Supporting Information

Hemin-based cell therapy via nanoparticle-assisted uptake, intracellular reactive oxygen species generation and autophagy induction

Abu Raihan Sarkar, Suman Pal, Ankan Kumar Sarkar and Nikhil R. Jana*

School of Materials Science, Indian Association for the Cultivation of Science,

Kolkata 700 032, India

*Address for correspondence to camnrj@iacs.res.in

Estimation of the number of primary amine per QD. Fluorescamine based titration method was used to measure the average number of primary amines per quantum dot. A series of known concentration of glycine (20-250 μM) was used as a standard and fluorescamine test was performed. In brief, various concentration of glycine solution in 1.8 mL borate buffer was prepared and 0.2 mL of freshly prepared fluorescamine (dissolve in acetone, $1x10^{-2}$ M) was added to it and stirred immediately. Next, fluorescence was measured at 493 nm by exciting at 400 nm. Then a linear calibration curve was drawn by plotting the concentration of glycine (μM) against fluorescence intensity. We get a linear equation as follows: Y=0.2553X-15.857 (Y=concentration of glycine in μM and X=fluorescence intensity) with R^2=0.9929. Next, fluorescamine test of polyacrylate coated quantum dot was done by quenching the emission of QD by concentrated HCl followed by neutralization. Fluorescamine test of the QD solution was done as described above and the number of primary amines was estimated using the calibration graph.

Estimation of the number of hemin per QD. UV-visible absorption spectral measurement method was used to calculate the average number of hemin per quantum dot. A series of solution with known concentration of hemin (5-40 μM) were prepared in 0.1 N NaOH and UV-visible

spectra were measured. Next, absorbance was measured for all the solutions at 386 nm. Then a linear calibration was drawn by plotting the concentration of hemin (μM) against absorbance. We get a linear equation as follows: Y = 20.66X+1.92 (Y=concentration of hemin in μM and X=absorbance) with R^2=0.997. In a separate vial, colloidal QD-hemin was taken and QD was dissolved by adding concentrated HCl followed by neutralization. Then a part of this solution was taken in a 0.1 N NaOH and absorbance at 386 nm was measured to estimate concentration of hemin by using the above mentioned calibration graph. Finally, the number of hemin per QD was measured by using concentration of hemin and concentration of QD.

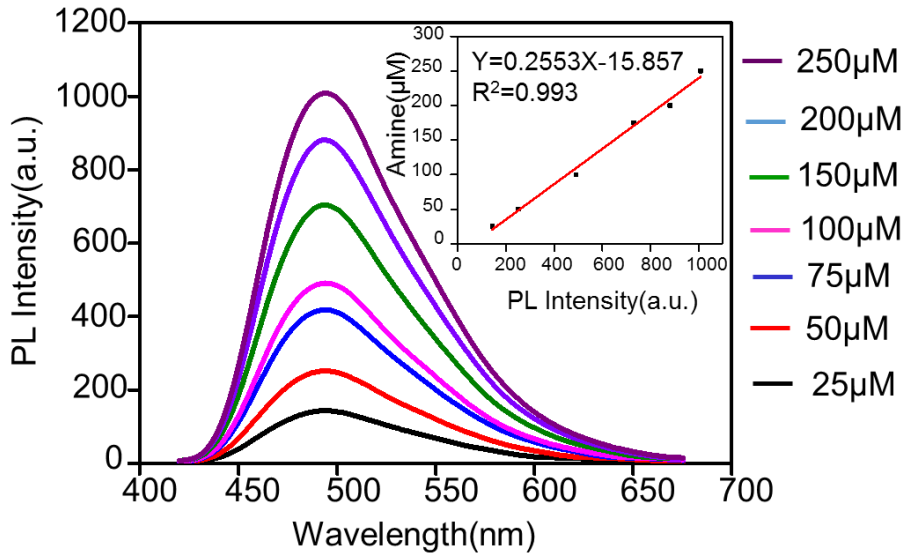

Figure S1. Fluorescamin test-based fluorometric titration using known concentrations of glycine. These data are used to make a linear equation as follows: Y = 0.2553X-15.857 with R^2 =0.993. (X = fluorescence intensity at 493 nm and Y = concentration of glycine/amine)

