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Electronic Supplementary Information

Chloride Accelerated Fenton Chemistry for Ultrasensitive and Selective Colorimetric Detection of Copper

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Materials and methods:

Materials: Hydrogen peroxide (H_2O_2 , $\geq 30\%$), 3,3,5,5-tetramethylbenzidine (TMB, $\geq 99\%$), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), diaminobenzidine (DAB), 2-(N-morpholino)ethanesulfonic acid (MES), were obtained from Sigma-Aldrich. Guaiacol, o-phenylenediamine (OPD), o-dianisidine (ODA), and terephthalic acid (TPA, $\geq 98\%$) were received from Alfa Aesar. $\text{Cu}(\text{NO}_3)_2$ of ultrahigh purity (99.999%) was from Alfa Aesar. NaF ($\geq 99\%$), NaCl ($\geq 99.5\%$), NaBr ($\geq 99.99\%$), Na_2SO_4 ($\geq 99\%$), NaNO_3 ($\geq 99\%$), NaH_2PO_4 ($\geq 99\%$), and sodium acetate (NaAC, $\geq 99\%$) were from Sigma-Aldrich. Cinnamyl alcohol was obtained from Matheson Coleman & Bell (Norwood, Ohio, USA). TMB was prepared in DMSO (50 mM) and stored in the dark at -70°C prior to use.

To ensure no trace contamination of other metal ions, NaCl (5 M) and HCl (10 mM) solutions were treated with Chelex100 resin (final concentration 1% w/v) overnight under room temperature before use. KCl, CoCl_2 , MnCl_2 , MgCl_2 , CaCl_2 , HgCl_2 , NiCl_2 , $\text{Pb}(\text{NO}_3)_2$, FeCl_2 , FeCl_3 , AgNO_3 , CrCl_3 , AlCl_3 , HAuCl_4 , and mannitol were of analytical reagent grade and obtained from Sigma-Aldrich; propanol (99.9%) was purchased from Fisher Scientific, and tert-butyl alcohol (TBA, 99.5%) was obtained from Alfa Aesar; all were used without further purification. Nanopure water (18 M Ω) prepared with a Barnstead NANO-pure system (Thermo Scientific) was used for all experiments. It is worth emphasizing that the use of the right buffer is critical for this research, since copper is known to interact with many different anions. MES buffer was used in our work due to its low interaction with copper¹ in Fenton reactions. Besides, lower concentration of MES was used due to lower depletion of free radicals, thus favoring for TMB- H_2O_2 reaction and achieving high sensitivity in copper detection (Fig. S21).

Instrumentation: Colorimetric and fluorescence measurements were performed by a M1000 Pro plate reader (TECAN, USA) using either round bottom clear 96-well polystyrene or flat bottom black 96-well polystyrene plates (COSTAR, USA). A DR 5000 UV-Vis spectrophotometer (HACH, Germany) was also used to obtain the absorbance (1 cm cuvette) for calculation of initial reaction rates. For naked eye Cu(II) detection, solution color was recorded with a digital camera. UV irradiation was performed using mineralight UVGL-25 lamp (San Gabriel, USA) at 366 nm. Identification of chlorinated compound was performed with an Agilent 6890 N GC coupled to a 5973 Inert Mass Spectrometer. Copper concentration in real

sample (tap water) was quantified through inductively coupled plasma mass spectrometry (ICP-MS, NexION 300D, PerkinElmer, USA).

Experimental procedure: Reaction kinetics were recorded by the plate-reader in kinetic mode by monitoring the absorbance change at 652 nm at room temperature. Unless stated otherwise, the experiments were performed in plate wells in 200 μL MES buffer (2 mM, pH 5.5) containing 0.5 mM TMB, 100 mM NaCl and differing concentrations of Cu(II), followed by the addition of H_2O_2 to a final concentration of 750 mM to start the reaction for 10 min.

To identify whether Na^+ or Cl^- contributed to the colorimetric assay, we compared several salts of similar concentrations, including NaCl (100 mM), KCl (100 mM), Na_2SO_4 (100 mM), CH_3COONa (100 mM), NaNO_3 (100 mM), NaH_2PO_4 (100 mM), MgCl_2 (50 mM) and CaCl_2 (50 mM), with the Cu(II) concentration maintained at 200 nM, where either CH_3COONa or NaH_2PO_4 was added in the form of their buffer (pH 5.5. Stock buffer concentration: 2 M) into the system to avoid pH variation.

To understand the universality of the assay to other chromogenic substrates, we evaluated colour development with ABTS (A_{415}), OPD (A_{420}), guaiacol (A_{470}), ODA (A_{460}), and DAB (A_{465}). Similarly, the reaction with these other chromogens was performed in the presence of 200 nM Cu(II) and 100 mM NaCl.

In the metal selectivity experiment, the catalytic activity of 0.1 μM Cu(II) was compared with that of 10 μM Co(II), Mn(II), Ca(II), Mg(II), Pb(II), Hg(II), Ni(II), Al(III), K(I), Fe(III), Fe(II) and Ag(I), and 1 μM Cr(III), and Au(III).

Effect of ROS (reactive oxygen species) scavengers on NaCl activated Cu(II) Fenton reaction was investigated using 600 mM propanol, 100 mM mannitol, or 500 mM tert-butyl alcohol (TBA) as scavengers; again, 200 nM Cu(II) was used with a 5 min reaction time. Hydroxyl radical concentration was monitored using the well-established TPA method² where 0.5 mM TPA was used to monitor $\cdot\text{OH}$ under differing NaCl concentrations (0-250 mM) and pH regimes (4.0-7.0) in MES solution. We observed that the fluorescence intensity of TPA-OH was pH dependent; therefore, the fluorescence intensity data of TPA-OH under different pHs were normalized to that under pH 5.5 to correct for the pH dependent variation.

To evaluate how hydroxyl radical/reactive chlorine transformation affected TMB oxidation, the experiment was performed in a photo-Fenton reaction system (UV/ H_2O_2 /TMB). The mixture of 0.5 mM TMB and 750 mM H_2O_2 in 200 μL MES buffer (2 mM, pH 5.5) in UV-transparent 96-well plate (Greiner, Bio One GmbH, Frickenhausen, Germany) was placed on a UV lamp and irradiated at 366 nm for 150 s; the reactions were then quickly recorded by plate reader at 650 nm. Similarly, Cl^- ([NaCl] = 100 mM) on $\cdot\text{OH}$ radical transformation was studied with the UV/ H_2O_2 /TPA system. After irradiation for 150 s, fluorescence was quickly recorded by excitation at 310 nm and emission at 420 nm.

