

**Supplementary Information**  
**for**  
**Enzymes as Bionanoreactors: Glucose Oxidase for the Synthesis of**  
**Catalytic Au Nanoparticles and Au Nanoparticle-Polyaniline**  
**Nanocomposite**

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### **Spectrophotometric assay for the ferrous oxidation in presence of Xylenol orange by H<sub>2</sub>O<sub>2</sub> using FOX reagent:**

The activity of the native enzyme and the enzyme after the synthesis of Au NPs was tested spectrophotometrically following a reported procedure<sup>1</sup> using the FOX method. The method is based on the oxidation of ferrous ions to ferric ions by hydrogen peroxide and simultaneous purple colored complex formation of the ferric ions with xylenol orange. Briefly, solutions of native enzyme and Au NP-GOx composite with enzyme concentration of 0.3 mg/ml were prepared. To both these solutions 0.84 mg of glucose was added and allowed to stand for 3 minutes. Finally 35 µl of the above solutions were added to 4 ml of FOX reagent taken in two separate vials and the mixture was incubated at room temperature for 40 minutes before recording the UV-visible spectrum.

### **Fluorescence study:**

The fluorescence study of the native enzyme and Au NP-enzyme composite was performed using an excitation wavelength of 295 nm. The enzyme concentration used for the fluorescence measurements was 0.3 mg/ml.

### **Modification of Thiol groups in glucose oxidase using DTNB:**

The Thiol groups of glucose oxidase were modified using DTNB in PBS buffer of strength 10 mM and pH 8.0, following a reported procedure.<sup>2</sup> Briefly, 1.75 mg of glucose oxidase was dissolved in 2 ml of phosphate buffer. To this solution 0.5 mL of 1 mM DTNB was added. After a few minutes, the color of the enzyme solution changed from almost colorless to yellow, indicating the modification of thiol groups.

## **Cyclic Voltammetry studies**

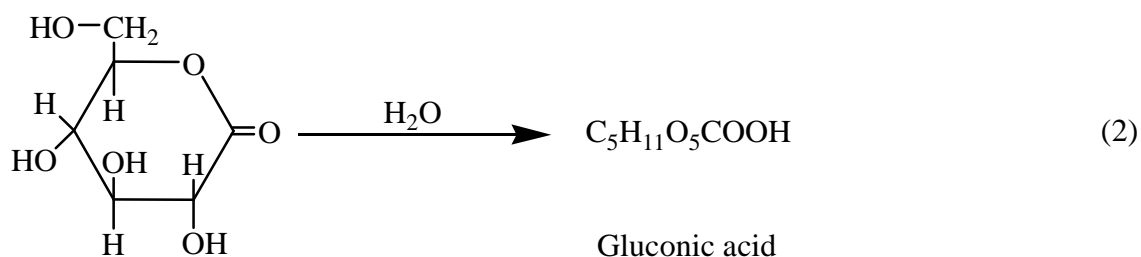
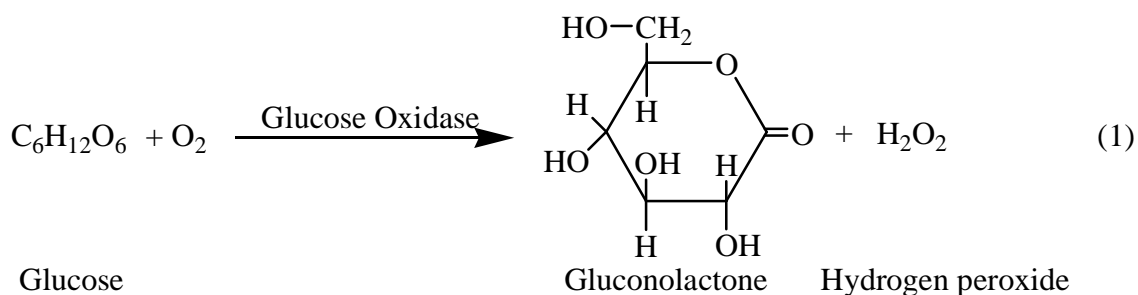
Cyclic voltammetry study was performed on a CHI620D electrochemical analyzer in 0.75 M HCl solution at a scan rate of 100 mV/s by using Pt as a working electrode, Pt wire as the counter electrode and Ag/AgCl as the reference electrode.

## **Instrumentation**

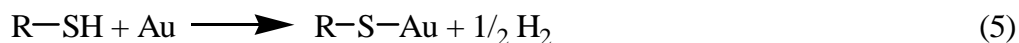
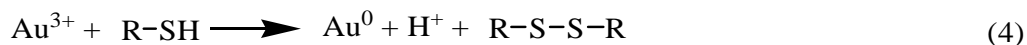
UV- visible spectra were recorded on a Varian Cary 100 Bio spectrophotometer. Powder X-ray diffraction spectra (XRD) were recorded on a Bruker D8 Advance diffractometer with Cu K $\alpha$  source (wavelength of X- rays was 0.154 nm). The samples for XRD were prepared by drop casting the sample solutions on glass slides and drying them at room temperature. FTIR spectra were recorded in KBr pellet using Bruker Tensor 27 instrument. The transmission electron microscopy (TEM) images were recorded on Technai G<sup>2</sup> 20 Ultra-Twin microscope. The samples for TEM were prepared by drop casting the sample solutions on carbon coated copper grid followed by room temperature drying. Circular Dichroism (CD) experiments were performed using a JASCO J-815 spectropolarimeter. Fluorescence measurement was performed on a fluoromax-4p fluorometer from Horiba (Model: FM-100). Cyclic voltammetry was carried out on a CHI620D electrochemical analyzer.

