Electronic Supplementary Material (ESI) for ChemComm.

Isomeric Effect of Mercaptobenzoic Acids on the Formation and Fluorescent Properties of Copper Nanoclusters

*Yi-Jyun Lin,⊥ Po-Cheng Chen,⊥ Zhiqin Yuan, Jia-Ying Ma, and Huan-Tsung Chang**

Department of Chemistry, National Taiwan University, 1, Section 4, Roosevelt Road, Taipei, 10617 (Taiwan)

⊥: the authors have equal contribution

Correspondence: Professor Huan-Tsung Chang, Department of Chemistry, National Taiwan University, 1, Section 4, Roosevelt Road, Taipei 106, Taiwan

Tel. and fax: 011-886-2-33661171

E-mail: changht@ntu.edu.tw

EXPERIMENTAL SECTION

Chemicals. Copper nitrate ($Cu(NO_3)_2 \cdot 3H_2O$) was obtained from Showa (Tokyo, Japan). TA was purchased from Acros (Morris Plains, NJ, USA). Dimethylformamide (DMF), 3-MBA, 4-MBA, and tetrahydrofuran (THF) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Monobasic, dibasic, and tribasic sodium salts of phosphate, as well as phosphoric acid were obtained from J.T. Baker (Phillipsburg, NJ, USA). Ultrapure water (18.25 MΩ cm) from a Milli-Q system of Millipore (Billerica, MA, USA) was used to prepare all aqueous and organic-aqueous solutions. All chemicals and solvents were of analytical grade and used without further purification.

Preparation of Cu NCs Aggregates. For the preparation of Cu NCs aggregates, TA, 3-MBA, or 4-MBA (0.1 M, 300 μL) in DMF was mixed with THF (600 μL) under vigorous shaking. Aliquots (100 μL) of 0.1 M $Cu(NO_3)_2$ in nitric acid (0.1 M) was then added to each of the mixtures. After vigorous shaking for 5 s, precipitates were formed in all of the three mixtures. The reaction was allowed to proceed for 30 min at 70 °C in the dark. After being cooled to ambient temperature (25 ^{o}C), TA-Cu NCs aggregates gradually became transparent. On the other hand, 3-MBA-Cu NCs and 4-MBA-Cu NCs aggregates were turbid. For simplicity, the concentrations of the three as-prepared Cu NCs aggregates are denoted as 1X.

Characterization of Cu NCs Aggregates. The three as-prepared Cu NCs aggregates were purified prior to conducting further analyses. To remove excess ligands, cooled Cu NCs aggregates were either separately subjected to dialysis against ultrapure water through a membrane with 100-500 Da molecular weight cutoff (Float-A-Lyzer G2) from Spectrum Laboratories (Rancho Dominiguez, CA, USA) for 48 h or centrifugation at 12000 rpm and wash with ultrapure water (5 × 1 mL) five times. These solutions were then purified through freeze-drying and re-dispersed in ultrapure water.

The fluorescence emission spectra of TA-Cu NCs, 3-MBA-Cu NCs, and 4-MBA-Cu NCs aggregates were recorded using a Cary Eclipse Varian spectrofluorometer (Walnut Creek, CA, USA) with excitation wavelengths at 338, 334, and 324 nm, respectively. A GBC Cintra 10e double-beam UV–Vis spectrophotometer (Victoria, Australia) was used to obtain the absorption spectra of these Cu NCs aggregates. For the determination of their Φ_f values, quinine sulphate ($\Phi_f = 0.54$) was used as a reference. The fluorescence lifetimes of these Cu NCs aggregates were measured using a photo-counting PicoHarp 300 system from PicoPicoQuant (Berlin, Germany) and a diode laser emitting at 375 nm (FluoTime 300) as the light source. Fluorescence images were recorded using an Olympus IX71 inverted microscope with a DP70 digital color camera from Olympus (Tokyo, Japan). As-prepared Cu NCs aggregates (10 μL) were placed separately on a thin glass slide that was then covered with a coverslip before their fluorescence images were taken.