To study the effect of NaCl concentration on the initial TMB oxidation reaction rates, the apparent steady-state reaction rates at different NaCl concentrations (0-250 mM) and Cu(II) concentrations (2, 20, and 200 nM, respectively) were obtained by measuring absorbance changes within 220 s after H_2O_2 addition, which is within the linear phase of the reaction kinetics. The slopes of linear kinetic trend-lines change were used to calculate the initial reaction rates, where concentration changes within the first 220 s were calculated using the Beer–Lambert Law with a molar absorption coefficient of $39\,000\text{ M}^{-1}\text{ cm}^{-1}$ for ox-TMB. The measured reaction rates for 2 nM Cu(II) were also reported as turnover frequencies (TOF) and are measured in molecules of ox-TMB produced per Cu(II) atom per second of reaction time. The same procedure was applied to study the effect of H_2O_2 concentration (0-1000 mM) on the initial rates of TMB oxidation reaction with different Cu(II) concentrations (10 and 100 nM, respectively).

To monitor the presence of the $[\text{CuCl}]^+$ complex, the UV absorbance spectra was recorded with 200 μM Cu(II) and increasing concentrations of NaCl (0-250 mM) in 2 mM MES solution with different pHs (4.0-7.0) within UV transparent 96 well plates.

To monitor the generation of active chloride species (ACSSs) and their functions, we identified chlorinated cinnamyl alcohol i.e., 2-chloroacetophenone in the presence of ≥ 100 mM NaCl. To prevent potential interference, the reaction was performed in 1 mL pure water with pH 4.0 adjusted using HCl solution. The concentration of the reagents were as follows: cinnamyl alcohol: 1.5 mM; Cu(II): 400 μM ; NaCl: 100 or 250 mM; H_2O_2 : 500 mM. The mixture was incubated under room temperature for 45 h. The products were extracted with 400 μL of dichloromethane for instrumental analysis. GC-MS analysis was performed on an Agilent 6890N Gas Chromatograph equipped with a capillary column (length 15 m, inner diameter 0.25 mm, film thickness 0.3 μm), connected to a 5973 inert mass-spectrometer. Helium was used as a carrier gas (1 ml min⁻¹), and the temperature programme was 3 min at a constant temperature of 45 °C followed by an increase to 300 °C at 10 °C/min.

Analysis of tap water samples was carried out using standard addition method. A water sample collected from our laboratory was filtered through 0.45 μm Teflon filter before analysis. Aliquots of this tap water were spiked with standard Cu(II) solutions (0–3 μM) that had been prepared in 2 mM MES solution with pH 5.5. The spiked samples were then analyzed separately using both ICP-MS and the present sensing technique.

All experiments were performed at room temperature in triplicate. Error bars in each figure represent standard deviations from three repeated experiments.

To ascertain the dominant copper chloride formed in the solution observed in the experiment, Cu species distributions were calculated using Phreeqc Interactive Version 3.0.6-7757 with Minteq.V4 thermodynamic database (USGS, Denver, Colorado: 2013).

To calculate the reaction order with respect to each starting reactant, the initial rates (r) were measured in a series of experiments. In each set of experiments, the concentration of one reactant [A] changed while the concentrations of all other reactants were kept constant in 5 mM MES buffer (pH 5.5) (detailed in table S1). The reaction order, which is defined as the regression slope (x) of $\ln r$ plotted against $\ln[A]$ based on the following equation:

$$\ln r = x \ln[A] + \text{constant}$$

Table S1. Experimental conditions for reaction order calculation.

Experimental Set #	NaCl (mM)	H ₂ O ₂ (mM)	Cu(II) (nM)	TMB (mM)
1	5 - 250	500	200	0.5
2	100	1 - 1000	100	0.5
3	100	500	2 - 200	0.5
4	100	750	200	0.025 - 1

Supporting Data:

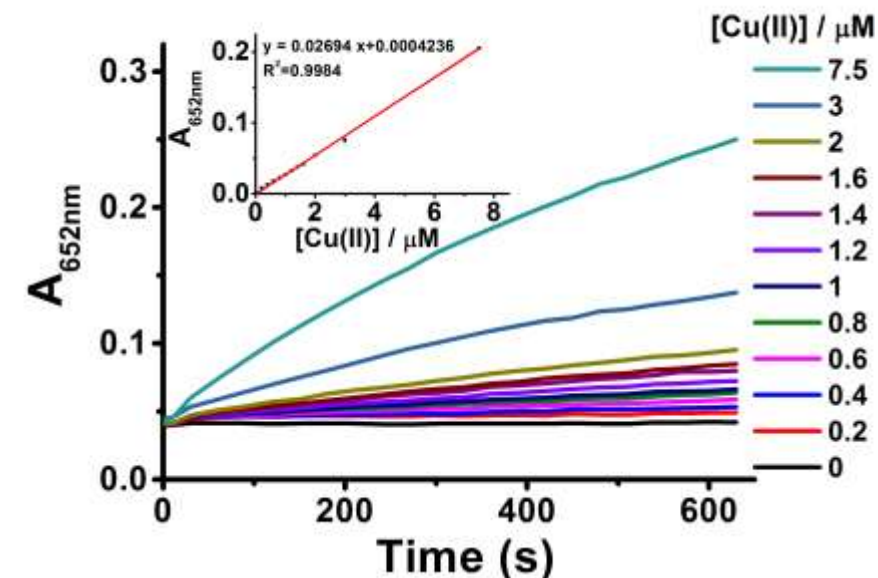


Fig. S1 TMB oxidation kinetics catalyzed by different concentration of Cu(II) via Cu-Fenton chemistry (No NaCl). Reaction conditions: 2 mM pH 5.5 MES with 0.5 mM TMB and 750 mM H₂O₂, reaction time 10 min. Inset: absorbance of ox-TMB versus [Cu(II)].

