Electronic Supporting Information:

Coumarin-based fluorescent probe for recognition of Cu^{2+} and for fast detection of histidine in hard-to-transfer cells by sensing ensemble approach

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Experimental

General

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Advance-III 400 MHz Spectrometer (at 400 and 100 MHz, respectively) using trimethylsilane (TMS) as an internal standard. High resolution mass spectra were performed on a Bruker Autoflex mass spectrometer (MALDI-TOF). Fluorescence emission spectra and UV-vis spectra were collected on a PE LS55 and a Cary UV-300 spectrometer, respectively. The melting point was determined with a MEL-TEMPII melting point apparatus (uncorrected). The pH measurements were performed on a Orion 420A pH mV temperature meter with a combined glass-calomel electrode. Double-distilled water was used throughout. The excitation wavelength was 430 nm. Fluorescence lifetime was measured using a PTI Time Master Model C-720. The fluorescence quantum yield Φ_F was calculated from Equation (1):

$$\Phi_{sample} = \Phi_{standard} \frac{F_{sample}}{F_{standard}} \frac{A_{standard}}{A_{sample}} \frac{\eta_{sample}^2}{\eta_{standard}^2} \quad (1)$$

Where the subscripts sample and standard denote reference standard and test sample, respectively, Φ is the fluorescence quantum yield, F denotes the integral of the corrected fluorescence spectrum, A is the absorbance at the excitation wavelength, and η is the refractive index of solvent.

All regents for synthesis were obtained commercially and were used without further purification. Solvents such as dichloromethane (DCM), ethyl acetate (EA), *n*-hexane were purchased from commercial sources and were the highest grade, dry dichloromethane was distilled in calsium hydride. Silica gel (200-300 mesh, MACHEREY-NAGEL GmbH & Co. KG) was used for column chromatography. Analytical thin-layer chromatography was performed using TLC silica gel 60 F254 (aluminium sheets, Merck KGaA). In titration experiments, all the cations in the form of perchlorate or chloride and other substrates were purchased from Sigma-Aldrich, USA and stored in a vacuum desiccator.

Sample preparation

The probe **CAQA** was dissolved in ACN as a stock solution (1 mM). Buffer solution was prepared by dissolving HEPES in water (20 mM). Slight variations in the pH of the solution were achieved by adding the minimum volumes of NaOH or HCl.

Absorption and fluorescence analysis

Absorption spectra and fluorescence emission spectra were obtained with 1.0 cm quartz cells. The detection procedures were as following: in 20% ACN-HEPES (20 mM, pH = 7.4) buffer solution containing 5 μM **CAQA** and 5 μM Cu^{2+}, a histidine

sample was gradually titrated into the solution, the mixture was equlibrated for 30 min before measurement. The fluorescence intensity was measured simultaneously at $\lambda_{ex/em}$ = 430/480 nm. The excitation and emission slits were set to 4.0 nm and 4.0 nm, respectively.

Cell culture

Human glioblastoma U87MG cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA), and cultured with RPMI-1640 (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin at 37 ºC and in a humidified 5% CO2 atmosphere.

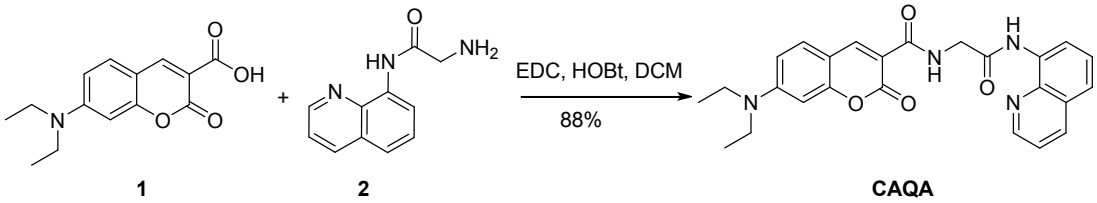
Cellular uptake study

Five thousand U87MG cells were seeded into the wells from a 24-well plate. After 12 h incubation, the culture medium in the wells was replaced with fresh medium with **CAQA** (5 μM or 10 μM) or premixed **CAQA** and $CuCl_2$ (equal 5 μM or 10 μM for each). After 30 min incubation, the cells were washed and fixed with 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA), and the fluorescence of cells was observed by a Nikon TE2000 fluorescence microscope through GFP channel. In another experiment, cells were pre-treated with *N*-ethyl maleimide (20 μM) and lipofectamine 2000 (4 μL/mL).[1]

Cytotoxicity study

The MTT assay was performed to determine cell viability. Five thousand U87MG cells were seeded into the wells from the 96-well plates.[2] After 12 h incubation, the medium in the wells was replaced with fresh medium with **CAQA** (5 μM or 10 μM) or premixed **CAQA** and $CuCl_2$ (5 μM or 10 μM equimolar for each). After 24 h incubation, 10 μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (5 mg/mL) was added to each well. After 3 h incubation, the medium was removed and formazan crystals were dissolved with 150 μL DMSO for 10 min on a shaker. The absorbance of each well was measured by a microplate reader at a wavelength of 540 nm. The relative cell viability (%) for each sample related to control well was calculated.

Synthesis



1 2 CAQA

To a solution of **1** (50 mg, 0.19 mmol), **2** (38 mg, 0.19 mmol) HOBt (26 mg, 0.19 mmol) and EDC (45 mg, 0.23 mmol) in dry DCM (4 mL) under N_2 atmosphere was added TEA (0.1 mL, 0.72 mmol) and the reaction mixture was stirred at r.t. overnight. H_2O (10 mL) was added to the reaction mixture and the aqueous phase was extracted with EA (2 × 10 mL). The organic phases were combined and washed with brine (10 mL) and dried with Na_2SO_4. After removal of solvent, the residue was purified by column chromatography on silica gel (DCM : EA = 5 : 1) to afford 74 mg of **CAQA** as a yellow solid. Yield: 88%.

m. p.: 217-218°C.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 10.30 (1H, s), 9.49 (1H, t, *J* = 5.8 Hz), 8.79 (1H, dd, *J* = 7.1 Hz, *J* = 1.9 Hz), 8.76 (1H, s), 8.74 (1H, dd, *J* = 4.2 Hz, *J'* = 1.7 Hz), 8.14 (1H, dd, *J* = 8.3 Hz, *J'* = 1.7 Hz), 7.56-7.49 (2H, m), 7.45 (1H, d, *J* = 9.0 Hz), 7.41 (1H, dd, *J* = 8.3 Hz, *J'* = 4.2 Hz), 6.67 (1H, dd, *J* = 9.0 Hz, *J'* = 2.4 Hz), 6.54 (1H, d, *J* = 2.2 Hz), 4.46 (2H, d, *J* = 5.8 Hz), 3.48 (4H, q, *J* = 7.1 Hz), 1.26 (6H, t, *J* = 7.1 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$): δ 167.4, 164.0, 162.6, 157.9, 152.8, 148.6, 148.4, 138.6, 136.2, 134.1, 131.3, 127.9, 127.3, 121.7, 121.5, 116.7, 110.0, 109.7, 108.4, 96.7, 45.1, 44.8, 12.4.

