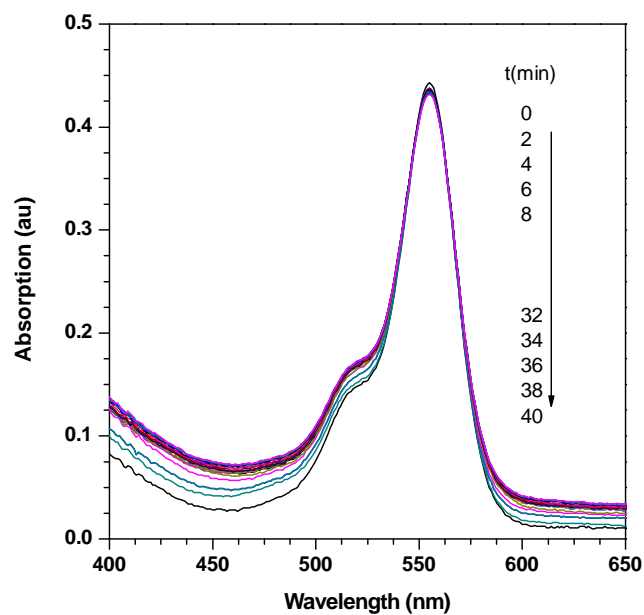


Figure S22. UV-vis spectra of **1**/ Cu^{2+} /BSA in Tris-HCl (10 mM, pH=7.0) buffer containing 50% (v/v) water/ CH_3CN . The concentration of **1** was 1.0×10^{-5} mol/L, $[\text{Cu}^{2+}] = 7.0 \times 10^{-6}$ mol/L, $[\text{BSA}] = 10 \mu\text{g/mL}$.

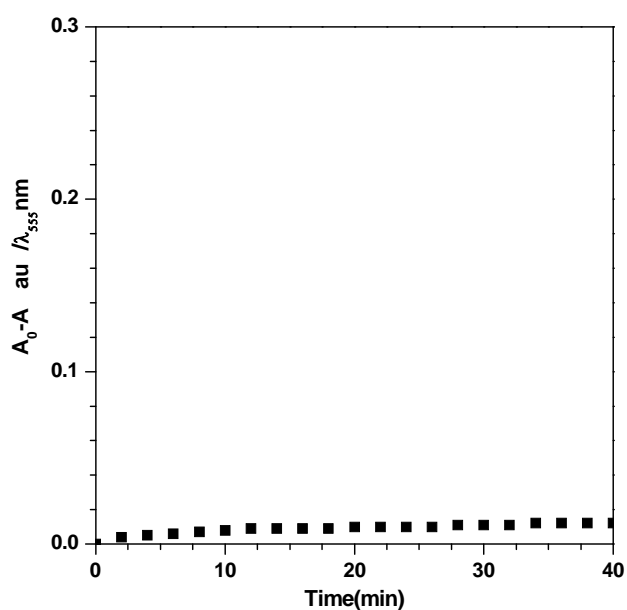


Figure S23. UV-vis spectra of **1**/ Cu^{2+} /BSA in Tris-HCl (10 mM, pH=7.0) buffer containing 50% (v/v) water/ CH_3CN . The concentration of **1** was 1.0×10^{-5} mol/L, $[\text{Cu}^{2+}] = 7.0 \times 10^{-6}$ mol/L, $[\text{BSA}] = 10 \mu\text{g/mL}$.