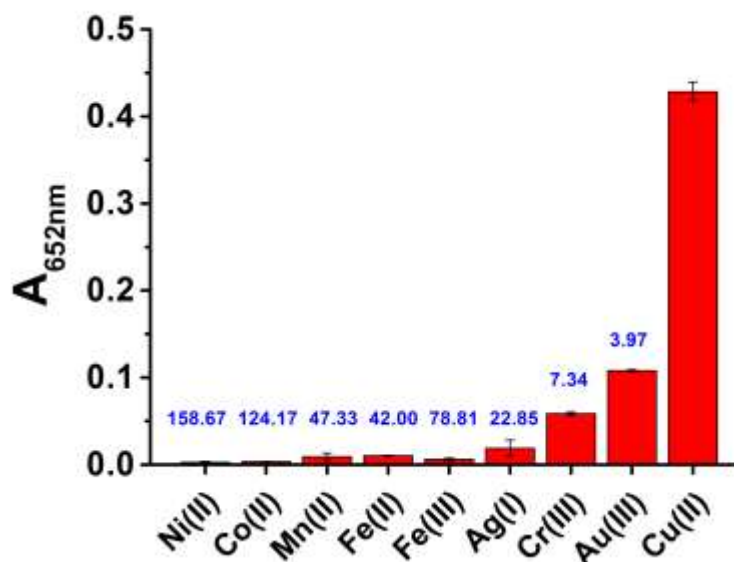


Fig. S2 $A_{652\text{nm}}$ (indicator of ox-TMB) generated in the presence of various metal ions (10 μM) in 2 mM MES buffer (pH 5.5); $[\text{H}_2\text{O}_2]$ was 750 mM and reaction time was 10 min (No NaCl). The number above each bar shows fold increase in A_{652} of Cu(II) catalyzed ox-TMB formation over that of the metal ions alone.

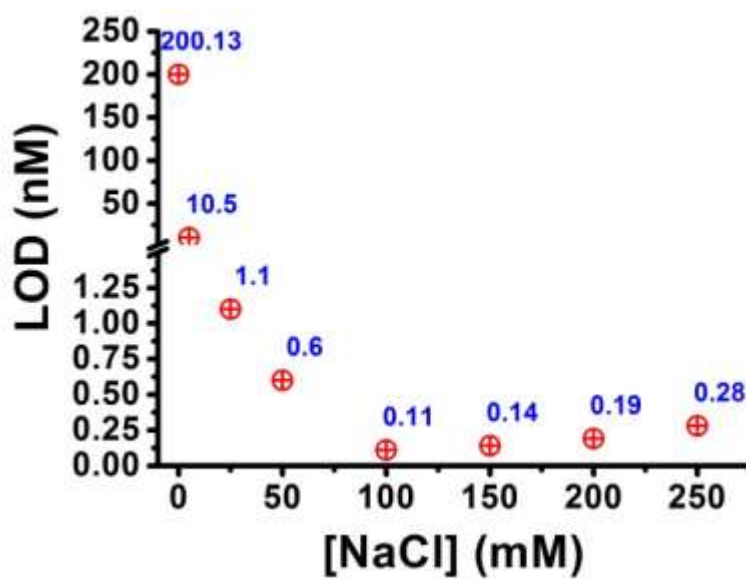


Fig. S3 Cu(II) detection limit relative to NaCl concentration. The best LOD of 0.11 nM was achieved at 100 mM NaCl. Reaction conditions: 2 mM MES, pH 5.5 with 0.5 mM TMB, 750 mM H_2O_2 , reaction time 10 min.

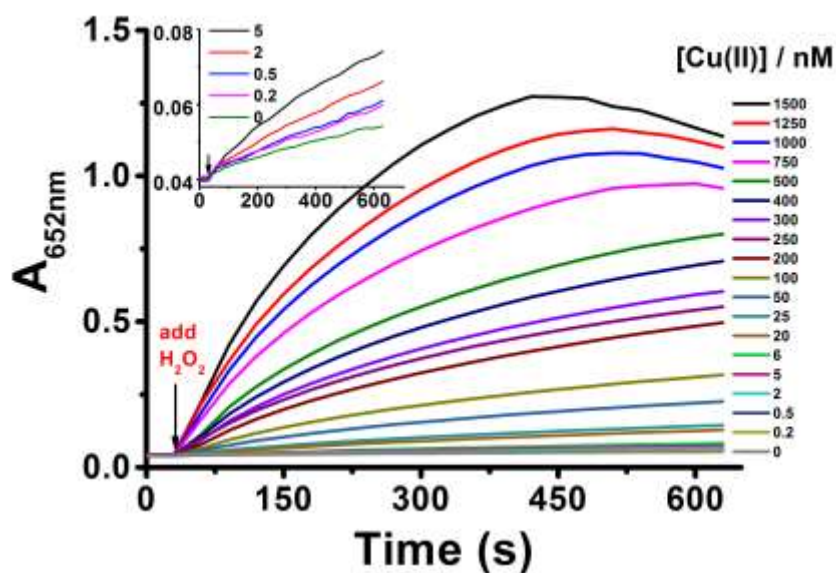


Fig. S4 Kinetics of TMB oxidation catalyzed by different concentrations of Cu(II) in the presence of 100 mM NaCl. Inset: responses at low Cu(II) levels. Reaction conditions: 2 mM MES, pH 5.5 with 0.5 mM TMB, 750 mM H₂O₂.

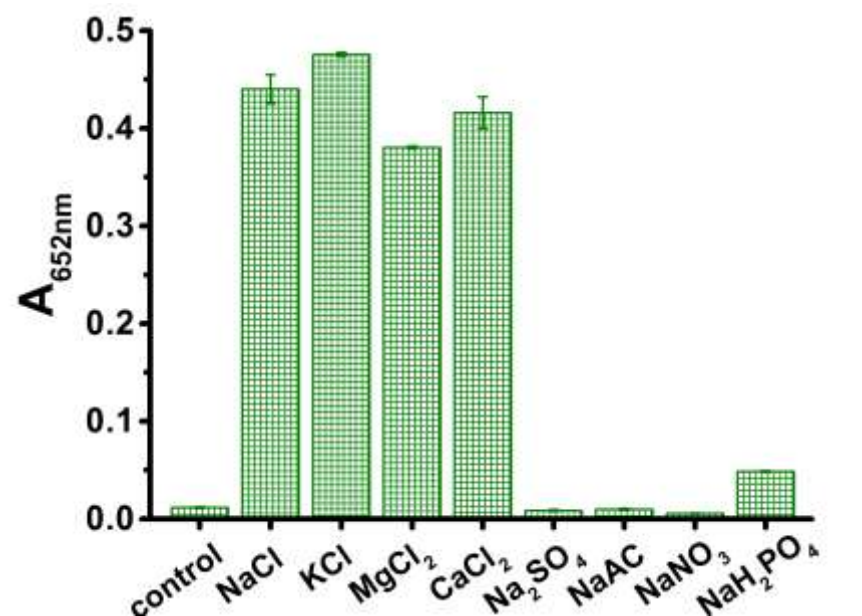


Fig. S5 Effects of different anions on the TMB oxidation catalyzed by 200 nM Cu(II). All anions were added as their sodium or potassium salts and were kept at 100 mM. Reaction conditions: 2 mM MES, pH 5.5 with 0.5 mM TMB, 750 mM H₂O₂.

