Supporting information:

Assembly, Two-Photon Absorption and Bioimaging Applications of A Cuprous Cluster

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1. Synthesis of (E)-(4-diethylanilinostyryl)pyridine (L):

A mixture of 11.30 mL (0.12 mol) of tert-butanol, 0.78 g (0.02 mol) of potassium was introduced into a 50 mL one-necked flask fitted with a condenser. After the reaction, a total of 0.98 mL (10 mmol) 4-picoline, 1.77 g (10 mmol) of 4-diethylaminobenzaldehyde were added. The mixture was heated for 2 hours at 80 °C under stirring; tert-butanol was evaporated, and then added to 200 mL dichloromethane, washed once with 200 mL ice water, and stirred. The aqueous phase was extracted three times with dichloromethane. The organic phase was dried on sodium sulfate, the solvent was evaporated, and the crude solid was recrystallized in toluene. Yield: 86.10%. ^{1}H NMR (500 MHz, DMSO-d_6, δ): 8.43 (d, J = 5.25 Hz, 2H, py H), 7.42 (t, J = 8.85 Hz, 2H, py H), 7.23 (d, J = 5.37 Hz, 2H, CH=CH), 7.03 (d, J = 16.07 Hz, 1H, Ar H), 6.70 (d, J = 16.07 Hz, 1H, Ar H), 6.27-6.66 (m, 2H, Ar H), 3.39 (q, 4H, CH_2), 1.13 (t, 6H, CH_3). ^{13}C NMR (500 MHz, $CDCl_3$, ppm): δ 148.56-146.96 (m, py), 134.57 (s, py), 128.86 (s, benzene), 120.51-119.95 (d, benzene), 111.56 (s, CH=CH), 44.47 (s, CH_2), 12.62 (s, CH_3). Anal. Calcd. for $C_{17}H_{20}N_2$ (EI-MS: 253.3): C, 80.91; H, 7.99; N, 11.10. Found: C, 80.76; H, 7.91; N, 11.01; IR data (KBr, cm^{-1}): 3026 w, 1581 s. Single crystals of the ligand suitable for X-ray analysis were obtained by slow evaporation of dichloromethane/benzene mixture solution at room temperature for five days.

2. Single-photon fluorescence quantum yield (Φ)

Quantum yield (Φ) of **$Cu_4I_4L_4$** and **L** in various solvents were determined with reference method. Rh6G (Φ = 0.90 in ethanol) was used as the reference standard while RhB was used to calibrate the system. In order to avoid possible aggregation and self-absorption, the concentrations of all solutions are 1.0×10^{-5} moL / L. The quantum yield was calculated according to the equation [S-(1)]

$$\Phi_S = \Phi_r \left(\frac{A_r(\lambda_r)}{A_s(\lambda_s)} \right) \left(\frac{I(\lambda_r)}{I(\lambda_s)} \right) \left(\frac{{n_s}^2}{{n_r}^2} \right) \frac{\int F_s}{\int F_r}$$

where, Φ is the quantum yield, n is the refractive index, $I(\lambda)$ is the relative intensity of the exciting light at wavelength λ, $A(\lambda)$ is the absorbance of the solution at the exciting wavelength λ, and $\int$F represents the integrated area under the corrected emission spectrum. Subscripts s and r refer to the sample and reference solutions, respectively. Quantificational measurements as illustrated in Table S5 that the quantum yields (Φ) of $Cu_4I_4L_4$ is enhanced compared with that of L.