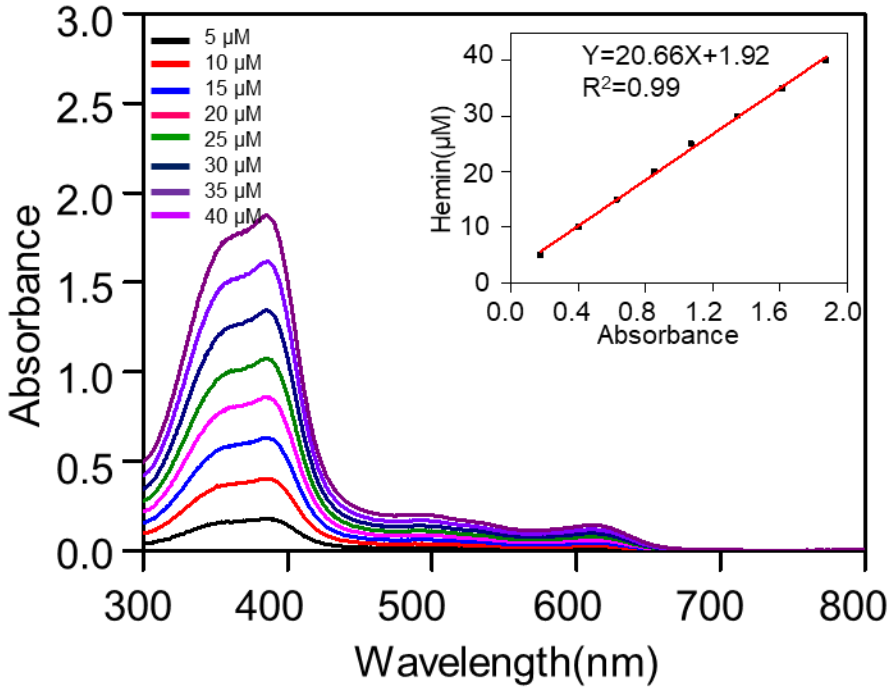

Figure S2. UV-visible absorption spectra of known concentration of hemin in 0.1 N NaOH. These data are used to make a linear equation as follows: Y = 20.66X+1.92 with R^2 =0.99. (X = absorbance at 386 nm and Y = concentration of hemin)

