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Metal-Assisted Selective Recognition of Biothiols with a Synthetic Receptor Array

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Electronic Supplementary Information

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1. General Information

Cavitand **1**[1] and guest **3**[2] were synthesized according to previous reports. DSMI **2** (trans-4-[4-(dimethylamino)styryl]-1-methylpyridinium iodide) was purchased from Sigma-Aldrich (St. Louis, MO) and used as received. All thiol compounds (2-mercaptoethanol (2-ME), mercaptoacetic acid (MAA), mercaptosuccinic acid (MSA), L-cysteine (Cys), L-Homocystine (Hcy), 2-Mercaptoethylamine (MEA), 1,4-dithiothreitol (DTT) and L-Glutathione (GSH), cystine (an oxidized dimer of cysteine), H_2O_2 and buffer salts were also purchased from Sigma-Aldrich (St. Louis, MO). Divalent metal salts (all chlorides except for $Pb(NO_3)_2$ and $Hg(OAc)_2$) were purchased from Aldrich (St. Louis, MO) and Alfa Aesar (Tewksbury, MA), and they were used as received. Ultrapure water with electric resistance > 18.2 MΩ was produced by the Millipore Milli-Q water purification system (Billerica, MA). Molecular modeling (semi-empirical calculations) was performed using semi-empirical calculations (AM1) in SPARTAN.

2. Experimental Procedures

Fluorescence sensor array. In a typical experiment, 10 μL cavitand 1 (200 μM for DSMI **2** or 40 μM for Guest **3**), 10 μL DMSI **2** (15 μM) or Guest **3** (30 μM), 10 μL of 100 μM metal salt solution and 60 μL of Tris buffer (pH 7.4, 20 mM) were added to each microwell (96 well plate). After adding 10 μL of the biothiol solution (1 mM), the mixture was incubated for 15 mins at room temperature. The fluorescence was measured by Perkin Elmer Wallac 1420 Victor 2 Microplate Reader with the Ex/Em wavelengths at 440/605 nm for DSMI **2** or 530/605 for Guest **3**.

Detection of spiked thiol compounds in cell lysate. The breast cancer cells of MCF-7 were first washed twice with ice cold PBS and then harvested by standard trypsin treatment. The harvested cells were diluted to 1 x 10^6/ mL with PBS, and were physically lysed by a sonicator for 5 mins with following settings: Pulse On 10 seconds, Pulse Off 10 seconds, Probe Size 50 mm, Frequency 20 KHz, Power Rate 100%. Cell breakage was con-firmed with an optical microscope. Cys, Hcy and GSH were spiked to the cell lysate and mixed well with the **1•2•**Cu^{2+} sensor before fluorescence measurement.

Data analysis. Principle component analysis (PCA) and Hierarchical clustering analysis (HCA) were completed with for R programs (version 3.3.2). PCA was performed using the standard "princomp()" function, with default settings. HCA was performed in two steps: the

Euclidean distance between two objects was calculated and stored in a matrix; then the matrix was input into the built-in HCA function “hclust()”, and the result was drawn with the R “plot()” function. The 95% confidence ellipses were drawn with the data obtained from PCA using Matlab (version R2016b) and a self-developed script. The full Matlab script is available upon request.

3. Supporting Figures

a) Screening of the effects of different metals on recognition of thiol compounds.

