Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry

The Switch-on Luminescence Sensing of Histidine-riched Proteins in Solution: A Further Application of a Cu^{2+} Ligand

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(Supporting Information)

Experimental Section

All chemicals were obtained from commercial suppliers and used without further purification. Mass spectra were measured on a QTRAP mass spectrometer (Applied Biosystems Sciex Foster city, USA). Fluorescence spectra were measured on a Shimadzu RF-5301PC fluorescence spectrophotometer. Infrared (IR) spectra were recorded on a Bruker Vertex 80V FTIR spectrophotometer.

1. Synthesis of target probe 1

The synthesis of **1** was started with free glycine (**2**), which was firstly phthaloylated with phthalic anhydride to give N-phthalimido-protected amino acid **3** in a high yield. The resulted pure **3** was coupled with amino methyl pyridine to give peptide analog **4** directly without further purification. Hydrazinolysis of the phthalimido protected **4** produced the crucial amine intermediate **5**, which was unstable to heat or prolonged storage at room temperature. Free amine **5** was immediately mixed with dansyl chloride in CH_2Cl_2 solution to give the molecule **1**.

1.1. Phthalimidoglycine (3).

Firstly, the mixture of phthalic anhydride (1.63g, 11 mM) and glycine (0.75g, 10mM) was fused at 140 °C for 0.5 h. And then it was cooled to room temperature and the resulted solid was recrystallized from ethanol/water solution. After filtrating, drying in vacuum produced compound **3** (1.95g, 95%) as a light yellow solid. ^{1}H NMR (500 MHz, d_6-DMSO): δ = 4.32 (s, 2H, CH_2), δ = 7.89-7.96 (m, 4H, Pht-H). Mass spectral data (ESI): for $C_{10}H_7NO_4$, calcd. 205.04, found 206.05

1.2. 2-(1,3-dioxoisoindolin-2-yl)-N-(pyridin-2-ylmethyl)acetamide (4) .

The obtained pale yellow solid **3** was dissolved in a mixture of 1:20 (v/v) DMF and CH_2Cl_2. To the solution, 1.5 equiv. Et_3N and 1.1 equiv. pyridin-2-ylmethanamine were added with stirring. When all the reagents dissolved, 1.5 equiv. EDC was added, and the mixture was stirred for 5-6 h at room temperature and the progress of the reaction was monitored by TLC. After dilution with CH_2Cl_2 the mixture was washed with saturated brine. The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The resulted

residue was recrystallized from ethanol to give a white solid **4**. ^{1}H NMR (500 MHz, d_6-DMSO): δ = 4.31 (s, 2H, $CH_2CONHC\mathit{H}_2$), δ = 4.31 (s, 2H, $C\mathit{H}_2CONHCH_2$), δ = 7.28-8.87 (m, 8H, Pht-H+ Py-H), δ = 8.51 (s, 2H, $CH_2CON\mathit{H}CH_2$). Mass spectral data (ESI): for $C_{16}H_{13}N_3O_3$, calcd. 295.10, found 296.1

1.3. 2-amino-N-(pyridin-2-ylmethyl)acetamide (5).

To a solution of pseudodipeptide **4** in EtOH, hydrazine monohydrate was added. The mixture was stirred at room temperature for 72 h, and then it was cooled to 0°C and phthalhydrazide was removed by filtering. The filtrate was concentrated and the resulted oil was redissolved in a minimum amount of EtOH. The solution was cooled to 0°C, filtered and evaporated under reduced pressure. The resulted pale yellow oil **5** was immediately used for the next reaction, without further purification.

1.4. 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-N-(pyridin-2-ylmethyl) acetamide (1).

In an ice bath, a solution of dansyl chloride in CH_2Cl_2 was added slowly to a mixture of **5** and triethylamine in CH_2Cl_2 (30ml) and then was stirred for 0.5 h. The reaction was kept stirring for 12 h at room temperature, and the progress of the reaction was monitored by thin layer chromatography (TLC). After dilution with CH_2Cl_2, the mixture was washed with saturated brine. The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. Purification of 1 was performed by using column chromatography on silica gel. ^{1}H NMR (500 MHz, d_6-DMSO): δ = 2.81 (s, 26, $C\mathit{H}_3NC\mathit{H}_3$), δ = 3.53 (s, 2H, $CH_2CONHC\mathit{H}_2$), δ = 4.25 (s, 2H, $C\mathit{H}_2CONHCH_2$), δ = 7.15-8.45 (m, 12H, dansyl-H+ Py-H+CON*H*+$SO_2N\mathit{H}$). ^{13}C NMR (500 MHz, d_6-DMSO): δ = 45.0 ($\mathit{C}H_2CONHCH_2$), δ = 46.5 ($\mathit{C}H_3N\mathit{C}H_3$), δ = 47.9 ($CH_2CONH\mathit{C}H_2$), δ = 118.0-156.2 (dansyl-C+ Py-C), δ = 172 ($CH_2\mathit{C}ONHCH_2$). Mass spectral data (ESI): for $C_{20}H_{22}N_4O_3S$, calcd. 398.14, found 399.1