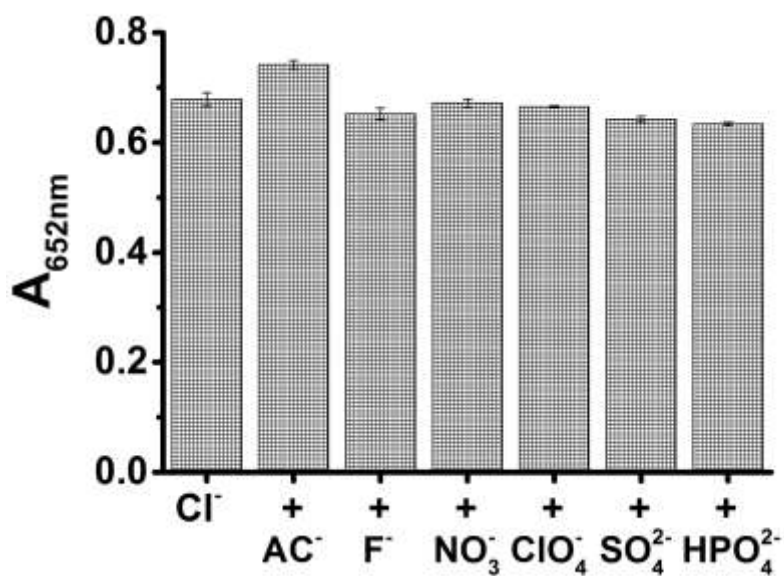


Fig. S6 Interference of co-existing anions on the signal amplification in Cl^- (100 mM) accelerated Fenton Chemistry. All anions were added as their sodium salts with concentration of 1 mM. $[Cu(II)]$ was 500 nM and reaction time was 5 min.

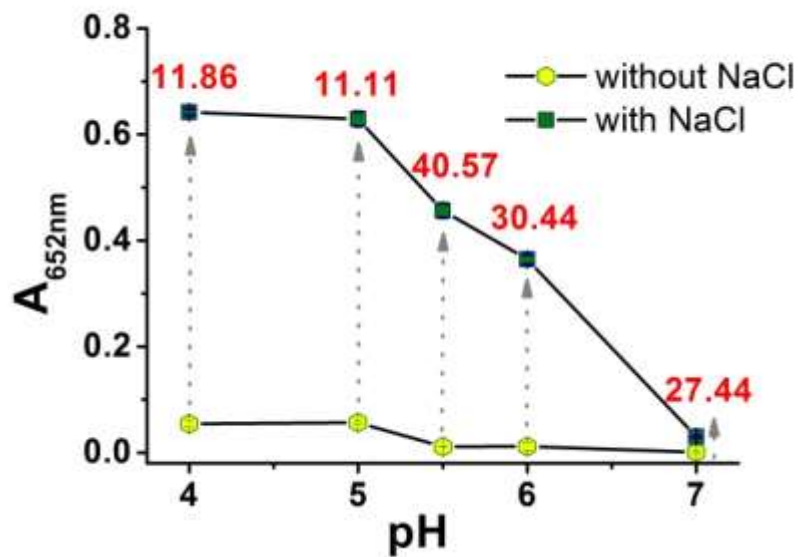


Fig. S7 The signal amplification of NaCl activated Cu-Fenton reaction under different pH regimes, $[Cu(II)] = 200$ nM, NaCl = 100 mM. The number on each data point with NaCl stands for the magnitude of signal amplification with Cl^- (A_{652} with NaCl/ A_{652} without NaCl).

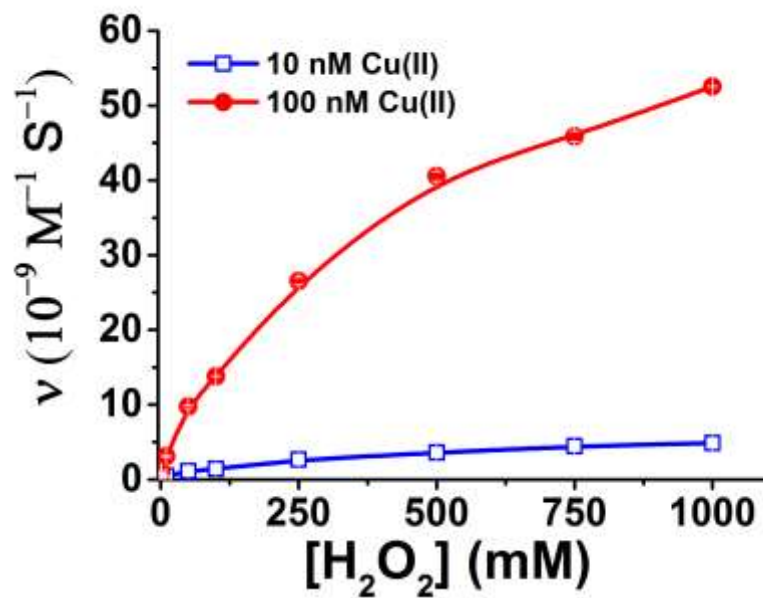


Fig. S8 Plot of the initial rate of TMB oxidation versus H_2O_2 concentration at different Cu(II) concentrations. Reaction conditions: 2 mM MES, pH 5.5 with 0.5 mM TMB and 100 mM NaCl.

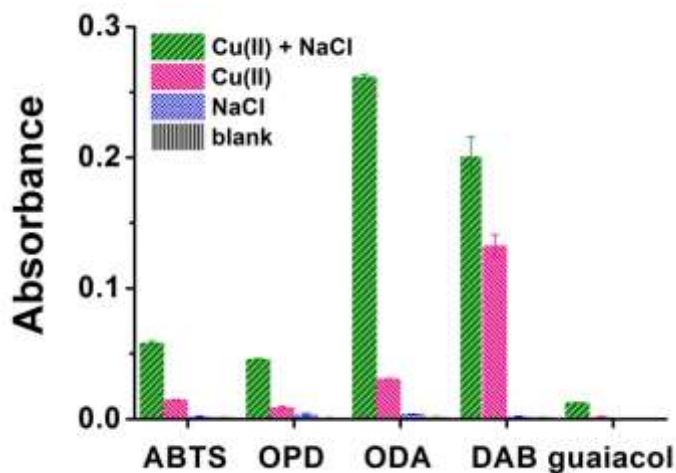


Fig. S9 The absorbance of different oxidized chromogenic substrates (0.5 mM) by 750 mM H_2O_2 for 10 min with 2 μM Cu(II) alone, 2 μM Cu(II) + 100 mM NaCl, 100 mM NaCl alone, or neither Cu(II) nor NaCl. The detecting wavelength was 415 nm for ABTS, 420 nm for OPD, 470 nm for guaiacol, 460 nm for ODA, and 465 nm for DAB, respectively.