### Pertinent equations:

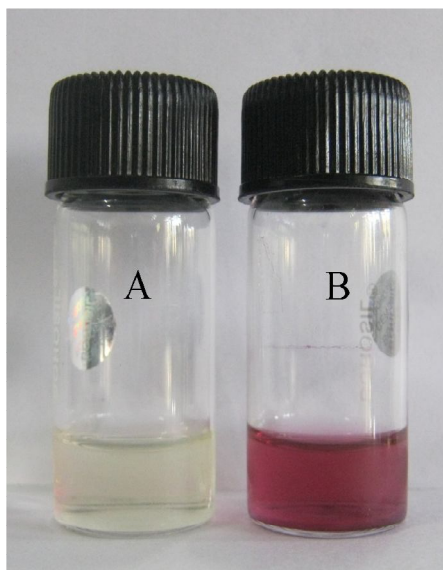
The reduction of oxygen, resulting in the formation of hydrogen peroxide can be achieved via enzymatic route using the enzyme glucose oxidase. The enzyme glucose oxidase catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide according to the following reactions:



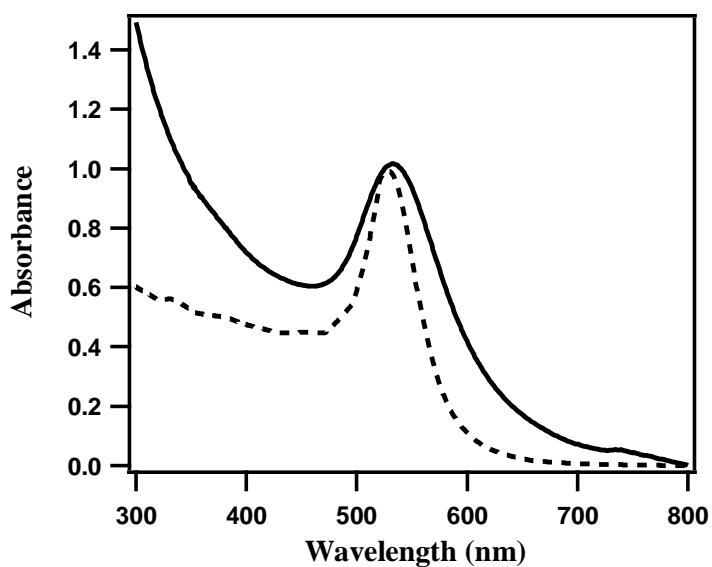
The reducing ability of the enzyme, presumably because of free thiols present in the cysteine residues function as follows during the synthesis of Au nanoparticles:



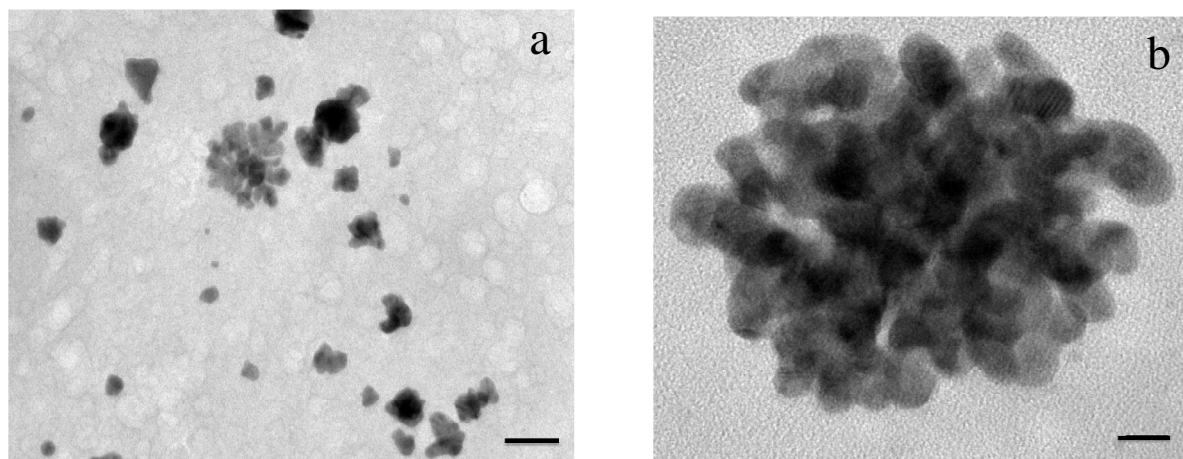
The free thiols provide the electrons to  $\text{Au}^{3+}$  for reduction and themselves get converted to disulfides.



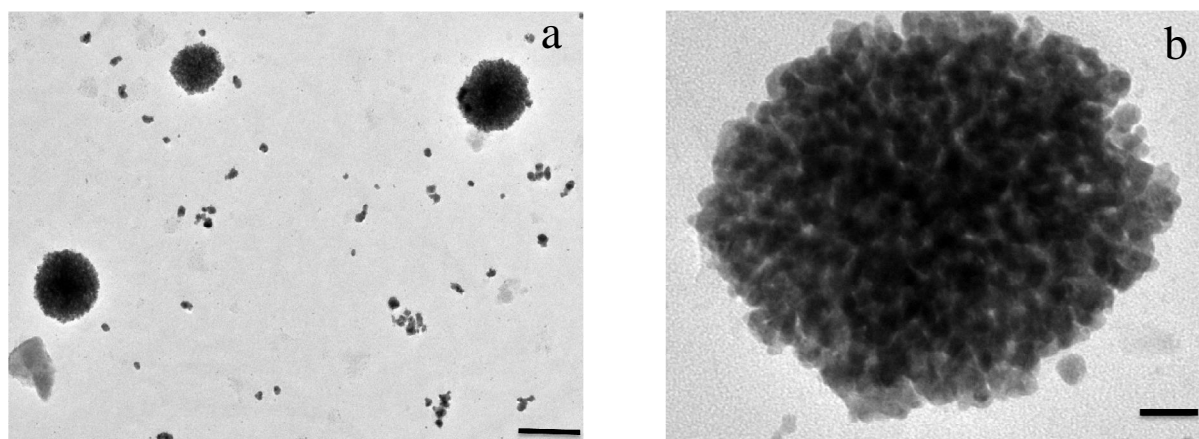
**Figure S1.** Digital image of GOx solution containing  $\text{HAuCl}_4$  (A) before reaction and (B) after incubation for 36 hours at  $37^\circ\text{C}$ .