Infrared spectra were recorded with a Nicolet iS5 Fourier transform infrared (FTIR) spectrometer from Thermo Scientific (Waltham, MA, USA). The binding energy spectra of as-prepared Cu NCs aggregates were collected with a VG ESCA210 X-ray photoelectron spectroscopy (XPS) from VG Scientific (West Sussex, UK), and all binding energies were calibrated by using C 1s (284.6 eV) as a reference. The samples for XPS measurements were prepared by depositing drops of these Cu NCs aggregates onto Si substrates and then evaporating the solvents at ambient temperature and pressure. Transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) images of as-prepared Cu NCs aggregates were taken using a JSM-1200EX II system from JEOL (Tokyo, Japan) and an FEI Tecnai-G2-F20 instrument equipped with a Philips

Technai G2 dispersive X-ray spectrometer, respectively. Thermogravimetric analysis (TGA) of Cu NCs aggregates was conducted on a TGA/DSC 1-Thermogravimetric Analyzer (Mettler Toledo, Switzerland) under N_2 atmosphere (flow rate of 50 mL min^{-1}).

Detection of Cyanide. TA-Cu NCs aggregates prepared at the Cu^{2+}-to-TA molar ratio of 7:3 were used for the detection of CN^-. The ratio allowed preparation of highly fluorescent TA-Cu NCs aggregates. To remove excess TA ligands, the TA-Cu NCs aggregate solution was concentrated to 4-fold using a rotary evaporator. Ultrapure water (5 mL) was used to transfer the TA-Cu NCs aggregates from the vial to a 100-500 D molecular weight cutoff membrane bag. Then the solution was subjected to dialysis against ultrapure water through the membrane for 2 h. The water was changed every 30 min. For simplicity, we denote the concentration of the as-prepared TA-Cu NCs aggregates as 4X. The water-dispersible TA-Cu NCs aggregates were stable in the dark at 4 °C for at least 4 weeks. Prior to use, TA-Cu NCs aggregates were diluted in the phosphate buffer solution.

A NaCN stock solution (0.1 M) was prepared, which was further diluted in phosphate buffer solutions (10 mM, pH 8.0) to 1 mM. Aliquots (0−100 μL) of the CN^- solution were mixed with phosphate buffer (950−850 μL, 10 mM, pH 8.0) and TA-Cu NCs aggregates (50 μL, 1 X), each with a final volume of 1.0 mL. The final concentrations of TA-Cu NCs aggregates in the mixtures were all 0.05X, while that for CN^- were 0−100 μM. After equilibration at ambient temperature for 1 h in the dark, the fluorescence spectra were recorded. To test the selectivity of the sensing system, the following anions (for simplicity, F^-, Cl^-, Br^-, I^-, Ac^-, $citrate^{3-}$, $EDTA^{2-}$, NO_2^-, NO_3^-, CO_3^{2-}, S^{2-}, BrO_3^-, SCN^-, SO_3^{2-}, SO_4^{2-}, IO_3^-, IO_4^{3-}, and ClO_4^- were denoted) at concentrations of at least 5 times higher than that of CN^- were added to the solutions with/without containing CN^- (0.1 μM).

Pond water samples collected from the NTU campus were filtered using a syringe filter (pore diameter 0.22 μm). Aliquots (500 μL) of the pond water samples spiked with standard CN^- solutions (1−100 μL), PB solution (499-400 μL) containing TA-Cu NCs aggregates were mixed, with final concentrations of 10 mM for PB, 0.01−1 μM for CN^-, and 0.05X for TA-Cu NCs aggregates. After equilibration at ambient temperature for 1 h in the dark, their fluorescence spectra were recorded.

Table S1. Zeta potential and sizes of TA-, 3-MBA-, and 4-MBA-Cu NCs aggregates

	TA-Cu NCs		**3-MBA-Cu NCs**		**4-MBA-Cu NCs**	
	Size* (nm)	Zeta potential (mV)	Size* (nm)	Zeta potential (mV)	Size* (nm)	Zeta potential (mV)
pH 3.0	409 ± 9	-3.4 ± 0.3	960 ± 52	-3.8 ± 0.5	4100 ± 87	-6.9 ± 0.3
pH 7.0	302 ± 8	-52.4 ± 3.6	507 ± 27	-42.7 ± 1.6	833 ± 83	-51.5 ± 1.9

*Sizes were determined from the DLS data.