2 Measurements of fluorescence spectra

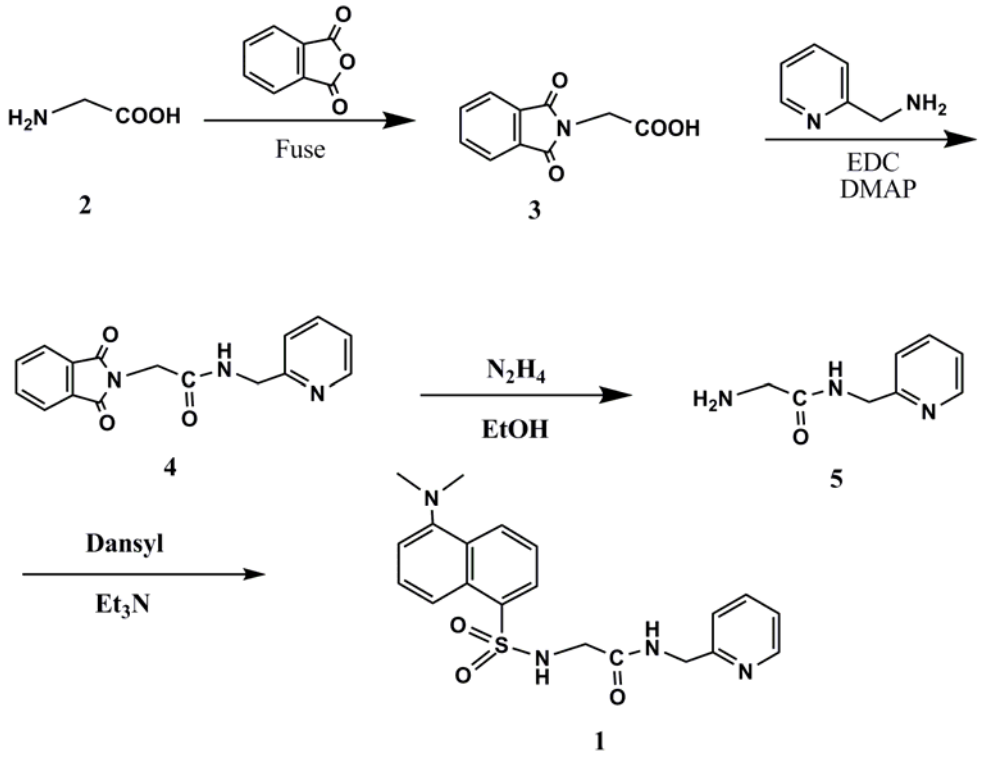
Fluorescence emission spectra were recorded on a Shimadzu (Japan) RF-5301PC fluorescence spectrophotometer. To reduce the fluctuation in the excitation intensity during measurement, the lamp was kept on for 1 h and the samples were stock-still for 3 min prior to the experiment. Samples for emission measurement were contained in a 1 cm × 1 cm quartz cuvettes (4 mL volume). All spectroscopic measurements of **1** were performed in 10.0 mM HEPES buffer solution (pH 7.4), and the concentrations of **1** in all the fluorescent experiments are 20.0 μM. A fixed excitation wavelength at 330 nm was used.

3 Quantum chemical simulations

To investigate the interaction of Cu^{2+} with probe **1** (dansly-Gly-Py), density functional theory calculations were performed with the B3LYP functional and the mixed "Double-ζ" quality basis sets 6-31G(d) for C, H, O, N, S and LANL2DZ for Cu. Optimization of the complexes was performed without any constraints for all the complexes. All calculations were carried out using Gaussian 09[1].

References:

1. Gaussian 09, Revision A.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.



Scheme S1. Synthesis route of probe **1**.