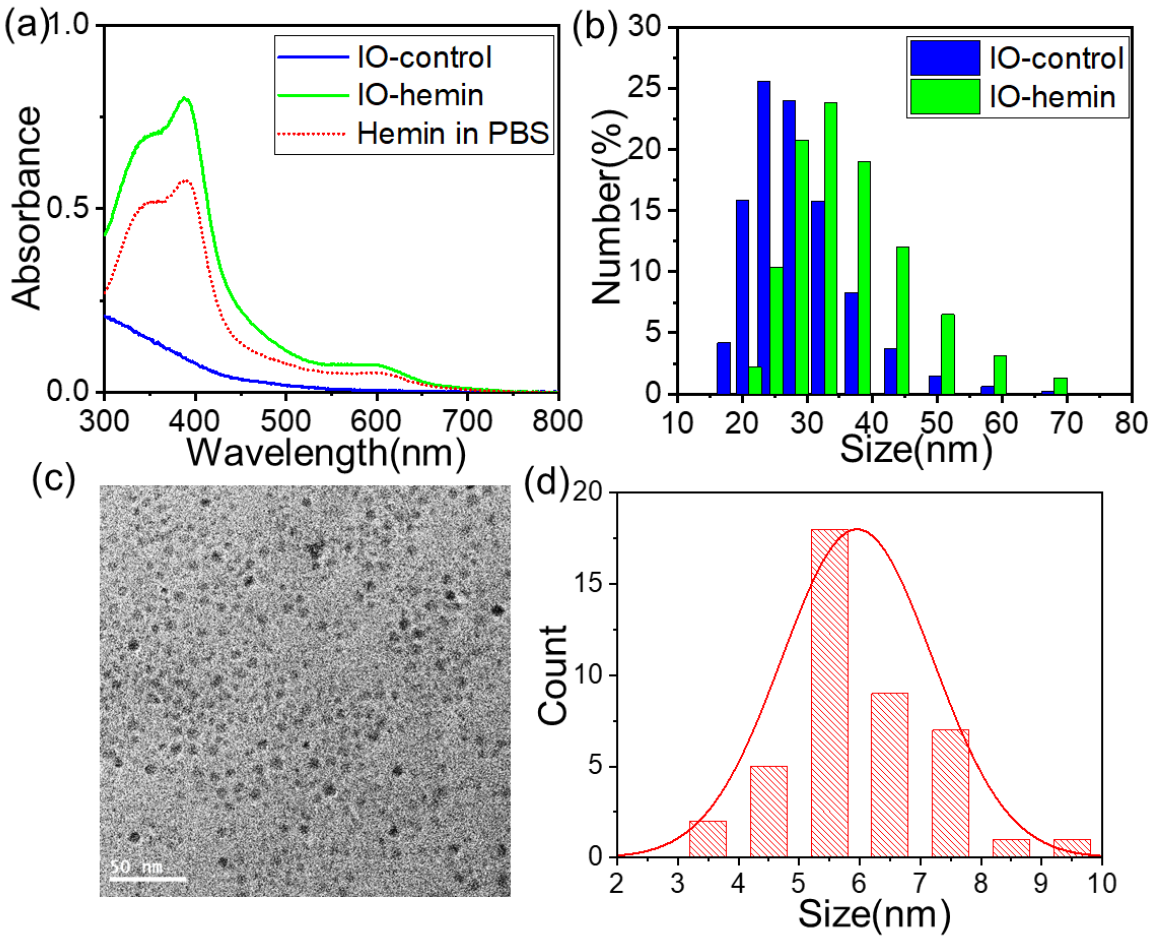

Figure S3. (a) UV-visible absorption spectra of IO-hemin, IO-control and molecular hemin in PBS 7.4 buffer. (b) Hydrodynamic size of IO-control and IO-hemin in PBS buffer. (c, d) TEM image of IO-hemin and corresponding size distribution histogram.

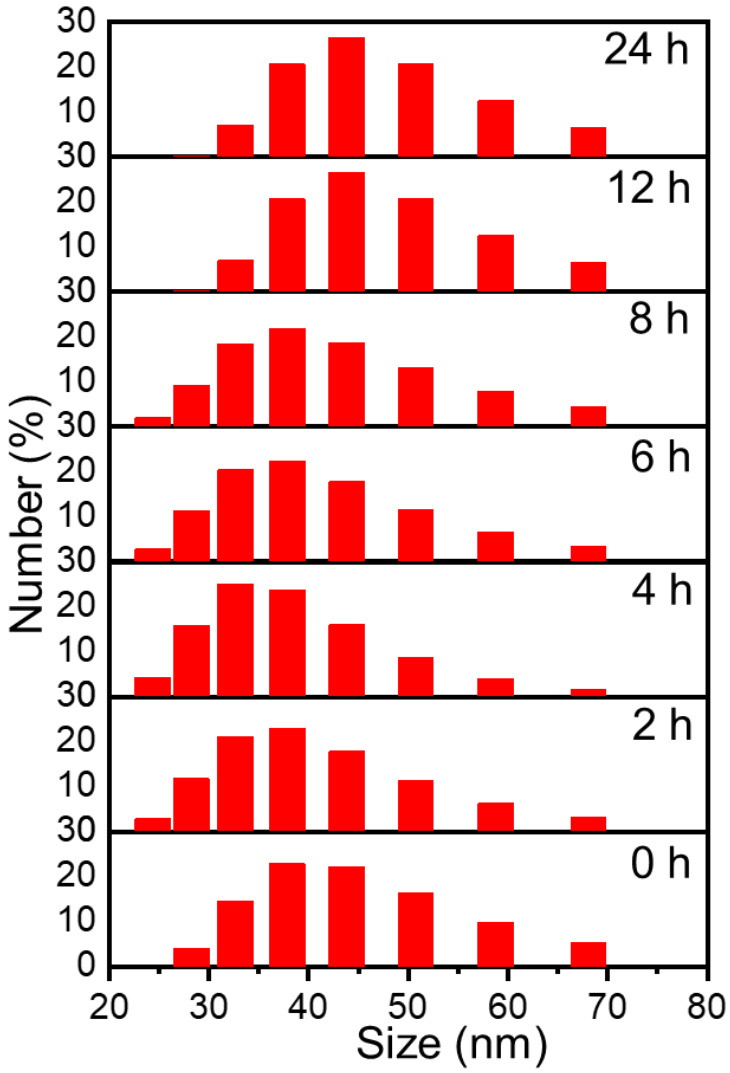

Figure S4. DLS size distribution of QD-hemin in cell culture medium at different time period. It is observed that the size of QD-hemin remains unchanged.