HRMS(MALDI-TOF): m/z calcd. for $C_{25}H_{24}N_4O_4$: $[M+H^+]$ = 445.1870, found: 445.1248, $[M+Na^+]$ = 467.1690, found: 467.1665.

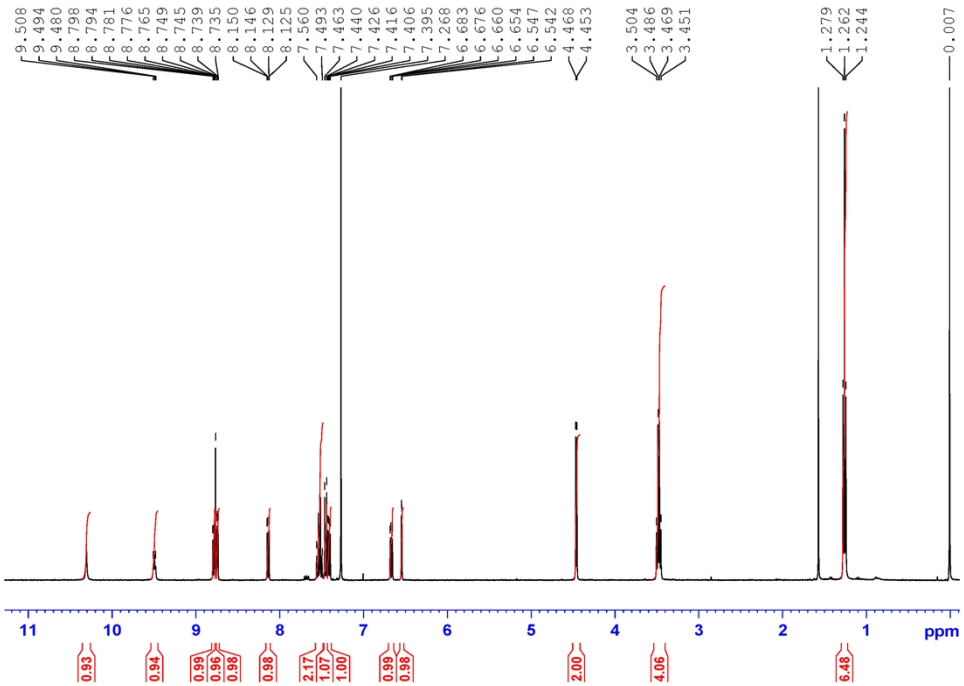


Fig. S1 [1]H-NMR spectrum of **CAQA**.

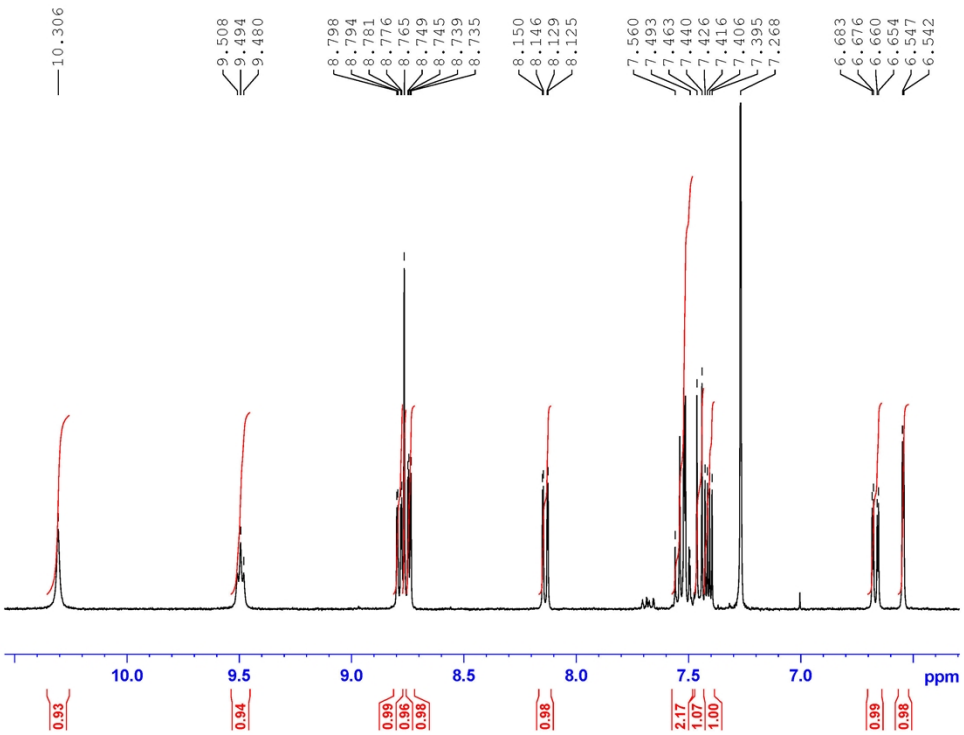


Fig. S2 Partial [1]H-NMR spectrum (6.3 – 10.5 ppm) of **CAQA**.