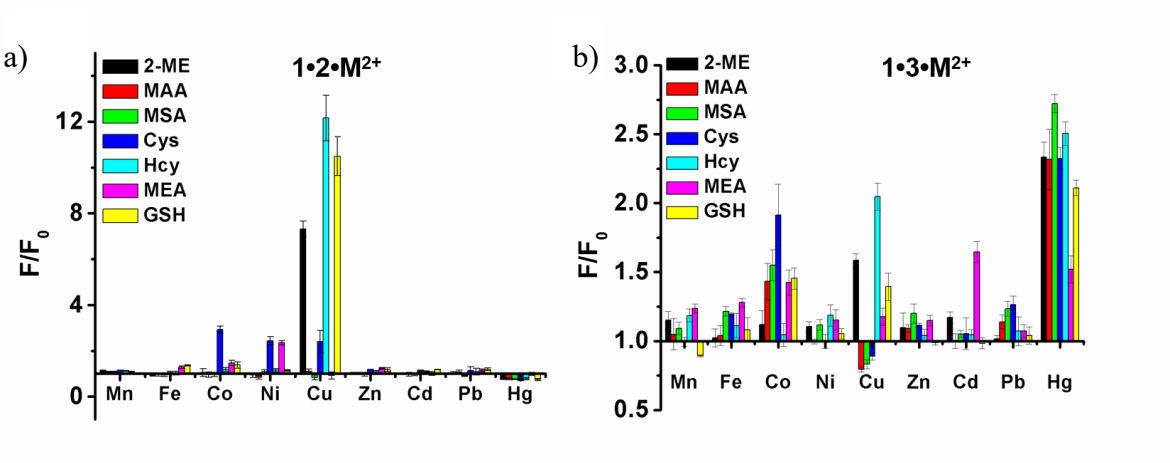


Figure S-1. Biothiol induced fluorescence change. a) **1•2** with 10 metals; b) **1•3** with 10 metals. Sensor **1•2•M**$^{2+}$: [**1**] = 20 μM, [**2**] = 1.5 μM, [metal] = 10 μM; Sensor **1•3•M**$^{2+}$: [**1**] = 4 μM, [**3**] = 3 μM, [M^{2+}] = 10 μM; [biothiol] = 100 μM for all, pH = 7.4.

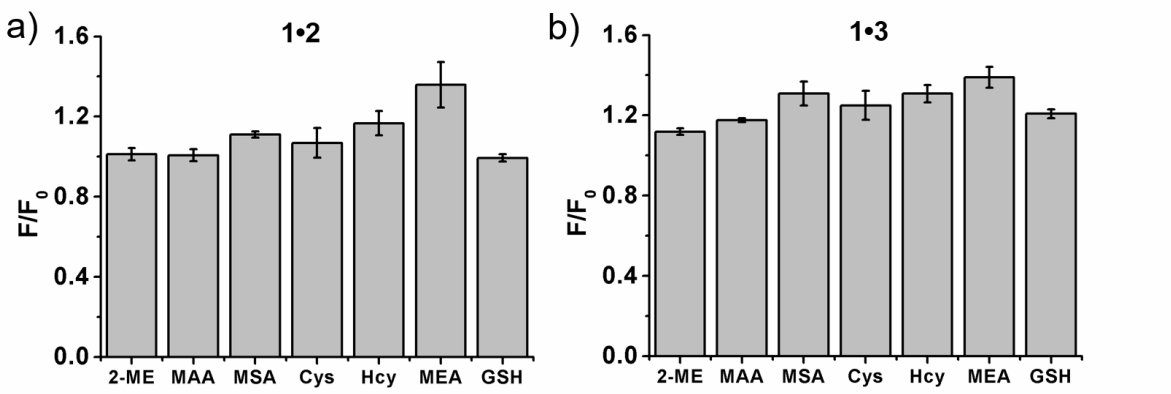


Figure S-2. Biothiol influence on cavitand+guest without metal. a) biothiol with **1•2**; b) biothiol with **1•3**. Sensor **1•2**: [**1**] = 20 μM, [**2**] = 1.5 μM; Sensor **1•3**: [**1**] = 4 μM, [**3**] = 3 μM; [biothiol] = 100 μM for all, pH = 7.4.

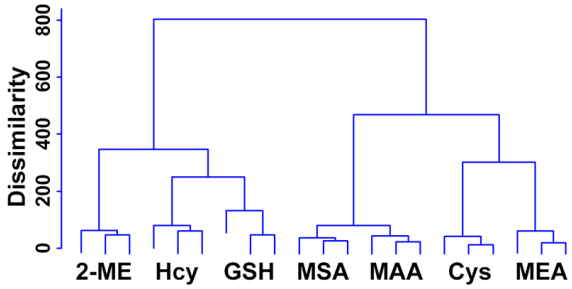


Figure S-3. Hierarchical cluster analysis of the LMW biothiols using a 4-compoinent array that added multiple metal ions to **1•2** and retained only **1•3•Cu**$^{2+}$.

b) MALDI-MS analysis of the effect of added thiols on [1•Cu$_2$] in solution.