Spectroscopic data

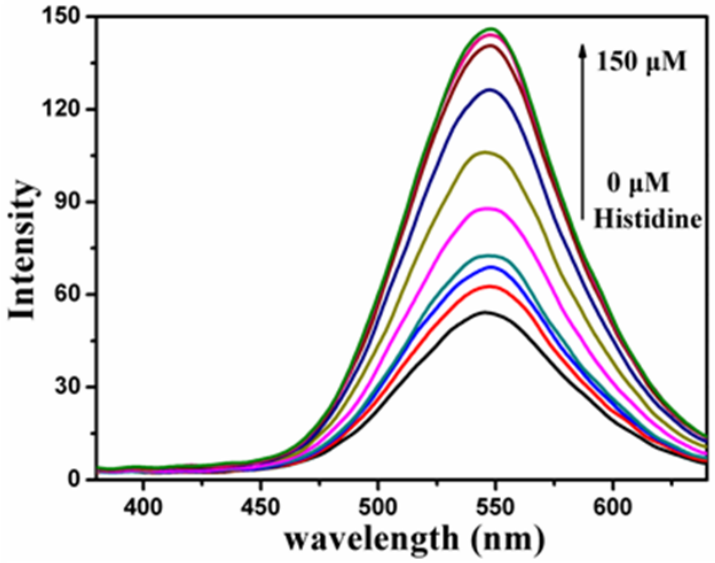


Fig. S1 The fluorescence intensity changes of **DGT**/Cu^{2+} (20 μM : 40 μM) upon the addition of histidine in buffer solution, indicating that the fluorescence intensity of **DGT** can be released after the coordinated Cu^{2+} were extracted by histidine.

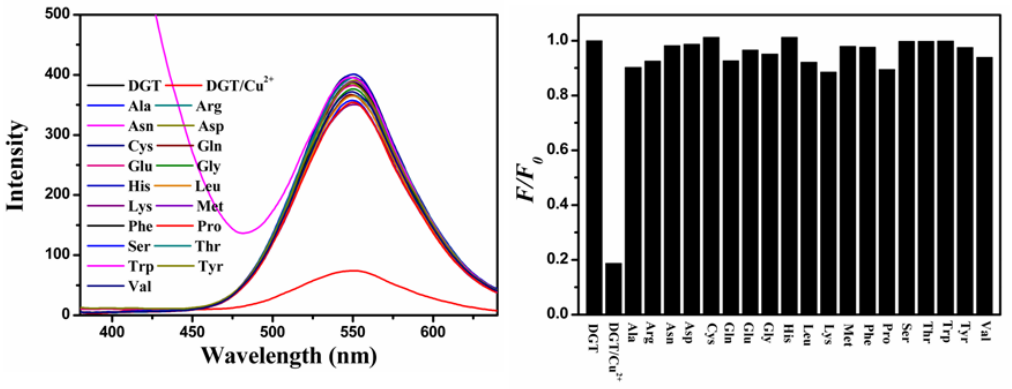


Fig. S2 The fluorescence intensity changes of **DGT** (20 μM : 40 μM) upon the addition of various amino acids in buffer solution, indicating that the fluorescence intensity of **DGT** can be released after the coordinated Cu^{2+} were extracted by amino acids.

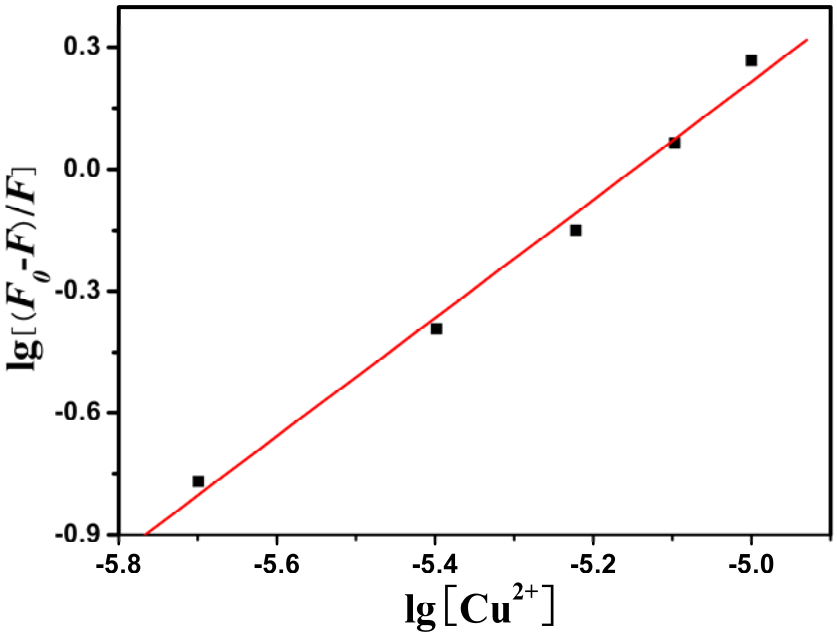


Fig. S3 Estimation of banding constant for **1** and Cu^{2+} in buffer solutions. The plot based on the fluorescence intensity changes at a maximal emission peak with a 1:1 bind model, supplying a banding constant of 3.98×10^{6} M^{-1} for the complex of **1**/Cu^{2+}.

