Electronic Supplementary Information (ESI)

Bacteria-Derived Topologies of Cu_2O Nanozymes Exert Variable Antibacterial Effect

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Materials and methods

Fluorine-doped tin oxide (FTO) coated glass (TECHINSTRO, Mumbai, India), potassium nitrate (KNO_3, SRL chemical), copper sulfate ($CuSO_4.xH_2O$, Sigma-Aldrich), citric acid (SRL chemical), horse heart cytochrome c (Sigma-Aldrich), sodium thiosulphate ($Na_2S_2O_4$, SRL chemical), sephadex G-25 PD-10 desalting column (Sigma-Aldrich), Luria-Bertani (Hi-Media, India), Luria-Bertani agar (Hi-Media, India) and Milli-Q grade water were used in procured from the respective vendors and used in experiments.

Instruments and accessories

Potentiostat-galvanostat (AutoLab instrument) was used for all electrochemical measurements, spin coater (Technoscience; model SC10k), FESEM (JEOL JSM-7900F), XRD (Bruker AXS, Germany), XPS (Thermofisher Scientific ESCALAB), Nebertherm furnace and incubator were used for various experiments and characterizations. Bacteria culture was performed using a shaker incubator.

Fabrication of copper oxide (CuO) thin film on FTO glass substrate

For fabrication of CuO electrodes, FTO coated glass substrates were used having dimensions of 25 mm×25 mm×1.1mm (L×B×H). Initially, the substrates were cleaned in an ultrasonic bath with a hot detergent solution, followed by consecutive washing with hot DI water (at 70 °C for 30 min) and ethanol solution (at 65 °C for 20 min). The copper oxide thin film electrodes were fabricated by a sol-gel method by modifying the protocol reported by Shukla et al.[S1] Sol was prepared by adding 0.25 M copper sulphate ($CuSO_4.5H_2O$) solution dropwise to the 0.25 M aqueous solution of citric acid. Subsequently, the solution was stirred for 12 h at room temperature on a magnetic stirrer and kept for 72 h for aging. Afterwards, 0.4 mL sol solution was drop casted on FTO substrate and accelerated at 300 rpm for 10 s and then spin-coated at 1500 rpm for 30 s. The above procedure was repeated 5 times for final synthesis of the CuO thin film. Spin-coated FTO substrates were then placed in a furnace at 450 °C temperature for 4 h. The CuO electrodes were allowed to cool slowly at room temperature in the furnace before using in experiments.

SEM image of synthesized CuO thin fim

The surface morphology of the synthesized CuO thin film was investigated using an FESEM analysis and the results are shown in Fig. S1. Groups of nanoparticles measuring approximately 50 nm were observed on the surface of a newly prepared WE, as depicted in the inset image. The corresponding EDAX analysis confirmed the presence of only Cu and O over fresh FTO substrate.

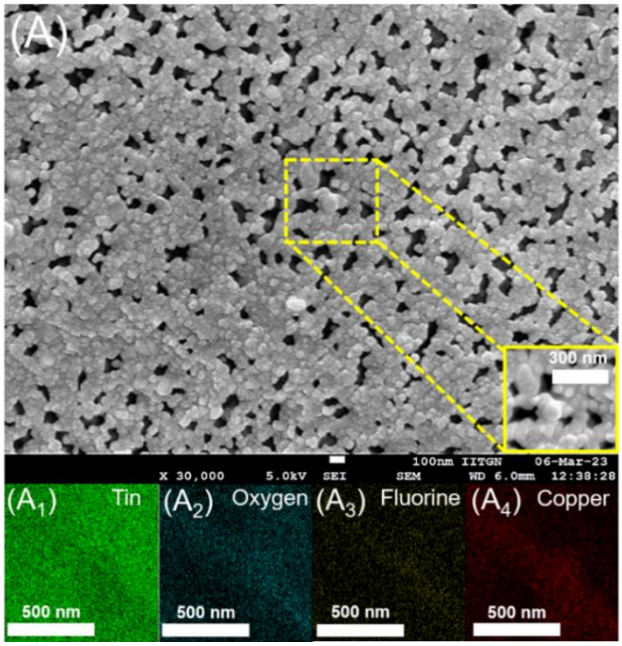

Fig. S1 (A) FESEM image of a synthesized CuO-coated FTO electrode. EDAX spectra taken at the region of interest in (A, inset) using elemental color mapping. Images (A_1), (A_2), (A_3) and (A_4) depict the elemental color maps at the selected region of interest.