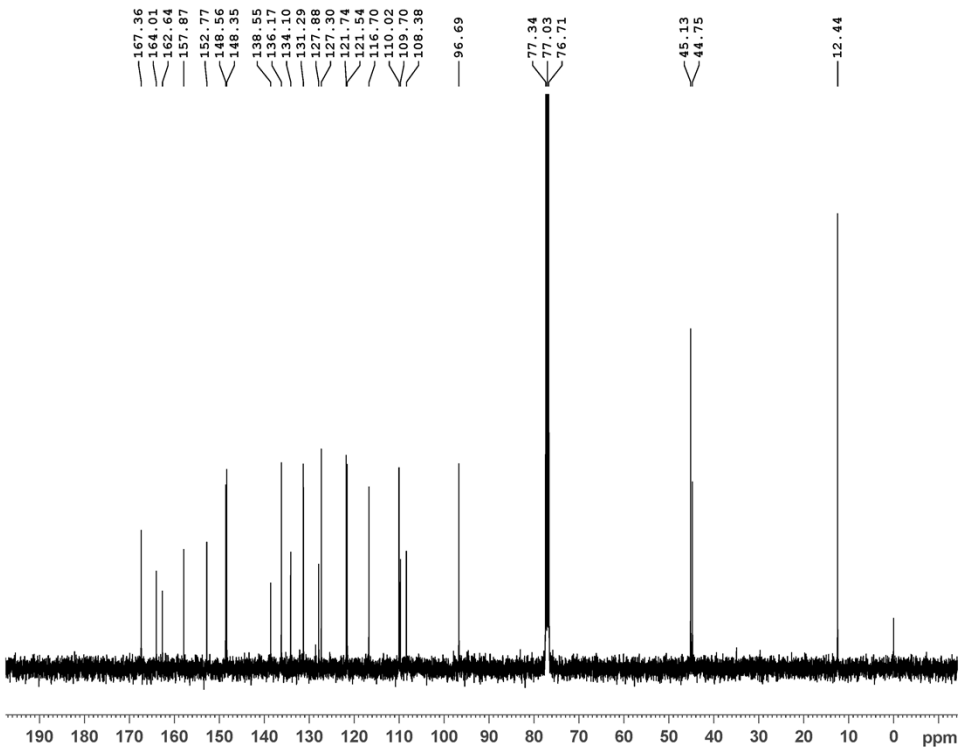


Fig. S3 ^{13}C-NMR spectrum of **CAQA**.

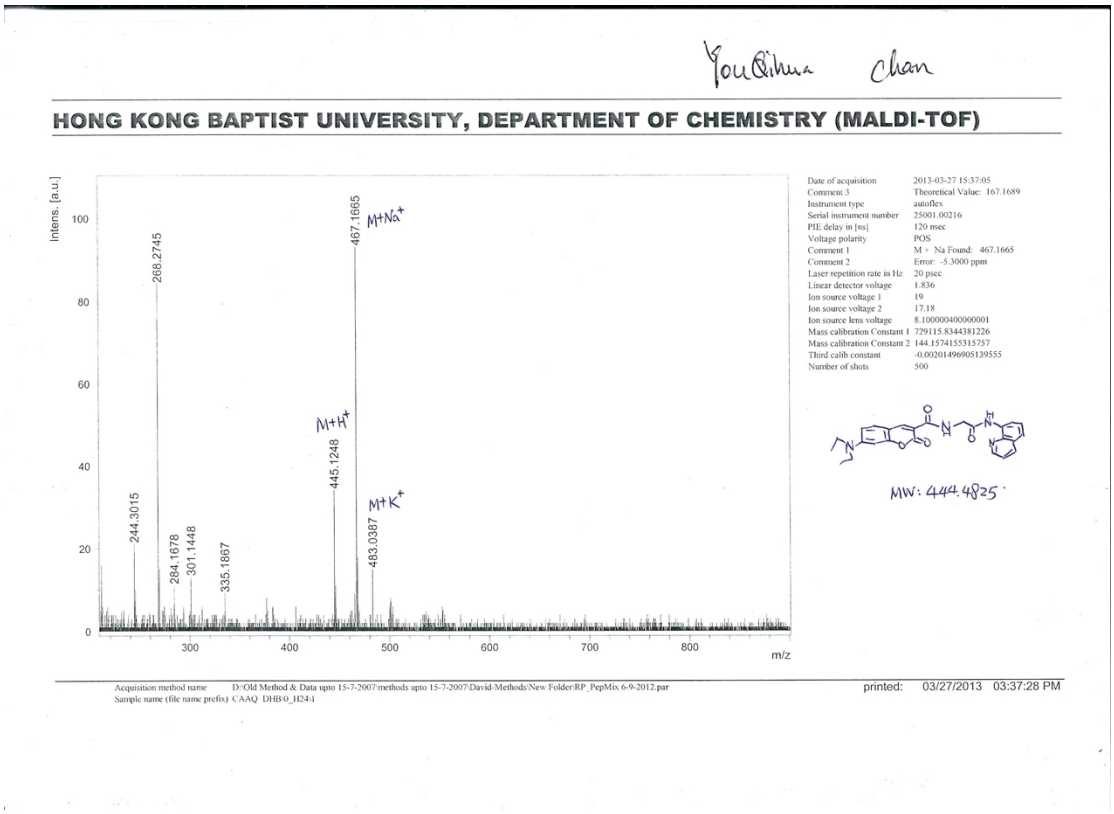


Fig. S4 MALDI-TOF HRMS spectrum of **CAQA**.

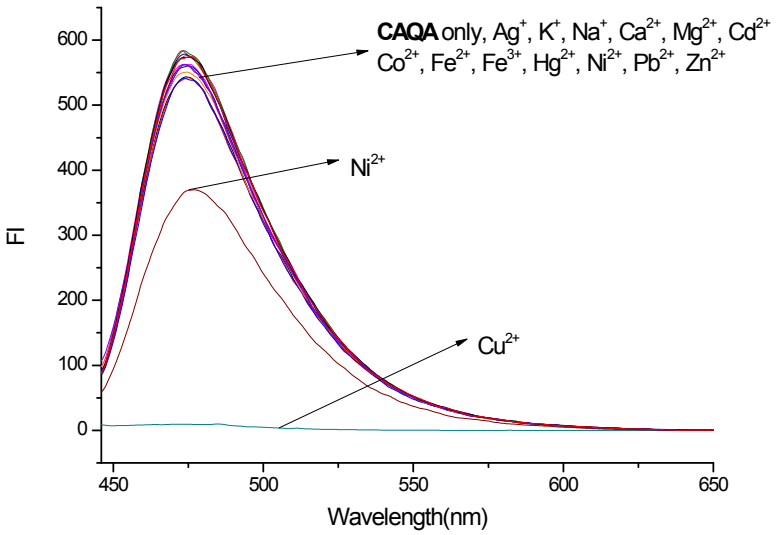


Fig. S5 Fluorescence intensity of **CAQA** (5 μM) in 20% ACN-HEPES (20 mM, pH=7.4) upon addition of different cations (1 equiv.).

