Supporting Information (SI)

Novel synthesis of mixed Cu/CuO-reduced graphene oxide nanocomposite with enhanced peroxidase-like catalytic activity for easy detection of glutathione in solution and using paper strip

*Preeti Singh,[a] Peuli Nath,[a] Ravi Kumar Arun,[a] Soumen Mandal,[a] and Nripen Chanda[a,b]**

[a]Micro System Technology Laboratory, CSIR-Central Mechanical Engineering Research Institute, MG Avenue, Durgapur 713209, India.

[b]Academy of Scientific and Innovative Research (AcSIR), CSIR Campus, CSIR Road, Taramani, Chennai 600113, India

Instrumentation

The surface morphologies of graphene stabilized copper nanoparticles (of Cu/CuO-rGO nanocomposite) were observed by a scanning electron microscope (Zeiss Sigma HD electron microscope), operated at an accelerating voltage of 200 kV. The core size was analyzed by transmission electron microscope (HRTEM, JEOL 3010). X-ray diffraction (XRD) measurements were carried out using a diffractometer (X'Pert PRO, PANalytical B.V., Netherlands) with CuK-α1 radiation (40 kV, 60 mA) to explore the crystallographic structure of the nanocomposite. The cyclic voltamogram was performed using PARSTAT (Potentiostat Galvanostat EIS Analyzer 4000 (Princeton Applied Research, AMETEK) to determine the oxidation state of copper in Cu/CuO-rGO nanocomposite. The Fourier transform-infrared spectroscopy spectra of GO, graphene, copper nanoparticles, and graphene stabilized copper nanoparticles were measured using a FT-IR spectrometer (Jasco 4700 FT/IR) to establish the interaction between CuNPs and graphene frame work. The copper electronic states and chemical composition of Cu/CuO-rGO were determined using X-ray photoelectron spectroscopy (XPS, PHI 5000 Versa Probe II, FEI Inc.). The UV-vis absorption spectra were recorded on a Cary 60 Agilent technologies spectrophotometer at room temperature. Centrifugation for sample purification was performed on a SORVALL RC 6+ centrifuge.

Analyses of glutathione on paper substrate

Analyses of GSH were performed on simple paper to test the practicality of Cu/CuO-rGO nanocomposite use. Whatman filter paper (Grade I) was used for the fabrication of straight strips. The paper was cut into the shape of straight strip with a circular head at one end by micro-machining method carried out by CO_2 laser engraving system (VLS 2.30, Universal Laser Inc., USA) at 3 Watt. The paper strip parameters were kept as follows i.e. height (H) = 0.1 mm, length (L) = 25 mm and width (W) = 3.75 mm and diameter of circular head = 7.5

mm. The paper strips were washed with hot HPLC water to remove unwanted impurities and dried. For GSH detection, one end was spotted with Cu/CuO-rGO nanocomposite solution (5.0 μL) followed by a drop of freshly prepared TMB-H_2O_2 solution mixture. Within few minutes, an intense blue color was appeared. Next, the opposite end of the paper strip was kept vertically in petridishes in such a way that small part of the strip remains in contact with GSH solutions of different concentrations. Due to capillary action of the paper strip, GSH solution reached to the spotted blue zone within few minutes and color change was observed. The bluish color of the channel heads became faded to different extents depending on concentration of GSH solution.