XRD spectra of CuO with Cu_2O nanoparticles

Figure S2 shows a detailed XRD analysis of fresh CuO electrode before using for synthesis of different topologies of the Cu_2O nanoparticles. The synthesized CuO thin film showed characteristic diffraction peak 2θ at 35.56º, 38.8º, and 68.03º in the diffraction pattern, which corresponds to reference card numbers 00-041-0254 of International Centre for Diffraction Data (ICDD).

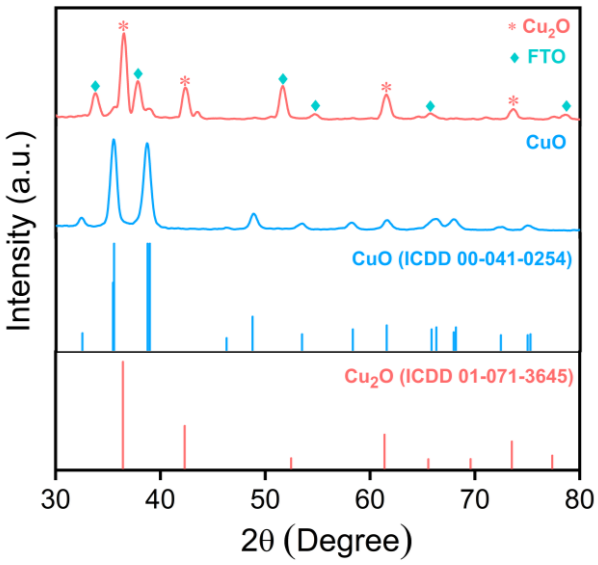

Fig. S2 XRD spectra of the fresh CuO thin film electrode before and after bacteria mediated redox reaction in the electrochemical setup. Image showed the presence of CuO before the redox reaction and formation of Cu_2O after the bacteria-mediated redox reaction on the thin film electrode.

E. coli bacteria culture

Primary culture of gram-negative bacteria *Escherichia coli* (*E. coli*, strain DH-5 Alpha) was prepared by overnight incubation of the culture tube containing trace amount of bacteria taken from prepared agar plate at 37 ^{0}C in 5 mL Luria-Bertani (LB) medium in a shaker incubator. The culture time was approximately 11-12 h. Aliquots of this primary solution were then diluted (400 µL primary culture solution in 4 mL LB media) and incubated again for 3 h to prepare the secondary bacterial suspensions. The secondary bacterial suspension was further diluted using LB medium to prepare 10^4 CFU mL^{-1} solution of the bacteria. Above volume of secondary bacterial solutions was slowly added to the electrolyte (KNO_3) solution to make the final volume 20 mL during experiments. Electrochemical measurements were performed to synthesis the different morphology of the Cu_2O nanoparticles.

Reduction of CuO nanoparticles with Cu_2O nanostructures

Fig. S3A depicts the mechanism of reduction of the CuO nanoparticles into Cu_2O nanostructures in the presence of *E. coli* (DH5- Alpha) bacteria. The fresh CuO thin film on the FTO substrate prior to the redox reaction is displayed in Fig. S3B. After undergoing the bacteria mediated redox reaction, the electrode was found to have a brick-colour, which is indicative of the formation of Cu_2O nanoparticles on FTO substrate.