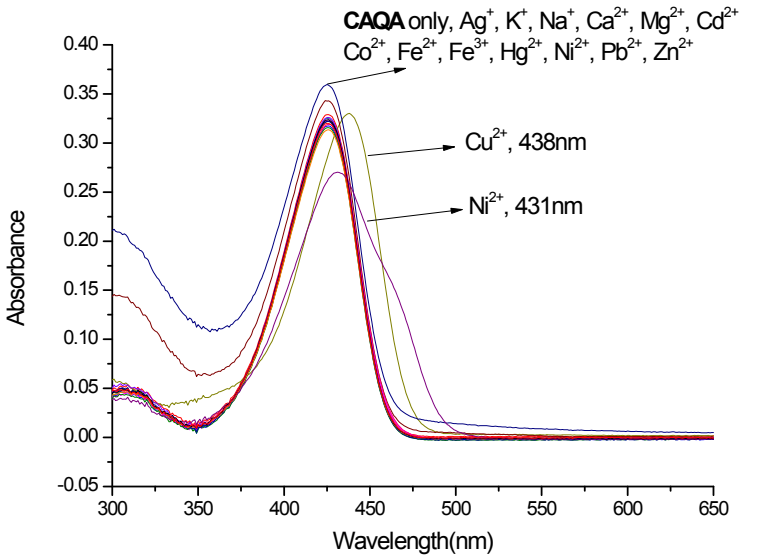


Fig. S6 Absorption spectra of **CAQA** (5 μM) in 20% ACN-HEPES (20 mM, pH=7.4) upon addition of different cations (1 equiv.).

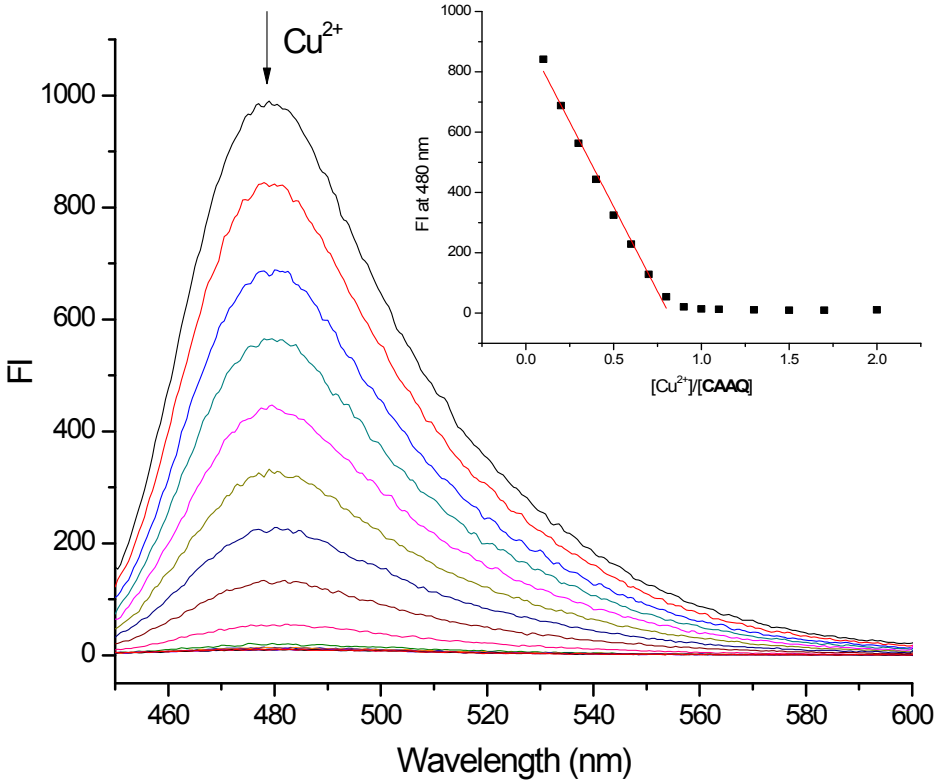


Fig. S7 Fluorescence spectra of **CAQA** (5 μM) in 20% ACN-HEPES (20 mM, pH = 7.4) upon addition of Cu^{2+}. Inset: Plot of fluorescence intensity at 480 nm of **CAQA** (5 μM) versus the concentration of Cu^{2+}.

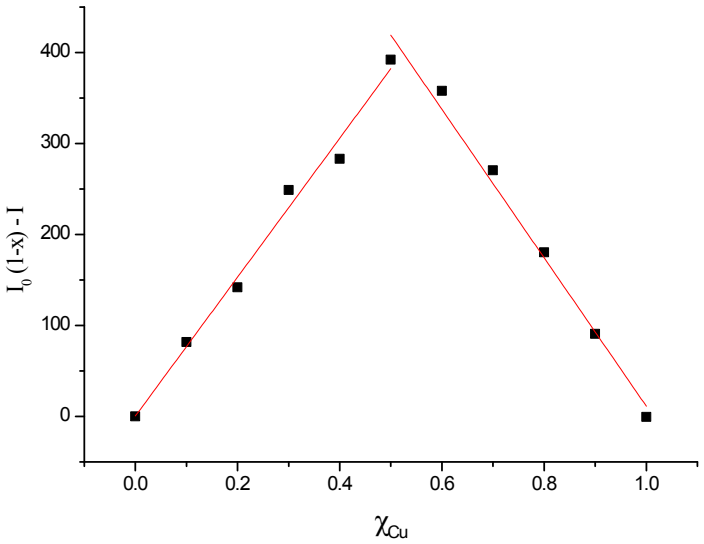


Fig. S8 Job's plot for determining the stoichiometry of **CAQA** and Cu^{2+} in 20% ACN-HEPES (20 mM, pH 7.4). The total concentration of **CAQA** and Cu^{2+} was 5 μM, where $\chi_{Cu} = [Cu^{2+}]/([Cu^{2+}] + [\mathbf{CAQA}])$.