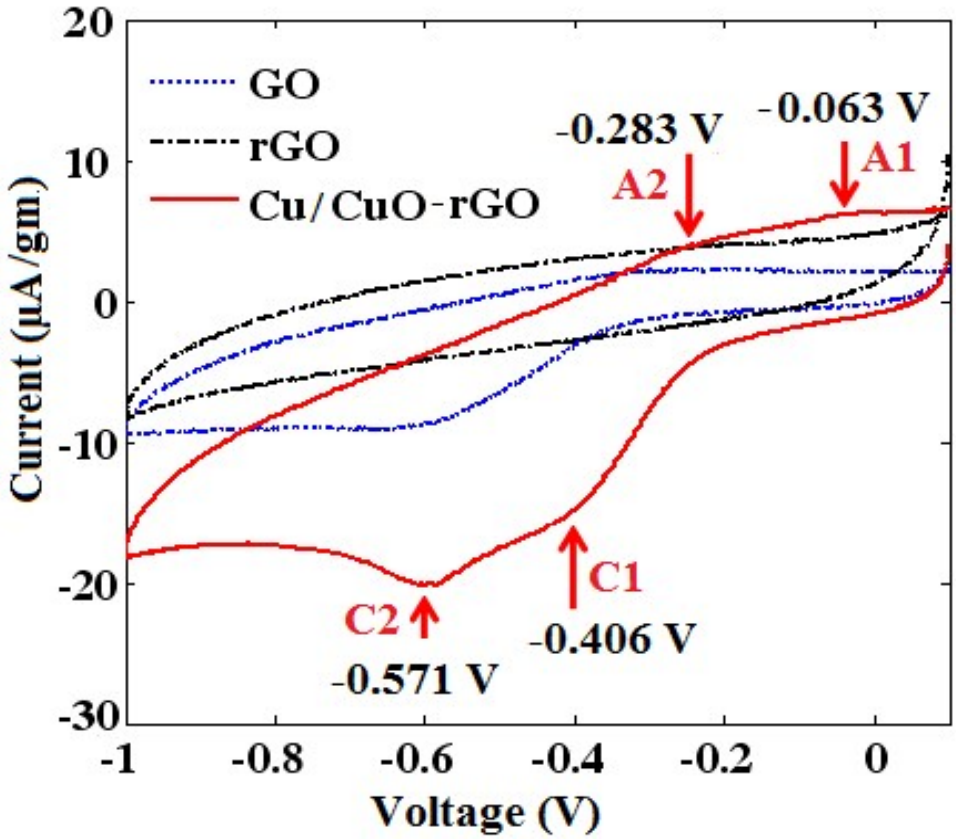


Fig. S1 Cyclic voltammograms of GO, rGO and Cu/CuO-rGO nanocomposites. The curve for nanocomposites shows two pairs of redox peaks at -0.063/-0.406 V (A1/C1) and -0.283/-0.571 V (A2/C2) due to Cu(II)/Cu(I)/Cu(0) and reverse transitions.

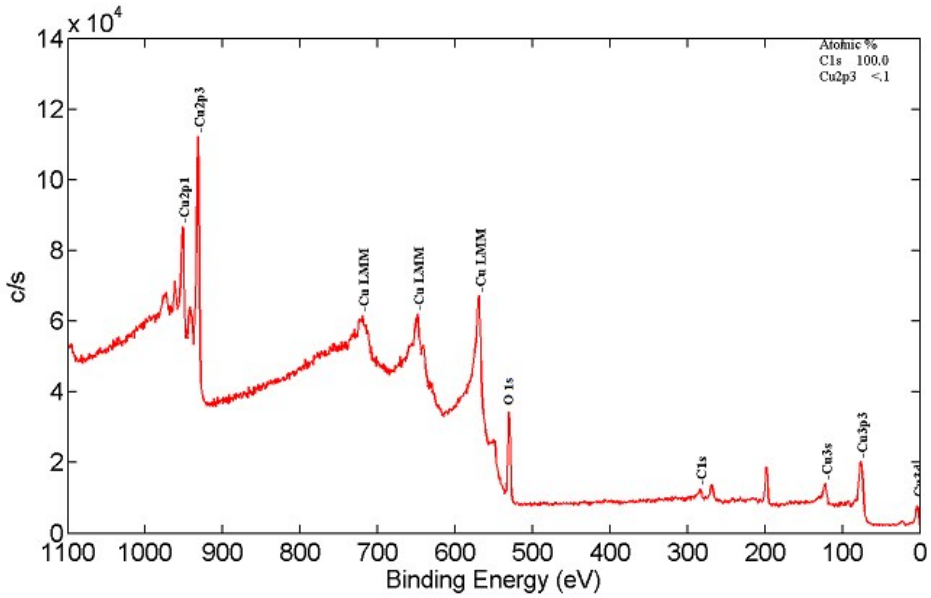


Fig. S2 The wide scan XPS spectrum of Cu/CuO-rGO showing the signals of Cu, C and O elements.