3. Photophysical Determinations of the Interactions with Calf-thymus DNA Between $Cu_4I_4L_4$ and the Ligand.

To gain further insights into $Cu_4I_4L_4$ stained the cell nucleus, photophysical determinations of the interactions of ct-DNA between the ligand and complex were carried out to investigate the DNA binding ability. The absorption spectra of these experimental samples were insensitive to compounds (L and $Cu_4I_4L_4$) concentrations in Tris–HCl buffer upon addition of calf-thymus DNA. However, the emission spectra of EB bound to DNA in the presence of the compounds $Cu_4I_4L_4$ in Tris–HCl buffer exhibit clearer enhancement than that of ligand L in Tris–HCl buffer near 600 nm. Moreover, $Cu_4I_4L_4$ could induce more evident disturbances on DNA base stacking than ligand (Figure S8), reflecting the impact of the moiety on DNA binding modes.

Table S1 Selected bond lengths (Å) and angles (°) for **the ligand** and cluster **$Cu_4I_4L_4$**.

L			
N(1)-C(1)	1.324(7)	N(1)-C(5)	1.338(7)
N(2)-C(11)	1.371(6)	N(2)-C(14)	1.459(7)
N(2)-C(16)	1.462(7)	C(3)-C(6)	1.465(6)
C(6)-C(7)	1.325(6)	C(7)-C(8)	1.455(7)
C(1)-N(1)-C(5)	115.2(5)	C(11)-N(2)-C(14)	121.8(5)
C(11)-N(2)-C(16)	122.7(5)	C(14)-N(2)-C(16)	115.0(4)
C(2)-C(3)-C(6)	120.2(5)	C(7)-C(6)-C(3)	126.8(5)
C(6)-C(7)-C(8)	128.0(5)	C(9)-C(8)-C(7)	124.0(5)
$Cu_4I_4L_4$			
Cu(1)-N(1)	2.058(10)	Cu(2)-N(3)	2.032(11)
Cu(1)-I(1)	2.627(2)	N(1)-C(1)	1.243(17)
Cu(1)-I(2)	2.683(2)	N(1)-C(5)	1.30(2)
Cu(2)-I(2)	2.616(2)	N(3)-C(18)	1.31(2)
Cu(2)-I(1)	2.612(2)	N(3)-C(22)	1.308(16)
Cu(1)-Cu(2)	2.640(2)	Cu(1)-Cu(1)$^{\#1}$	2.659(3)
Cu(2)-Cu(2)$^{\#1}$	2.721(4)	Cu(2)-Cu(1)$^{\#1}$	2.732(3)
N(1)-Cu(1)-I(1)	111.9(3)	C(1)-N(1)-C(5)	118.7(13)
N(1)-Cu(1)-I(2)	108.5(4)	C(1)-N(1)-Cu(1)	119.1(11)
I(1)-Cu(1)-I(2)	107.97(7)	C(5)-N(1)-Cu(1)	122.1(10)
Cu(2)-I(1)-Cu(1)	60.53(5)	N(1)-C(1)-C(2)	120.0(15)
Cu(2)-I(2)-Cu(1)	59.76(5)	C(2)-C(3)-C(4)	113.9(14)

Symmetry transformations used to generate equivalent atoms: #1 x+1/2,-y+3/2,z; #2 x-1/2,-y+3/2,z.

Table S2 Crystallographic data and structure refinement summary for the ligand and **$Cu_4I_4L_4$.**

compound	**L**	**$Cu_4I_4L_4$**
formula	$C_{17}H_{20}N_2$	$C_{34}H_{40}Cu_2I_2N_4$
formula weight	252.35	885.58
T/K	298	298
Crystal system	Orthorhombic	Monoclinic
space group	*P*na2_1	$C_{2/c}$
a [Å]	12.717(8)	31.674(1)
b [Å]	15.016(9)	10.142(5)
c [Å]	7.578(5)	24.001(7)
β [°]	90	115.291(6)
V [Å^3]	1447.1(2)	6972(5)
Z	4	8
D_{calc} (gcm^{-3})	1.158	1.687
θ range [°]	2.10-25.01	1.81-25.02
Total no. of data	7135	15359
No. of unique data	1379	5490
No. of para refined	174	379
R_1	0.0462	0.0795
wR_2	0.1009	0.2434
GOF	1.082	1.012

