Dopamine coated Fe_3O_4 nanoparticles as enzyme mimics for the sensitive detection of bacteria

Shazia Mumtaz,[a] Li-Sheng Wang,[b] Syed Zajif Hussain,[a] Muhammad Abdullah ,[a] Zill-e- Huma,[a] Zafar Iqbal,[a] Brian Creran,[b] Vincent M. . Rotello[b*] and Irshad Hussain[a*].

[1]Department of chemistry, Syed Babar Ali School of Science and Engineering, Lahore University of Management Sciences (LUMS), DHA, Lahore Cantt – 54792, Lahore, Pakistan.

[2] Department of Chemistry, University of Massachusetts Amherst, 710 North Pleasant Street, Amherst, Massachusetts 01003, United States.

We report a simple and economical turn-off colorimetric bacterial sensing strategy catalytically amplified with dopamine capped iron oxide (Dop-Fe_3O_4) nanoparticles. The cationic Dop-Fe_3O_4 nanoparticles catalyze the oxidation of chromogenic substrate 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) in the presence of H_2O_2 into a green colored product. The nanoparticles catalytic activity is inhibited in the presence of bacteria resulting in the naked eye detection of bacteria up to 10^4 cfu/mL and by spectrophotometric detection down to 10^2 cfu/mL.

Materials

Ferric chloride hexahydrated ($FeCl_3$. $6H_2O$, 98%), Ferrous chloride tetrahydrated ($FeCl_2$. 4 H_2O, 99%), diethylene glycol (DEG, 99.5%), Sodium hydroxide powder (NaOH, 97%), sodium acetate trihydrate (CH_3COONa. $3H_2O$, 99%), glacial acetic acid (CH_3COOH, 99.7%), dopamine hydrochloride ($(HO)_2C_6H_3CH_2CH_2NH_2 \cdot HCl$, analytical grad) and hydrogen peroxide (H_2O_2, 30%) were purchased from Sigma-Aldrich.

Synthesis of nanoparticles

Synthesis of Dopamine capped Fe_3O_4 was done by slight modification in Haiou Qu's method.[1] In a typical synthesis, 0.5 mmol (99.4 mg) of $FeCl_2$ $4H_2O$ and 1 mmol (270.3 mg) of $FeCl_3$ $6H_2O$, were dissolved in 20 mL of diethylene glycol (DEG) and were subsequently added to nitrogen-protected three-necked flask. Separately, 4 mmol (160 mg) of NaOH was dissolved in 10mL of DEG and added to the flask. The mixture was then heated to 220 °*C* and the temperature was maintained at 220 °*C* for 2 h followed by cooling the system down to room temperature. The solid product was isolated by centrifugation at 4500 rpm and the particles were re-dispersed in water and 1 mmol of dopamine was added to replace the labile DEG ligand. The dopamine modified iron oxide nanoparticles were purified by dialysis using 12000 D dialysis tubing and stored at room temperature for further use. These nanoparticles were stable for a period of at least 06 months. The synthetic scheme of dopamine functionalized iron oxide nanoparticles is shown in **Fig. S1**.

Reference
1. H. Qu, D. Caruntu, H. Liu and C. J. O'Connor, *Langmuir*, 2011, 27, 2271-2278.