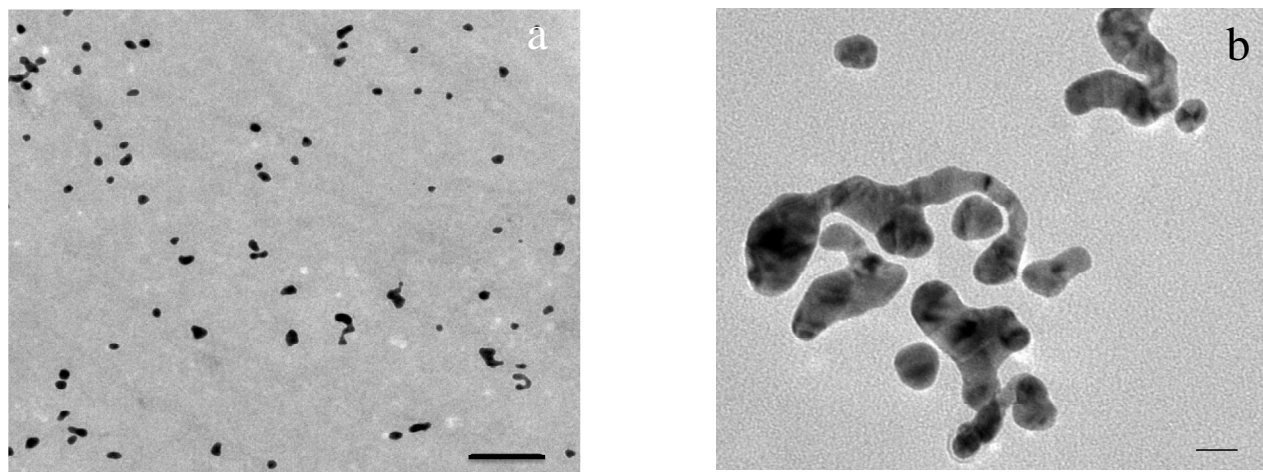
**Figure S2.** UV-visible absorption spectrum of Au nanoparticles. Experimental absorption spectrum (Solid line) and calculated absorption spectrum (Dashed line) as calculated using Mieplot v4304 software for Au nanoparticles with average diameter 9.9 nm.



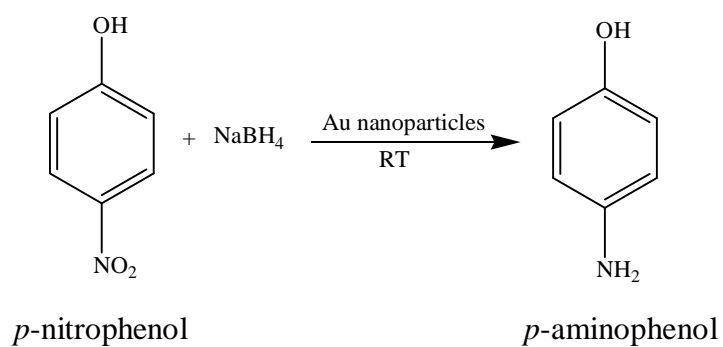
**Figure S3.** (a) TEM image; Scale bar 20 nm and (b) HRTEM image; scale bar 5 nm of Au nanostructures synthesized using glucose oxidase with an enzyme concentration of 1.75 mg/ml and  $\text{HAuCl}_4$  concentration  $6 \times 10^{-4}$  M.