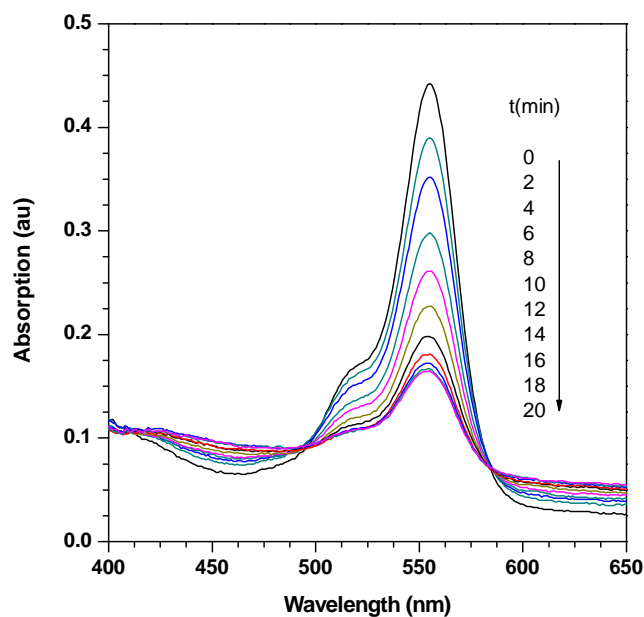


Figure S24. UV-vis spectra of **1**/ Cu^{2+} /BSA as a function of trypsin digestion time in Tris-HCl (10 mM, pH=7.0) buffer containing 50% (v/v) water/ CH_3CN . The concentration of **1** was 1.0×10^{-5} mol/L, $[\text{Cu}^{2+}] = 7.0 \times 10^{-6}$ mol/L, $[\text{BSA}] = 10 \mu\text{g/mL}$, $[\text{trypsin}] = 3.0 \mu\text{g/mL}$.

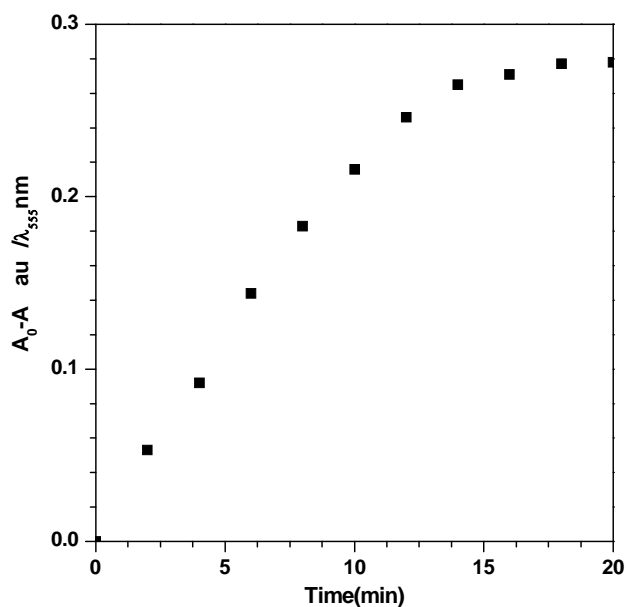


Figure S25. Absorption difference of **1**/ Cu^{2+} /BSA as a function of trypsin digestion time at 555 nm in Tris-HCl (10 mM, pH=7.0) buffer containing 50% (v/v) water/ CH_3CN . The concentration of **1** was 1.0×10^{-5} mol/L, $[\text{Cu}^{2+}] = 7.0 \times 10^{-6}$ mol/L, $[\text{BSA}] = 10 \mu\text{g/mL}$, $[\text{trypsin}] = 3.0 \mu\text{g/mL}$.