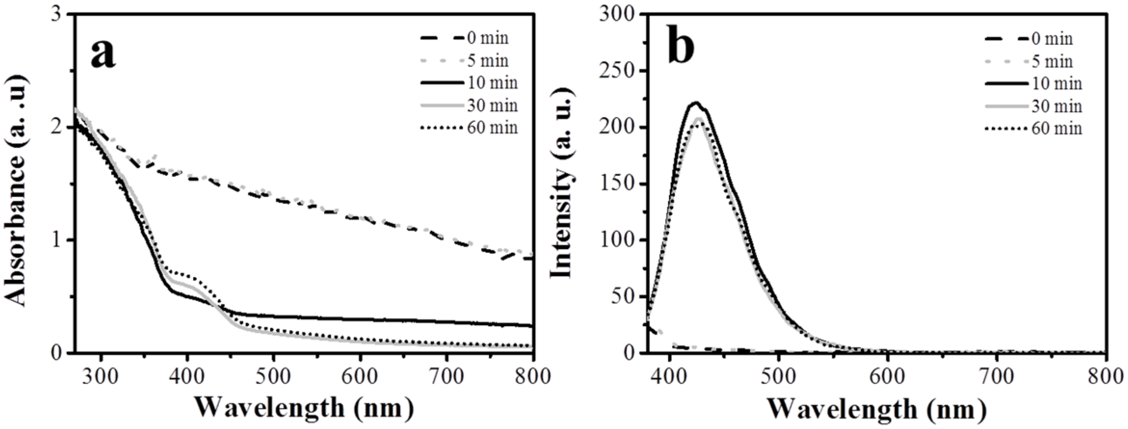

Figure S1. Time-evolution (a) absorption and (b) fluorescence spectra of TA-Cu NCs aggregates prepared at 70 °C. Excitation wavelength was set at 338 nm in (b).

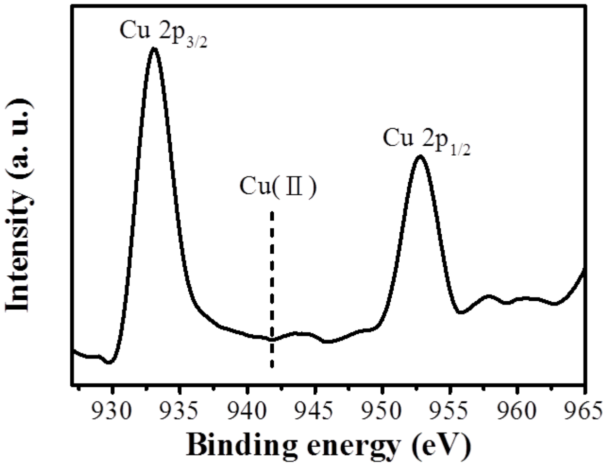

Figure S2. XPS spectrum (Cu 2p) of TA-Cu NCs aggregates. The dashed line shows the binding energy of Cu^{2+} 2p electrons. No satellite peak confirms that Cu^{2+} was not detected.

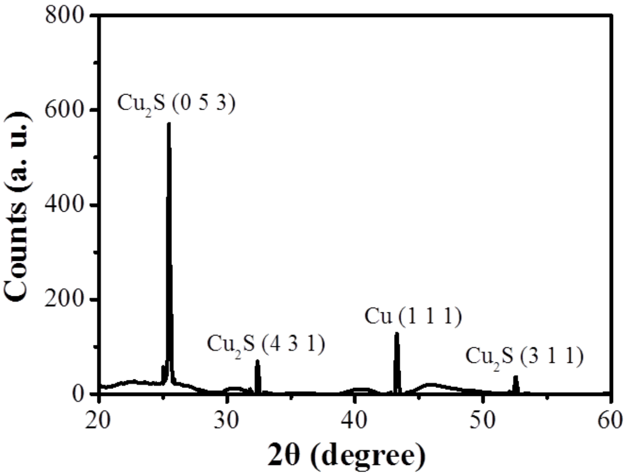

Figure S3. XRD pattern of the as-prepared TA-Cu NCs aggregates.