$R_1=\Sigma \| Fo- Fc \| / \Sigma Fo ;\ wR_2{}^b=[\Sigma(w(Fo^2-Fc^2)^2)/\Sigma(w(Fo^2)^2)]^{1/2/K}$

Table S3. Cytotoxicity Data (MCF-7 cell, 2 h) [a]

Concentration	control	100 μM	200 μM	300 μM	400 μM
% Cell survival ($Cu_4I_4L_4$)	100 ±2	94 ±3	83 ±1	75 ±5	64 ±2
% Cell survival (L)	100 ±2	55 ±7	39 ±4	26 ±3	11 ±5

[a] Cell viability was quantified by the MTT assay (mean (SD).

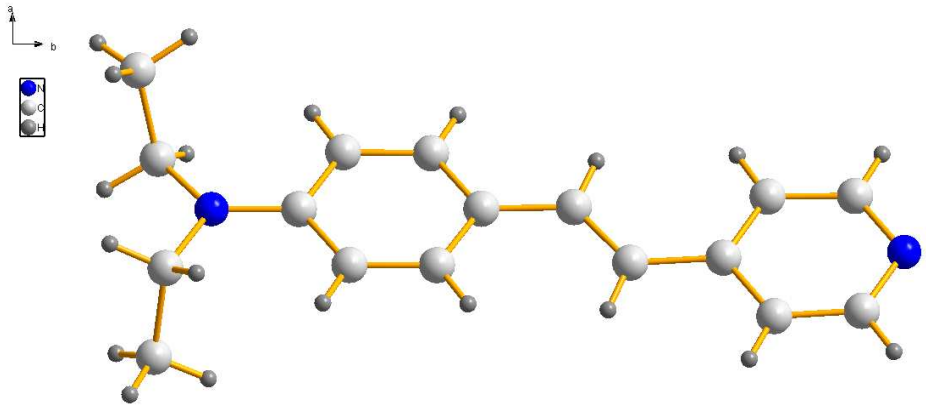

Figure S1. Crystal structure of L

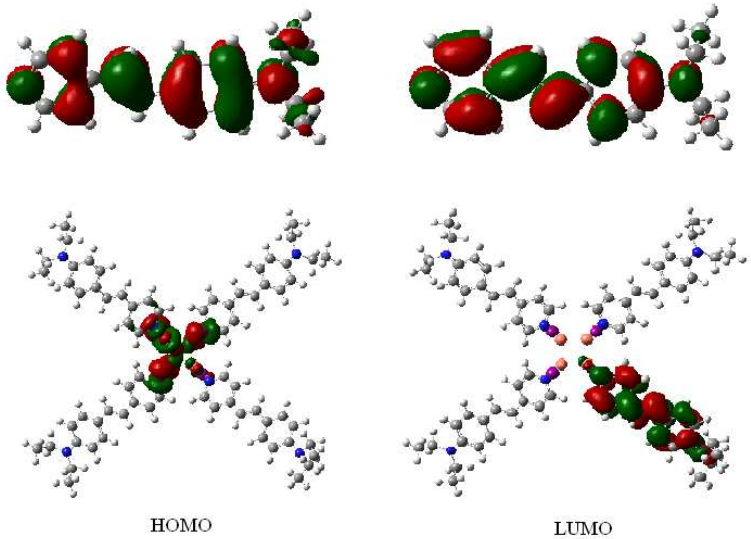

Figure S2. DFT computed frontier orbitals of L and $Cu_4I_4L_4$ obtained at the B3LYP level.