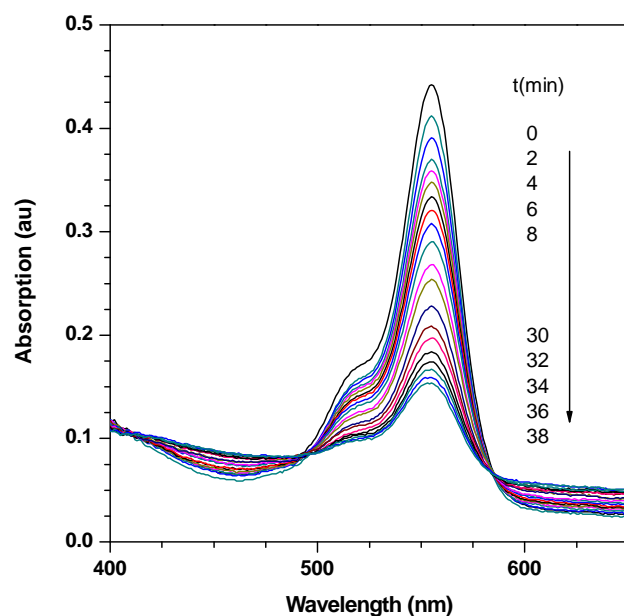


Figure S26. UV-vis spectra of **1**/ Cu^{2+} /BSA as a function of trypsin digestion time in Tris-HCl (10 mM, pH=7.0) buffer containing 50% (v/v) water/ CH_3CN . The concentration of **1** was 1.0×10^{-5} mol/L, $[\text{Cu}^{2+}] = 7.0 \times 10^{-6}$ mol/L, $[\text{BSA}] = 10 \mu\text{g/mL}$, $[\text{trypsin}] = 1.0 \mu\text{g/mL}$.

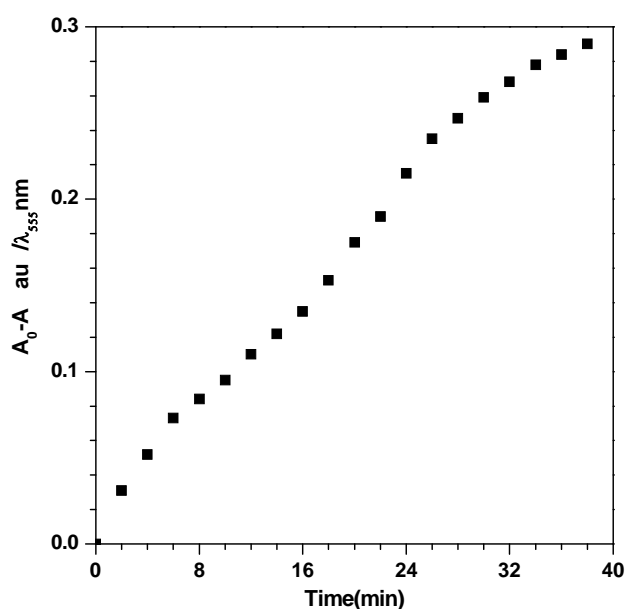


Figure S27. Absorption difference of **1**/ Cu^{2+} /BSA as a function of trypsin digestion time at 555 nm in Tris-HCl (10 mM, pH=7.0) buffer containing 50% (v/v) water/ CH_3CN . The concentration of **1** was 1.0×10^{-5} mol/L, $[\text{Cu}^{2+}] = 7.0 \times 10^{-6}$ mol/L, $[\text{BSA}] = 10 \mu\text{g/mL}$, $[\text{trypsin}] = 1.0 \mu\text{g/mL}$.

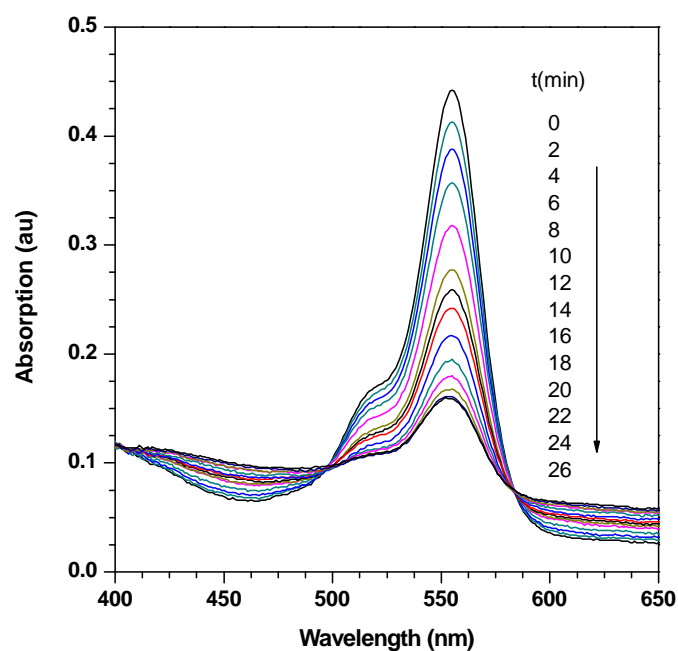


Figure S28. UV-vis spectra of **1**/ Cu^{2+} /BSA as a function of trypsin digestion time in Tris-HCl (10 mM, pH=7.0) buffer containing 50% (v/v) water/ CH_3CN . The concentration of **1** was 1.0×10^{-5} mol/L, $[\text{Cu}^{2+}] = 7.0 \times 10^{-6}$ mol/L, $[\text{BSA}] = 10 \mu\text{g/mL}$, $[\text{trypsin}] = 2.0 \mu\text{g/mL}$.