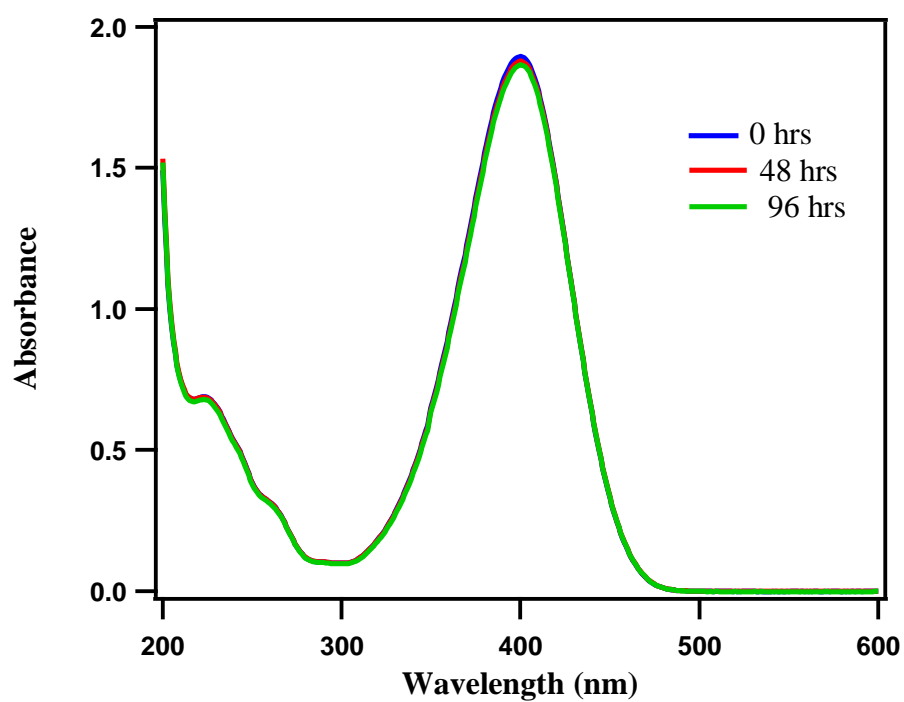
**Figure S4.** (a) TEM image; Scale bar 100 nm and (b) HRTEM image; scale bar 10 nm of Au nanostructures synthesized using glucose oxidase with an enzyme concentration of 0.7 mg/ml and  $\text{HAuCl}_4$  concentration  $1.5 \times 10^{-3}$  M.



**Figure S5.** (a) TEM image; Scale bar 100 nm and (b) HRTEM image; scale bar 10 nm of Au nanostructures synthesized with a glucose oxidase concentration of 0.7 mg/ml and  $\text{HAuCl}_4$  concentration  $3 \times 10^{-3}$  M.

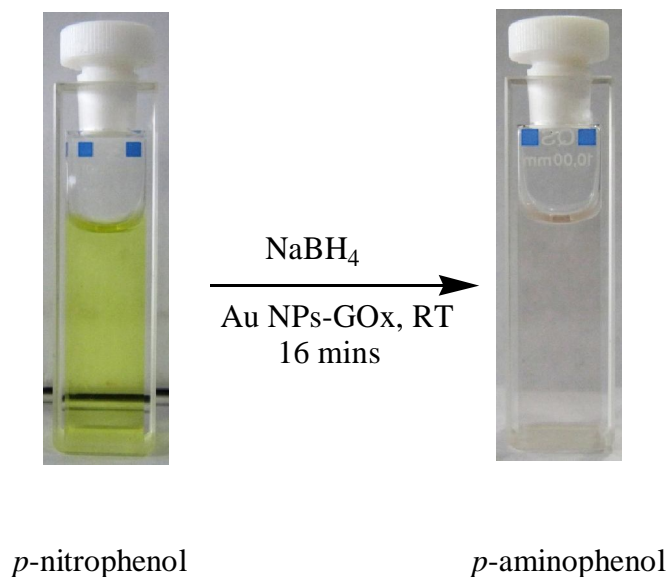


**Scheme 1.** Scheme for the reduction of *p*-nitrophenol to *p*-aminophenol by NaBH<sub>4</sub> using glucose oxidase reduced Au nanoparticles as catalyst.

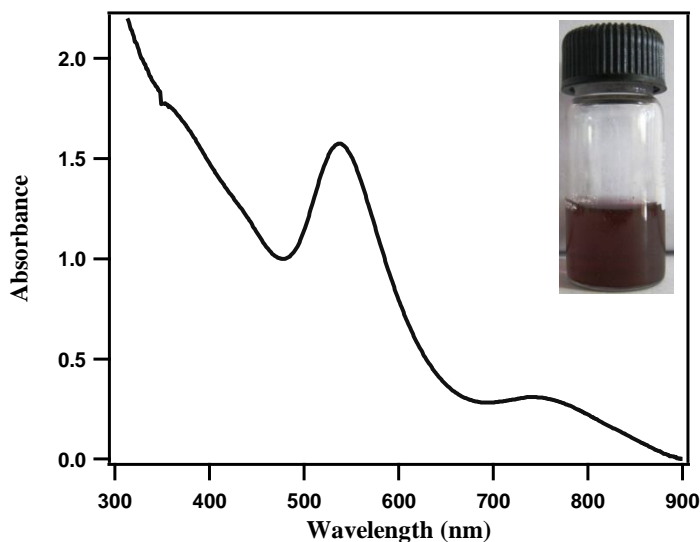


**Figure S6.** UV-visible spectrum for the reduction of *p*-nitrophenol by NaBH<sub>4</sub> in the absence of GOx reduced Au nanoparticles.