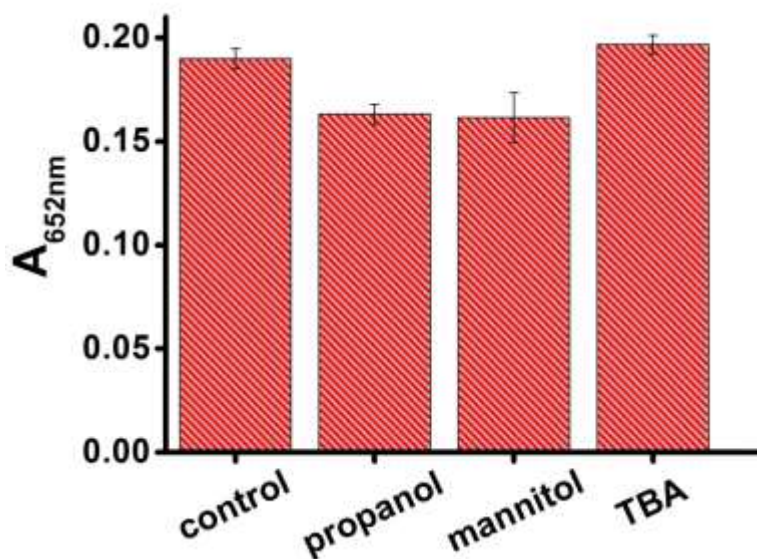
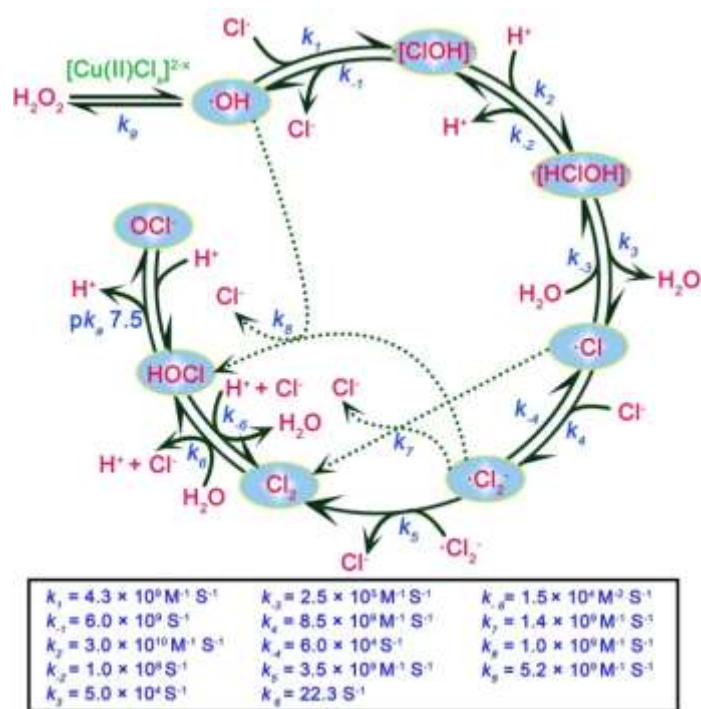


Fig. S10 The scavenging effect of propanol (600 mM), mannitol (100 mM) and tert-butyl alcohol (TBA) (500 mM) on TMB oxidation in CA Cu-Fenton systems. 200 nM Cu(II) was used and the reaction time was 5 min.



Scheme S1 The chemical reaction of relevant species with reaction constants or pK_a .³

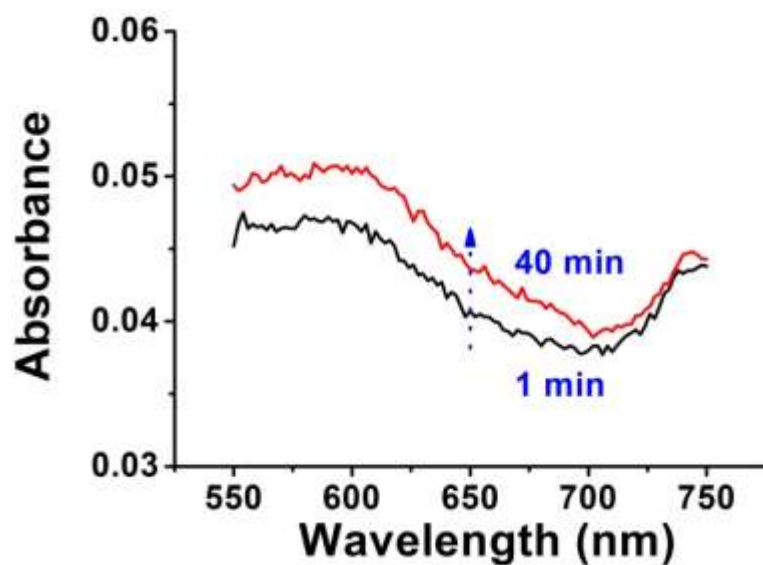


Fig. S11 The UV-vis spectra of 0.5 mM TMB at 1- and 40-mins of incubation with 3 M H₂O₂ in 2 mM MES, pH 5.5.

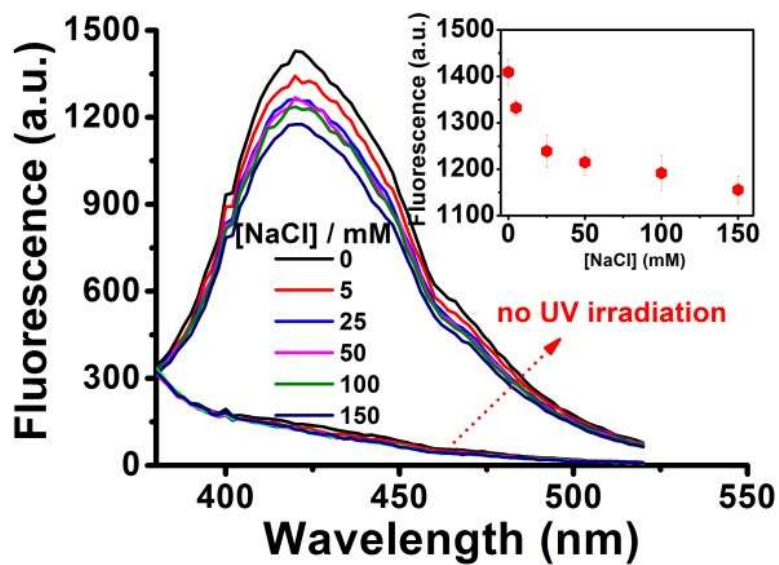


Fig. S12 The fluorescence spectra of TPA/H₂O₂ irradiated at 365 nm for 150 s at the presence of different concentration of NaCl; inset, plot of the emission at 420 nm (indication of the ·OH concentration) versus the concentration of NaCl.