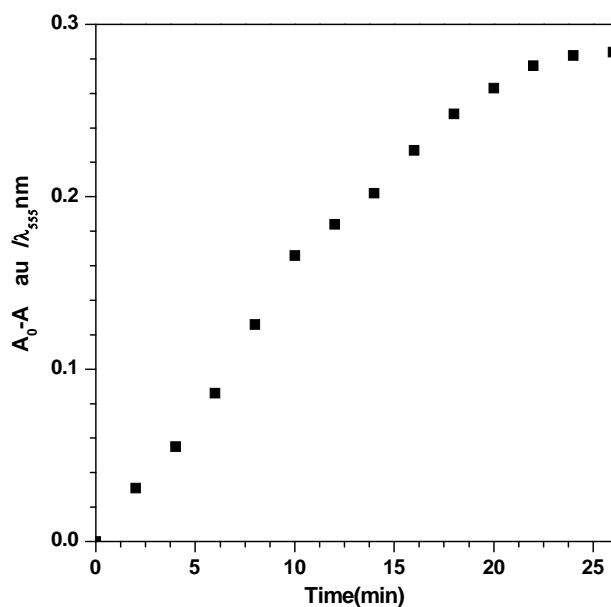


Figure S29. Absorption difference of **1**/ Cu^{2+} /BSA as a function of trypsin digestion time at 555 nm in Tris-HCl (10 mM, pH=7.0) buffer containing 50% (v/v) water/ CH_3CN . The concentration of **1** was 1.0×10^{-5} mol/L, $[\text{Cu}^{2+}] = 7.0 \times 10^{-6}$ mol/L, $[\text{BSA}] = 10 \mu\text{g/mL}$, $[\text{trypsin}] = 2.0 \mu\text{g/mL}$.

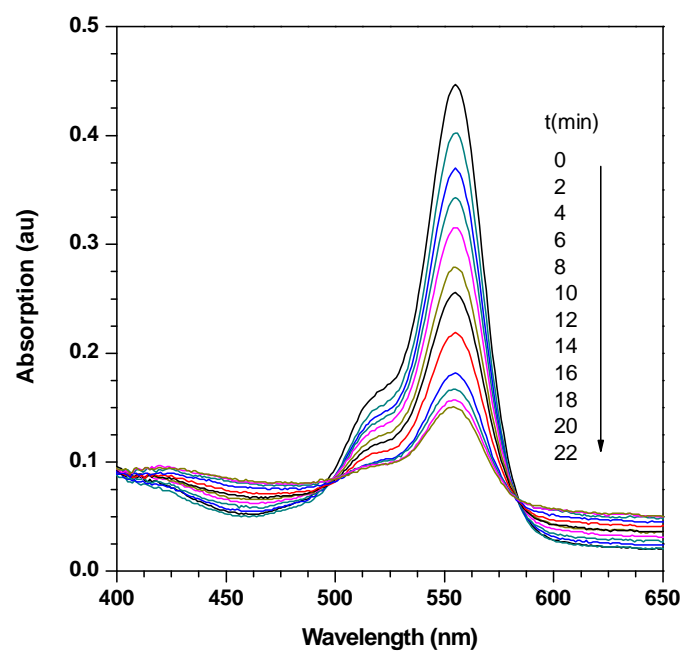


Figure S30. UV-vis spectra of **1**/ Cu^{2+} /BSA as a function of trypsin digestion time in Tris-HCl (10 mM, pH=7.0) buffer containing 50% (v/v) water/ CH_3CN . The concentration of **1** was 1.0×10^{-5} mol/L, $[\text{Cu}^{2+}] = 7.0 \times 10^{-6}$ mol/L, $[\text{BSA}] = 10 \mu\text{g/mL}$, $[\text{trypsin}] = 5.0 \mu\text{g/mL}$.