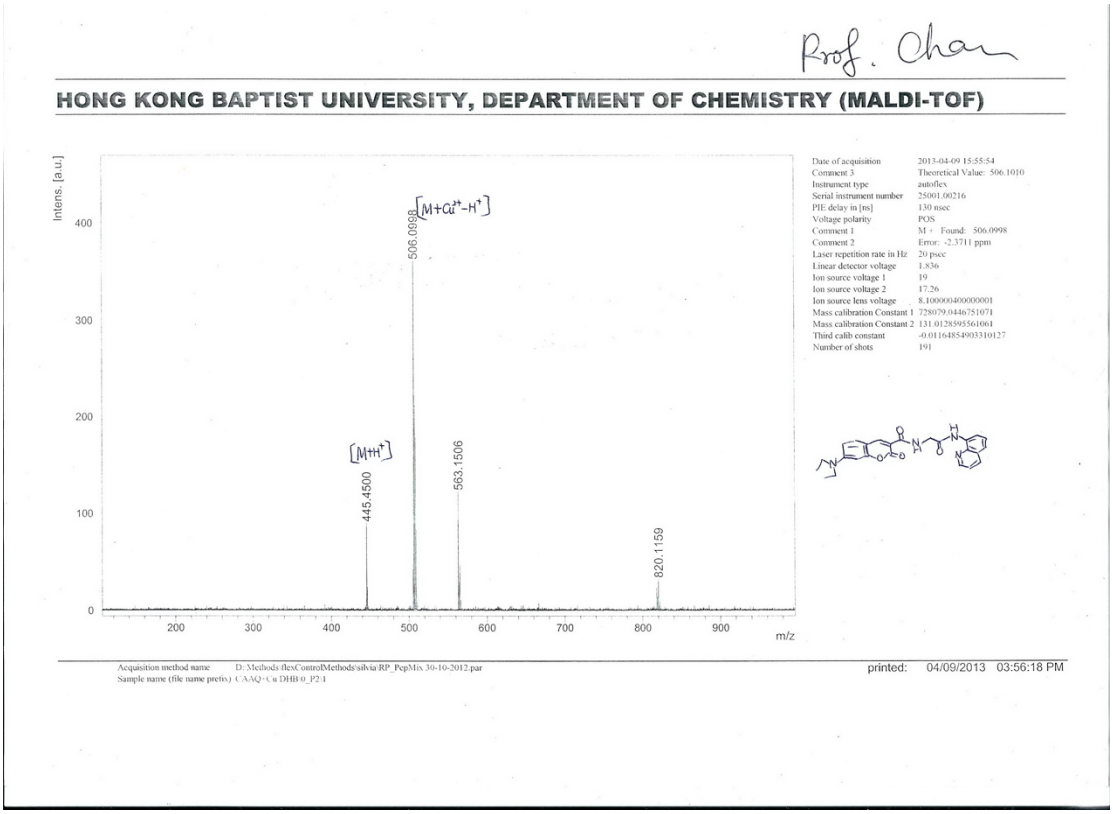


Fig. S9 MALDI-TOF HRMS of **CAQA** + $Cu(ClO_4)_2$.

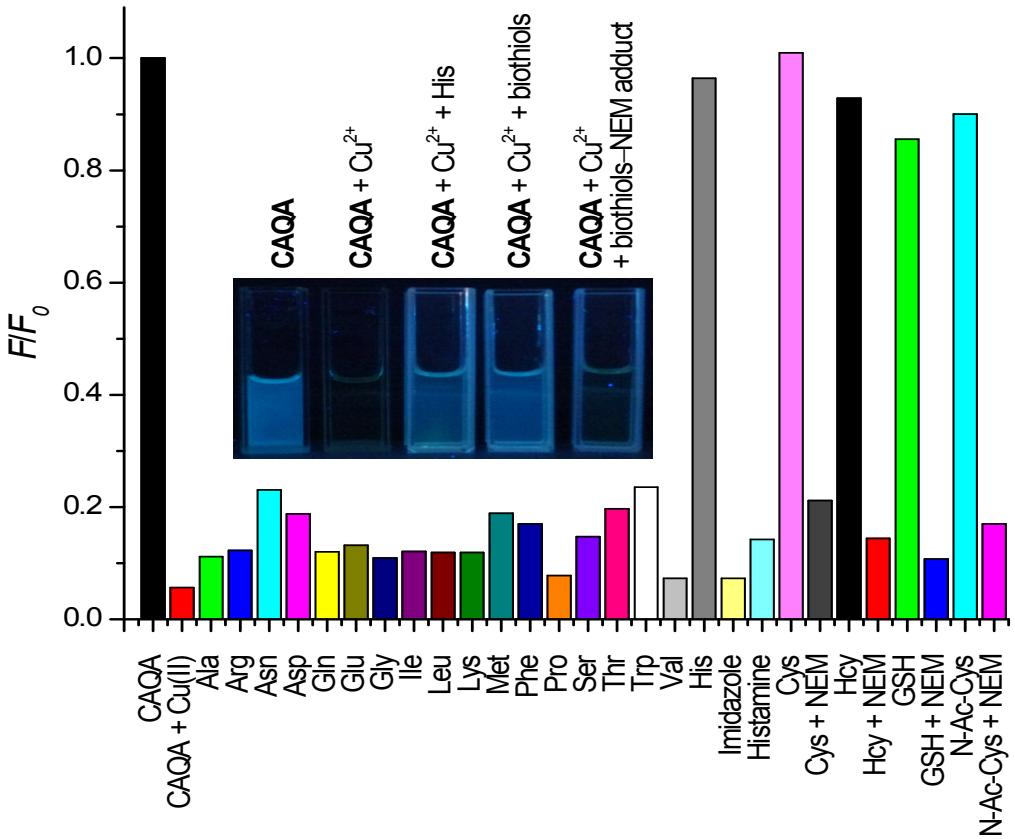


Fig. S10 Fluorescence response ratio of **CAAQ**-Cu^{2+} ensemble (5 μM) towards 20 equiv of various bio-analytes.

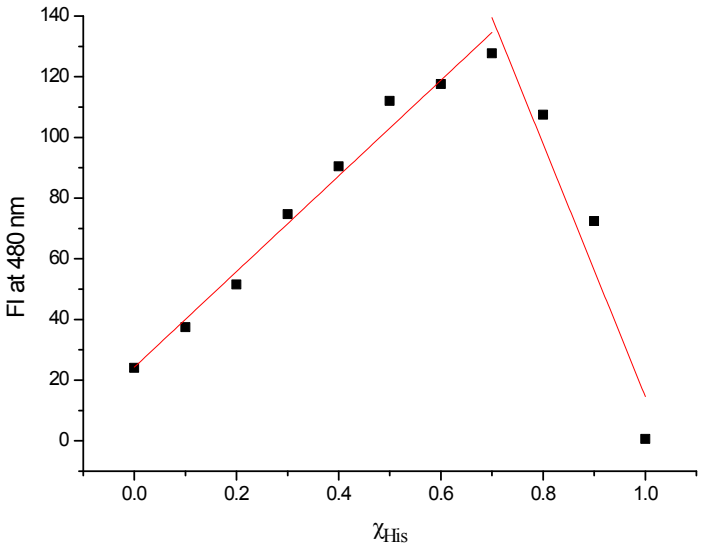


Fig. S11 Job's plot for determining the stoichiometry of **CAQA**-Cu^{2+} ensemble and histidine in 20% ACN-HEPES (20 mM, pH 7.4). The total concentration of **CAQA**-Cu^{2+} ensemble and Histidine was 5 μM, where χ_{His} = [histidine]/([histidine] + [**CAQA**-Cu^{2+}]).