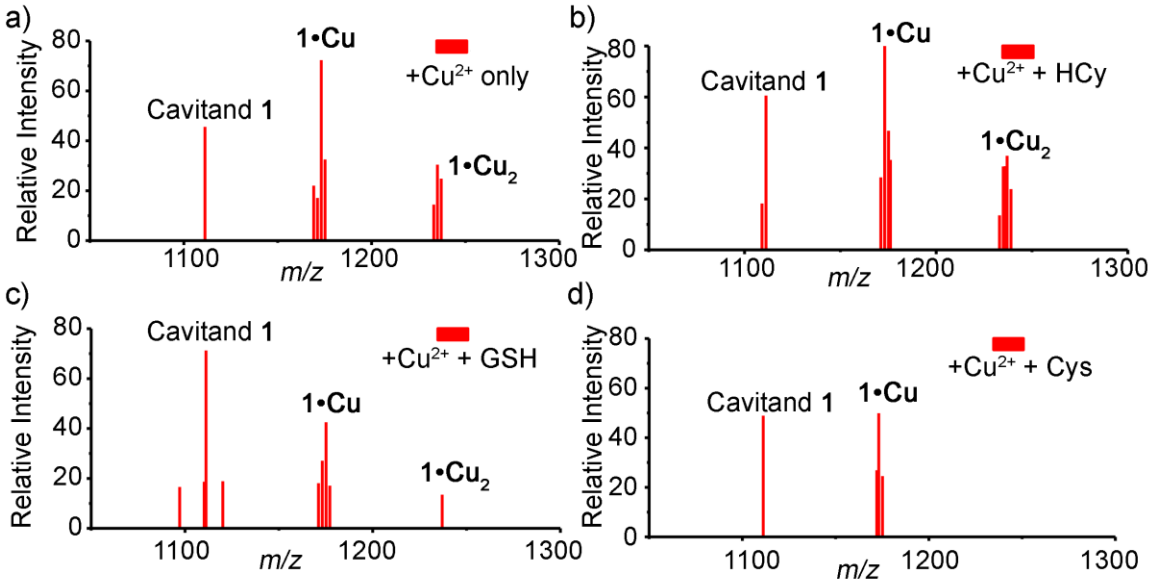


Figure S-4. SELDI-MS spectra of the cavitand **1** complex with Cu^{2+} ions in the presence of thiols. SELDI spectra of **1** and a) Cu^{2+} only, indicating presence of ion peaks for **1**, **1•Cu** and **1•Cu$_2$**; b) Cu^{2+} + Hcy; c) Cu^{2+} + GSH; d) Cu^{2+} + Cys. Host:RSH ratio 1:5, [**1**] = 20μM, pH 7.4, 20 mM Tris buffer.