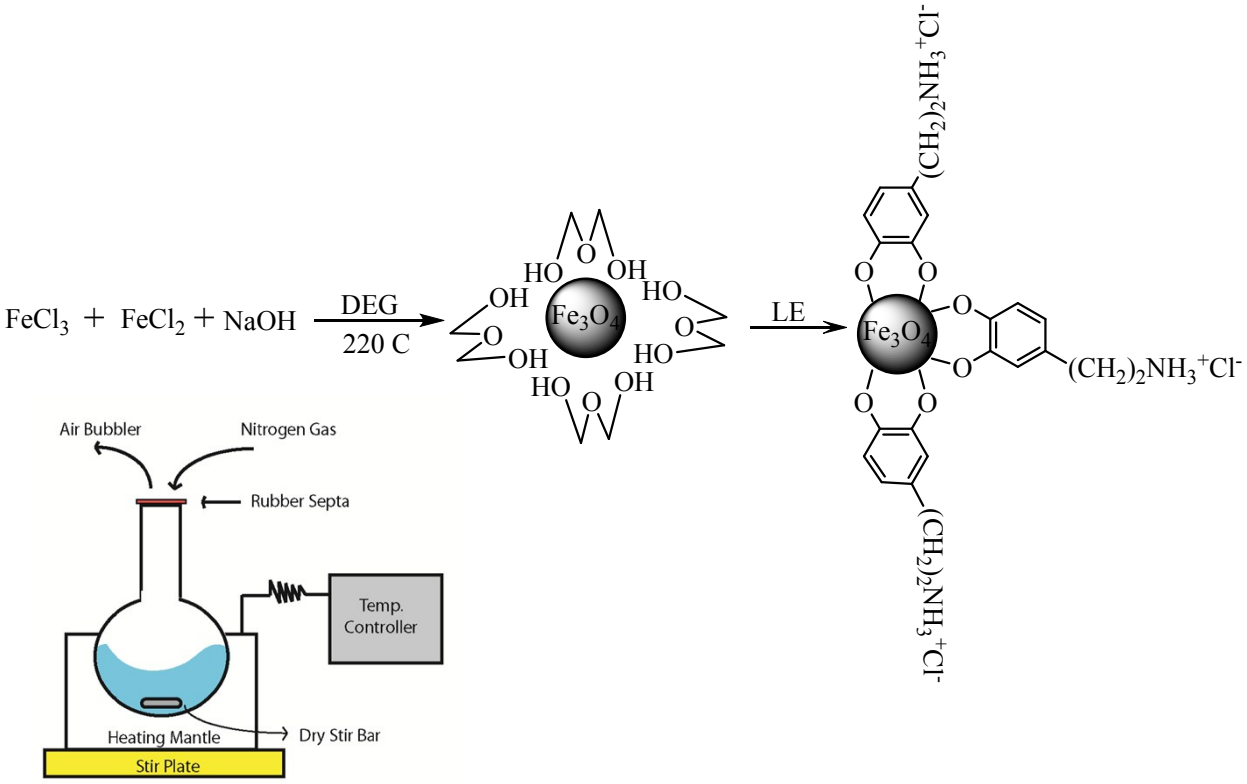


Figure S1: A scheme showing the formation of DEG-Fe_3O_4 nanoparticles followed by the formation of Dop-Fe_3O_4 nanoparticles by ligand exchange (LE)

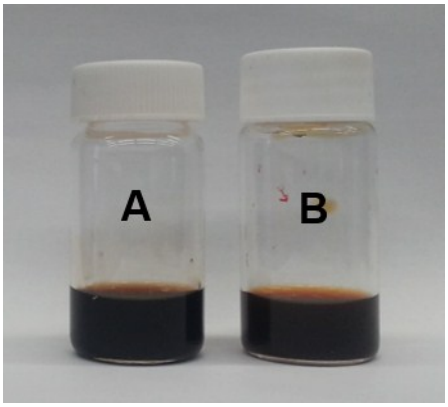


Figure S2: Stable aqueous dispersion of Dop-Fe_3O_4 nanoparticles (**A)** freshly prepared, (**B)** after 06 months of preparation

Table S1: Dynamic light scattering studies of Dop-Fe_3O_4 nanoparticles to determine their hydrodynamic diameter and zeta potential to ensure stability after six months

Sample	Hydrodynamic size D_h (nm)	Zeta-potential ζ (mV)
Dop-Fe_3O_4 NPs (freshly prepared)	36.89	+31.3
Dop-Fe_3O_4 NPs (After 06 months)	87.69	+28.3