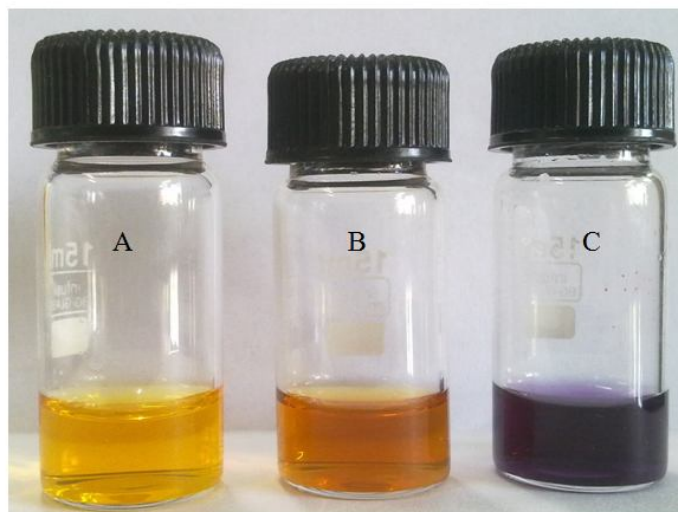




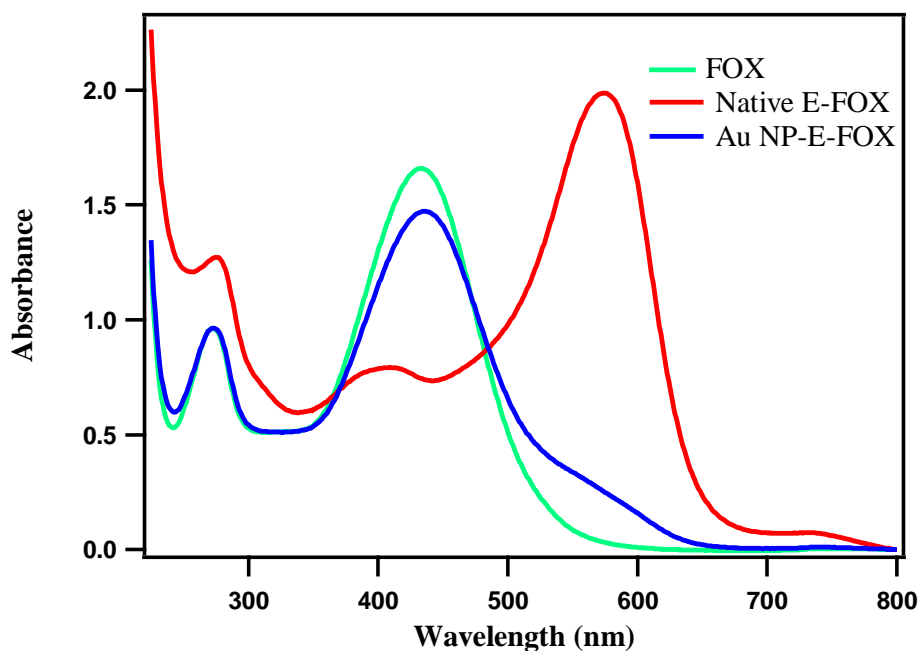
**Figure S7.** Digital image showing the reduction of *p*-nitrophenol to *p*-aminophenol by  $\text{NaBH}_4$  in presence of GOx reduced Au nanoparticles as catalyst. The intense yellow color due to formation of *p*-nitrophenolate ion becomes colorless after the reduction due to the formation of *p*-aminophenol.



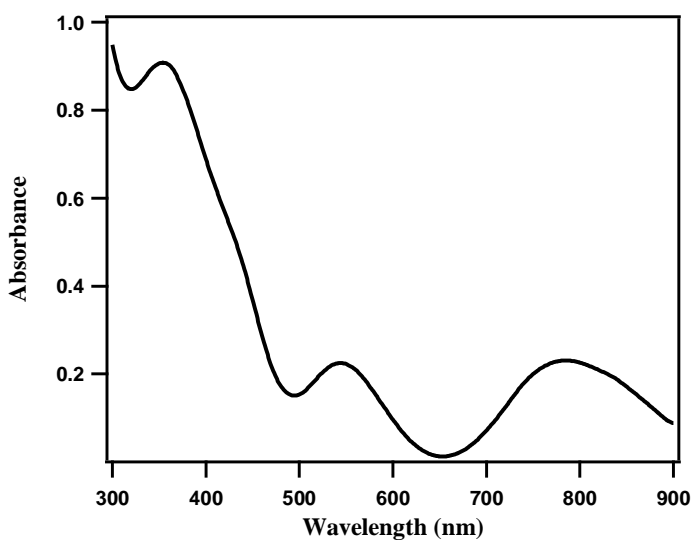
**Figure S8.** UV-visible spectrum of Au NP-GOx-PANI composite indicating the decrease in the activity of GOx after its involvement in the synthesis of Au nanoparticles and digital image of Au NP-PANI composite (inset).



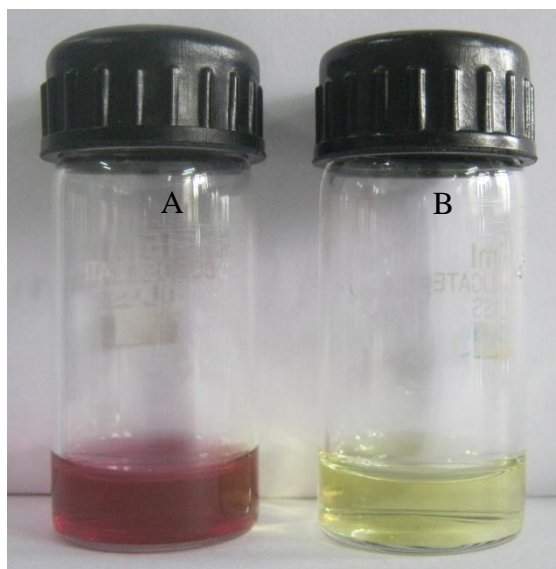
**Figure S9.** Photograph showing the reaction of native GOx and the Au NP- GOx composite with FOX reagent after addition of glucose, indicating the inhibition of activity of Au NP-GOx composite. (A) Blank FOX reagent, (B) Au NP- GOx- FOX after addition of glucose and (C) Native GOx- FOX after addition of glucose.