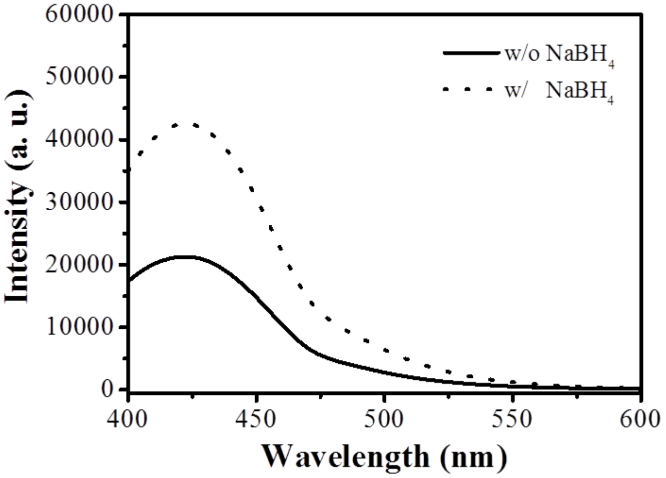

Figure S4. Fluorescence emission spectra of TA-Cu NCs aggregates in the absence and presence of 10 mM $NaBH_4$. Excitation wavelength was set at 338 nm.

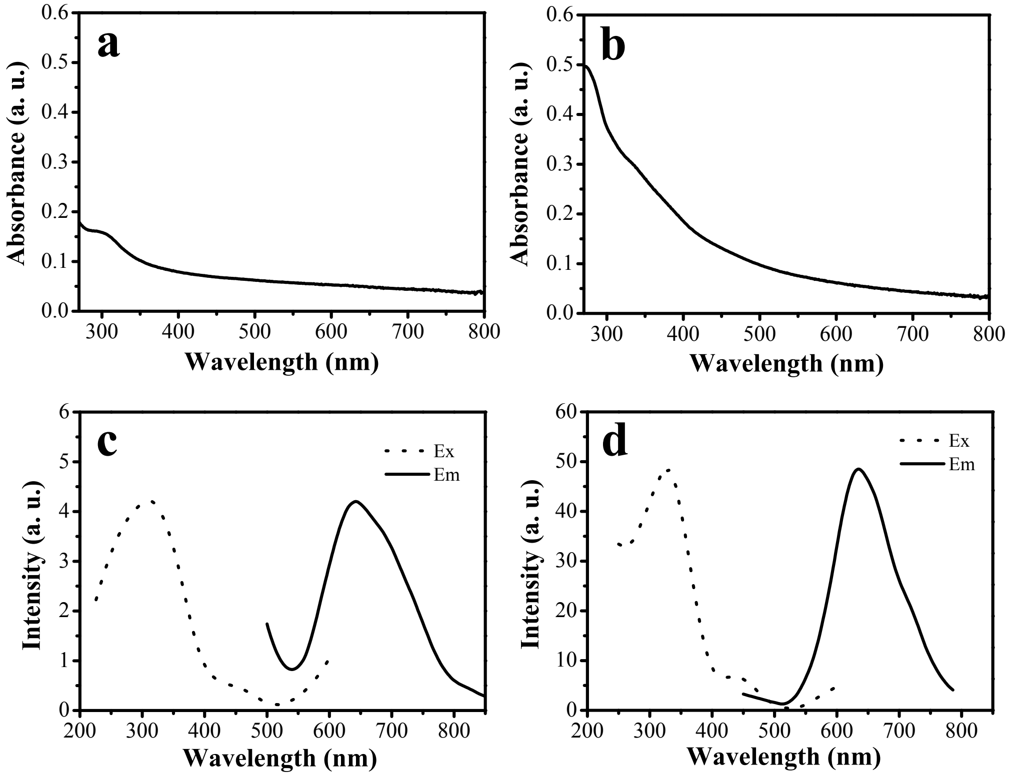

Figure S5. (a and b) Absorption and (c and d) emission and excitation spectra of 3-MBA- and 4-MBA-Cu NCs aggregates. (c, d) Excitation wavelengths of 3-MBA- and 4-MBA-Cu NCs were set at 334 and 324 nm, respectively; emission wavelengths of 3-MBA- and 4-MBA-Cu NCs were set at 668 and 646 nm, respectively.

