# **Electronic Supporting Information**

# Synthesis of novel luminescent copper nanoclusters with substituent driven self-assembly and aggregation induced emission (AIE)

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# Materials and Methods

Synthesis of Copper nanoclusters (CuNCs) Synthesis of CuNCs@MI Synthesis of CuNCs@MMI and CuNPs@DMMI Quantum yield Cell culture conditions Cell viability assay Confocal microscopy **Figures S1-S10** 

#### **Materials and Methods:**

2-mercapto-1-methylimidazole ( $C_4H_6N_2S$ ) was obtained from Alfa Aesar. 2mercaptoimidazole and 1,3-diphenylisobenzofuran (DPBF) were purchased from Sigma Aldrich. Copper(II) sulfate pentahydrate (CuSO<sub>4</sub>.5H<sub>2</sub>O), hydrazines hydrate (NH<sub>2</sub>-NH<sub>2</sub>.H<sub>2</sub>O) and methanol were purchased from SDFCL. All reagents were of analytical grade and used as received. De-ionised water was used in all experiments.

FT-IR spectrum was recorded on Bruker Alpha. The spectra were obtained with OPUS software 7.0. <sup>1</sup>H NMR experiments were done using 400 MHz Bruker Advance NMR Spectrometer. Fluorescence spectra and UV-Visible spectra were recorded using Fluromax-4 spectrofluorometer and Shimadzu UV-Vis Spectrophotometer respectively. MALDI-TOF data were recorded on an UltrafleXtreme MALDI TOF/TOF (Bruker Daltonics). Transmission electron microscopy (TEM) images were recorded on JEOL transmission electron microscope operating at 200 KV. X-ray photoelectron spectroscopy (XPS) was acquired on a MULTLAB 2000 THERMO SCIENTIFIC, UK.

#### Synthesis of Copper nanoclusters (CuNCs):

Synthesis of CuNCs@MI: In a typical synthesis, 2-mercaptoimidazole (100 mg, 0.998 mmol) was added to CuSO<sub>4</sub>.5H<sub>2</sub>O (249.68 mg, 0.998 mmol) in 15 mL methanol solvent. The solution was allowed to stir under dark condition for one hour at 25°C, after which the solution color turns blue. The reduction of copper complex was carried out in the presence of hydrazine hydrate (485.04  $\mu$ L, 9.98 mmol) and the stirring was further continued for one hour. During the course of reaction the solution color changes from blue to pale green and finally dirty white. The resulting solution was washed with methanol and water to remove excess reactants and impurities. After washing, the nanoclusters were centrifuged, lyophilized and then stored in an amber colored vial at 4°C. This CuNCs was used for further characterization. Yield=30%. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz):  $\delta$  (ppm) = 6.55 (1H, s), 6.79 (1H, s), 12.00 (1H, s). MALDI-TOF: m/z [Cu<sub>4</sub>(MI)<sub>3</sub>-4H]<sup>+</sup> calculated: 549.713; found: 549.388.

Synthesis of CuNCs@MMI and CuNPs@DMMI: CuNCs@MMI and CuNPs@DMMI nanoclusters were also prepared by following the same protocol with varying capping ligands such as 2-Mercapto-1-methylimidazole and 2-Mercapto-1,3-dimethylimidazole respectively. CuNCs@MMI: Yield = 21 %. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz):  $\delta$  (ppm) = 3.49 (3H, s), 6.55 (1H, s), 6.91 (1H, s). MALDI-TOF: m/z [Cu<sub>4</sub>(MMI)<sub>3</sub>-3H]<sup>+</sup> calculated: 592.770; found:

592.579. CuNPs@DMMI was poorly characterized because it is feebly soluble in most of the solvents.

#### Quantum Yield:

Quantum yield of CuNCs were determined relative to the standard flurophores with reported quantum yield (QY). We have used Quinine sulfate in 0.5 M sulfuric acid (QY=54.6%) for determining the quantum yield of CuNCs@MMI and Rhodamine 6G in ethanol (QY=95%) for CuNCs@MI. Quantum yield was calculated using the following equation:

$$\begin{array}{c} Q_{CuNCs} = Q_{std} \frac{A_{std} F_{CuNCs} \prod^2_{CuNCs}}{A_{CuNCs} F_{std} \prod^2_{std}} \end{array}$$

Where  $Q_{CuNCs}$  is the quantum yield of copper nanoclusters,  $Q_{std}$  is the quantum yield of the standard compounds,  $F_{CuNCs}$  and  $F_{std}$  are the integrated areas of the fluorescence of the CuNCs and the standards respectively,  $A_{std}$  and  $A_{CuNCs}$  are the absorbance of the standard and the CuNCs at the excitation wavelength (340 nm) respectively,  $\Pi$  is the refractive index of the solvents used. All the samples were diluted to ensure that the absorbance was less than 0.05 nm. The fluorescence quantum yield of CuNCs@MI and CuNCs@MMI is 11.42% and 22.45% respectively.

#### **Cell culture conditions:**

Human liver hepatocellular carcinoma cells (HepG2) were obtained from ATCC (American Type Culture Collection), Washington, DC, USA. HepG2 cells were grown in minimum essential medium (MEM) and supplemented with 2.2 g/L sodium bicarbonate, 10% supplemental fetal bovine serum (FBS), penicillin G (100 unites/mL) and streptomycin (100 mg/mL). The cells were cultured at approx. 80-90% confluence and further sub-cultured as adherent monolayer in different sized cell culture dishes/ plates depending on the type of experiment in a humidified incubator at 37 °C with 5% CO<sub>2</sub>.

