Supplementary Information

Materials and Methods:

AFM measurement:

The Atomic force microscopy measurements were performed with Park System XE 100. AFM operated in frequency modulation mode in the ambient condition. Silicon cantilever OMCL-AC160TS with force constant 26N/m with 300 KHz typical resonance frequency used as force sensor. The AFM images processed in Gwyddion 2.55. G-CoONP centrifuged for 20 minutes at 9000rpm. Highly concentrated portion from the solution dried on Si-wafer for measurement. Each spot (10 x 10 μm) measured under contact mode operation.

MTT assay for Cell viability analysis

MTT assay was used to check the cell viability at different exposure time and dose treatment of G-CoO NP and C-CoO NP. Harvested Cells in the exponential growth phase were seeded at a density of 1.0×10^4 cells/well into 96 well plates in 100μl of DMEM containing 10% (vol/vol) NC serum. The cells were allowed to adhere for 24hours at 37^0C. The medium was replaced by fresh DMEM containing 10% (vol/vol) NC serum after 24h, with the addition of silver nanoparticles samples in a series of increasing concentration of 1μg/ml to 100μg/ml. After incubation for 24 to 48 hrs, the medium was removed and the wells were washed three times with 0.01 Phosphate Buffered Saline (PBS). Followed by washing, 150 μl of 0.1mg/ml MTT (3-(4, 5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) in PBS were added to each well and the plate was kept for 4 hrs incubation. Finally, the media was replaced by 150μl MTT solvent (11gm SDS in 50mL of 0.02M HCl and 50mL Isopropanol) to dissolve the formazan crystal formed by live cells. The absorbance (OD value) was then taken at 570 nm using ELISA reader (Epoch, Biotek, Germany). Cytotoxicity was estimated by calculating the percentage of the OD value with respect to control experiment. Graphical presentation was then done to compare the effects of different concentration. All the experiments were performed at least three times in triplicates.

Nanoparticles uptake estimation by flow cytometry

Estimation of uptake of nanoparticles by HCT116 cells was done by measuring the change in granularity of the cells exposed to nanoparticles. A cell density of $50x10^4$ cells/well were seeded

in 24 well cell culture plates and kept for proper adherence at 24h. The cells were treated with different concentration of G-CoO NP for 24h. Followed by incubation, cells were trypsinized, centrifuged at 135g for 10 min, re-suspended in 500µl of medium and kept in ice. The data acquisition was done with 10000 cell count by Attune acoustic focusing cytometer (Applied Biosystem, Life technologies) equipped with 488nm argon laser. The cytometer was setup to measure FSC and SSC logarithmically. 1 mg /ml of nanoparticles were used to set the standard FSC and SSC. The data were processed in FCS express 7 (DeNovo, Los Angeles, CA). Mean side scatter of each sample was measured and presented in form of histogram.

Cell cycle analysis

HCT116 Cells were treated with G-CoO NP for 24h in a set of cells seeded with density of 1×10^6cells per well in 24 well plate. After treatment, they were trypsinized and stored on ice. For cell cycle analysis, incubated of cells was done with 1:2 dilution in 0.5% NP-40 non-ionic detergent made up with PBS without $Ca^{2+}$and Mg^{2+}. Cells were stained with Propidium Iodide (20µg/ml, MP Biomedical, USA) after PBS washing. Measurement of nuclei was performed using BL3 filter (640LP) of Attune focusing Flow cytometer. Nuclei subpopulations were analyzed by FCS express7 (Denovo, Los Angeles, CA) software and the cells with different phases were calculated from histogram using Area parameter.

Zebrafish maintenance and embryo culture

Adult zebrafishes were maintained in overflow system (Aquaneering, USA) equilibrated with fish water containing 75 g NaHCO3, 18 g sea salt, 8.4 g CaSO4 per 1000 ml. Feeding of fishes were done thrice a day with fish food containing dried bloodworm. Photoperiodism conditions was kept as 12h light and 12h dark. Fishes were breed in breeding container having net partition with male and female zebrafish at the ratio of 2:1. Followed by breeding, obtained viable embryos were separated, and rinsed with Holtfreter (HF) buffer. Toxicology assessment experiments were performed with embryos reared in filter sterilized in the HF medium. All chemicals used for buffer preparation were purchased from Merck. The Institutional Animal Ethics Committee (IAEC) of KIIT University approved all animal procedures. All experiments were performed in accordance with the relevant animal practice guidelines and regulations of IAEC, KIIT University.