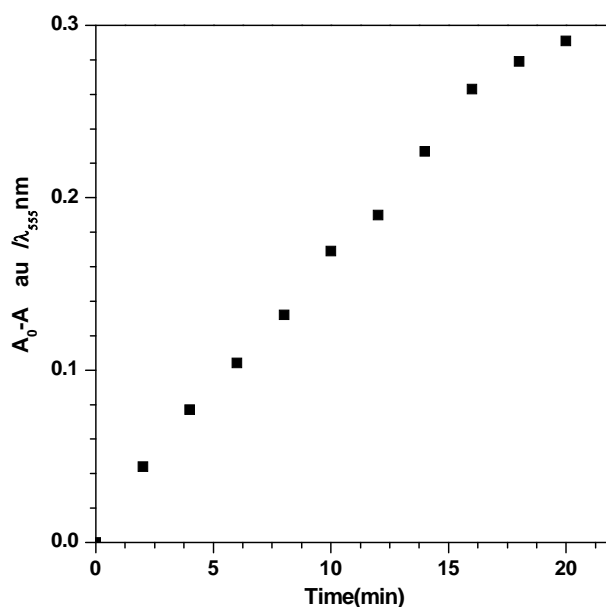


Figure S31. Absorption difference of **1**/ Cu^{2+} /BSA as a function of trypsin digestion time at 555 nm in Tris-HCl (10 mM, pH=7.0) buffer containing 50% (v/v) water/ CH_3CN . The concentration of **1** was 1.0×10^{-5} mol/L, $[\text{Cu}^{2+}] = 7.0 \times 10^{-6}$ mol/L, $[\text{BSA}] = 10 \mu\text{g/mL}$, $[\text{trypsin}] = 5.0 \mu\text{g/mL}$.

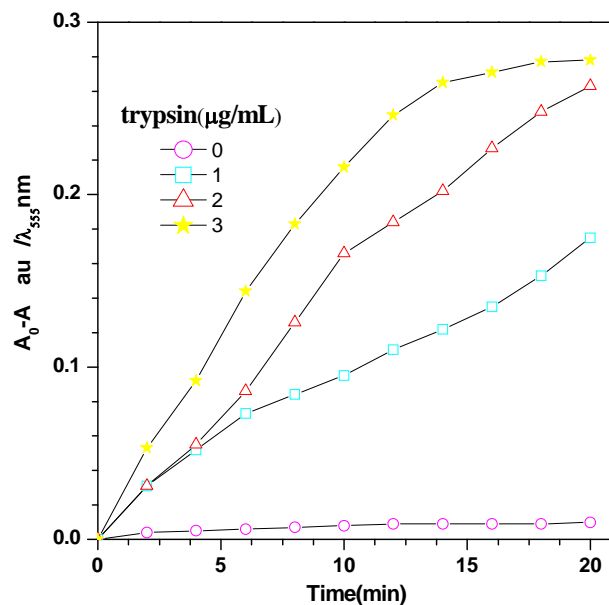


Figure S32. Absorption difference at 555 nm on the concentration of trypsin with fixed concentrations of **1**, Cu^{2+} ions, and BSA in Tris-HCl (10 mM, pH=7.0) buffer containing 50% (v/v) water/ CH_3CN . The concentration of **1** was 1.0×10^{-5} mol/L, $[\text{Cu}^{2+}] = 7.0 \times 10^{-6}$ mol/L, $[\text{BSA}] = 10 \mu\text{g/mL}$, $[\text{trypsin}] = 0 \sim 3.0 \mu\text{g/mL}$.