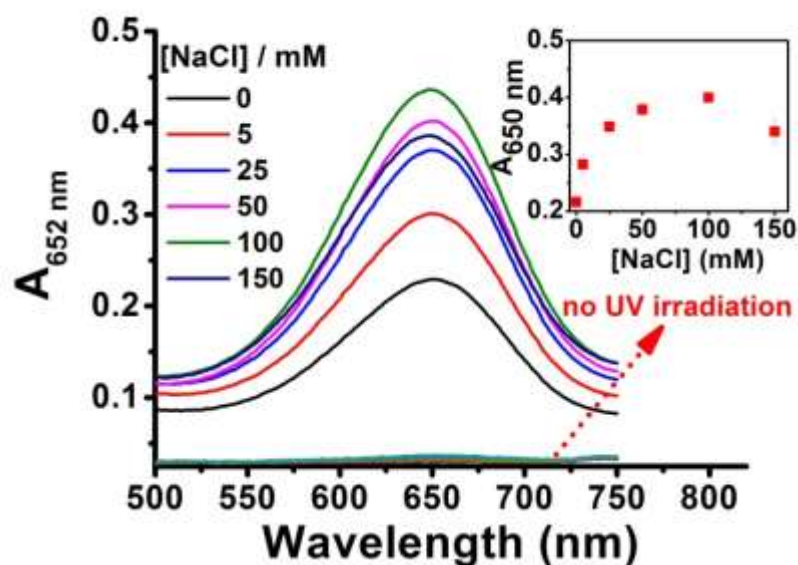


Fig. S13 UV-vis absorbance spectra of TMB oxidation by H_2O_2 in photo-Fenton reaction where the samples were irradiated at 365 nm for 150 s with different concentration of NaCl; inset, plot of the A_{650} (indication of the ox-TMB concentration) versus the concentration of NaCl. The decrease of ox-TMB at 150 mM NaCl is due to its instability in higher salt concentrations.

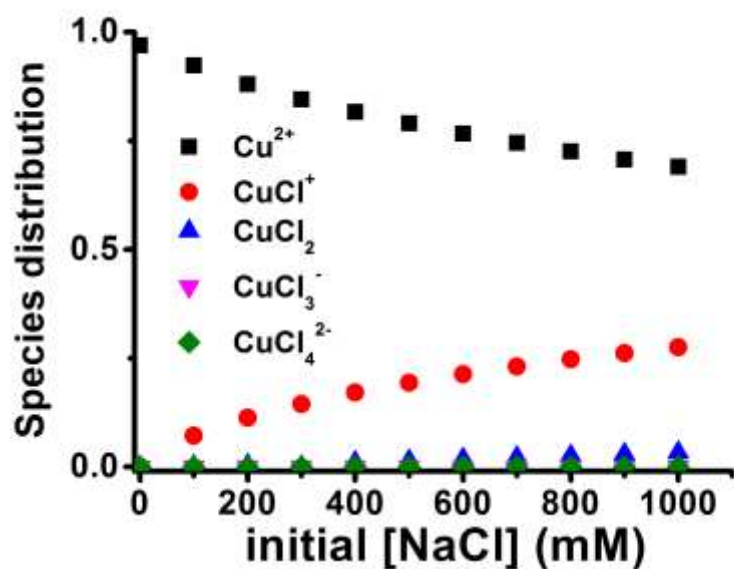


Fig. S14 Calculated Cu(II) species formation and distribution at pH 5.5 under variable initial NaCl concentrations from 0 – 100 mM using PhreeqC modelling code.

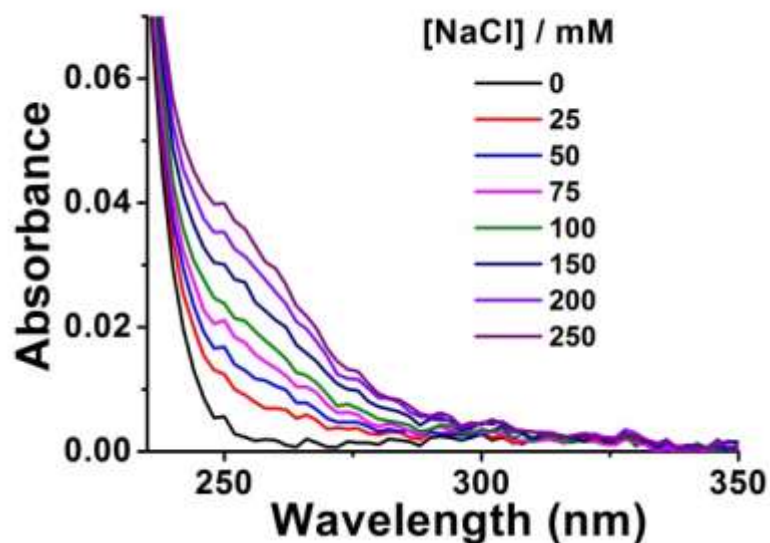


Fig. S15 UV-vis spectra of Cu(II)-chloride complex in 2 mM MES buffer (pH 5.5). The experiment was performed by mixing varying NaCl concentrations with a 200 μM of Cu(II) for UV absorbance measurement.

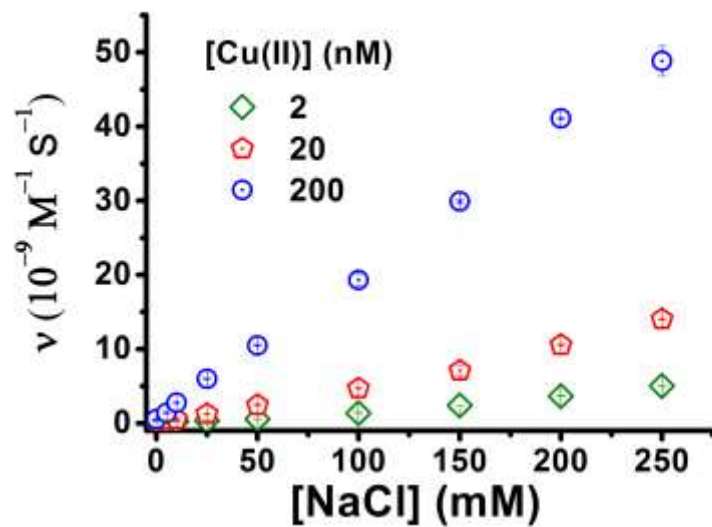


Fig. S16 Plot of the initial rate of TMB oxidation versus NaCl concentration at different Cu(II) concentrations. Reaction conditions: 2 mM MES, pH 5.5 with 0.5 mM TMB and 750 mM H_2O_2 .