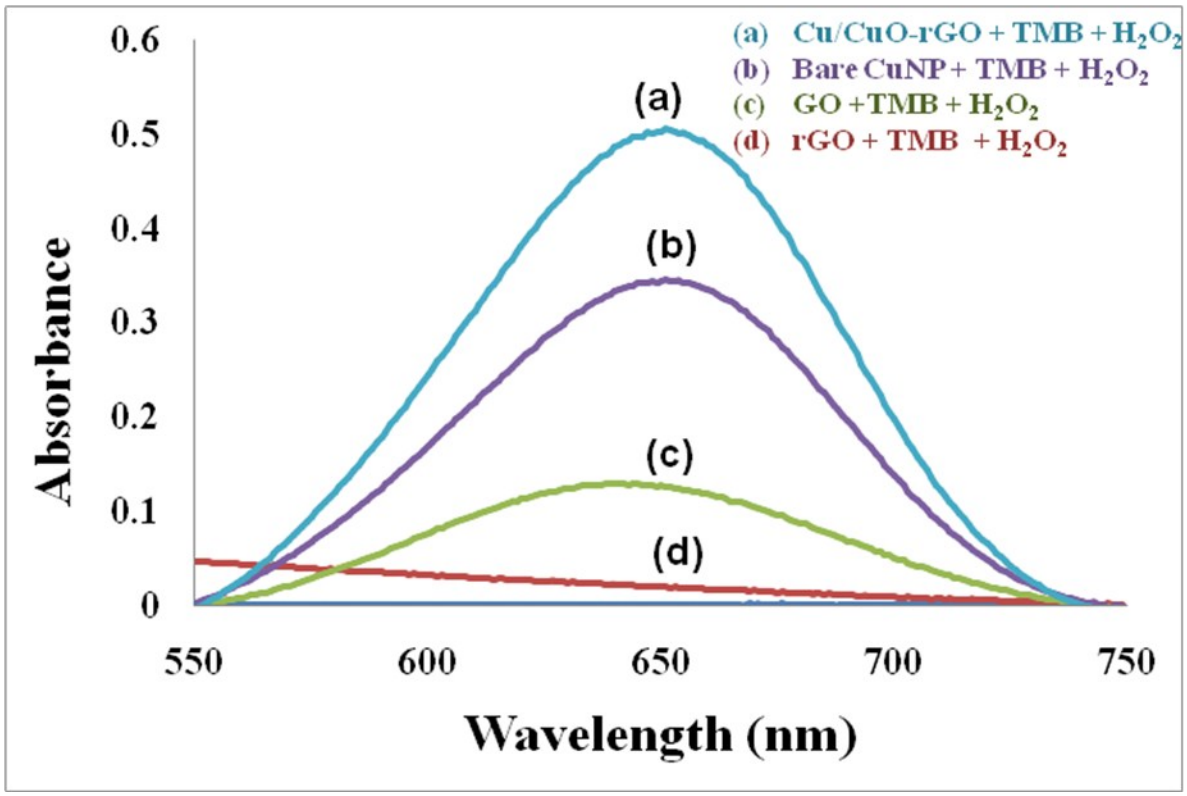


Fig. S3 UV-Vis spectra of TMB-H_2O_2 reaction performed with Cu/CuO-rGO and its various components i.e. GO, rGO and bare copper nanoparticles. GO and bare copper nanoparticles minimally contribute to the blue color formation while Cu/CuO-rGO shows maximum color after the reaction.