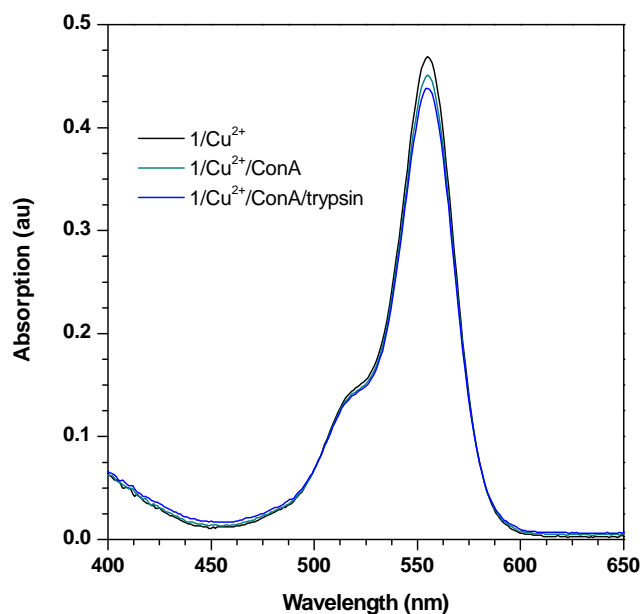


Figure S33. UV-vis spectra of **1**/ Cu^{2+} /ConA before and after incubation with trypsin in Tris-HCl (10 mM, pH=7.0) buffer containing 50% (v/v) water/ CH_3CN . The concentration of **1** was 1.0×10^{-5} mol/L, $[\text{Cu}^{2+}] = 7.0 \times 10^{-6}$ mol/L, $[\text{ConA}] = 10 \mu\text{g/mL}$, $[\text{trypsin}] = 3.0 \mu\text{g/mL}$.

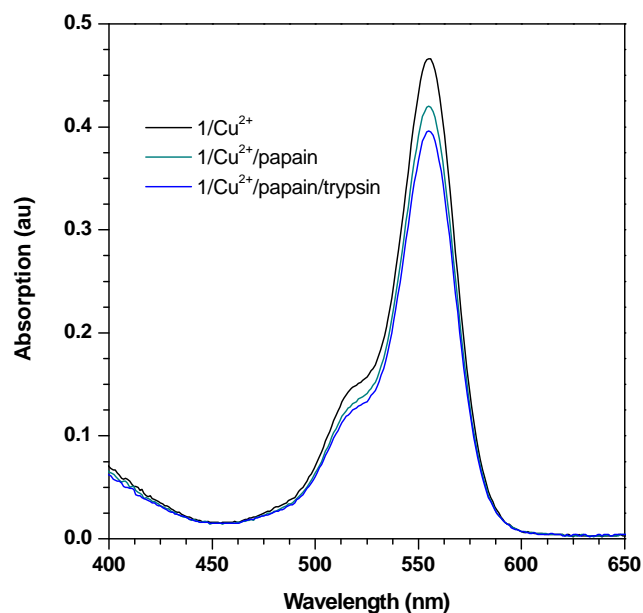


Figure S34. UV-vis spectra of **1**/Cu²⁺/papain before and after incubation with trypsin in Tris-HCl (10 mM, pH=7.0) buffer containing 50% (v/v) water/CH₃CN. The concentration of **1** was 1.0×10^{-5} mol/L, [Cu²⁺] = 7.0×10^{-6} mol/L, [papain] = 10 μ g/mL, [trypsin] = 3.0 μ g/mL.

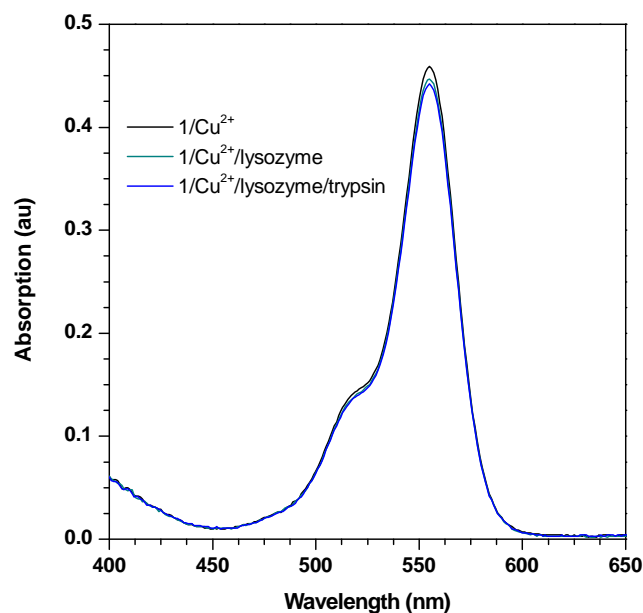


Figure S35. UV-vis spectra of **1**/Cu²⁺/lysozyme before and after incubation with trypsin in Tris-HCl (10 mM, pH=7.0) buffer containing 50% (v/v) water/CH₃CN. The concentration of **1** was 1.0×10^{-5} mol/L, [Cu²⁺] = 7.0×10^{-6} mol/L, [lysozyme] = 10 μ g/mL, [trypsin] = 3.0 μ g/mL.