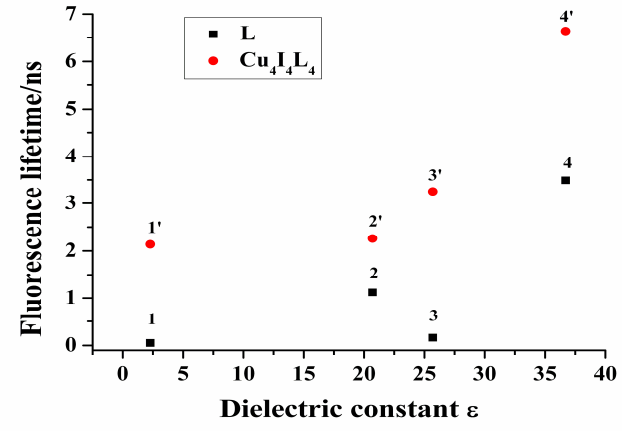

Figure S3. Fluorescence lifetimes of the ligand and $Cu_4I_4L_4$ (1.0×10^{-5} mol L^{-1}) in four solvents: benzene (1, 1'); Acetone (2, 2'); Ethanol (3, 3'); DMF (4, 4').

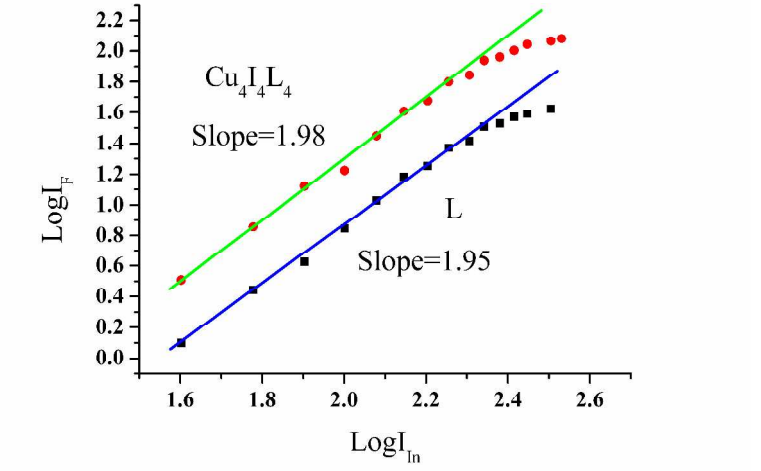

Figure S4. Log-Log linear of squared dependence of induced fluorescence signal of **$Cu_4I_4L_4$** and **L** in DMF solvent (1.0×10^{-4} mol L^{-1}) and incident irradiance intensity.

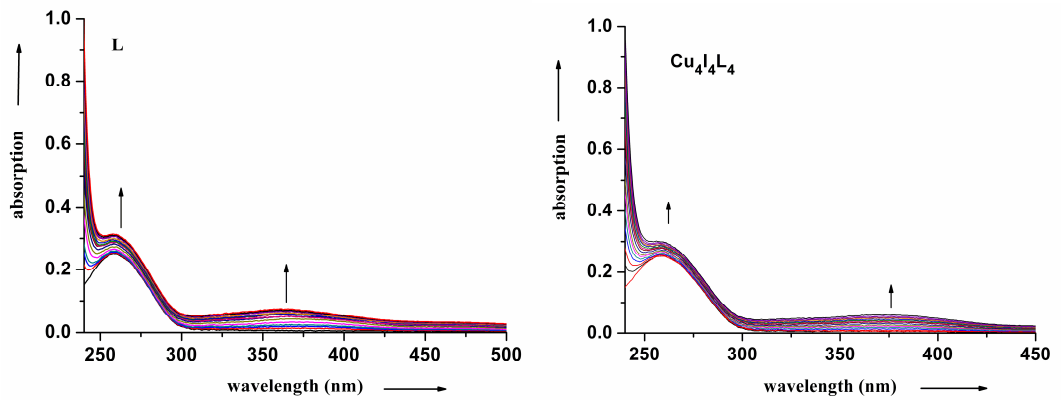

Figure S5. Absorption spectra of the compound **L** (left) and compound **$Cu_4I_4L_4$** (right) in Tris–HCl buffer (NaCl, pH = 7.2) in the presence of calf-thymus DNA. [DNA] = 2.0×10^{-5} mol L^{-1}, [compound] = 1.0×10^{-5} mol L^{-1}.