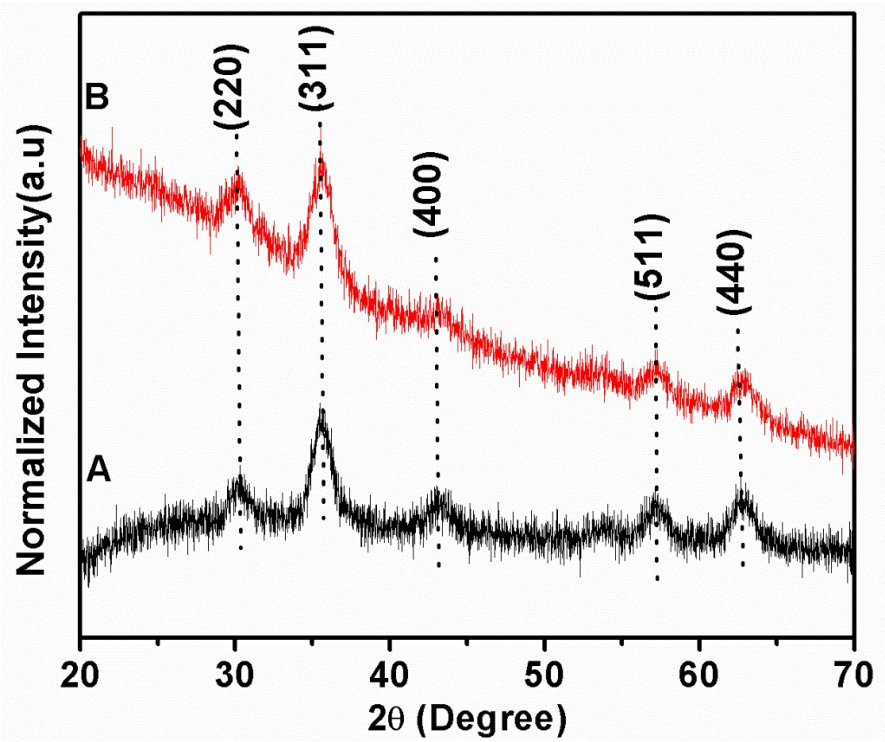


Figure S3: X-ray diffraction spectra of (**A)** DEG capped iron oxide nanoparticles and (**B)** dopamine capped iron oxide nanoparticles.

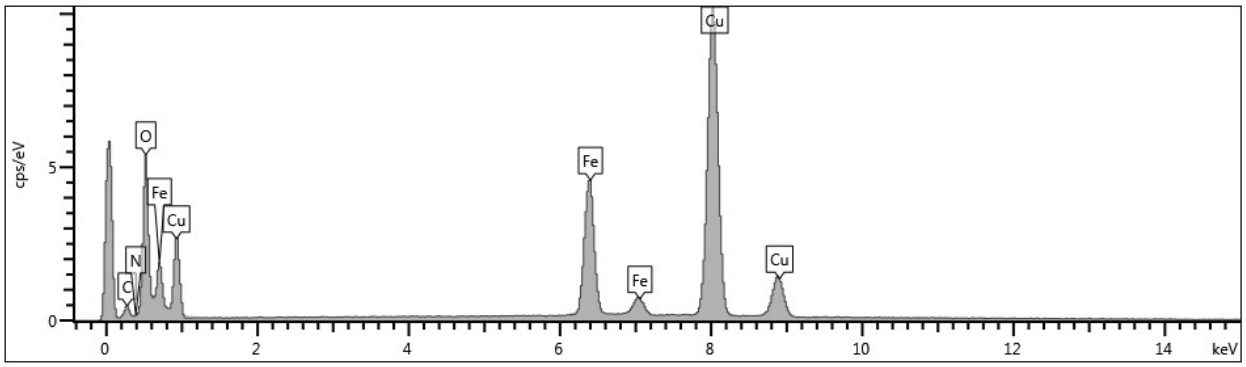
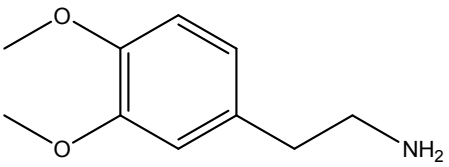
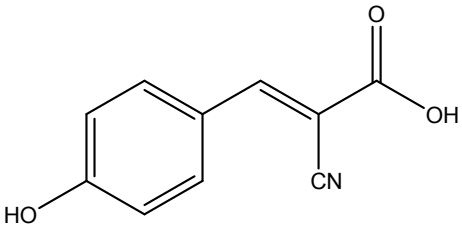


Figure S4: Energy dispersive X-ray (EDX) spectra of dopamine capped iron oxide nanoparticles