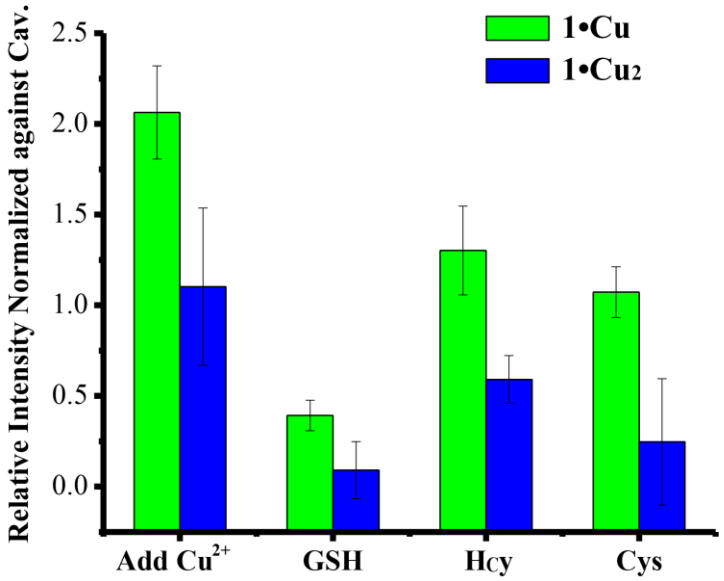


Figure S-5. Tabulated relative intensities of **1•Cu** and **1•Cu$_2$** from the MALDI experiments in Fig S-4, normalized to intensity of **1** alone, indicating lower proportions of **1•Cu$_{1/2}$** in the presence of RSH.

c) Response curves for different thiols by individual sensors.

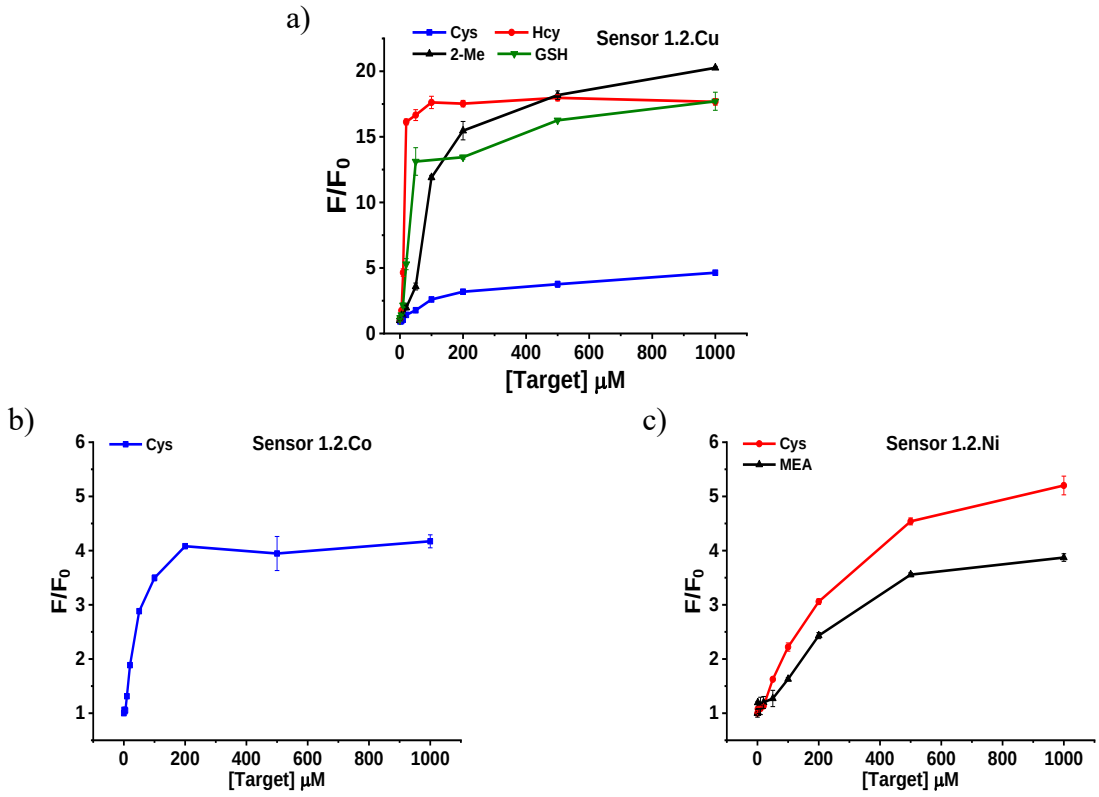


Figure S-6. Fluorescence response of biothiols with **1•2•M**$^{2+}$ in tris buffer. Sensor **1•2**: [**1**] = 20 μM, [**2**] = 1.5 μM. In all three sensors, [M^{2+}] = 10 μM, pH = 7.4.