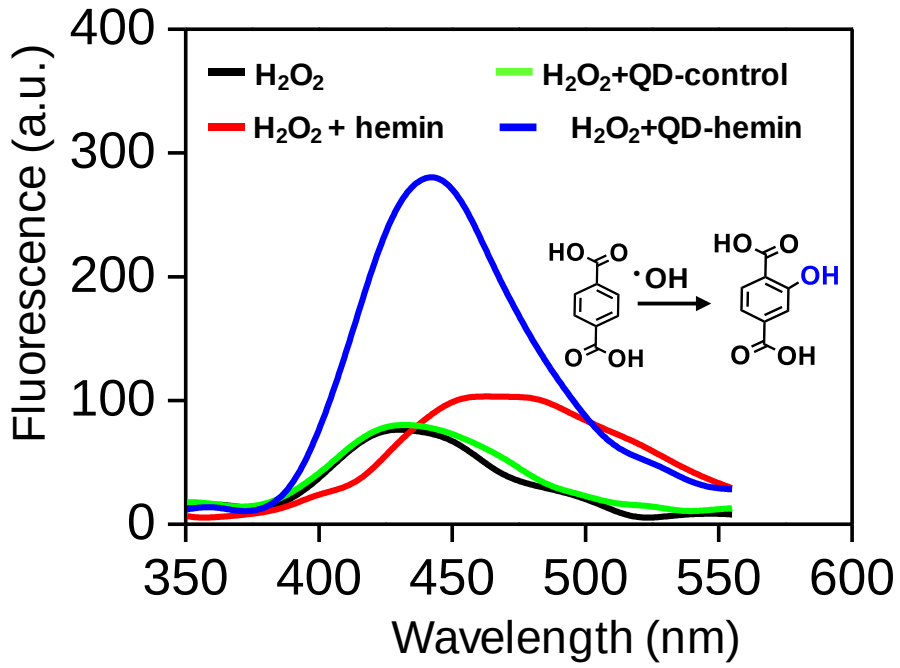

Figure S5. Reactive oxygen species generation by QD-hemin or molecular hemin in presence of H_2O_2 as measured by fluorescent probe terephthalic acid. Appearance of distinct fluorescence at 430 nm indicates oxidation of terephthalic acid to fluorescent 2-hydroxyterephthalic acid and confirms •OH radical generation. Fluorescence spectra confirms QD-hemin produces higher amount of ROS than that of molecular hemin. Control experiment confirms insignificant ROS generation by QD-control and H_2O_2. [Conditions: H_2O_2 - 10 mM, hemin - 50 μM, hemin in QD-hemin - 50 μM, terephthalic acid – 0.5 mM].

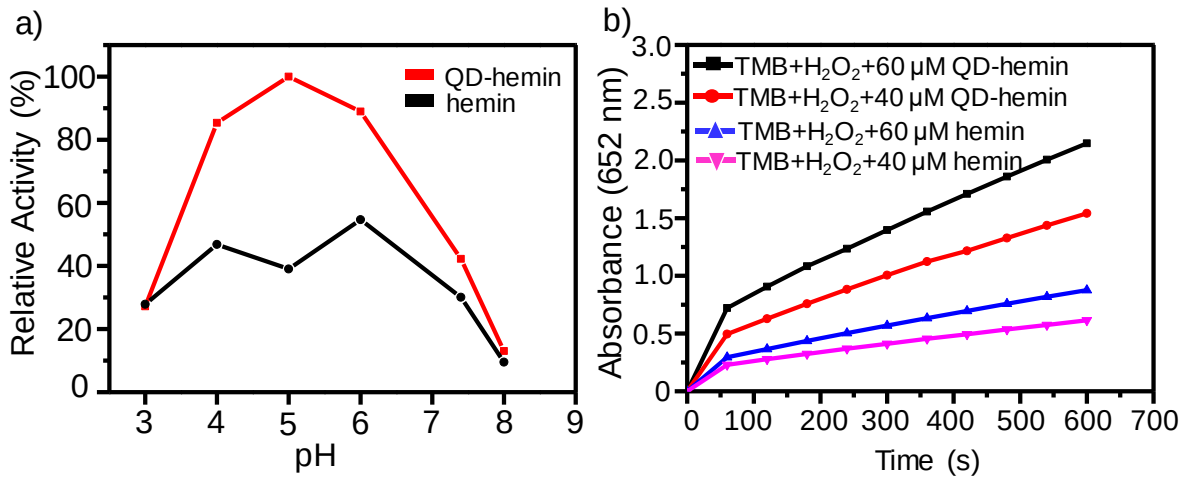

Figure S6. a) Peroxidase activity of QD-hemin and molecular hemin in buffer solutions of different pH, using TMB oxidation. Conditions: H_2O_2 - 2 mM, hemin - 50 μM, hemin in QD-hemin - 50 μM. b) Time-dependent absorbance changes of TMB solution (0.5 mM) at 652 nm in the presence of QD-hemin or molecular hemin.