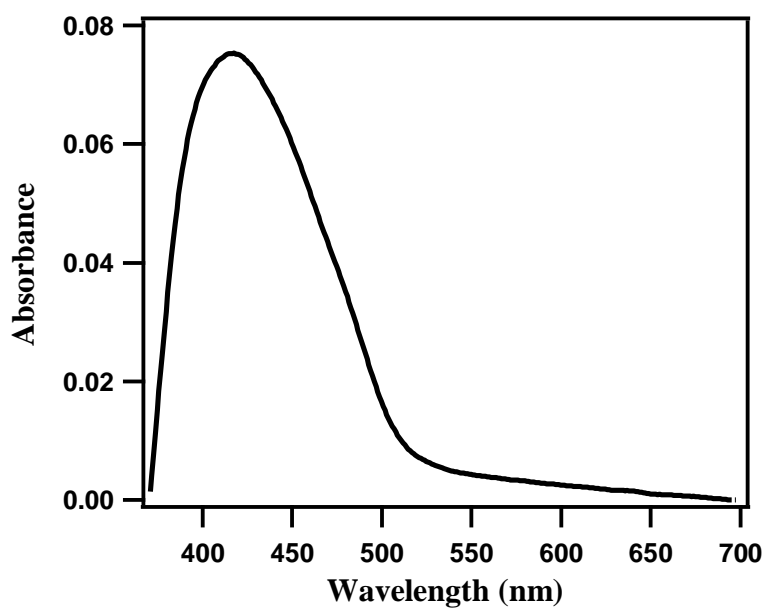
**Figure S10.** UV-visible spectrum, showing the reduced ability of the Au NP-GOx composite to synthesize  $\text{H}_2\text{O}_2$ . The peak at 570 nm in the red spectrum is due to the complex o-cresolsulfone-phthalein- 3'-3''-bis(methylimino) diacetate, formed by the reaction of  $\text{Fe}^{3+}$  and xylenol orange. The  $\text{H}_2\text{O}_2$  produced on the decomposition of glucose oxidized the  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$ .



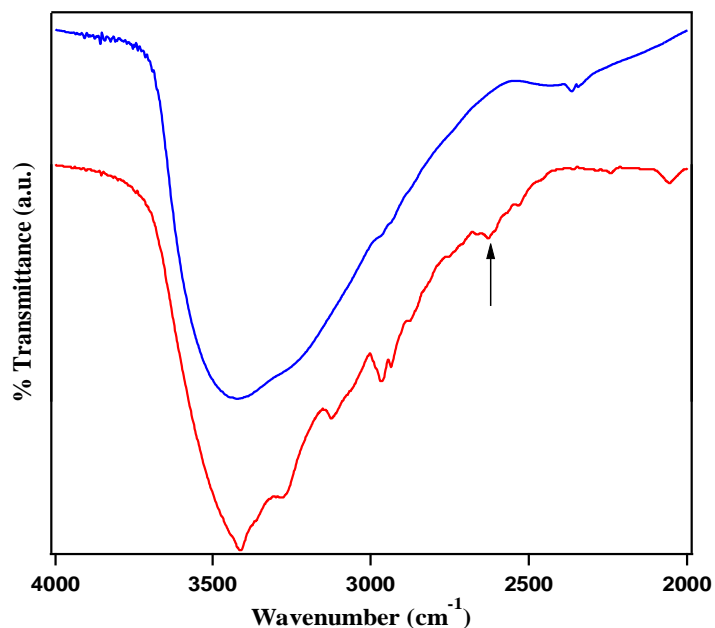
**Figure S11.** UV-visible spectrum of Cit. capped Au NP-PANI composite synthesized by GOx, after its adsorption on citrate Capped Au nanoparticles.



**Figure S12.** Digital image showing reaction of glucose oxidase with  $\text{HAuCl}_4$  in PBS (A) unmodified glucose oxidase and (B) after blocking the thiol groups in glucose oxidase by DTNB.

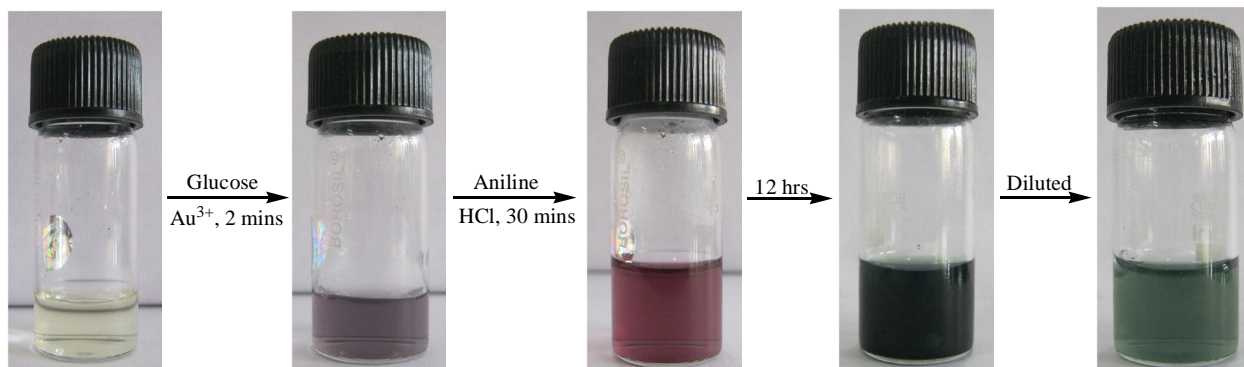


**Figure S13.** UV- visible spectrum of DTNB modified glucose oxidase after incubation with  $\text{HAuCl}_4$  for 60 hours, showing the absence of SPR band of Au nanoparticles.