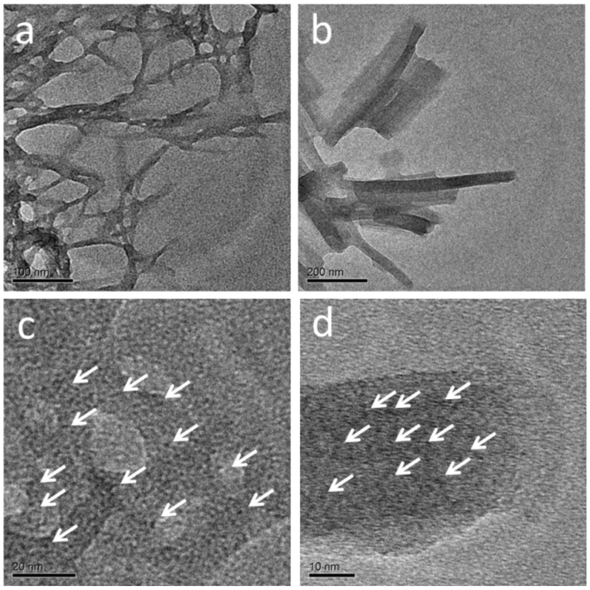

Figure S6. TEM images of (a) 3-MBA- and (b) 4-MBA- Cu NCs aggregates. Their corresponding enlarged images are displayed in (c) and (d), respectively. The individual Cu NCs are identified by the arrows.

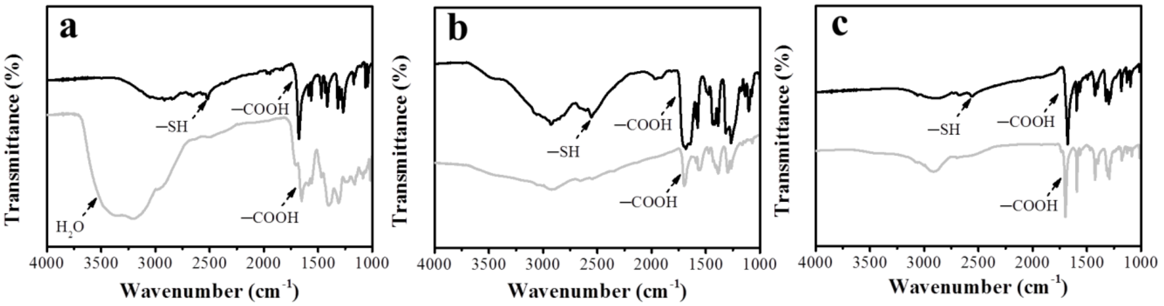

Figure S7. FTIR spectra of (a) TA-, (b) 3MBA- and (c) 4-MBA and their corresponding Cu NCs aggregates, respectively. Dark and gray curves are for organic acid and NCs aggregates, respectively.