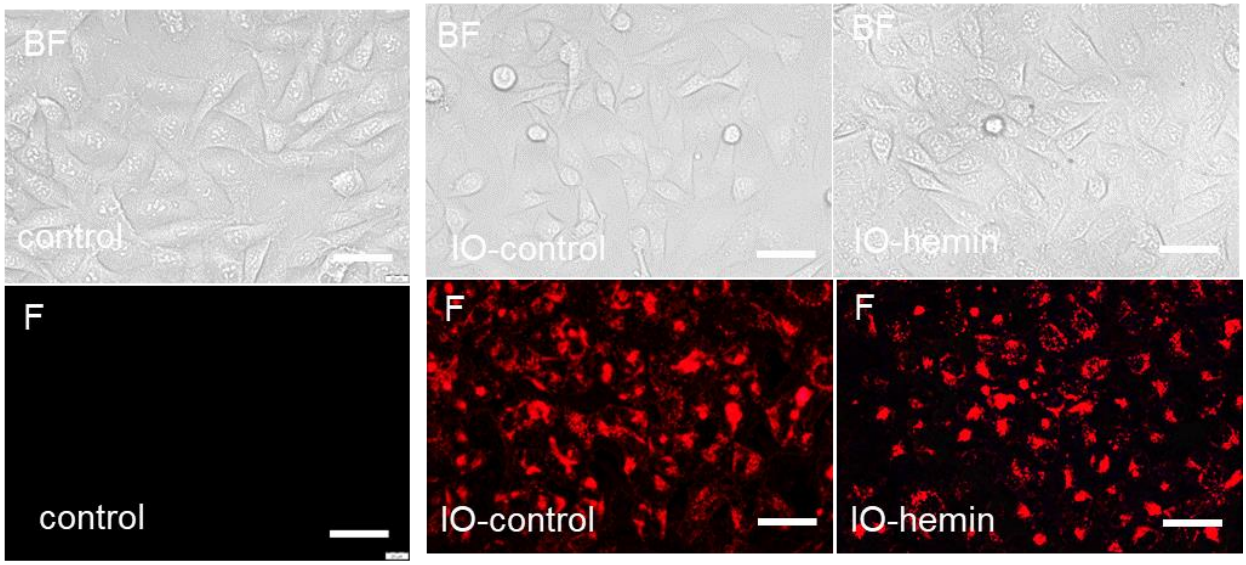

Figure S7. Evidence of high cell uptake of rhodamine tagged IO-hemin in KB cells. Cells are incubated with colloidal IO-hemin/IO-control/control for one hour and washed cells are used for imaging under bright field (BF) or fluorescence (F) mode. Results show significant labeling/uptake of IO before and after hemin conjugation. Scale bars are 50 μm.