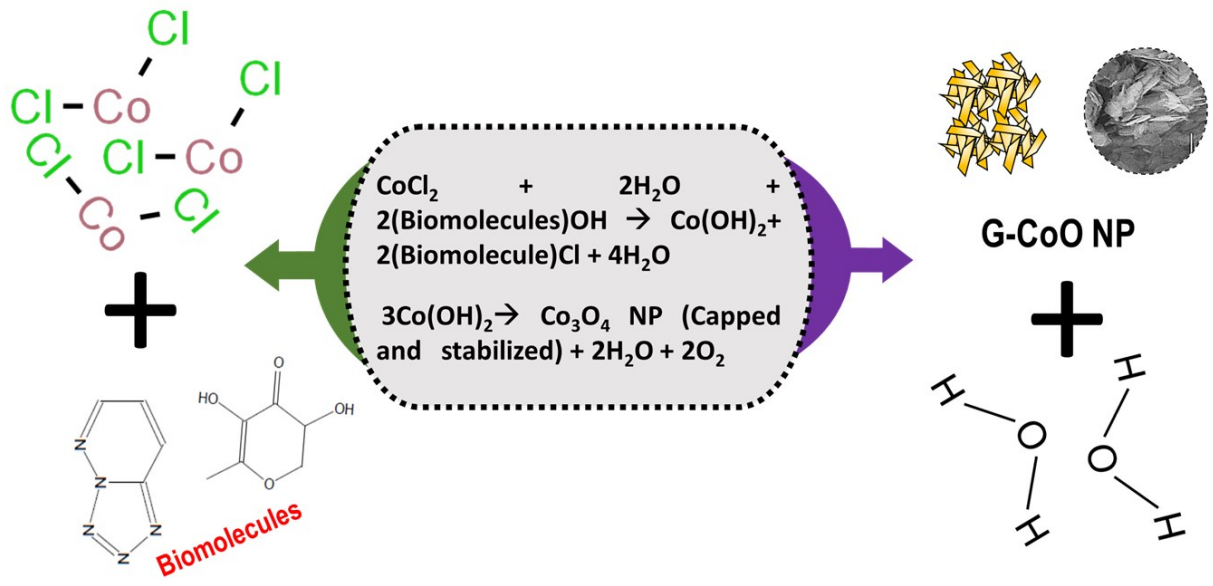


Figure S1: Schematic presentation of reaction for green synthesis of G-CoONP from *Calotropis gigantea* biomolecules**.**

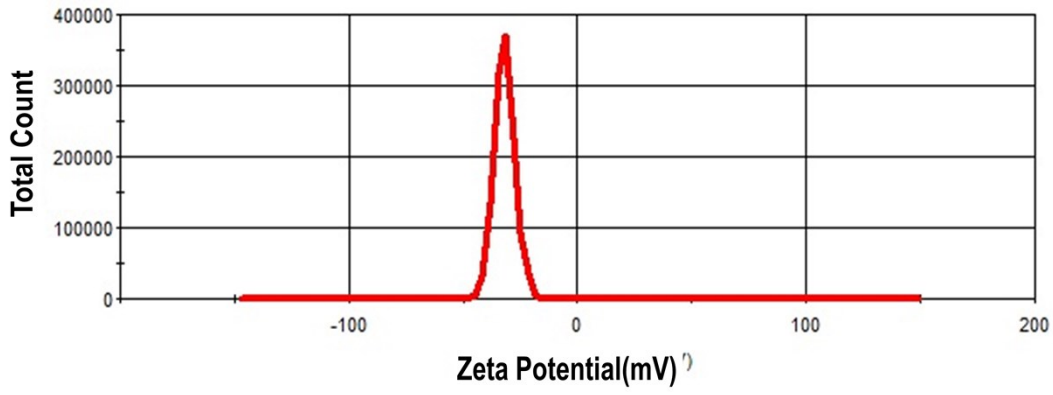


Figure S2: Zeta potential of G-CoONP determined by dynamic light scattering. The nanoparticles were suspended in aqueous medium for the measurement. The measurement was taken 3 times. The total number of 20counts were done for the measurement.