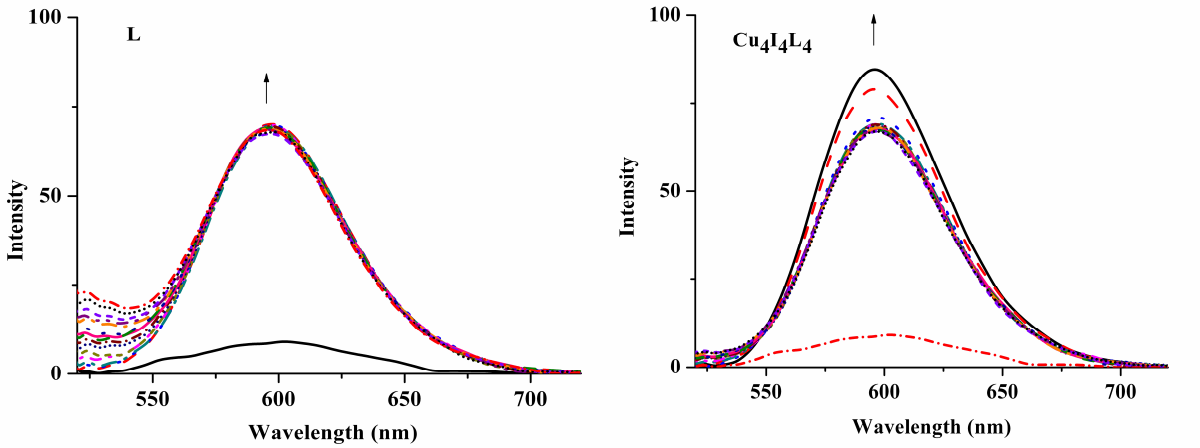

Figure S6. Emission spectra of EB bound to DNA in the absence and presence of the compounds **L** (left) and **$Cu_4I_4L_4$** (right) in Tris–HCl buffer (NaCl, pH =7.2). [EB] = 1×10^{-6} M; [DNA] = 2×10^{-5} mol L^{-1}; [compound] = 1×10^{-5} mol L^{-1}.

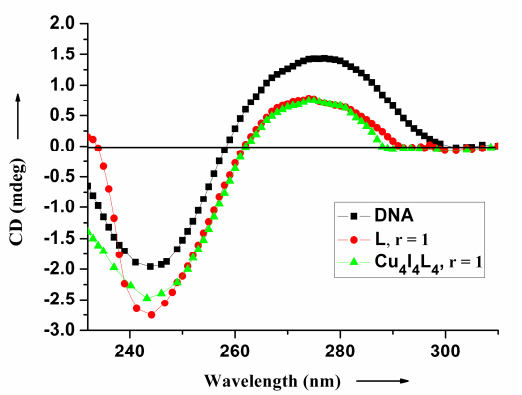

Figure S7. Induced CD spectra upon addition of ct-DNA to L and $Cu_4I_4L_4$ (1.0×10^{-5} mol L^{-1}) in fixed ratios (r = [DNA]/[L] or [$Cu_4I_4L_4$]), r = 1).

Circular dichroism spectra of the complexes, in 20 mM Hepes buffer pH 7 containing 30 mM NaCl, were measured using a JASCO J-820 CD spectrophotometer. Each sample solution (3000 μL, 3 mL) was obtained by adding the required volume of stock solution of calf thymus DNA and metal complex dissolved in the above Hepes buffer, and finally topping up the solution with an additional volume of the same buffer. Two sets of complex–DNA solutions were used: the ratio of the complex : DNA = 40 μM : 40 μM (1 : 1) in each solution.