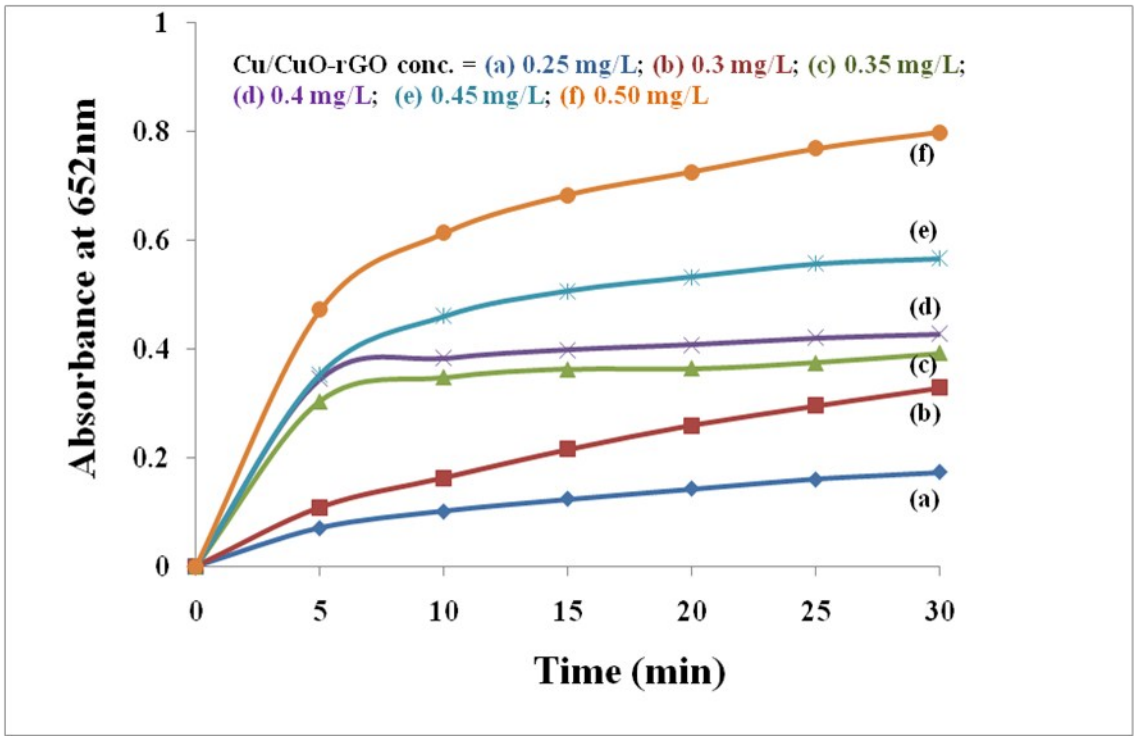


Fig. S4 Time course curves of different TMB oxidations catalyzed by Cu/CuO-rGO at various concentrations.

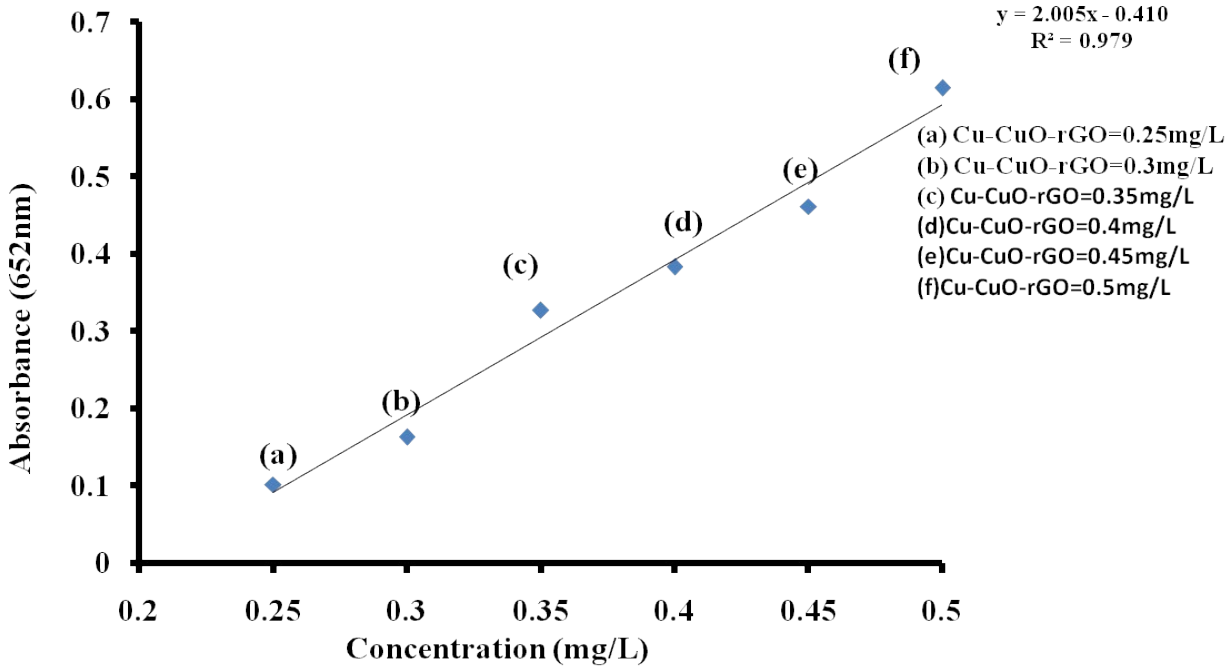
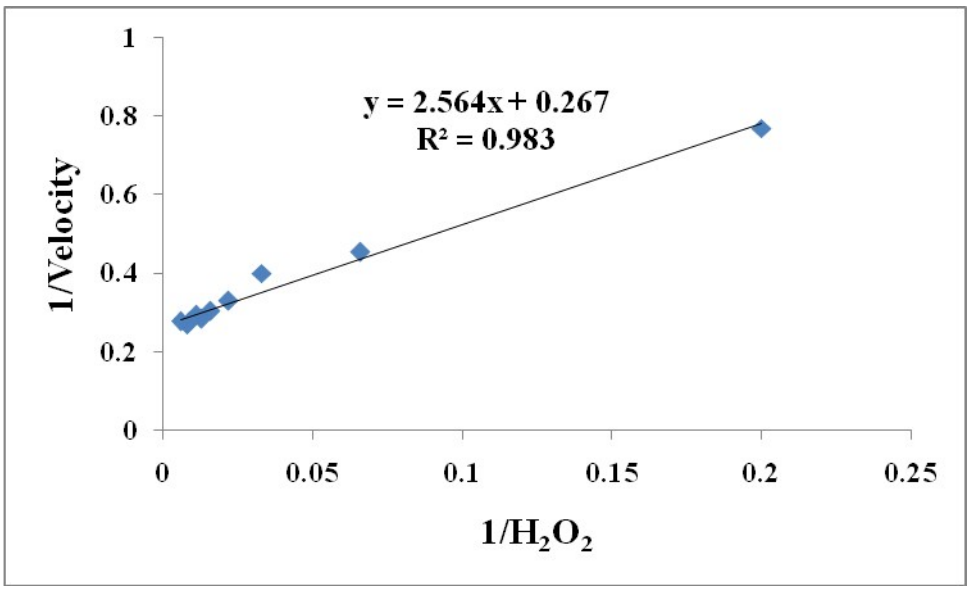