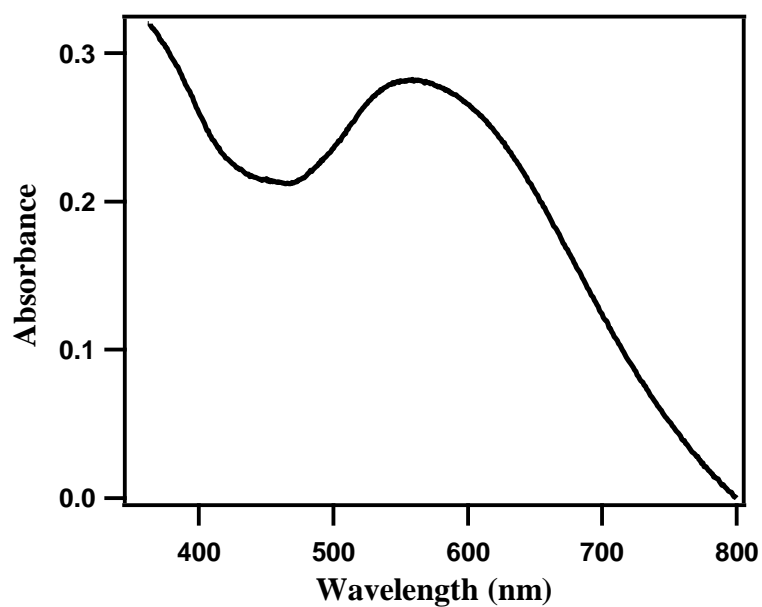


**Figure S14.** FTIR spectrum of native GOx (Red line) and Au-NP-GOx composite (Blue line).

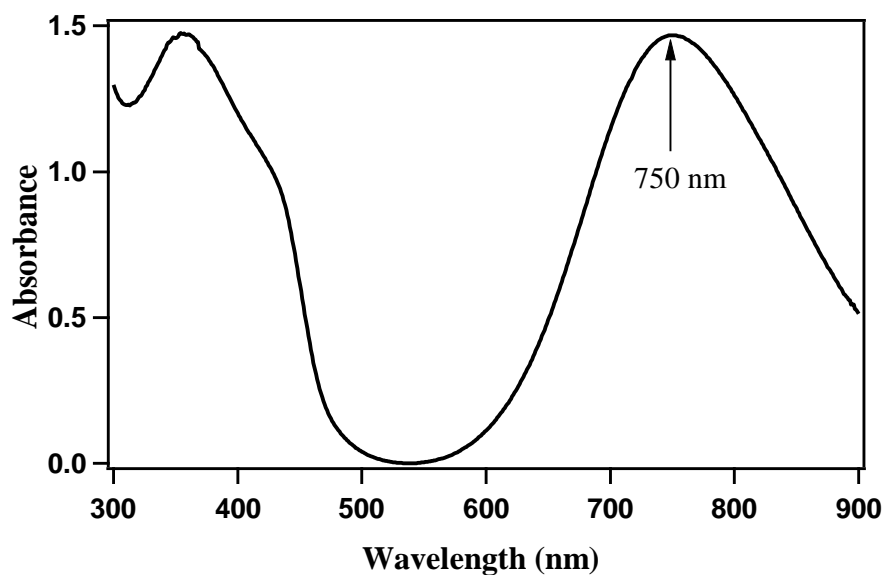
Arrow in red line at  $2625\text{ cm}^{-1}$  is due to S-H stretching which is absent in blue line.



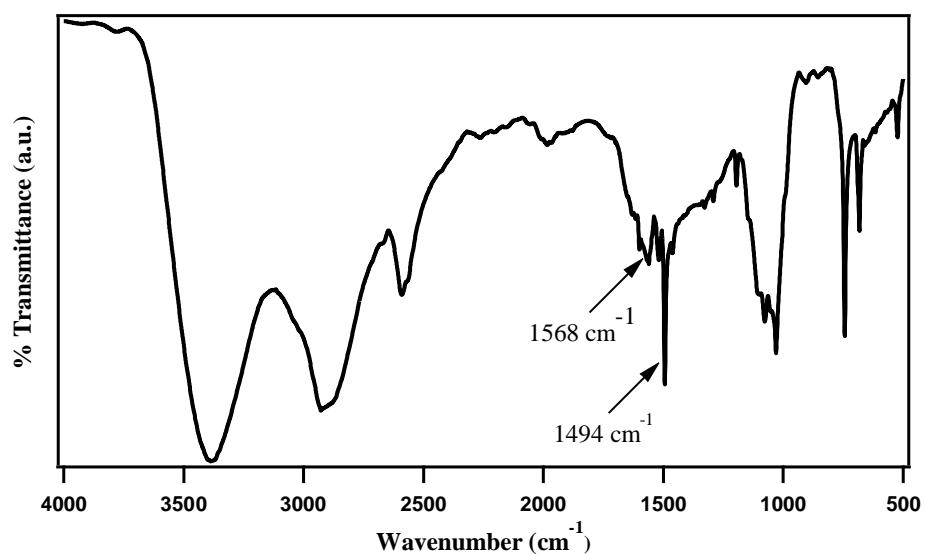
**Figure S15.** Digital images showing the sequence of reactions involved in the formation of Au NP- polyaniline composite by making use of the activity of glucose oxidase.