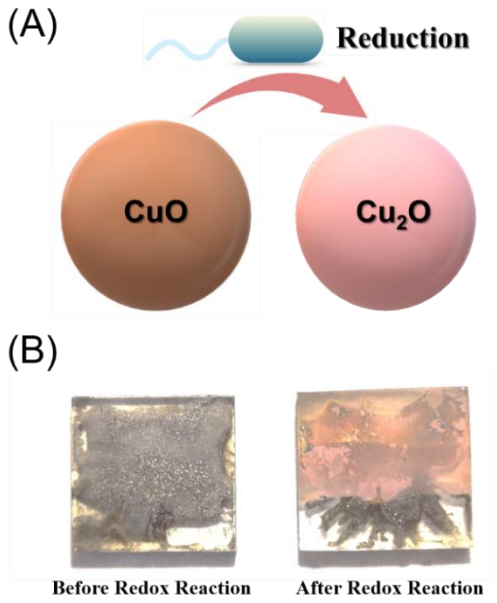

Fig. S3 Image (A) showing the reduction of CuO nanoparticles with Cu_2O nanostructures in presence of bacteria. In the image (B) prior to the redox reaction, only a thin film of CuO was visible, after the reaction however, a brick-colored coating was seen on the electrode, confirming the presence of a Cu_2O film caused by the enzymatic activity of bacteria.

FESEM of the electrode at variable CV cycles

We also performed the experiments at 5, 15 and 25 CV cycles and recorded the FESEM images of the respective electrodes. As shown in Fig. S4, we observed intermediate morphologies of the Cu_2O NP emerging after 5, 15 and 25 cycles. It should be noted that the outcomes from a certain number of CV cycles also depends upon the specific concentration of the bacteria and electrolyte (KNO_3) used and the exposed surface area of CuO electrode used during the reaction.

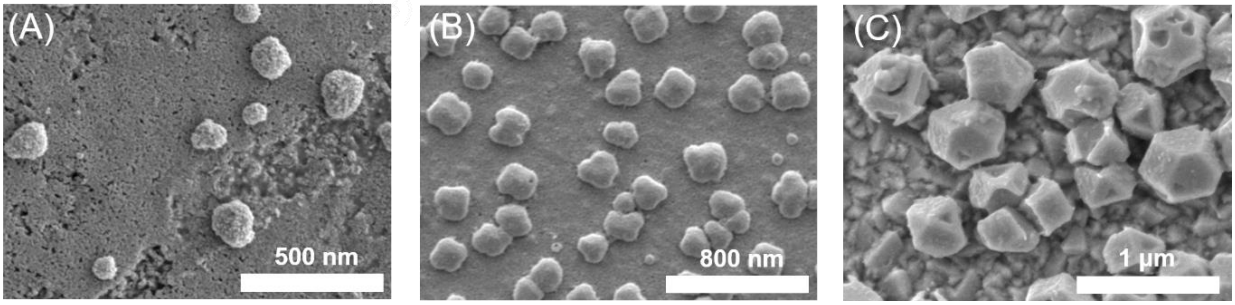

Fig. S4 SEM images of the working electrodes after 5, 15 and 25 cycles of the cyclic voltammetry measurements.

X-ray Photoelectron Spectroscopic (XPS) analysis

We performed XPS measurements to confirm the oxidation state of Cu in the nanoparticles pre- and post-redox reaction in our setup. Figure S5 (A, B) present survey scans of the XPS spectra of the electrode before and after the electrochemical redox reaction. The survey scan of the electrode contains peaks characteristic of Cu 2p, and O 1s. To determine the binding energy peak of oxygen elements narrow scan XPS spectra were recorded within the BE ranges (525 to 545 eV) for both cases (Fig. S5 C, D).