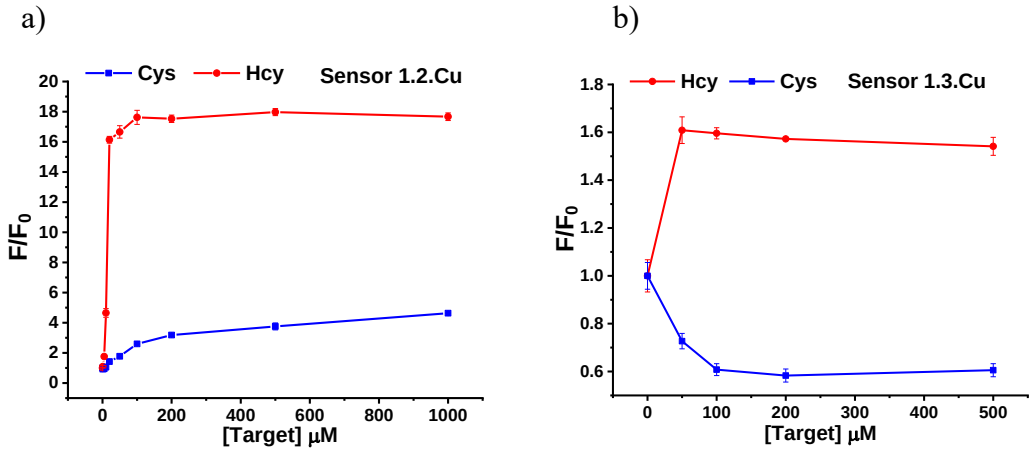


Figure S-7. Fluorescence response of Cys and Hcy with sensor **1•2•Cu**$^{2+}$ and **1•3•Cu**$^{2+}$. Sensor **1•2**: [**1**] = 20 μM, [**2**] = 1.5 μM; Sensor **1•3**: [**1**] = 4 μM, [**3**] = 3 μM. In both sensors, [Cu^{2+}] = 10 μM, pH = 7.4.

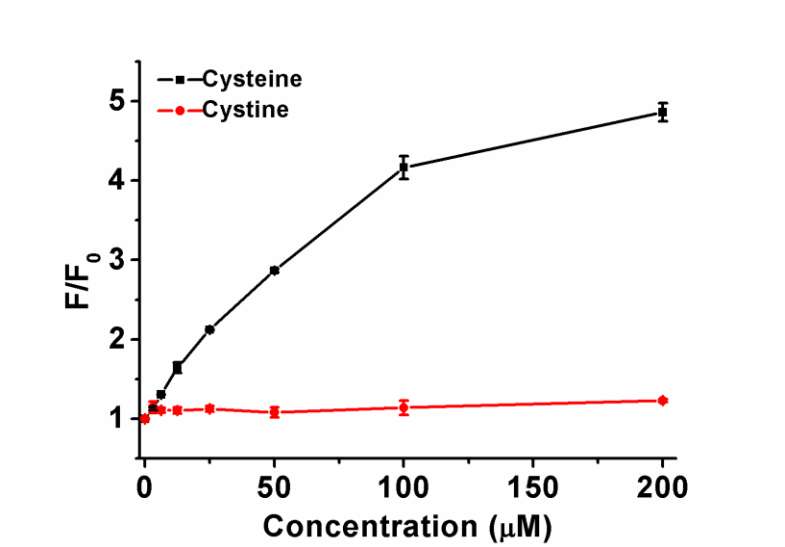
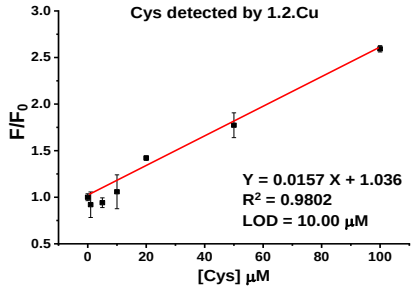