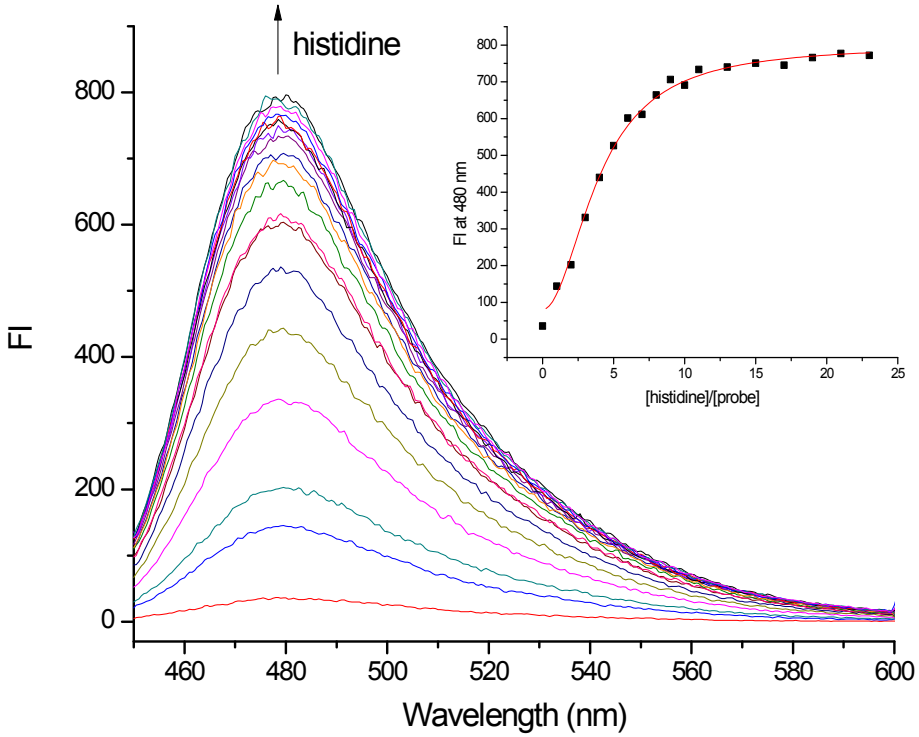


Fig. S12 Fluorescence spectra of **CAQA**-Cu^{2+} ensemble (5 μM) in 20% ACN-HEPES (20 mM, pH 7.4) upon the addition of histidine.

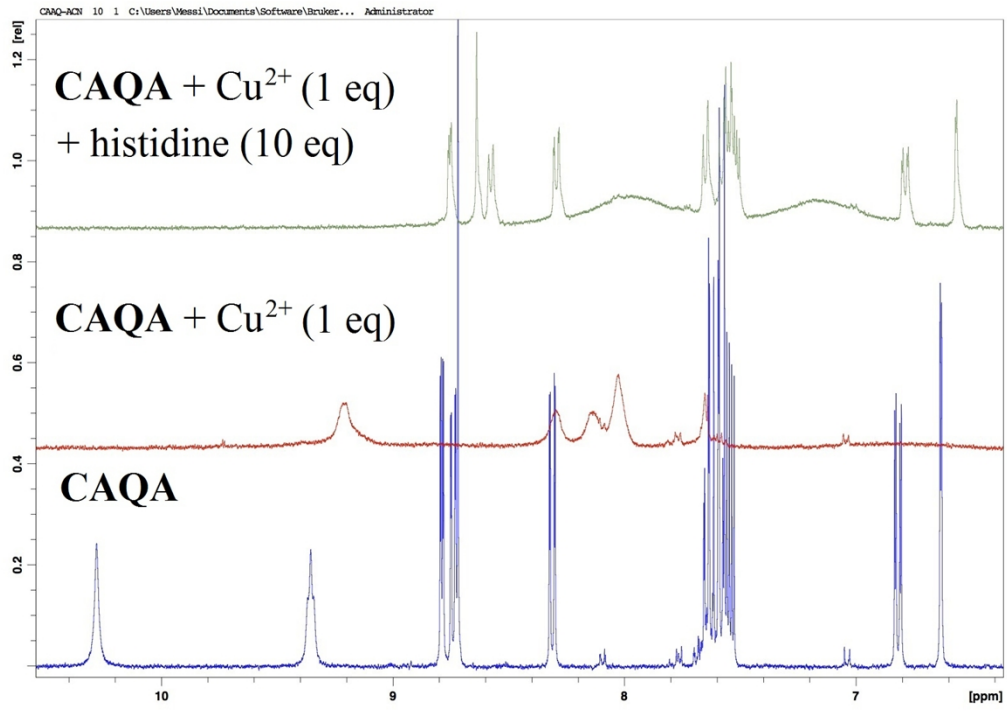


Fig. S13 Partial ^{1}H-NMR titration spectrum (6 – 11 ppm) of **CAQA** in ACN-d_4 with 1 equiv. of Cu^{2+} followed by 10 equiv. of histidine.

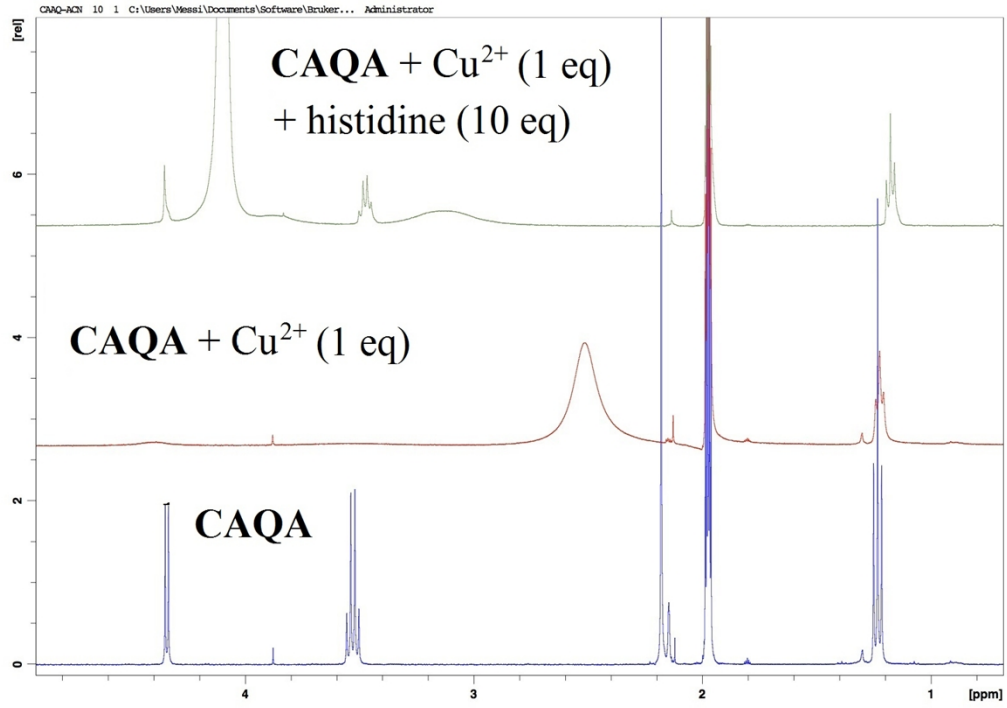


Fig. S14 Partial ^{1}H-NMR titration spectrum (0 – 5.5 ppm) of **CAQA** in ACN-d_4 with 1 equiv. of Cu^{2+} followed by 10 equiv. of histidine.