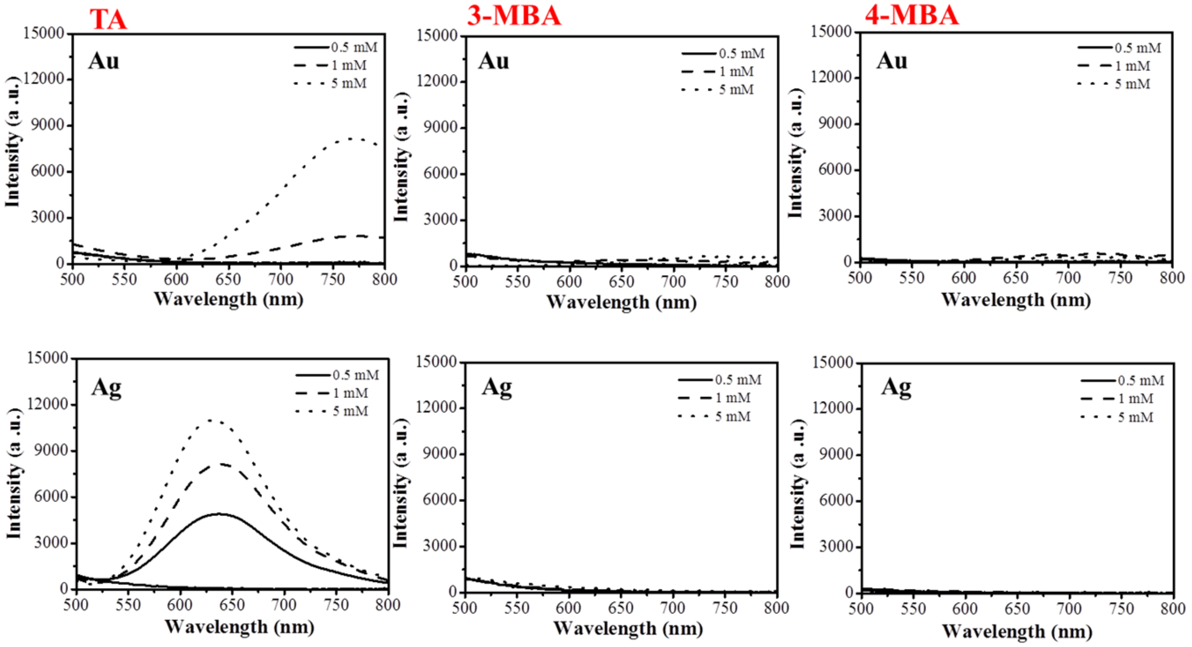

Figure S8. Fluorescence spectra of the products after reactions of Au^{3+} or Ag^{+} at various concentrations separately with TA, 3-MBA, and 4-MBA. The concentrations of TA, 3-MBA, and 4-MBA were all 30 mM.

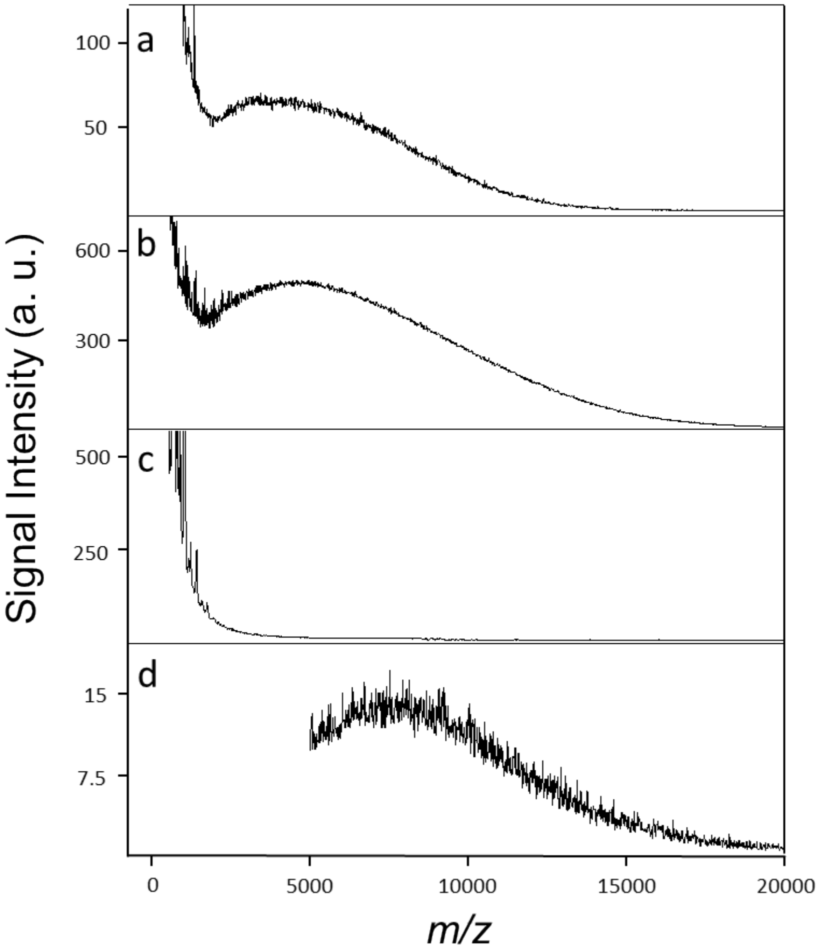

Figure S9. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) spectra of (a) TA-Cu NCs aggregates, (b) TA-Cu complexes, (c) TA molecules, and (d) TA-Cu NCs aggregates after they reacted with 10 mM $NaBH_4$.