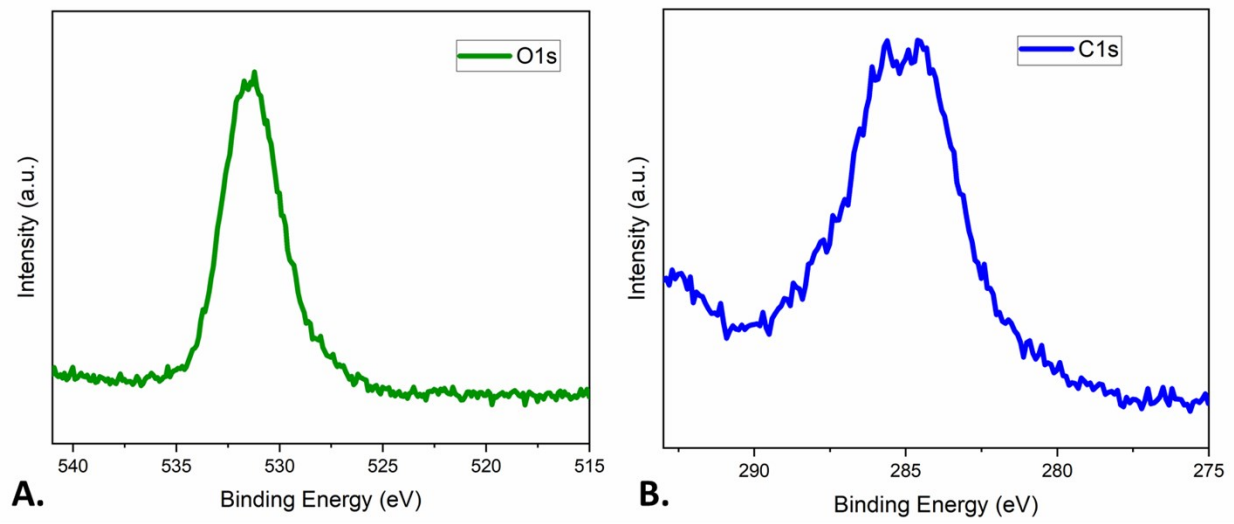


Figure S3: (A) and (B) core level XPS spectra for O1s and C1s after calibration by adventitious carbon peak position at binding energy of 285eV. The O1s peaks appears at ~531 eV, which corresponds to Cobalt oxide and non-stoichiometric oxygen.

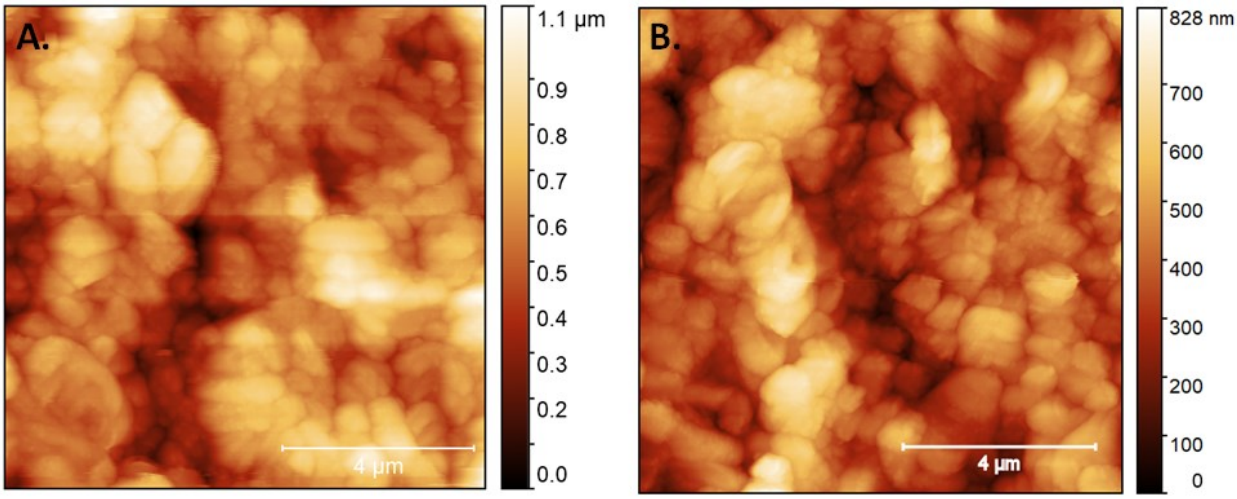


Figure S4: Two AFM images (A) and (B) measured at two different spots from G-CoONP sample. Each spot area of 100 μm^2 measured under contact mode operation. Because of high rough surface distribution of powder over silicon substrate, AFM images measured at significant low resolution compared to TEM. AFM measured surfaces can be compared as agglomeration of nanostructured flakes by 2-dimentional view.