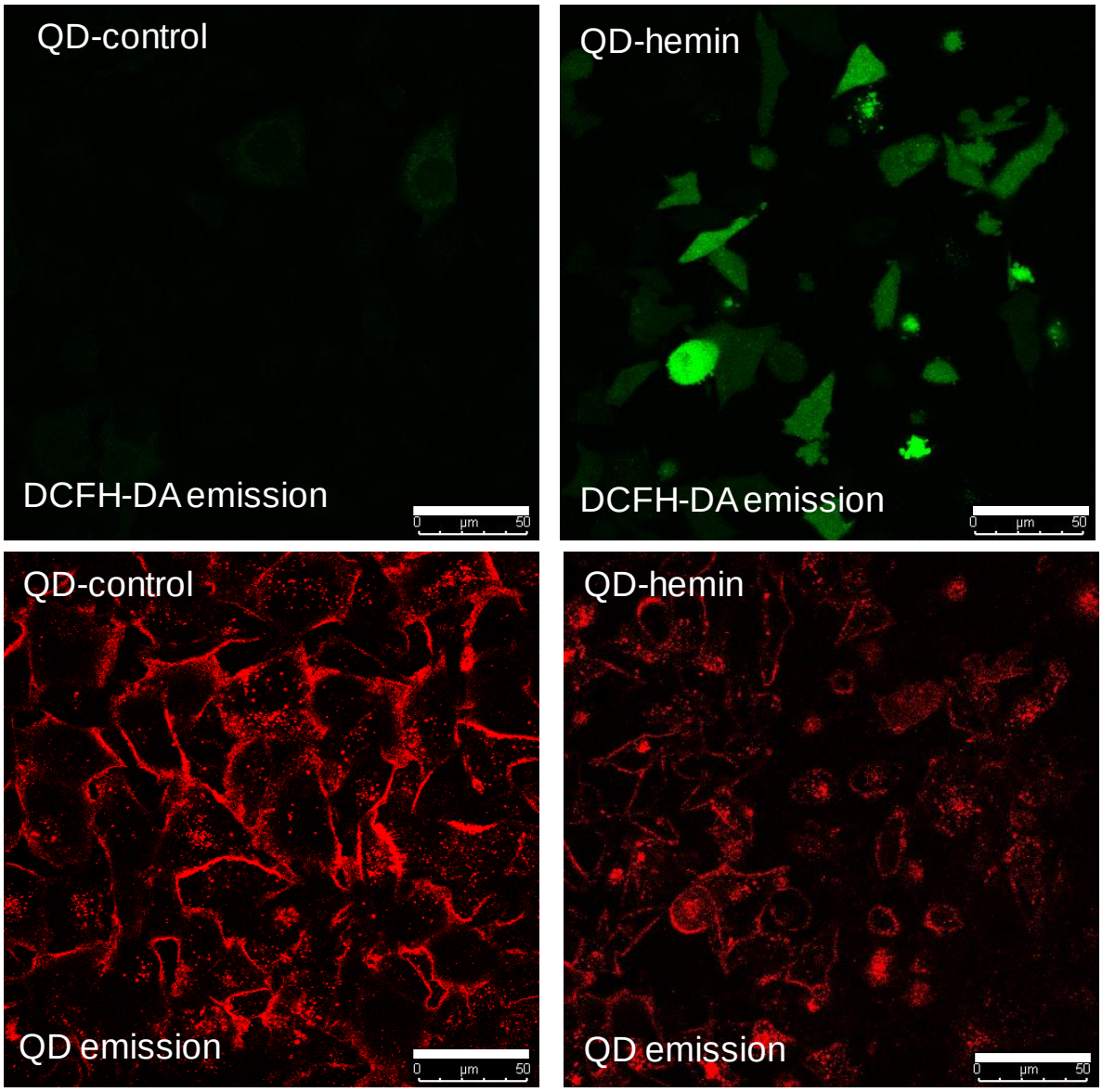

Figure S8. Evidence of insignificant interferences of QD red emission during the collection of green emission of DCFH-DA. Top panels represents emission of DCFH-DA after excitation at 488 nm and emission is collected using filter at 505 - 530 nm. Bottom panels represents emission of QD at the same area after excitation at 561 nm and emission is collected using filter at 575 - 620 nm. Labeling pattern of DCFH-DA and QD are different and there is insignificant DCFH-DA emission for QD-control. This result confirms that emission from top panels are due to DCFH-DA and represents ROS signals. Scale bars are 50 µm.