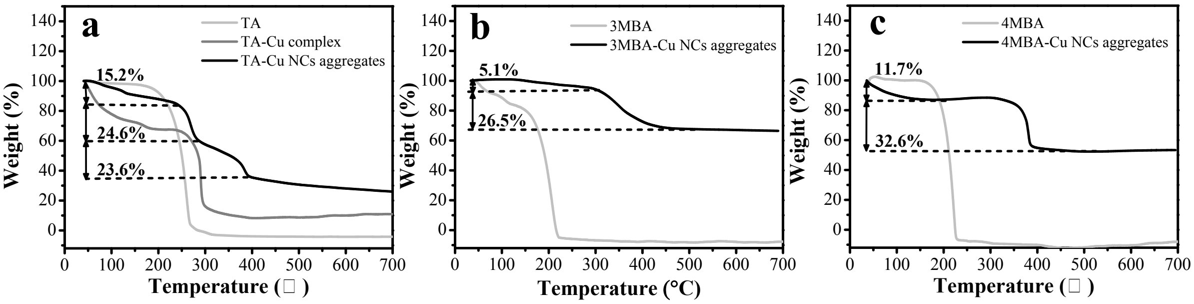

Figure S10. TGA curves of (a) TA-, (b) 3-MBA-, and (c) 4-MBA Cu NCs aggregates**.** As controls, TA, 3-MBA, 4-MBA and Cu-TA complexes were investigated.

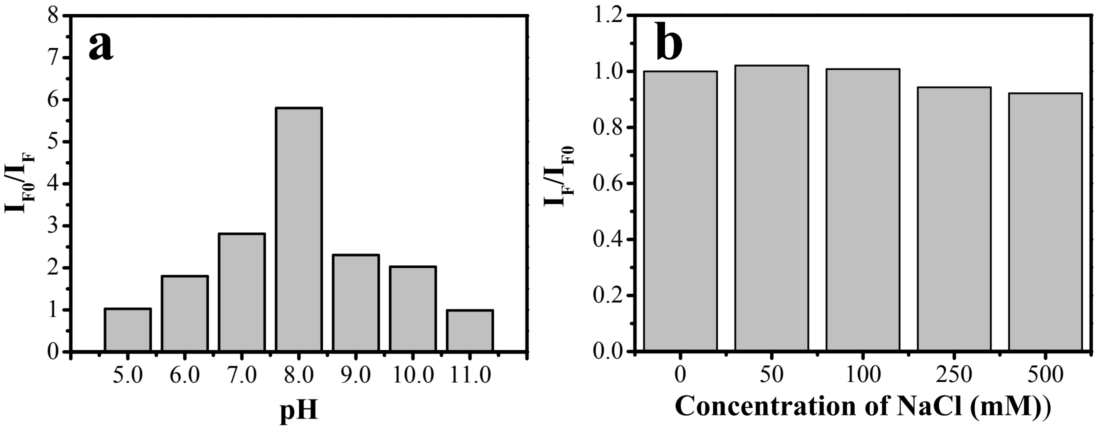

Figure S11. Effects of (a) pH for CN^- (10 μM) detection and (b) NaCl concentration on the fluorescence of TA-Cu NCs aggregates (0.05X) at 420 nm. (a) PB solutions (10 mM) at various pH values and (b) PB solutions (10 mM, pH 8.0) containing various concentrations of NaCl.