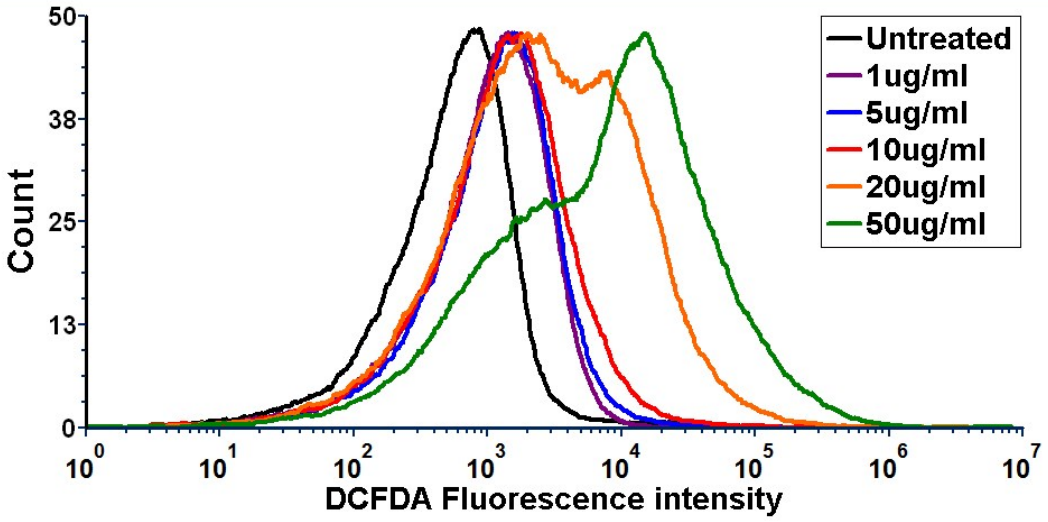


Figure S5: Oxidative stress analysis by ROS measurement obtained by DCFDA staining to the zebrafish embryos exposed to G-CoO NP.

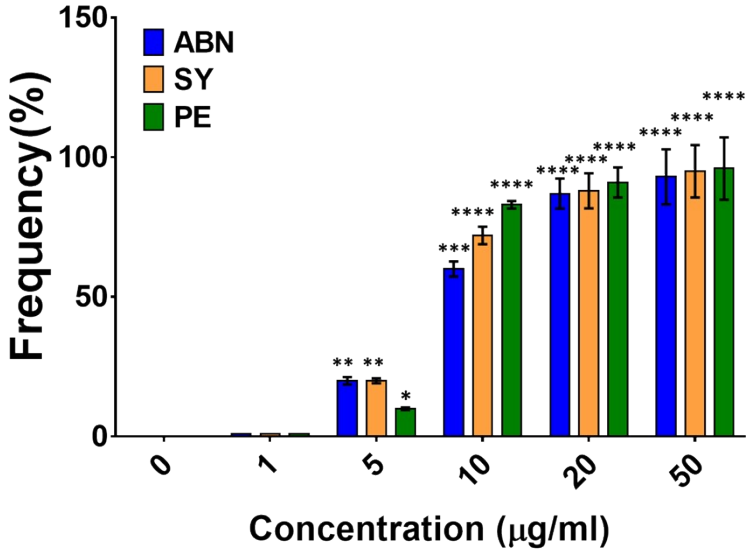


Figure S6: Frequency of morphological abnormalities in Zebrafish embryos (72h) exposed to C-CoO NP. The abnormalities ABN, SY, PE represent abnormal notochord, Swollen yolk and pericardial edema. The values show Mean ± SD of 20 embryos in triplicates. *P<0.05 denotes significant change from untreated embryos as obtained from ANOVA analysis. Number of * presents the degree of significance.

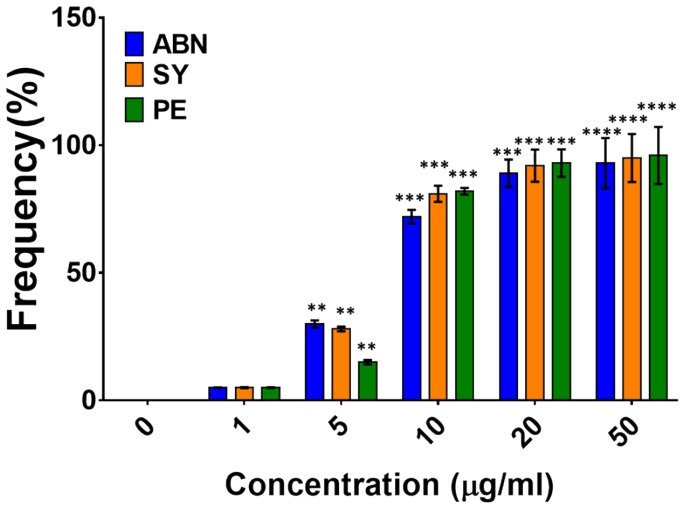


Figure S7: Frequency of morphological abnormalities in Zebrafish embryos (72h) exposed to G-CoONP. The abnormalities ABN, SY, PE represent abnormal notochord, Swollen yolk and pericardial edema. The values show Mean ± SD of 20 embryos in triplicates. *P<0.05 denotes significant change from untreated embryos as obtained from ANOVA analysis. Number of * presents the degree of significance.