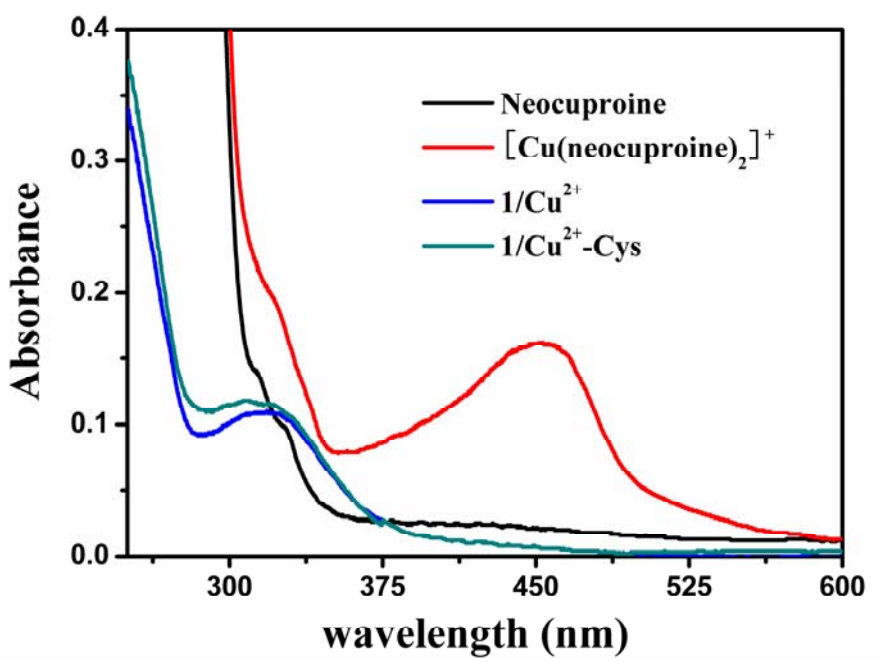


Fig. S4 The absorption spectra of **1** (20 μM) and 1 equiv. Cu^{2+} in the absence or presence of 1 equiv. DTT in 10 mM pH 7.4 HEPES buffer, and the further addition of neocuproine. For comparison, the absorption spectrum of neocuproine was also displayed.

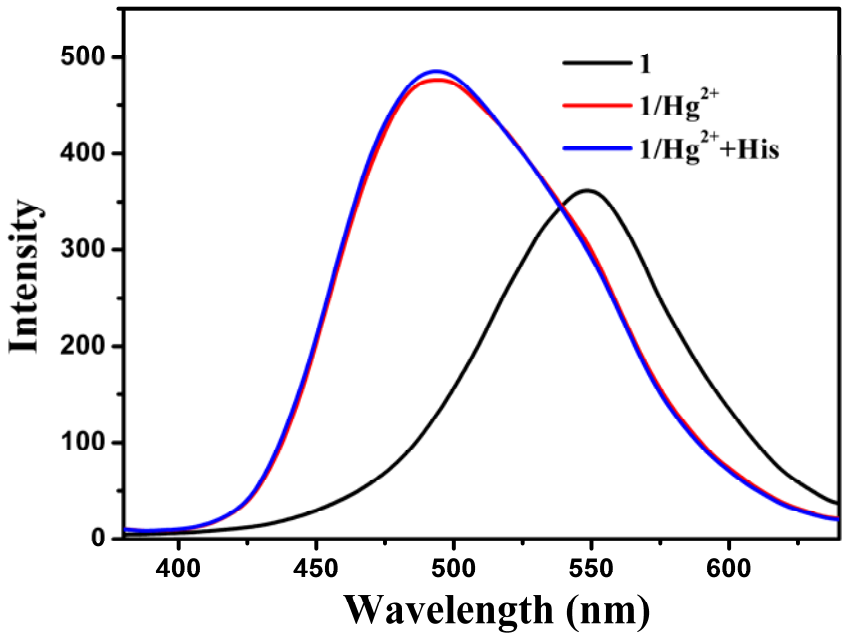


Fig. S5 The fluorescence intensity changes of **1**/Hg^{2+} (20 μM : 20 μM) upon the addition of histidine in buffer solution, indicating that histidine can not extract Hg^{2+} from **1**/Hg^{2+} complex.