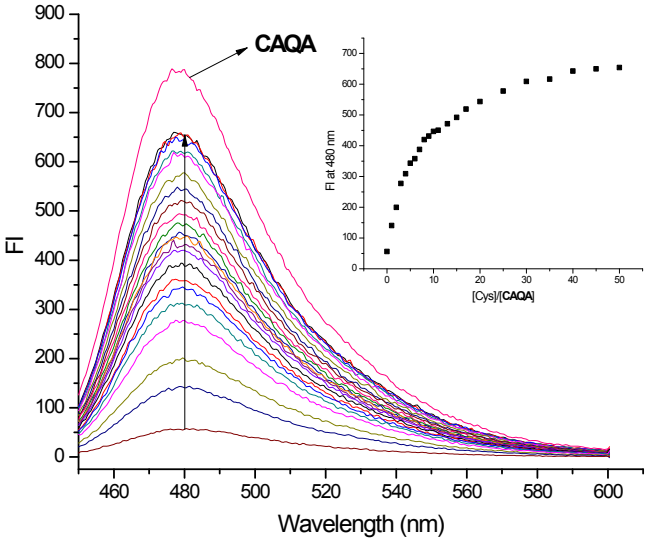


Fig. S15 Fluorescence spectra of **CAQA** (5 μM) in the presence of 1 equiv of Cu^{2+} upon the addition of Cys. Inset: Plot of fluorescence intensity at 480 nm of **CAQA** (5 μM) in the presence of 1 equiv of Cu^{2+} versus the concentration of Cys.

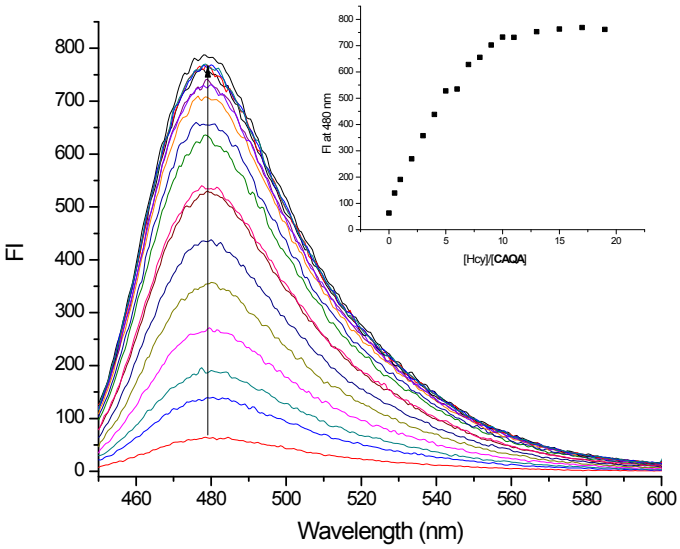


Fig. S16 Fluorescence spectra of **CAQA** (5 μM) in the presence of 1 equiv of Cu^{2+} upon the addition of Hcy. Inset: Plot of fluorescence intensity at 480 nm of **CAQA** (5 μM) in the presence of 1 equiv of Cu^{2+} versus the concentration of Hcy.

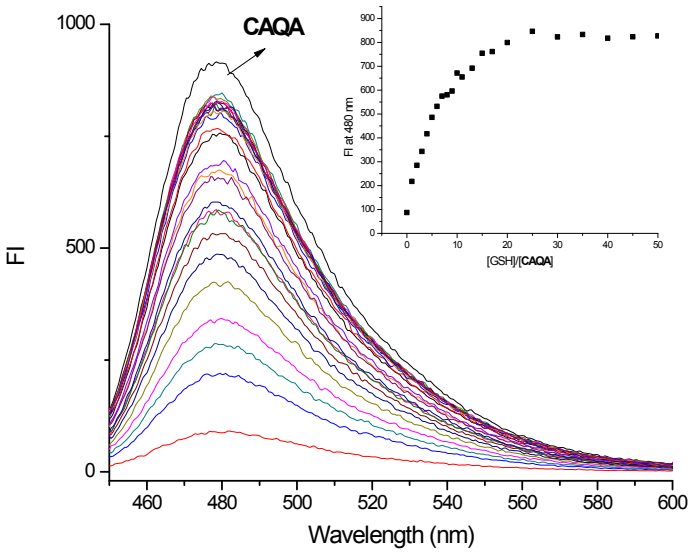


Fig. S17 Fluorescence spectra of **CAQA** (5 μM) in the presence of 1 equiv of Cu^{2+} upon the addition of GSH. Inset: Plot of fluorescence intensity at 480 nm of **CAQA** (5 μM) in the presence of 1 equiv of Cu^{2+} versus the concentration of GSH.