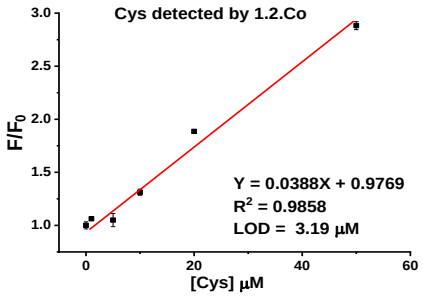
Figure S-8. Detection of Cysteine and Cystine using sensor **1•2•Co^{2+}**. [**1**] = 20 μM, [**2**] = 1.5 μM, [Co^{2+}] = 10 μM, pH = 7.4.

d) Calibration curves for detection of specific thiol compounds using various sensors.

a)



b)



c)

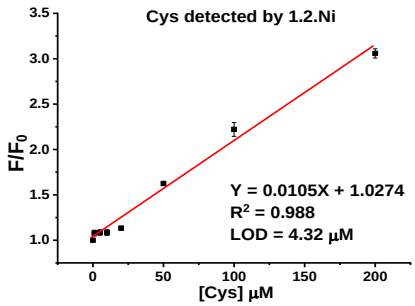


Figure S-9. Calibration curves of Cys with a) **1•2•Cu^{2+}**; b) **1•2•Co^{2+}**; c) **1•2•Ni^{2+}**. Sensor **1•2**: [**1**] = 20 μM, [**2**] = 1.5 μM; in all three sensors, [M^{2+}] = 10 μM, pH = 7.4.

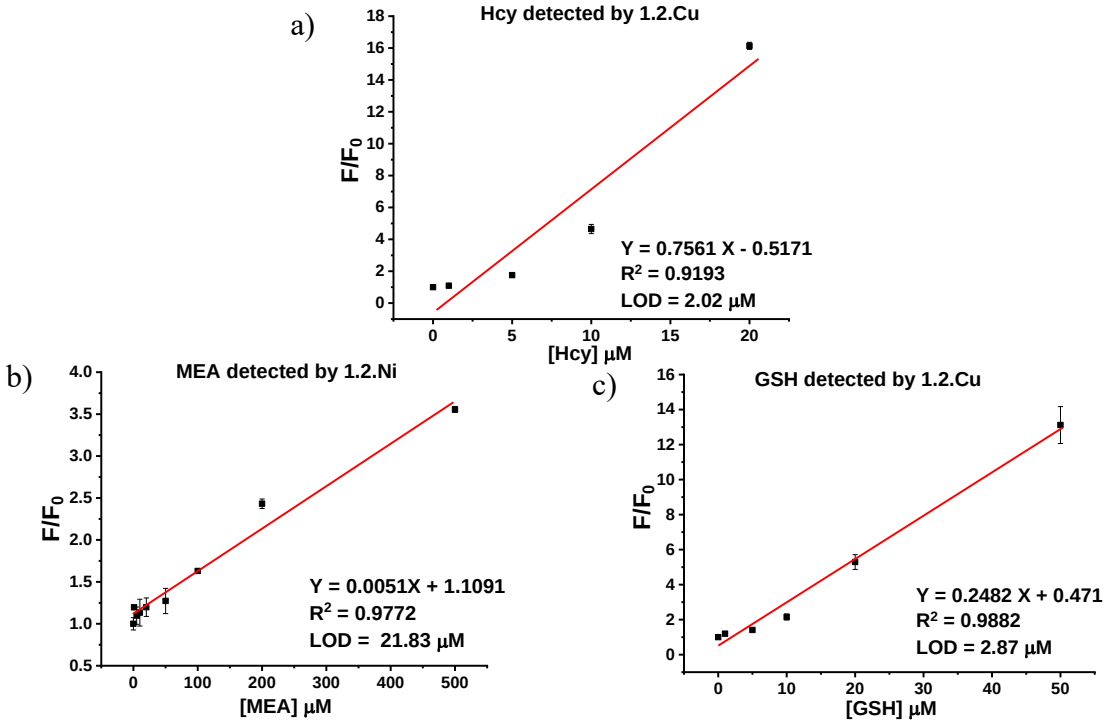


Figure S-10. Calibration curves of other biothiols with **1•2•M**$^{2+}$. a) Hcy with **1•2•Cu**$^{2+}$; b) GSH with **1•2•Cu**$^{2+}$; c) MEA with **1•2•Ni**$^{2+}$. Sensor **1•2**: [**1**] = 20 µM, [**2**] = 1.5 µM; in all three sensors, [M^{2+}] = 10 µM, pH = 7.4.