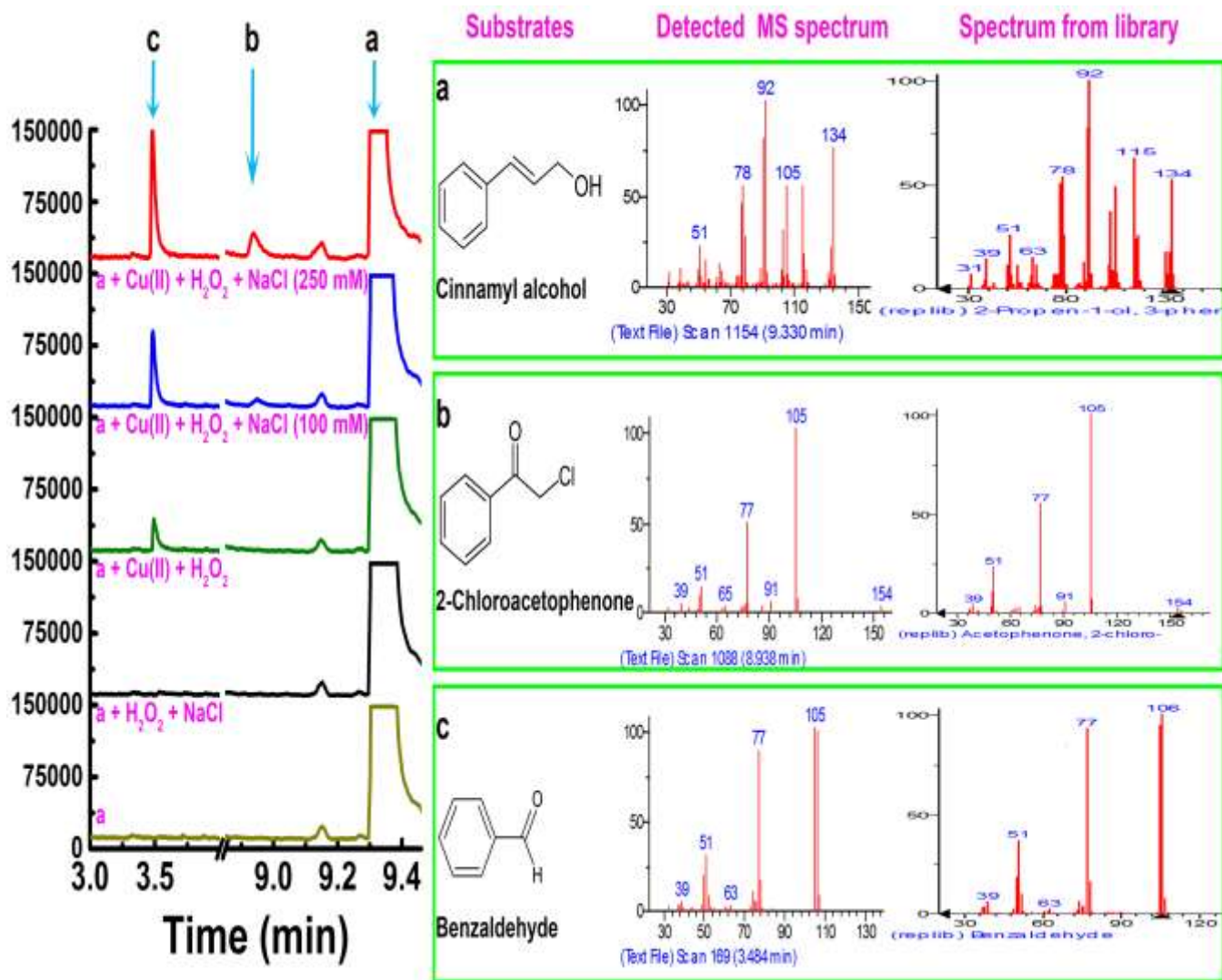


Fig. S17. Chloride accelerated oxidation and chlorination of cinnamyl alcohol.