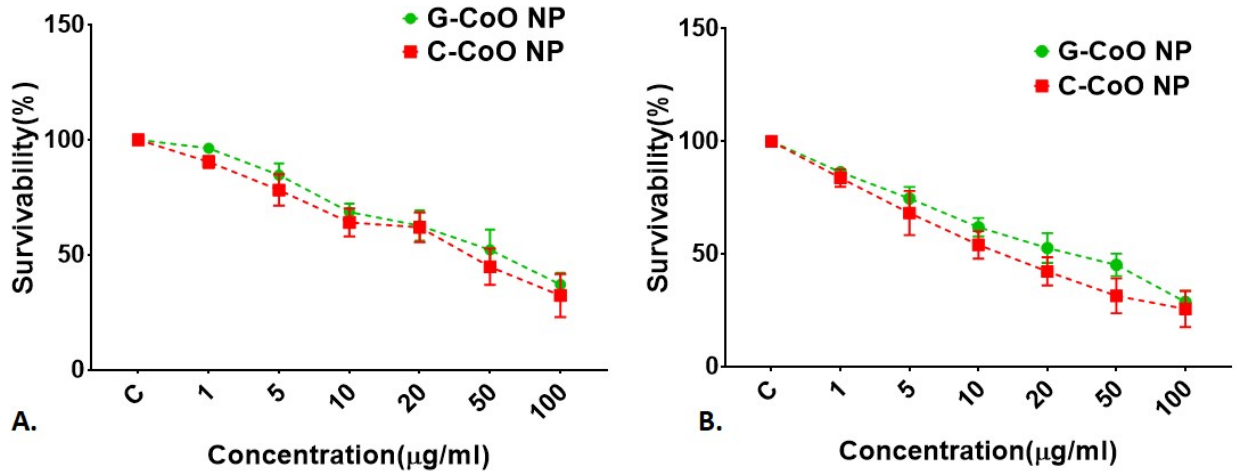


Figure S8: Survivability of HCT 116 cells exposed to G-CoONP and C-CoONP obtained after (A) 1st exposure recycle (B) 2nd exposure cycle. The nanoparticles were retrieved from the DMEM culture medium after the exposure through density centrifugation. The values represent the mean ± SD of three independent experiments. *P >0.5, **P >0.01, ****P >0.001 denotes compared significant change at each exposed concentration as obtained from post hoc analysis after one-way ANOVA

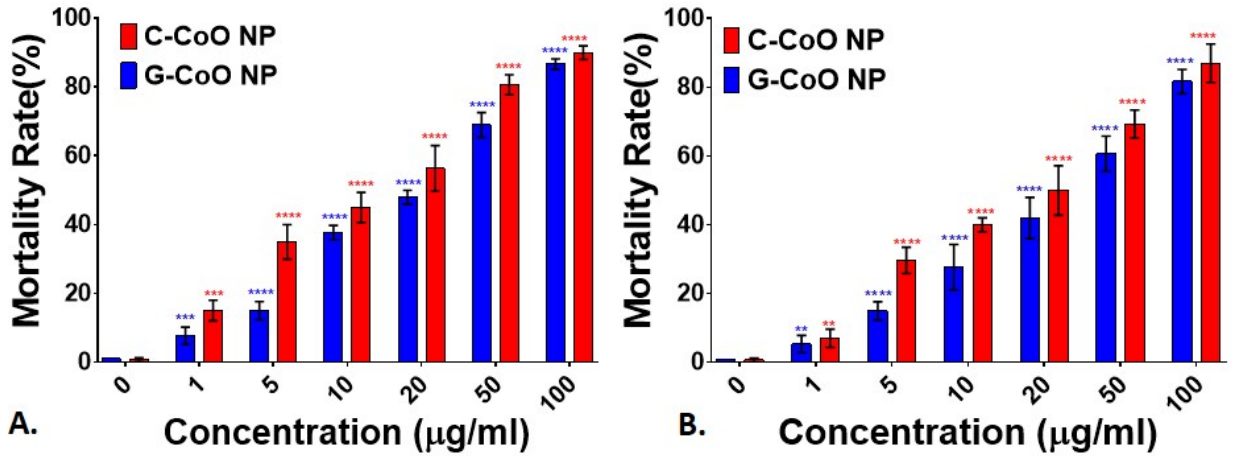


Figure S9: Mortality rate of Zebrafish embryos exposed to G-CoONP and C-CoONP obtained after (A) 1st exposure recycle (B) 2nd exposure cycle. The nanoparticles were retrieved from the HF fish medium after the exposure through density centrifugation. The values show Mean ± SD of 20 embryos in triplicates. *P<0.05 denotes significant change from untreated embryos as obtained from ANOVA analysis. Number of * presents the degree of significance.