Fig. S5 Plot of absorbance versus Cu/CuO-rGO nanocomposites concentrations ranging from 0.25 to 0.50 mg/L showing a linear relationship.

(A)



(B)

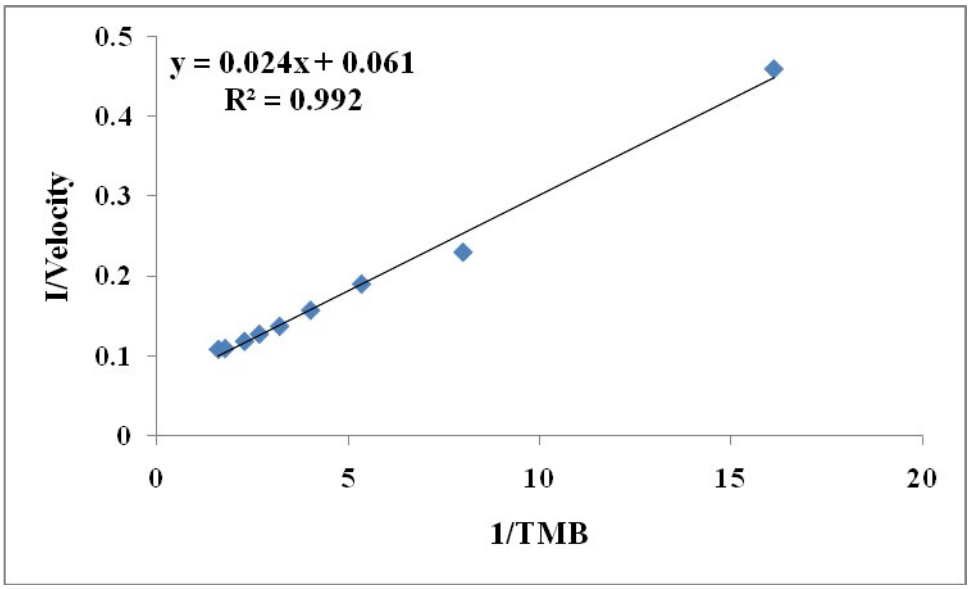


Fig. S6 Lineweaver-Burk plots for (A) various H_2O_2 concentrations (reciprocal) and (B) various TMB concentrations (reciprocal) to obtain Michaelis-Menten constant (K_m).