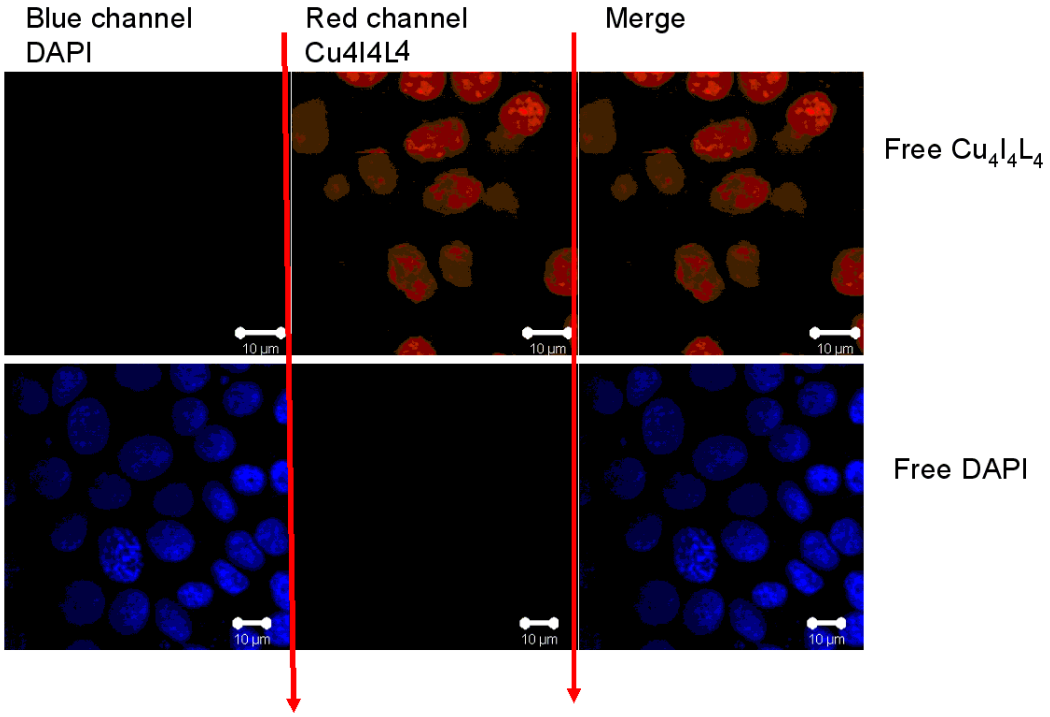

Figure S8. Control experiment for **$Cu_4I_4L_4$** and DAPI image indicted there is no affection between the **$Cu_4I_4L_4$** and DAPI.

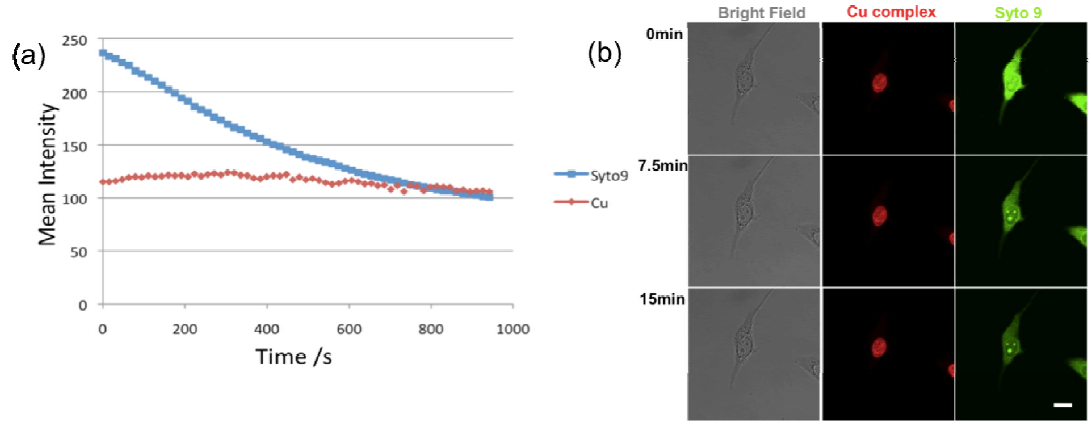

Figure S9. (a) Time series showing luminescence intensity of $Cu_4I_4L_4$ in a MCF-7 nucleus region (200μM, 2hours) under laser exposure over 15mins, compare with Syto 9, there is no obvious photon bleaching observed, data point obtain every 20 seconds. (b) Images from time point 0, 7.5 and 15 minutes, scale bars represent 10 μM.