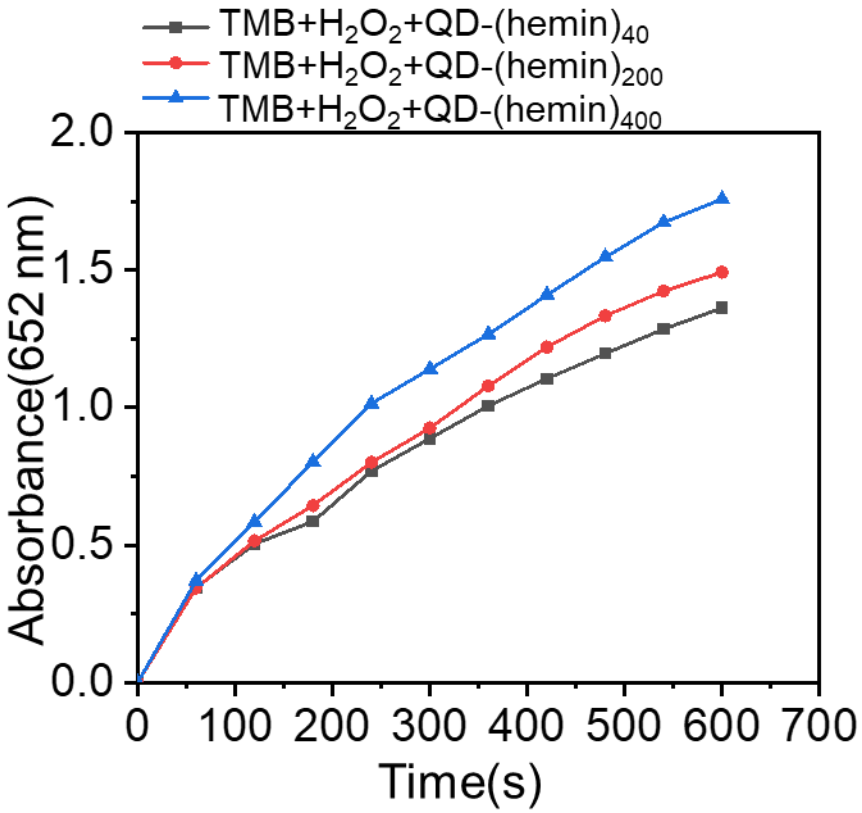

Figure S9. Comparison of catalytic activity among QD-(hemin)$_{40}$, QD-(hemin)$_{200}$, QD-(hemin)$_{400}$ using the time-dependent absorbance changes of TMB solution (0.5 mM) at 652 nm in the presence of 2 mM H_2O_2.

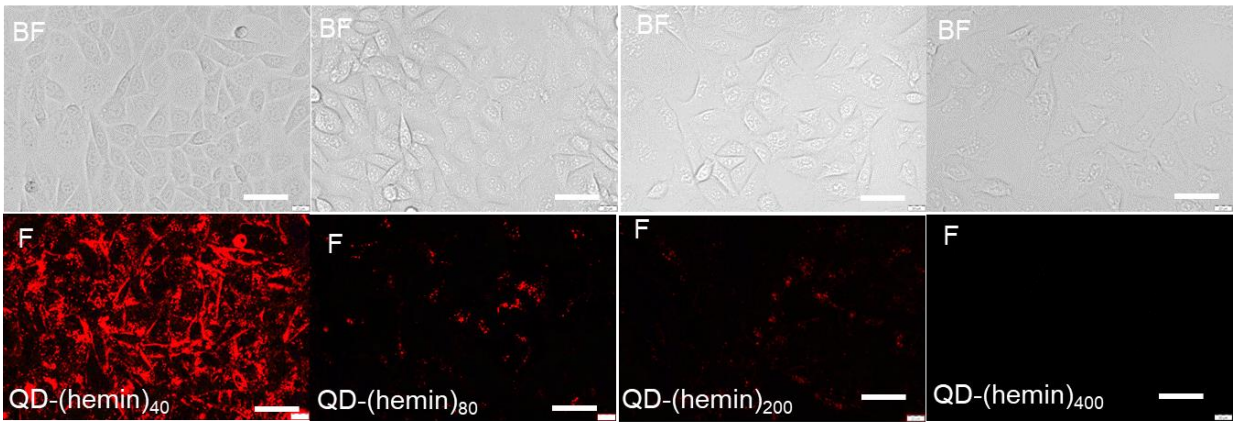

Figure S10. Fluorescence (F) and bright field (BF) image of KB cells labelled with QD-$(hemin)_{40}$, QD-$(hemin)_{80}$, QD-$(hemin)_{200}$ and QD-$(hemin)_{400}$. Typically, cells are incubated with set of QD-hemin for 2 h, then washed cell are imaged under bright field (BF) and fluorescence (F) microscopy. Image showed that with increasing the number of hemin per QD, cellular uptake decreases. Scale bars are 50 μm.