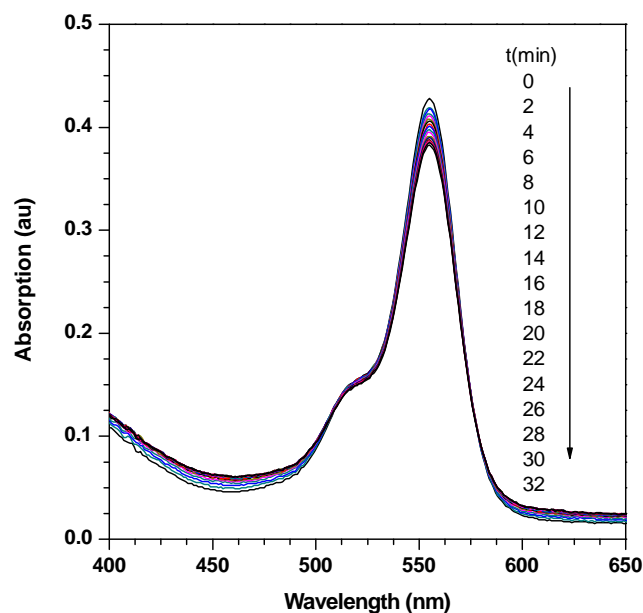


Figure S36. UV-vis spectra of **1**/ Cu^{2+} /BSA as a function of pepsin digestion time in Tris-HCl (10 mM, pH=7.0) buffer containing 50% (v/v) water/ CH_3CN . The concentration of **1** was 1.0×10^{-5} mol/L, $[\text{Cu}^{2+}] = 7.0 \times 10^{-6}$ mol/L, $[\text{BSA}] = 10 \mu\text{g/mL}$, $[\text{pepsin}] = 3.0 \mu\text{g/mL}$.

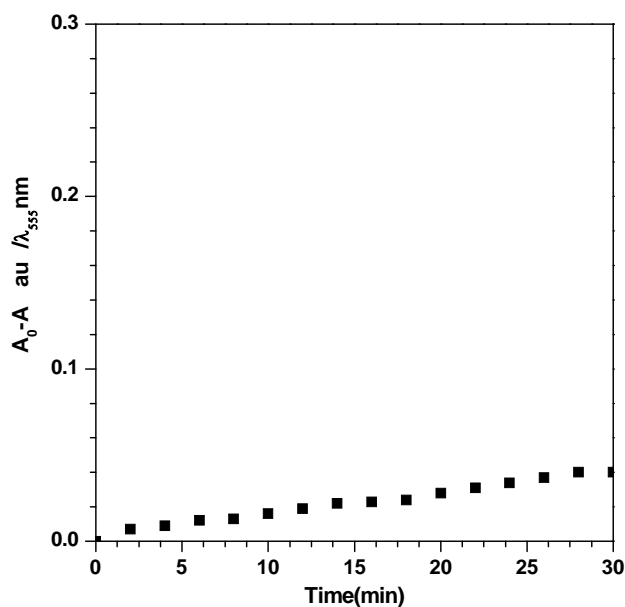


Figure S37. Absorption difference at 555 nm on the concentration of pepsin with fixed concentrations of **1**, Cu^{2+} ions, and BSA in Tris-HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/ CH_3CN . The concentration of **1** was 1.0×10^{-5} mol/L, $[\text{Cu}^{2+}] = 7.0 \times 10^{-6}$ mol/L, $[\text{BSA}] = 10 \mu\text{g/mL}$, $[\text{pepsin}] = 3.0 \mu\text{g/mL}$.