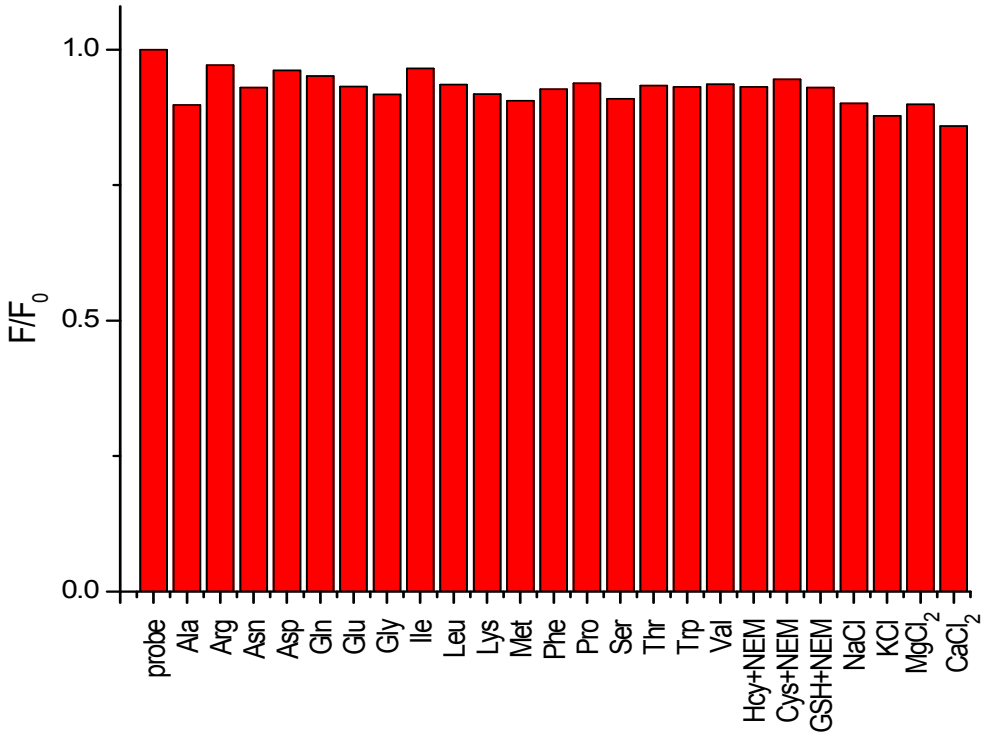


Fig. S18 Fluorescence response ratio of **CAQA**-Cu^{2+} ensemble (5 μM) in the presence of various amino acids (20 equiv), biothiols-NEM adducts (20 equiv) and cations (Na^+, K^+, Ca^{2+}, Mg^{2+}: 10 mM) upon addition of histidine (20 equiv).

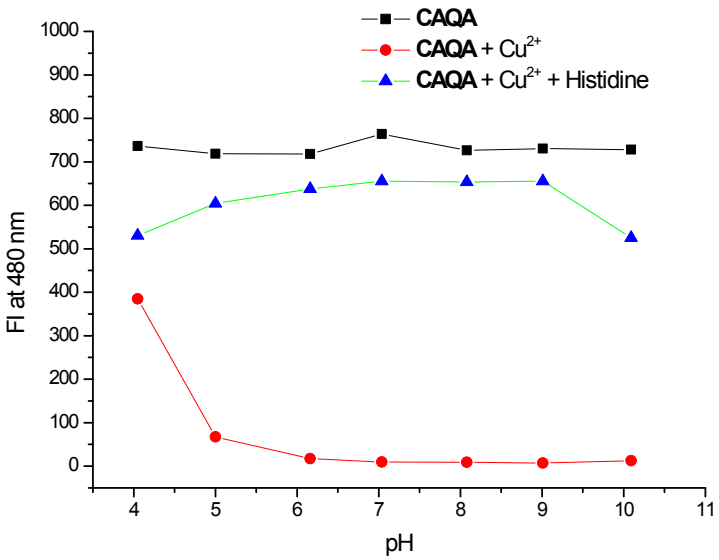


Fig. S19 Plot of fluorescence intensity at 480 nm of **CAQA** (5 μM) (Square), **CAQA**-Cu^{2+} ensemble (5 μM) (Circle) and **CAQA**-Cu^{2+}-histidine (1:1:20) (Triangle) in 20% ACN-HEPES(20 mM) as a function of pH, respectively.

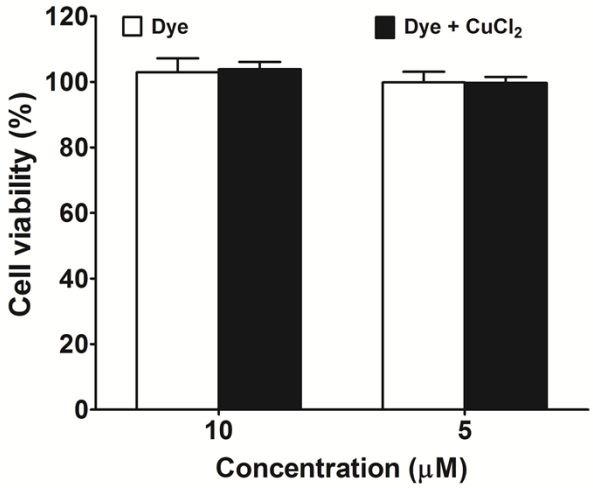


Fig. S20 MTT cell viability of human glioblastoma cells U87MG incubated with the **CAQA** with and without Cu^{2+} ion at 5 or 10 μM.

Reference:

1 (a) S.-H. Xuan, S.-F. Lee, J. T.-F. Lau, X. Zhu, Y.-X. J. Wang, F. Wang, J. M. Y. Lai, K. W. Y. Sham, P.-C. Lo, J. C. Yu, C. H. K. Cheng and K. C.-F. Leung, *ACS Appl. Mater. Interfaces*, 2012, **4**, 2033. (b) S.-F. Lee, X.-M. Zhu, Y.-X. J. Wang, S.-H. Xuan, Q. You, W.-H. Chan, C.-H. Wong, F. Wang, J. C. Yu, C. H. K. Cheng and K. C.-F. Leung, *ACS Appl. Mater. Interfaces*, 2013, **5**, 1566. (c) K. C.-F. Leung, C.-P. Chak, S.-F. Lee, J. M. Y. Lai, X.-M. Zhu, Y.-X. J. Wang, K. W. Y. Sham, C.-H. Wong and C. H. K. Cheng, *Chem. Asian J.*, 2013, **8**, 1760. (d) D.-W. Wang, X.-M. Zhu, S.-F. Lee, H.-M. Chan, H.-W. Li, S. K. Kong, J. C. Yu, C. H. K. Cheng, Y.-X. J. Wang and K. C.-F. Leung, *J. Mater. Chem. B*, 2013, **1**, 2934. (e) K. C.-

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2 (a) X.-M. Zhu, Y.-X. J. Wang, K. C.-F. Leung, S.-F. Lee, F. Zhao, D.-W. Wang, J. M. Y. Lai, C. Wan, C. H. K. Cheng and A. T. Ahuja, *Int. J. Nanomed.*, 2012, **7**, 953. (b) X.-M. Zhu, J. Yuan, K. C.-F. Leung, S.-F. Lee, K. W. Y. Sham, C. H. K. Cheng, D. W. T. Au, G.-J. Teng, A. T. Ahuja and Y.-X. J. Wang, *Nanoscale*, 2012, **4**, 5744. (c) K. C.-F. Leung, C.-P. Chak, S.-F. Lee, J. M. Y. Lai, X.-M. Zhu, Y.-X. J. Wang, K. W. Y. Sham and C. H. K. Cheng, *Chem. Commun.*, 2013, **49**, 549. (d) W. K. W. Ho, S.-F. Lee, C.-H. Wong, X.-M. Zhu, C.-S. Kwan, C.-P. Chak, P. M. Mendes, C. H. K. Cheng and K. C.-F. Leung, *Chem. Commun.*, 2013, **49**, 10781.