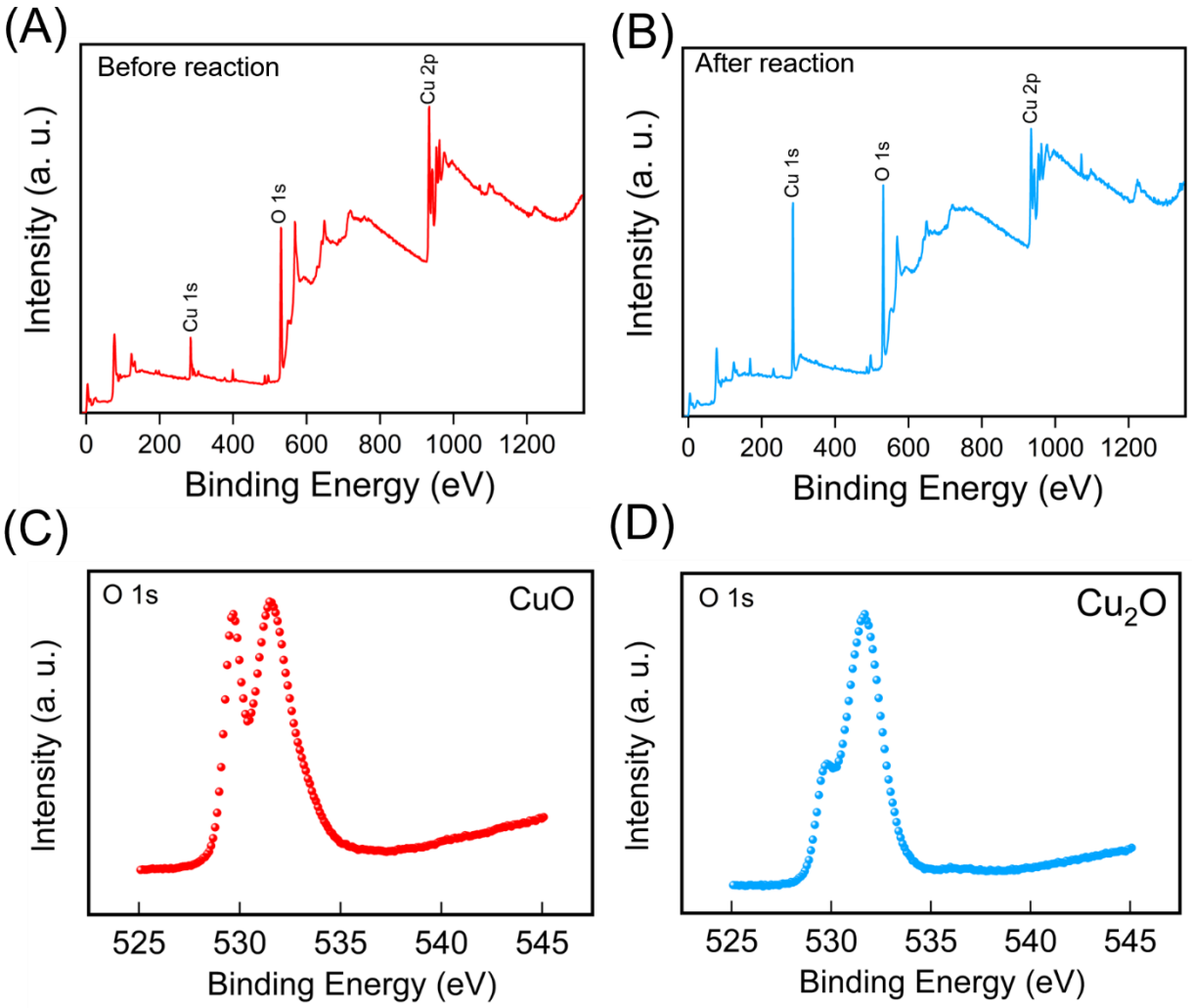

Fig. S5 XPS survey spectra of the working electrode (A) before and (B) after the redox reaction. (C) and (D) show oxidation state of oxygen in case of CuO and Cu_2O nanoparticles, respectively.

Preparation of ferrous cytochrome c (Cyt c)

To check the catalytic activity of different morphology of the Cu_2O nanoparticles, we prepared ferrous Cyt c by adding 10 mM of sodium thiosulphate ($Na_2S_2O_4$) to the solution of ferric Cyt c in

phosphate buffer (pH 7.4). After reduction, excess amount of $Na_2S_2O_4$ was removed by passing through a Sephadex G-25 PD-10 desalting column followed by centrifugation at 2000 rcf for 2 min.