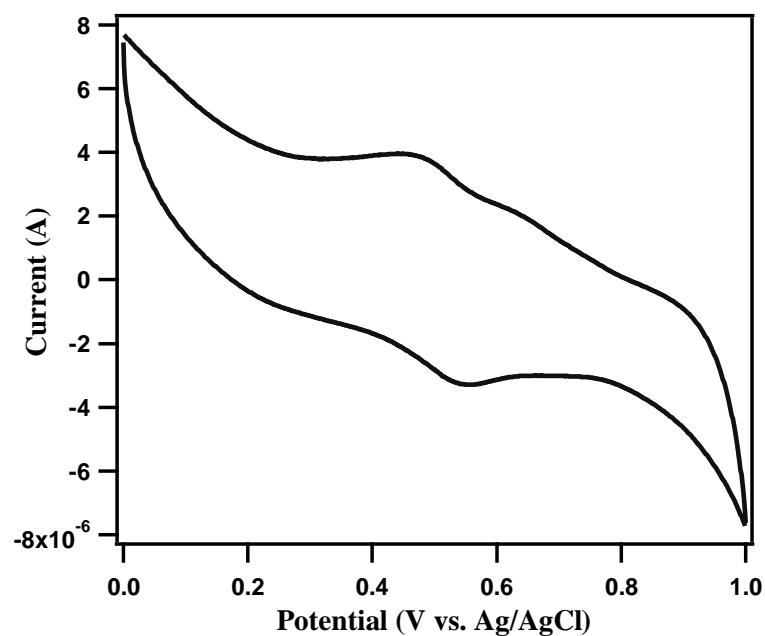
**Figure S16.** UV-visible spectrum of Au nanoparticles synthesized through the reduction of  $\text{Au}^{3+}$  by  $\text{H}_2\text{O}_2$  generated by the oxidation of glucose by glucose oxidase.



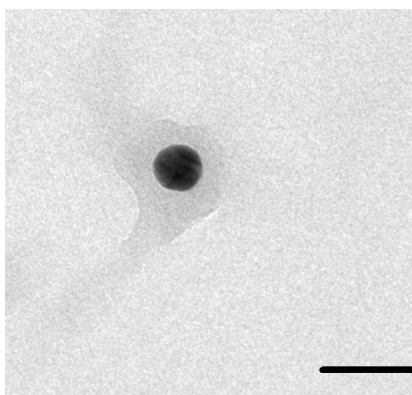
**Figure S17.** UV-visible spectrum of polyaniline synthesized using the activity of glucose oxidase in the absence of Au nanoparticles.



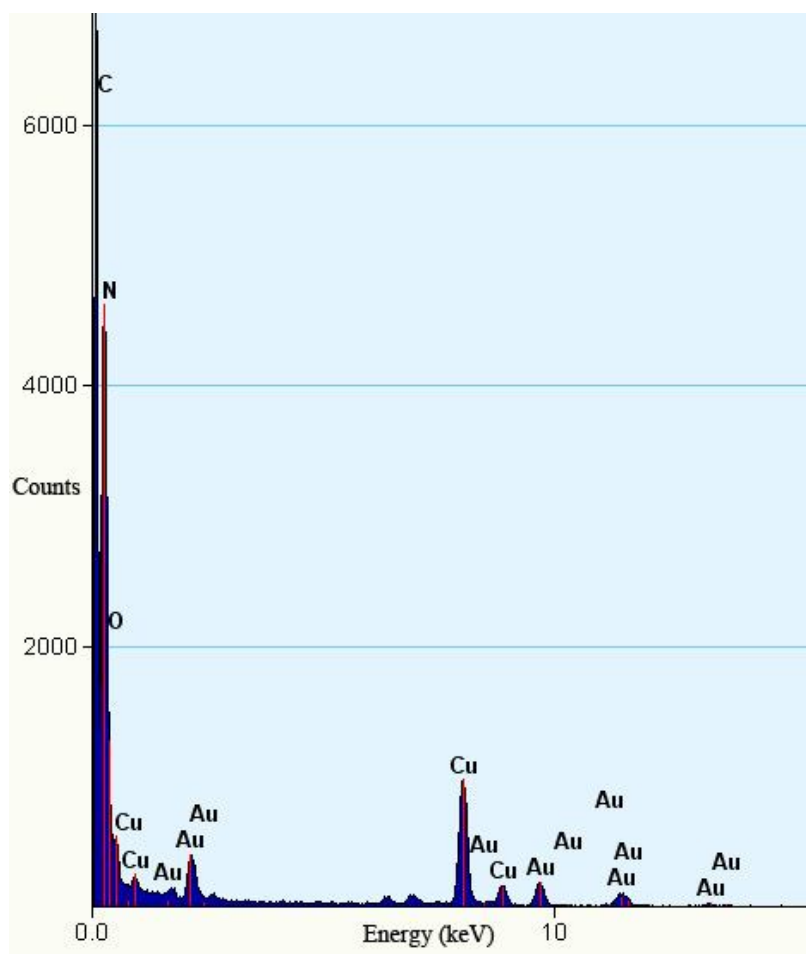
**Figure S18.** FTIR spectrum of polyaniline synthesized utilizing the activity of glucose oxidase. Arrows at 1568 cm<sup>-1</sup> and 1494 cm<sup>-1</sup> indicate the peaks due to the quinoid and benzenoid ring deformations respectively.



**Figure S19.** Cyclic voltammogram of Au NP-PANI nanocomposite at a scan rate of 100 mV/s



**Figure S20.** TEM image of Au NP-PANI composite showing core-shell morphology with a single Au nanoparticle being encapsulated by PANI. (scale bar 50 nm).



**Figure S18.** EDS spectrum of Au NP- polyaniline nanocomposite.



## References

1. S. G. Rhee, T. S. Chang, W. Jeong and D. Kang, *Mol. Cells*, 2010, **29**, 539-549.
2. G. L. Ellman, *Arch. Biochem. Biophys.*, 1959, **82**, 70-77.