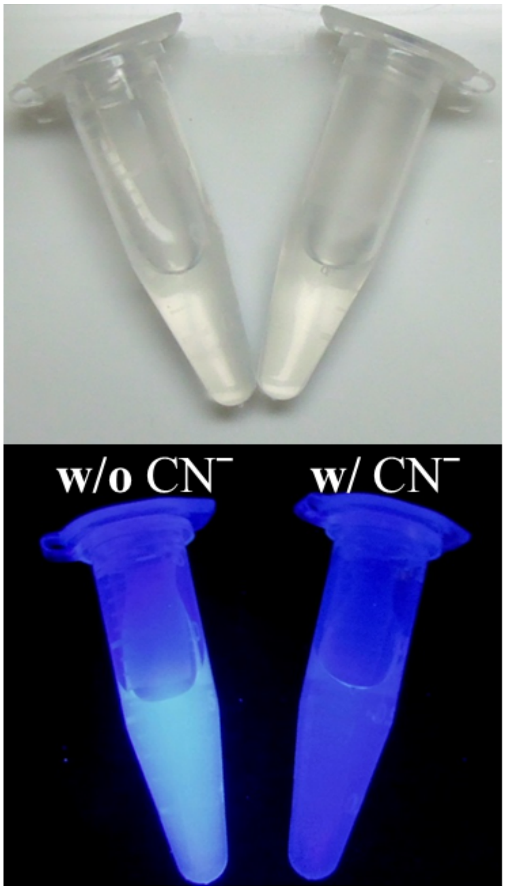

Figure S12. Photographs of TA-Cu NCs aggregates in the absence and presence of CN^- ions (1 uM) under room light (top) and UV irradiation (bottom).

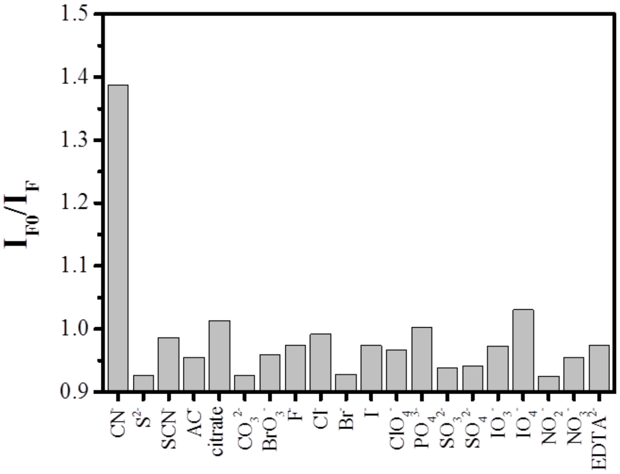

Figure S13. Selectivity of the TA-Cu NCs aggregates (0.05X) toward CN^-over other anions. The concentrations of CN^- and potential interferences are 100 and 500 nM, respectively. Mixtures were prepared in phosphate buffer (10 mM, pH 8.0) containing 20 mM Zn^{2+} ions. I_{F0} and I_F are the fluorescence intensities of the TA-Cu NCs aggregates in the absence and presence of CN^-or the potential interference, respectively.

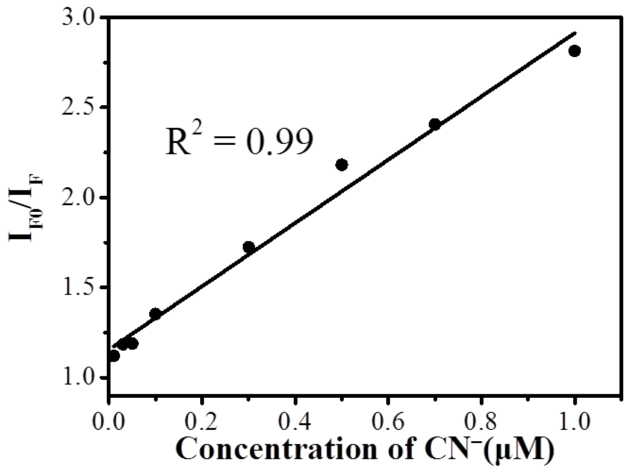

Figure S14. Detection of CN⁻ in pond water sample using TA-Cu NCs aggregates (0.05X). Aliquots of pond water were spiked with 0.01−1.0 μM CN⁻. Excitation wavelength was set at 338 nm.