#### Cell viability assay:

The viability of the cells was determined using sulforhodamine B (SRB) colorimetric assay. Briefly, 20,000 cells per well were seeded in the 96-well microtiter cell culture plates for 24 hours before adding different concentration of CuNCs@MI, CuNCs@MMI and CuNPs@DMMI. The CuNCs were first dissolved in DMSO and then diluted with MEM to achieve the working concentrations. The concentration of DMSO in all the stock solution was maintained < 0.5% v/ v. The cells were exposed with different concentration of CuNCs for 24 hours with a final volume of 200  $\mu$ L. After this, 25  $\mu$ L of cold trichloroacetic acid (50% wt/vol) was added to each well and then the plate was incubated at 4°C for 1 hour. The plate was washed four times with slow running tap water. The excess water was removed by tapping the plate gently on a paper towel. Then the plate was allowed to dry completely at room temperature. After complete drying, 50 $\mu$ L of 0.04% (wt/ vol) SRB was added in to each well and the incubation was continued for 1 hour at room temperature. Finally the excess SRB was removed by washing the plate with 1% acetic acid for four times and the plate was allowed to air dry at room temperature. 100  $\mu$ L of 10 mM Tris base solution (pH 10.5) was added to each well and the plate was determined at 510 nm using multi-cell plate reader. The percentage of cell survival was calculated by comparing the absorbance of treated cells relative to the untreated control cells. The half maximal inhibitory concentrations (IC<sub>50</sub>) values were determined as the average of triplicates and their standard deviations.

#### **Confocal microscopy:**

HepG2 cells were seeded on glass cover slips in 12-well cell culture plates (5 x  $10^5$  cells per well). The cells were allowed to adhere and proliferate for 24 hours. 50  $\mu$ M of CuNCs were incubated with cells for four hours. After the treatment, the cells were washed twice with PBS and fixed with paraformaldehyde (4%) for 15 minutes followed by washing with PBS buffer. Cover slips having fixed cells were mounted on microscopic slides using fluoromount (F4680, Sigma). Images were obtained with Zeiss LSM880 (Airyscan) confocal microscopy and the images were extracted using Zen v2.3 (blue edition) software.



FTIR spectra of MI and CuNCs@MI

**Figure S1**: FTIR spectra of a) 2-Mercaptoimidazole (MI) and b) CuNCs@MI showing the presence of N-H stretching even after CuNCs formation indicating the presence of one free N-H group in CuNCs@MI.



FTIR spectra of MMI and CuNCs@MMI

**Figure S2**: FTIR spectra of a) MMI and b) CuNCs@MMI indicating the disappearance of NH stretching and bending vibrations after CuNCs formation.

## **MALDI-TOF mass spectrum**



**Figure S3:** MALDI-TOF mass spectrum of CuNCs@MMI indicating the fragmentation and aggregation of clusters.



**Figure S4:** MALDI-TOF mass spectrum expanded region of CuNCs@MI depicting the presence of  $[Cu_8(MI)_7+Na]^+$ ,  $[Cu_{12}(MI)_9+Na]^+$  and  $[Cu_{15}(MI)_{10}+Na]^+$  with m/z 1222.462, 1678.203, and 1967.281 respectively.





**Figure S5**: UV-Vis spectra of CuNCs@MI, CuNCs@MMI and CuNPs@DMMI. CuNPs@DMMI shows surface plasmon resonance (SPR) band, which is absent in CuNCs@MI and CuNCs@MMI.



Aggregation induced emission of CuNCs@MMI

**Figure S6:** a) Emission spectra of CuNCs@MMI in different ratios of methanol and dichloromethane respectively. b) AIE of CuNCs@MMI in solution state shows bright bluish green emission in aggregated state after exciting it with 365 nm hand held UV light.



XPS of nitrogen and sulfur in CuNCs

**Figure S7**: a), b) Nitrogen and sulfur XPS spectra of CuNCs confirm the binding of copper with nitrogen and sulfur atom. The binding energy of sulfur atom  $(S2p_{3/2})$  in mercaptoimidazole is ~ 164 eV. However, after metal coordination, the binding energy value of Cu–S is observed between 161.77-162.81 eV. Similarly, the binding energy of copper coordinated nitrogen is observed at 399.07-400.24 eV. <sup>1, 2</sup>

# TEM and XPS of CuNPs@DMMI



**Figure S8**: a) TEM image of CuNPs@DMMI showing the formation of nanoparticles with diameter  $\approx 15$  nm. b) XPS spectrum of CuNPs@DMMI showing the absence of Cu<sup>2+</sup> satellite peak.





Figure S9: Cell viability SRB colorimetric assay for CuNPs@DMMI

# Confocal laser scanning microscope images



**Figure S10:** Confocal laser scanning microscope images of HepG2 cells in the absence of CuNCs. a) Emission in yellow channel. b) Emission in green channel. c) Bright field image. d) merged image of a), b) and c).

## **References:**

1) F. -L. Meng, H. -X. Zhong, Q. Zhang, K. -H. Liu, J. -M. Yan and Q. Jiang, *J. Mater. Chem. A*, 2017, **5**, 18972-18976.

2) N. Kovacevic, I. Milosev, A. Kokalj, Corrosion Science, 2015, 98, 457-470.