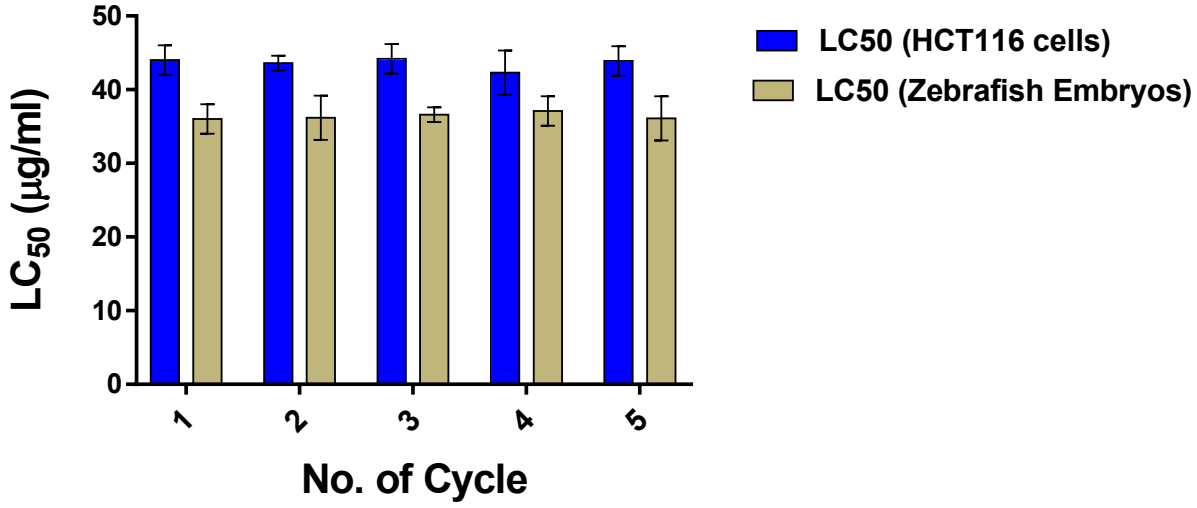
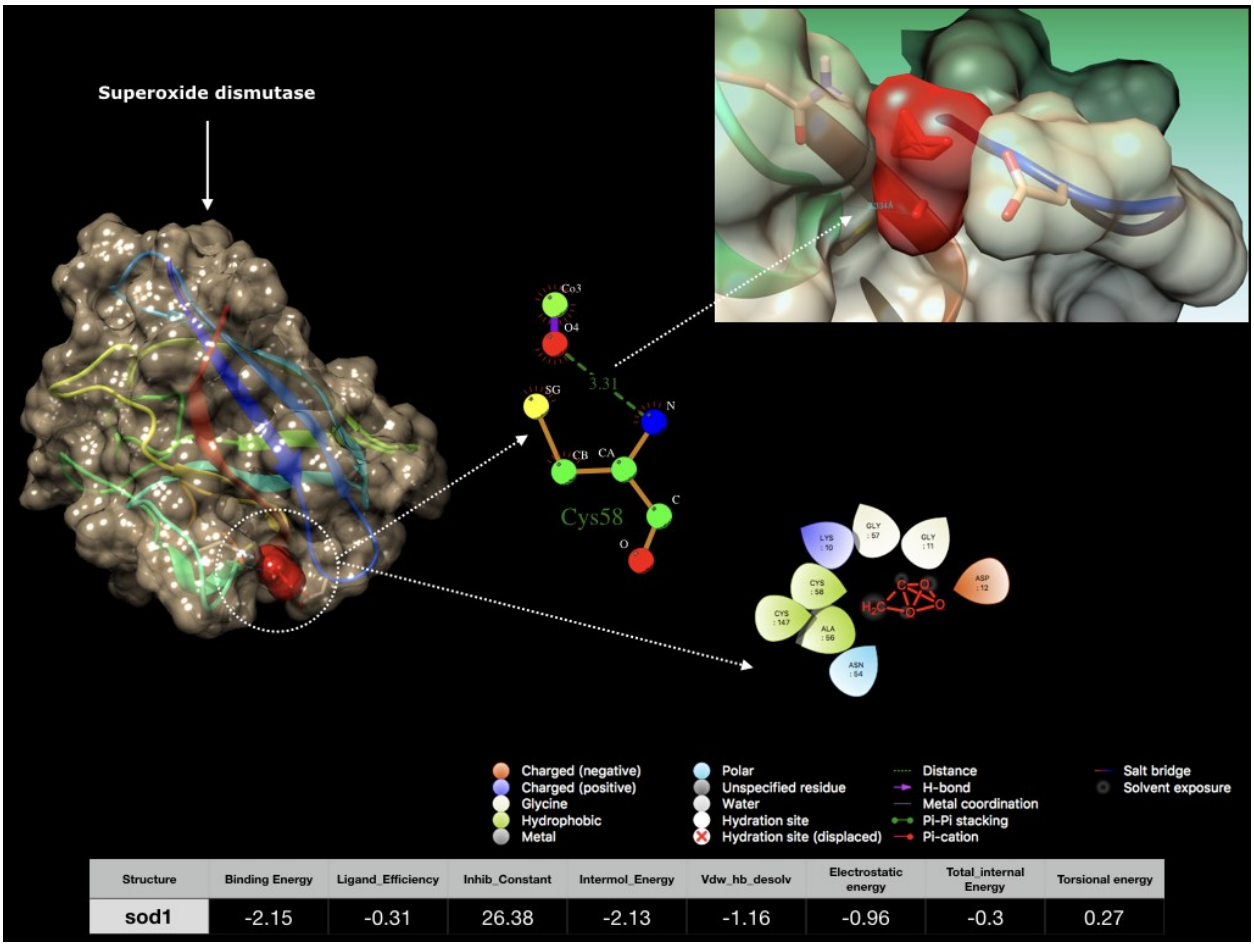