M = Dopamine ligand attached on the surface of iron oxide nanoparticles



m= ∞-Cyano-4-hydroxycinnamic acid used as a matrix for MALDI-TOF spectra

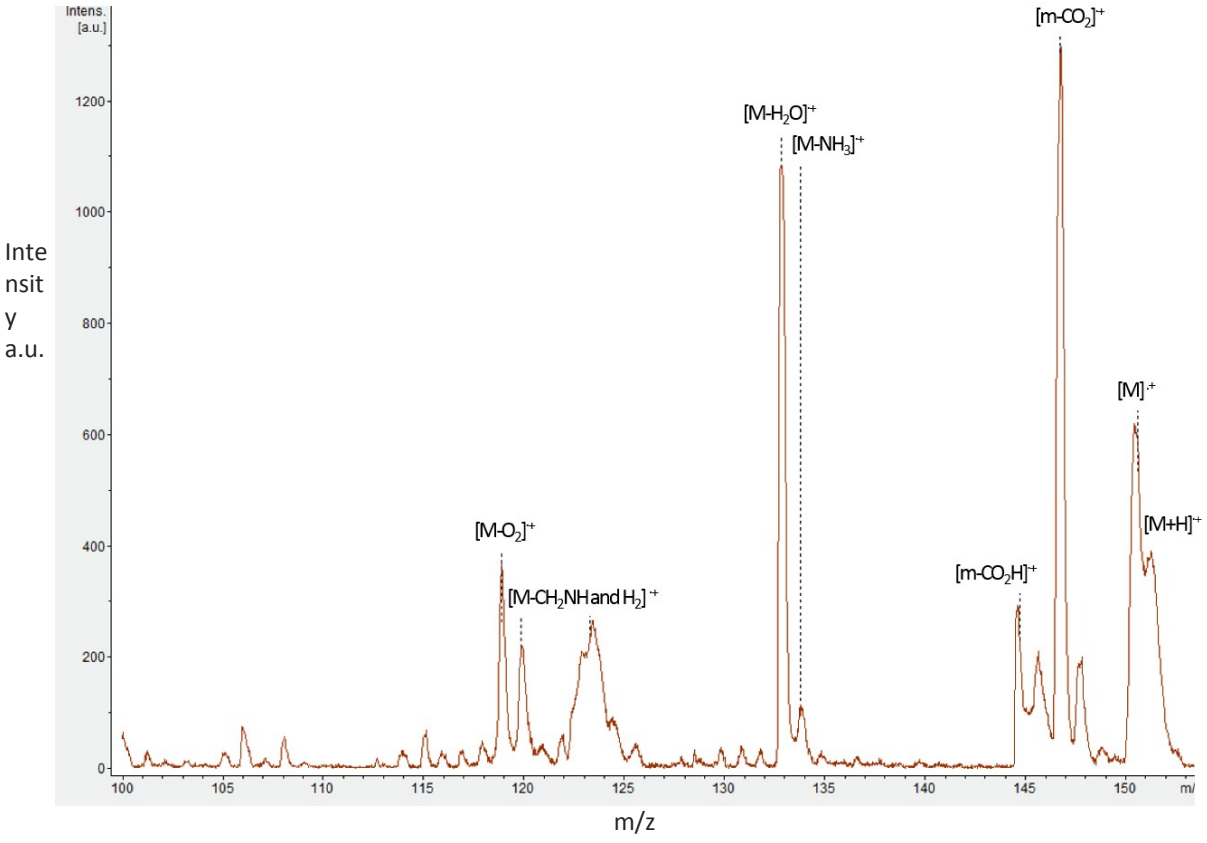


Figure S5: MALDI- TOF mass spectra of dopamine capped iron oxide nanoparticles (Dop-Fe_3O_4)