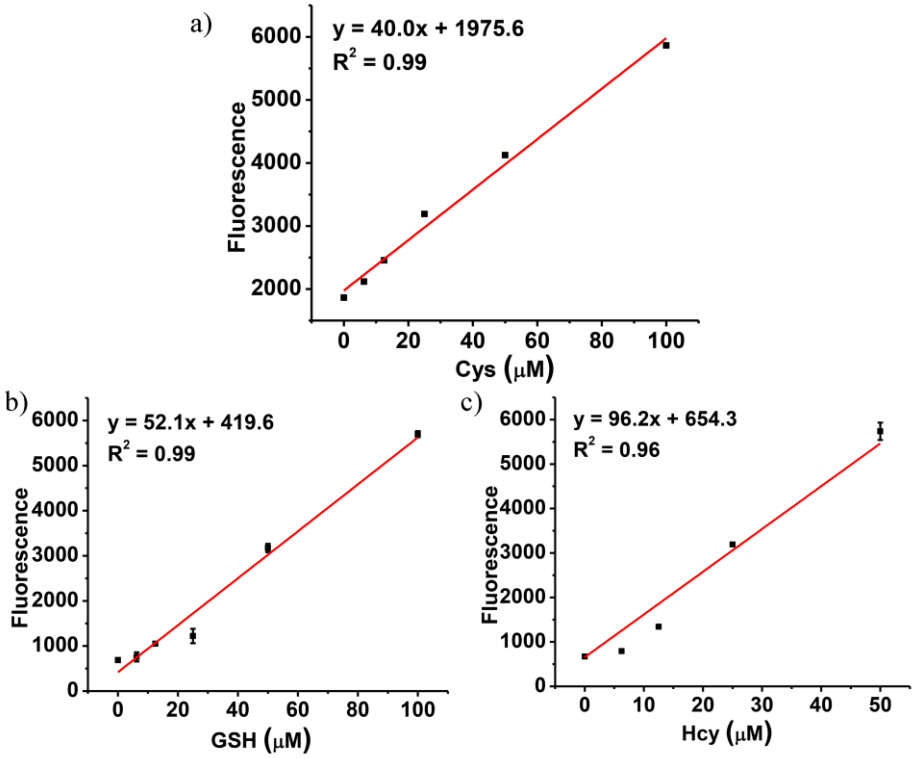


Figure S-11. Calibration curves of a) Cys with **1•2•Co**$^{2+}$, b) GSH with **1•2•Cu**$^{2+}$ and c) Hcy with **1•2•Cu**$^{2+}$ in an MCF-7 cell lysate (1x10^6 cells/mL). [**1**] = 20 µM, [**2**] = 1.5 µM, [M^{2+}] = 10 µM, pH = 7.4.

4. Supporting Tables

Table S-1. Factor loadings table for Figure 1 data.

Factor loadings	PC1 (45.8%)	PC2 (32.7%)
Guest 2 with Co^{2+}	-0.523	-0.118
Guest 2 with Ni^{2+}	-0.506	-0.113
Guest 2 with Cu^{2+}	0.327	-0.55
Guest 3 with Co^{2+}	-0.457	-0.316
Guest 3 with Cu^{2+}	0.338	-0.552
Guest 3 with Cd^{2+}	-0.201	-0.515

5. References

1. S. M. Biros, E. C. Ullrich, F. Hof, L. Trembleau and J. Rebek, *J. Am. Chem. Soc.*, 2004, **126**, 2870.

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