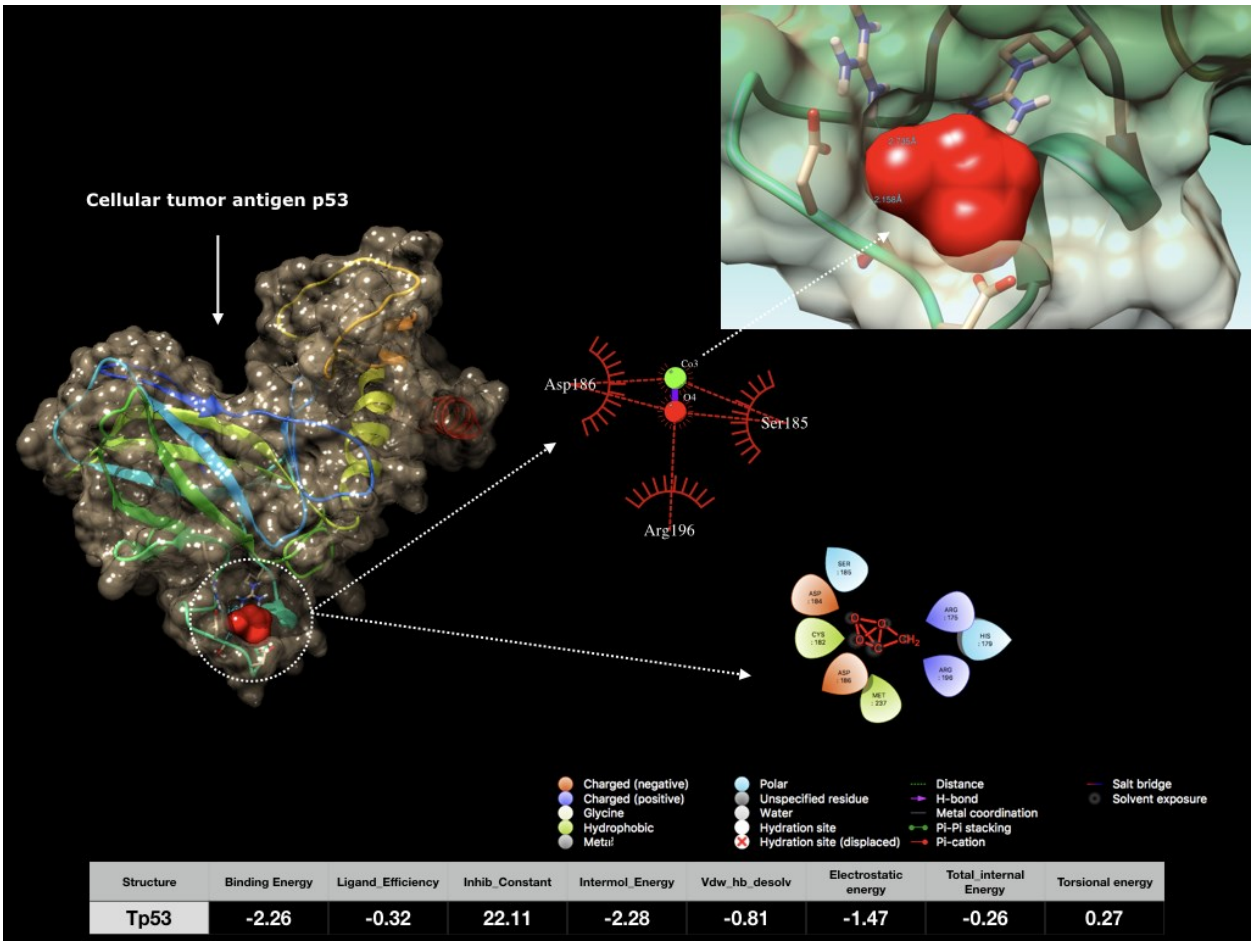