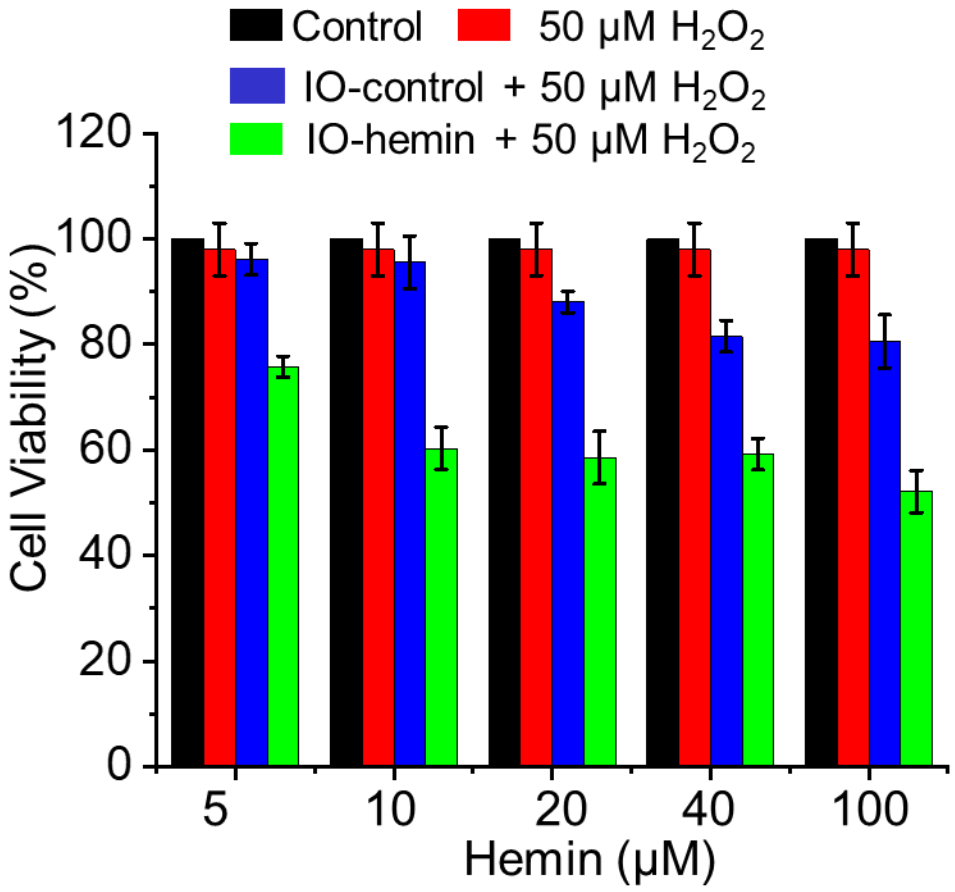

Figure S11. Dose dependent cytotoxicity of IO-hemin nanoparticle in comparison with IO-control. KB cells are incubated with various concentration of IO-hemin nanoparticle or same concentration of IO-control along with H_2O_2 (50μM), and cytotoxicity study was carried out after 24 h via MTT assay.

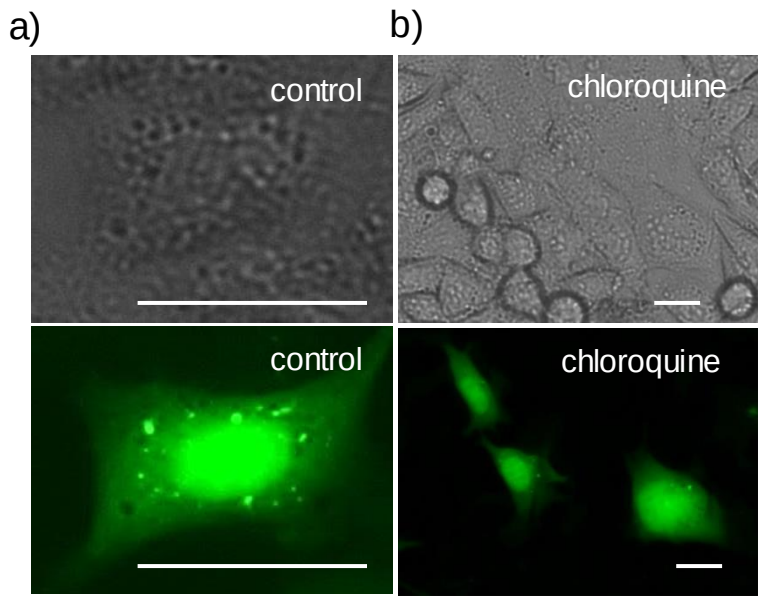

Figure S12. Suppression of autophagy in presence of autophagy inhibitor chloroquine. (a) Nano-hemin in absence of chloroquine (control). (b) Nano-hemin in presence of chloroquine (50 μM). Typically, KB cells are transfected with LC3-GFP plasmid for 12 h and then treated with chloroquine (50 μM) for 1 h and finally treated with nano-hemin. Next LC3-GFP-colocalized autophagosomes are imaged under a fluorescence microscope. The absence of chloroquine treated cells indicates autophagy inhibition. The scale bar represent 25 μM.