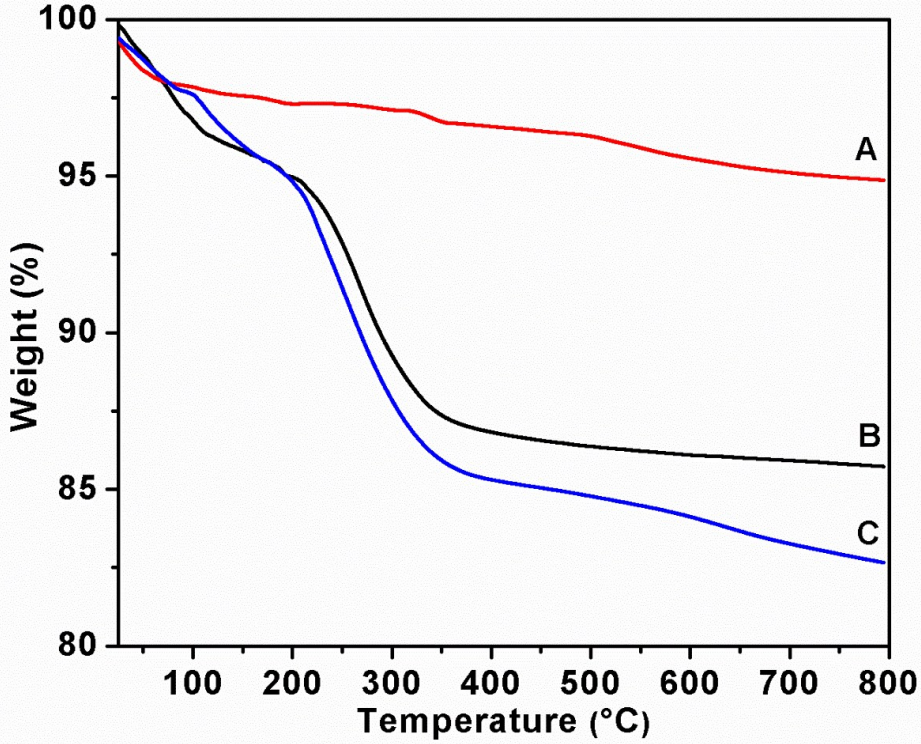


Figure S6: Thermogravimetric analysis of A, B and C naked, DEG capped and dopamine capped iron oxide nanoparticles respectively.

Sensing of Bacteria: For sensing experiment, bacterial strain *E.coli* (*XL1*) and *B.sub* (DH_{∞}) were chosen as a model analytes. These bacteria were grown in LB growth media by incubating at 37 °C in an orbital shaker at 200 rpm. The cultured bacteria were cleaned by centrifugation at 2000 rpm. The clean bacteria were dispersed in deionized water and used immediately for colorimetric sensing. Iron oxide nanoparticles mimic peroxidases and catalyze the reaction of ABTS with hydrogen peroxide resulting in a green color product. In the presence of different concentration of bacteria chromogenic substrate (ABTS) oxidation into green color product is inhibited due to electrostatic interaction of bacteria with dopamine iron oxide nanoparticles, resulting in decrease in the observed absorption spectra at λ_{max} value of 420 nm figure S8.

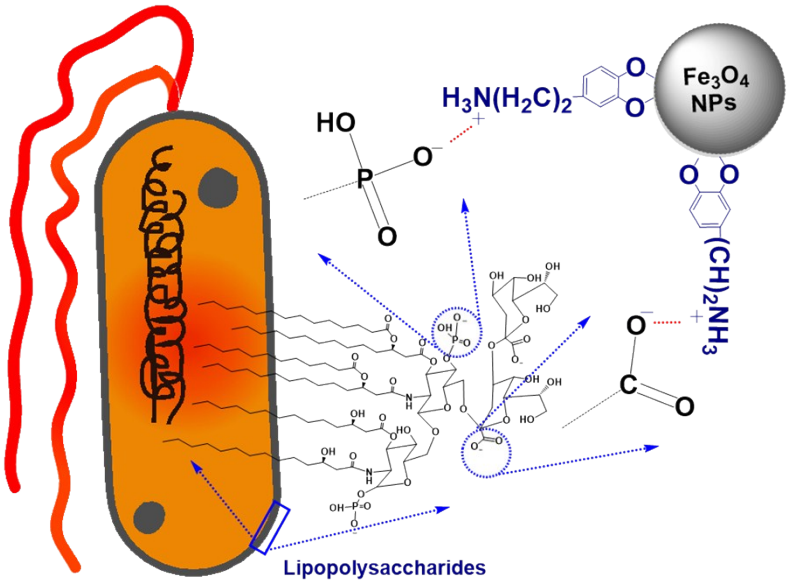


Figure S7: Proposed electrostatic interaction (shown by dotted red lines) between negatively charged bacterial (gram negative) cell surface and positively charged Dop-Fe_3O_4 NPs.