Figure S10: LC50 of the G-CoO NP after exposure to 5 cycles. The 5 cycles present the order of exposure of G-CoONP nanoparticles retrieved after each treatment procedure.



Structure	Binding Energy	Ligand_Efficiency	Inhib_Constant	Intermol_Energy	Vdw_hb_desolv	Electrostatic energy	Total_internal Energy	Torsional energy
sod1	-2.15	-0.31	26.38	-2.13	-1.16	-0.96	-0.3	0.27

Figure S11: *In silico* analysis of Co_3O_4 nanoparticles interaction with Sod1 enzyme of mammalian cells determined by molecular docking. Autodock 4.2 was used to study the interaction with Co3O4 as a ligand with sod1 and tp53 of both human and zebrafish as receptor proteins. Visualization and Post-docking analysis were performed with the help of conformational clustering, Chimera, and Discovery Studio Visualizer.



Structure	Binding Energy	Ligand_Efficiency	Inhib_Constant	Intermol_Energy	Vdw_hb_desolv	Electrostatic energy	Total_internal Energy	Torsional energy
Tp53	-2.26	-0.32	22.11	-2.28	-0.81	-1.47	-0.26	0.27

Figure S12: *In silico* analysis of Co_3O_4 nanoparticles interaction with tp53 enzyme of mammalian cells determined by molecular docking. Autodock 4.2 was used to study the interaction with Co3O4 as a ligand with sod1 and tp53 of both human and zebrafish as receptor proteins. Visualization and Post-docking analysis were performed with the help of conformational clustering, Chimera, and Discovery Studio Visualizer.

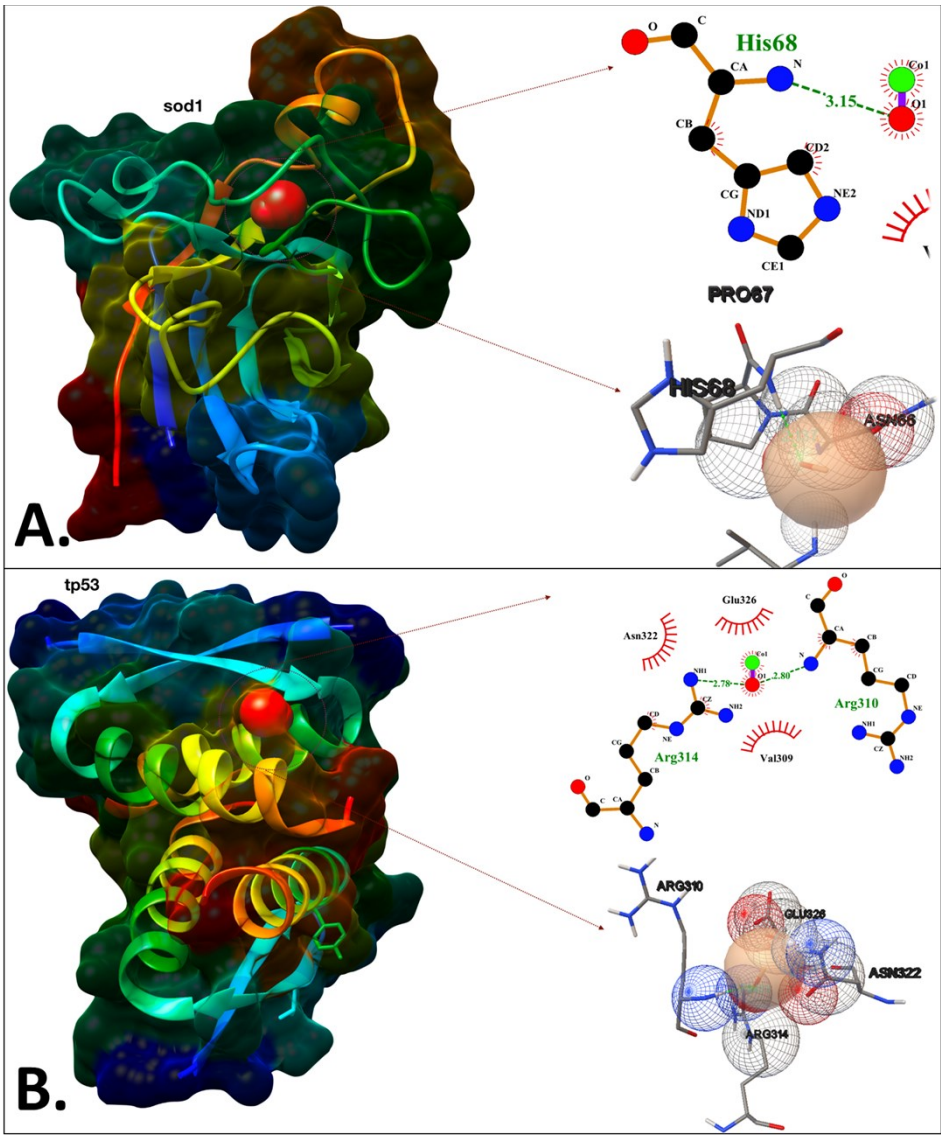


Figure S13: *In silico* analysis of Co_3O_4 nanoparticles interaction with (A) Sod1 and (B) tp53 enzyme of zebrafish determined by molecular docking. Autodock 4.2 was used to study the interaction with Co3O4 as a ligand with sod1 and tp53 of both human and zebrafish as receptor proteins. Visualization and Post-docking analysis were performed with the help of conformational clustering, Chimera, and Discovery Studio Visualizer.