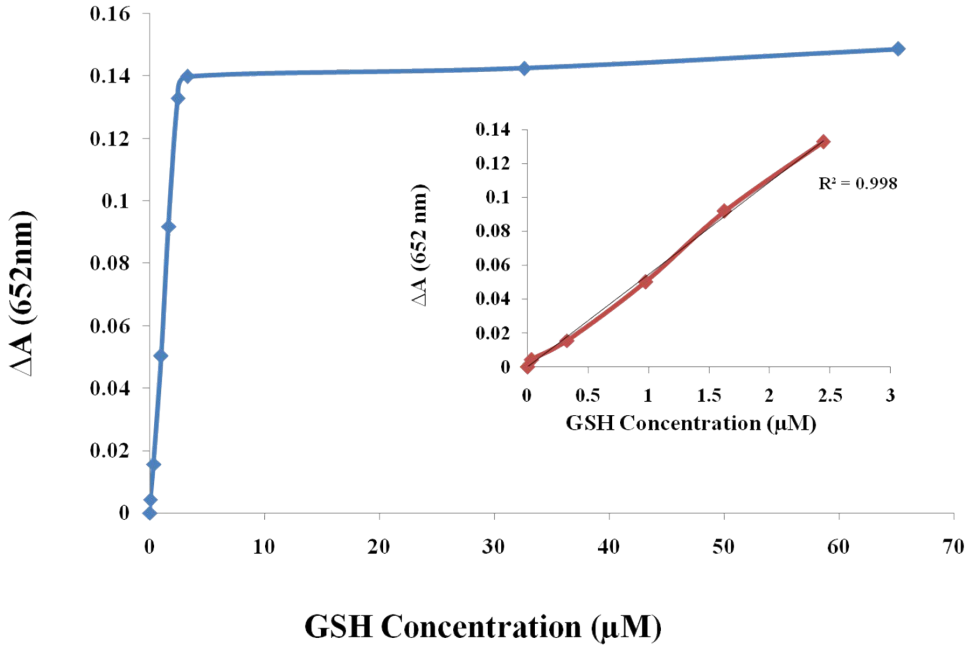


Fig. S7 Relationship between the ΔA (ΔA = $A_{blank} - A_{GSH}$) and GSH concentration. Inset shows a good linear relationship with GSH concentration in the range of 0.03 µM to 3.25 µM.

Catalyst	K_m/mM		Reference
	H_2O_2	TMB	
HRP	3.7	0.434	Zheng et al. (2015)
Cu/CuO-rGO	9.21	0.39	This Work

Table S1 Comparison of kinetic parameters of Cu/CuO-rGO (enzyme-mimic) and HRP (natural enzyme).

Probe	Mode	Detection Limit	Detection Time/min	Reference
Au NPs-ppzdtc	Colorimetry	8nM	30	Li et al. (2011)
CQDs-Au NPs	Colorimetry	Not given	5	Shi et al.(2014)
Au NPs-HG (II)	Colorimetry	17nM	21	Xu et al. (2012)
Fe_3O_4-ABTS-H_2O_2	Colorimetry	Not given	20	Ma et al.(2012)
MnO_2 NPs-TMB	Colorimetry	0.1μM	15	Liu et al. (2013)
Ag^+-TMB	Colorimetry	0.05μM	10	Ni et al. (2015)
Graphene dots	Colorimetry	0.5μM	10	Zheng et al. (2013)
RGO_Fe_3O_4-Pd nanohybrids	Colorimetry	8.6×10^{-8} M	Not given	Zheng et al. (2015)
(GO)-hemin	Colorimetry	$8.2\times10^{(-11)}$ M	Not given	Bi et al. (2014)
Cu/CuO-rGO	Colorimetry	0.0325μM	~ 5 min	This work

Table S2 Lowest detection limit of reported peroxidise/oxidase- mimics including the present Cu/CuO-rGO-TMB-H_2O_2 system.