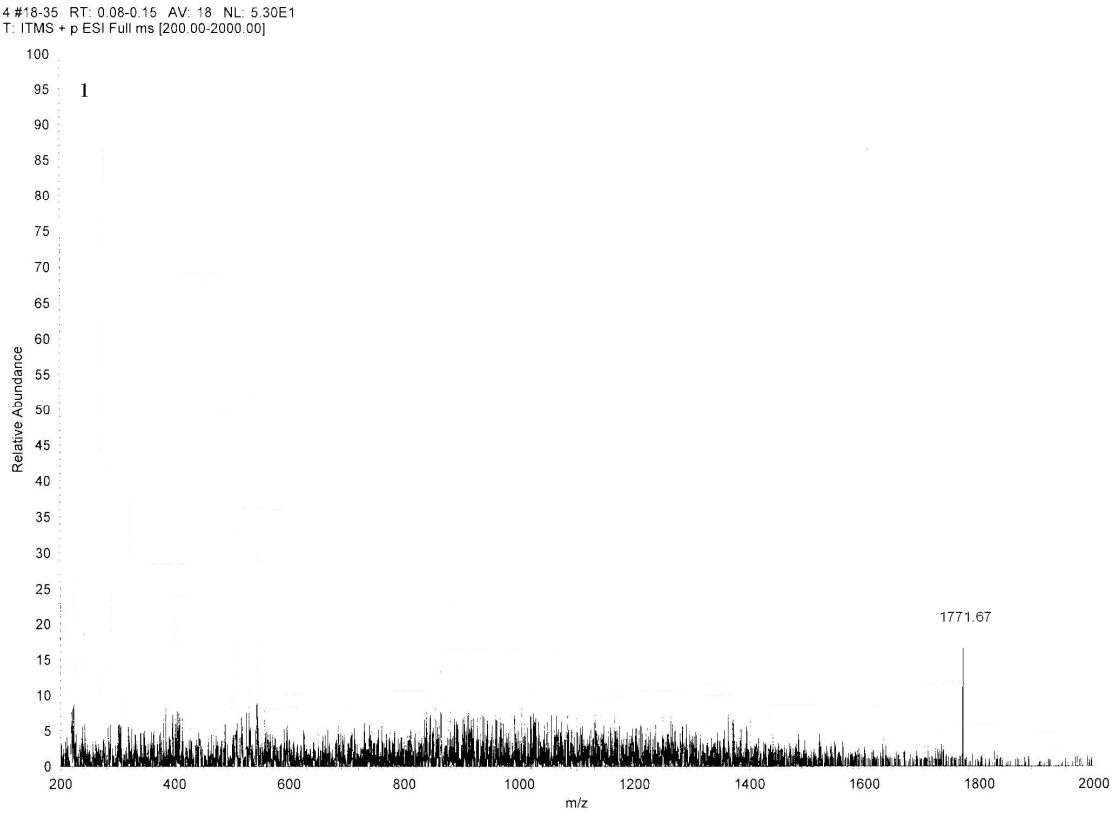

Figure S10. MALDI-TOF Mass spectrometry of $Cu_4I_4L_4$.

s-(1) Woo H. Y.; Liu B.; Kohler B.; Korystov D.; Mikhailovsky A.; Bazan G. C. *J. Am. Chem. Soc.* **2005**, *127*, 14721-14729.