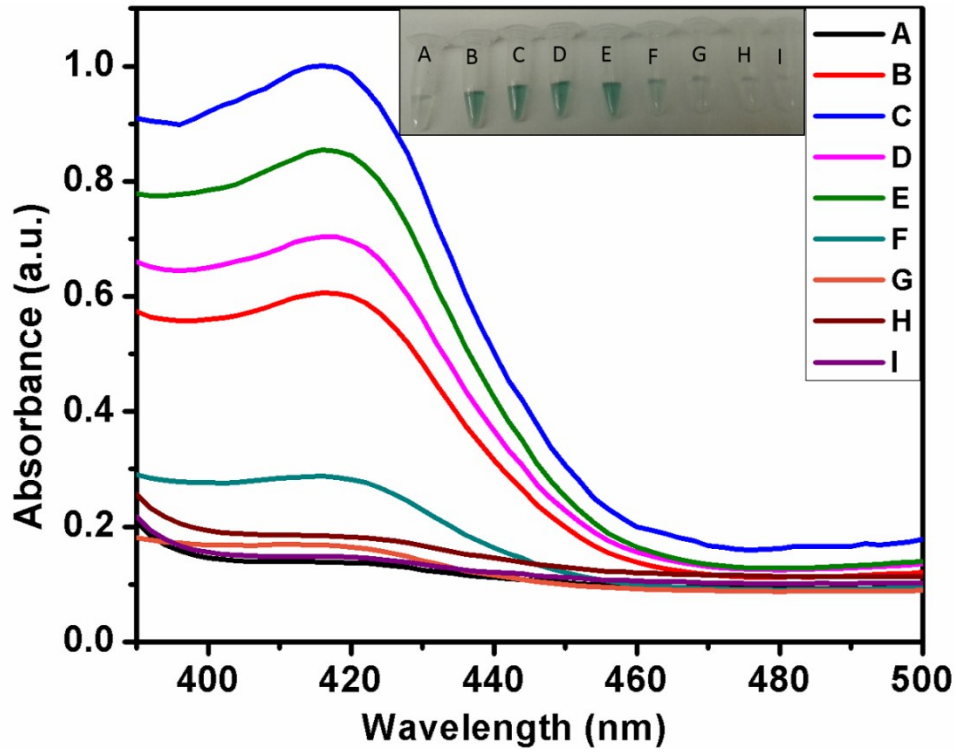


Figure S8: Absorption spectra of green colored production formed by the reaction/oxidation of ABTS with hydrogen peroxide, (A), (B) in the absence and in the presence of Dop-Fe_3O_4 nanoparticles without bacteria. (C—I) The inhibition of the catalytic activity of Dop-F_3O_4 nanoparticles with 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, and 10^8 cfu/ml of *E.coli* concentrations respectively.

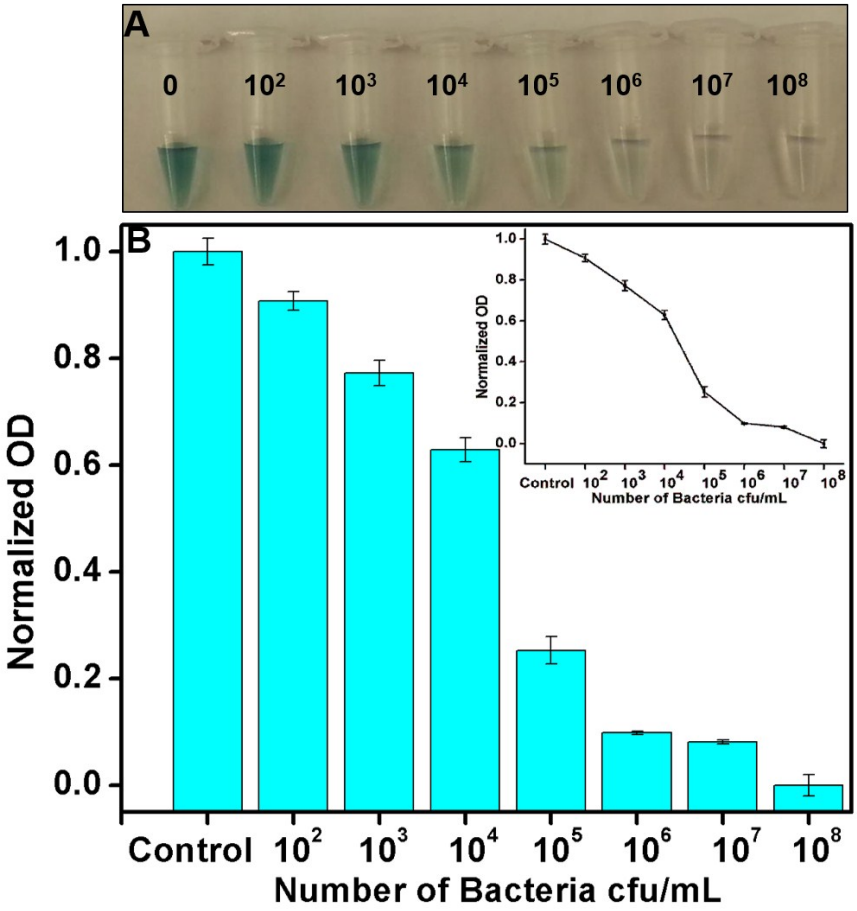


Figure S9: (A) Colorimetric response when ABTS is reacting with H_2O_2 catalysed by Dop-Fe_3O_4 NPs in the presence of different concentrations of *B.sub (DH$_\infty$)*; (B) Bar graph and inset showing gradual decrease in optical density at 420 nm by different conc. of *B.sub*.