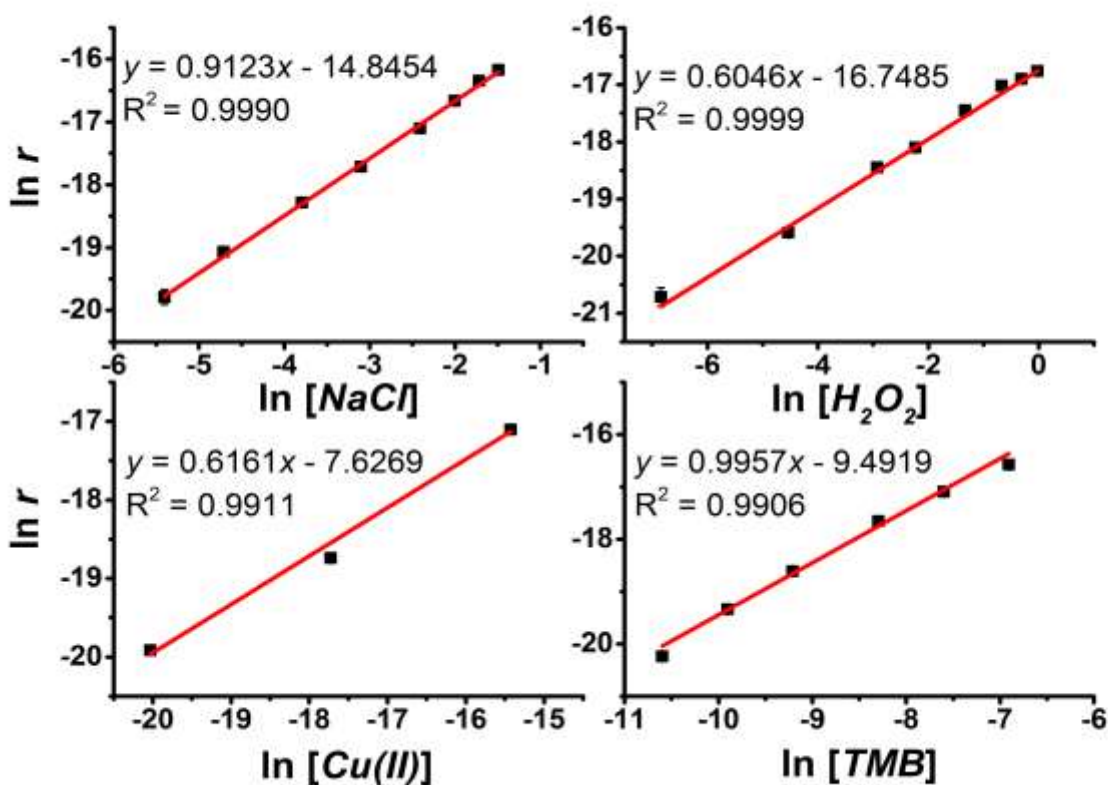


Fig. S18 Regression of $\ln r$ (initial rate) values as a function of $\ln [A]$ (substrate concentration) to calculate the reaction order (the regression slope) with respect to each substrate. The overall rate (for TMB oxidation) = $1.2 \cdot [\text{NaCl}]^{0.9} \cdot [\text{Cu}^{2+}]^{0.5} \cdot [\text{H}_2\text{O}_2]^{0.6} \cdot [\text{TMB}]^1$.

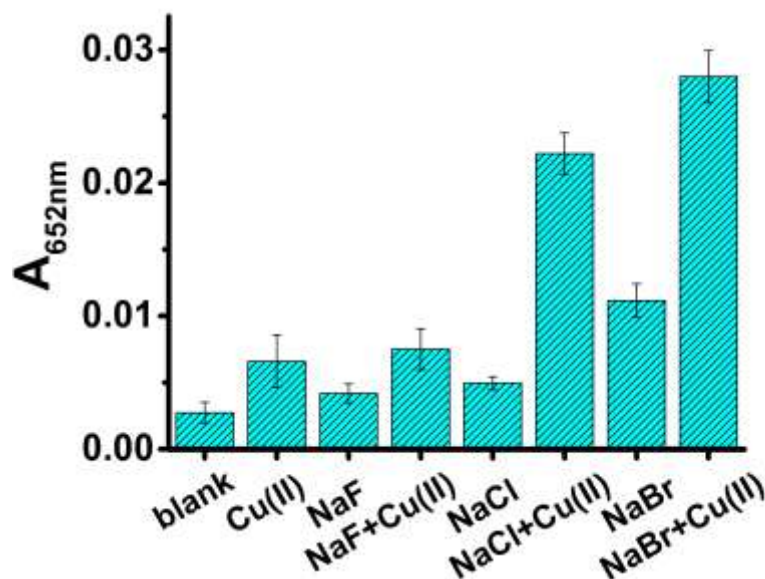


Fig. S19 Effect of different halide ions on Cu(II) catalyzed TMB oxidation. $[\text{Cu(II)}]$ and halide ions were 200 nM and 2 mM, respectively. Reaction was conducted in 2 mM MES (pH 5.5) with 0.5 mM TMB and 750 mM H_2O_2 and reaction time was 10 min.

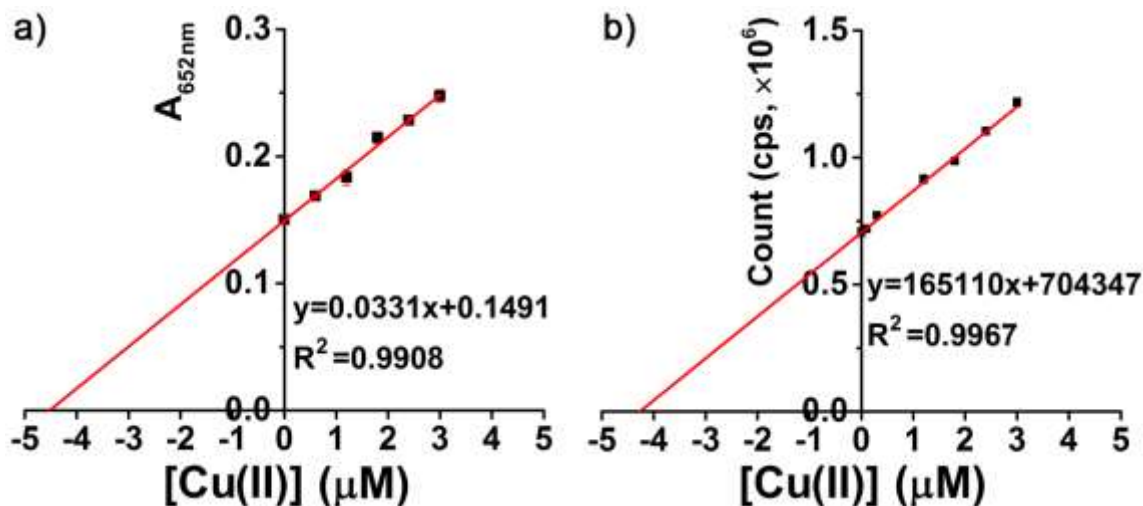


Fig. S20 Standard addition method for the calibration of Cu(II) concentrations in the real sample using (a) our method and (b) ICP-MS.

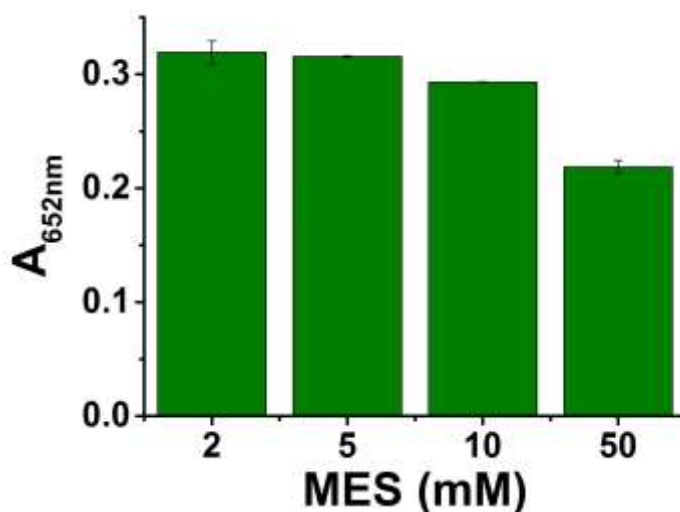


Fig. S21 MES buffer (pH 5.5) concentration on TMB- H_2O_2 reaction in the CA Cu-Fenton system. Reaction conditions: 100 nM Cu(II), 750 mM H_2O_2 , 0.5 mM TMB.

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