Colour of the Cu_2O nanostructures

The suspension of freshly synthesized different morphology Cu_2O NPs was used for comparing optical images of spherical (176 nm), cubic and (320 nm) and truncated octahedron (518 nm), as shown in Fig. S6.

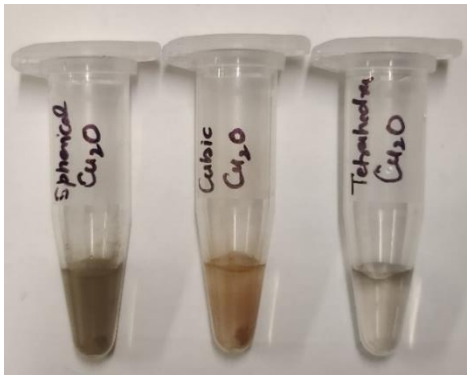

Fig. S6 Comparison of color of different morphologies of the synthesized Cu_2O NPs.

Circular dichroism (CD) spectroscopy of ferrous and ferric cytochrome c (Cyt c)

The CD spectroscopy of the Cyt c was performed to find the change of ferrous to ferric Cyt c in experiments. Fig. S7 shows the CD spectra of the ferrous Cyt c, and after addition of the 1 mg mL^{-1} Cu_2O NPs. The change in CD spectra indicate conformational changes of ferrous Cyt c to ferric Cyt c due to its interaction with Cu_2O NPs.

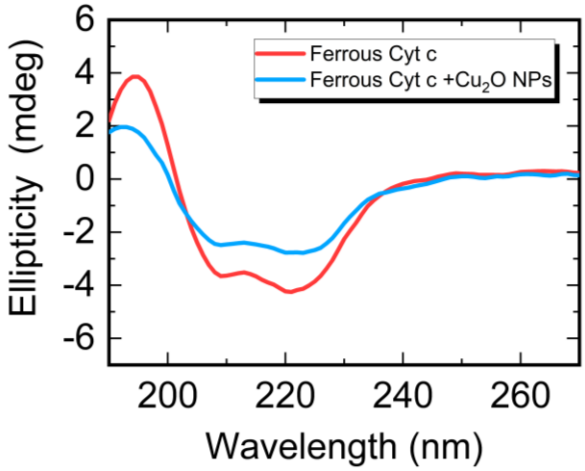

Fig. S7 CD spectroscopy profiles for ferrous Cyt c to ferric Cyt c solutions. During experiments, samples were measured using 0.1 cm cuvettes at 25 °C temperature and pH 7.4.

Descriptions of the bactericidal experiments

The antibacterial effect of the Cu_2O NPs was tested following the previous reported protocols.[S1-S3] We performed bactericidal experiments using different topologies (spherical, cubic and truncated octahedron) of Cu_2O NPs at a concentration of 1 mg mL^{-1}. These NPs were incubated with *E. coli* (1×10^5 CFU mL^{-1} DH-Alpha) bacteria in LB media. After a 15 min incubation period of the bacteria with Cu_2O NPs, the solutions were cultured on agar plates, as shown in Figure 5(B-D). The plates were incubated overnight in a shaker incubator for approximately 12-13 h to facilitate colony formation.

References:

[S1] A. K. Shukla, S. Mitra, S. Dhakar, A. Maiti, S. Sharma K. K. Dey, *ACS Appl. Bio Mater.* 2023, **6**, 117–125.

[S2] A. K. Shukla, S. Bhandari, and K. K. Dey, *Mater. Today Commun.* 2021, **28**, 102504.

[S3] A. K. Shukla, S. Mitra, S. Dhakar, A. Maiti, S. Sharma, and K. K. Dey, *ACS Appl. Energy Mater.* 2022, **5**, 12916-12924.