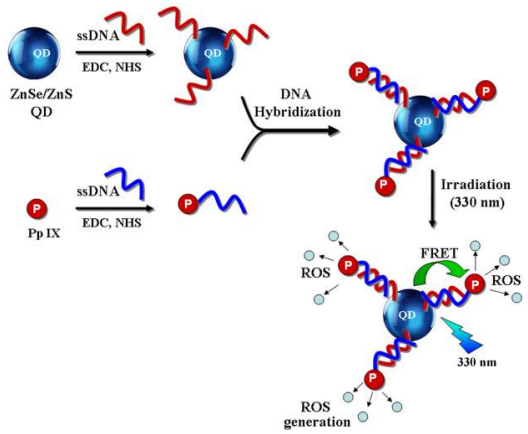
Quantum Dot-Protoporphyrin IX FRET Probe and Effect of FRET Efficiency on ROS Generation

For the purpose of achieving controlled generation of ROS (reactive oxygen species), a ZnSe/ZnS quantum dots (QDs) donor and protoporphyrin IX (Pp IX) acceptor based fluorescence resonance energy transfer (FRET) probe is fabricated. The QDs and Pp IX are assembled either by direct conjugation or through DNA hybridization. Complimentary DNA strands are individually conjugated to the QDs and Pp IX by amide coupling. Due to the overlap of the emission spectrum of QDs and the absorption spectrum of Pp IX, efficient transfer of energy from QD-DNA conjugate to Pp IX-DNA conjugate was observed. The FRET efficiency was quantitatively evaluated by steady- state and time- resolved spectroscopy and compared between QD-Pp IX direct conjugate and QD-DNA-Pp IX assembly at various donor to acceptor ratio.



Scheme 1: Scheme for conjugation of DNA with QD and Pp IX and subsequent generation of ROS from Pp IX by FRET from QD conjugated through DNA assembly.

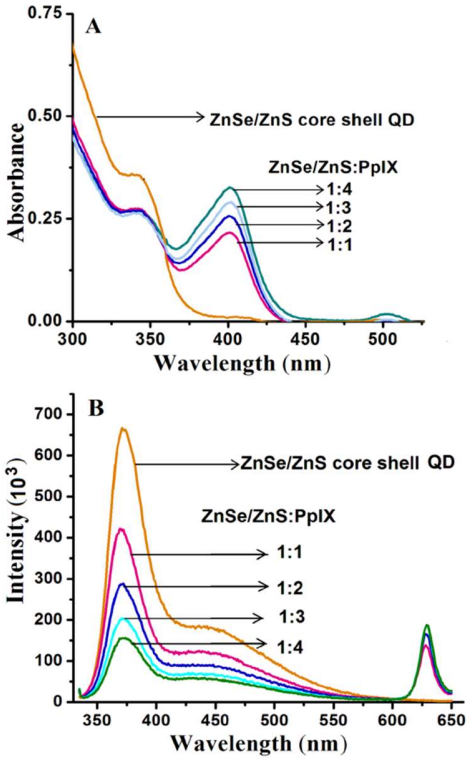


Fig. 1
A. UV-Visible absorption and B. Fluorescence Spectra of QD-Pp IX direct conjugate

Since a single QD can harbor multiple number of Pp IX-DNA counterpart through DNA hybridization, FRET efficiency was found to increase with the increase in the number of Pp IX

acceptors. ROS generation from Pp IX was studied for the highest efficient FRET pair and was found to be affected by the irradiation time of the QD donor.

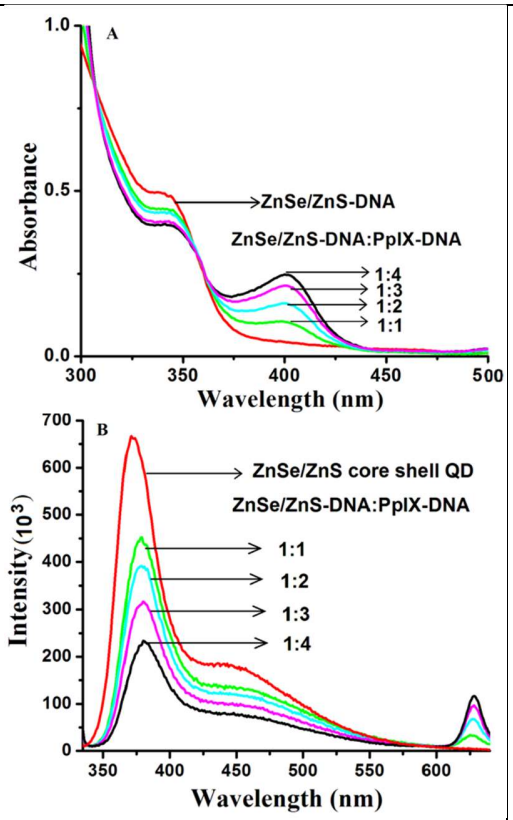


Fig. 2 A. UV-Visible absorption and B. Fluorescence Spectra of QD-DNA-Pp IX assembly

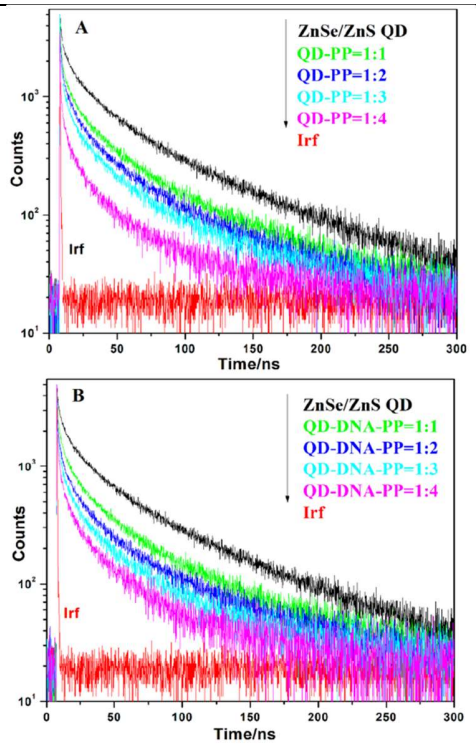


Fig. 3 Time-resolved fluorescence decay of **A.** QD-Pp IX Conjugate and **B** QD-DNA-Pp IX assembly recorded for solutions with varying ratio of donor and acceptor. Irf: Instrument Respose Function.

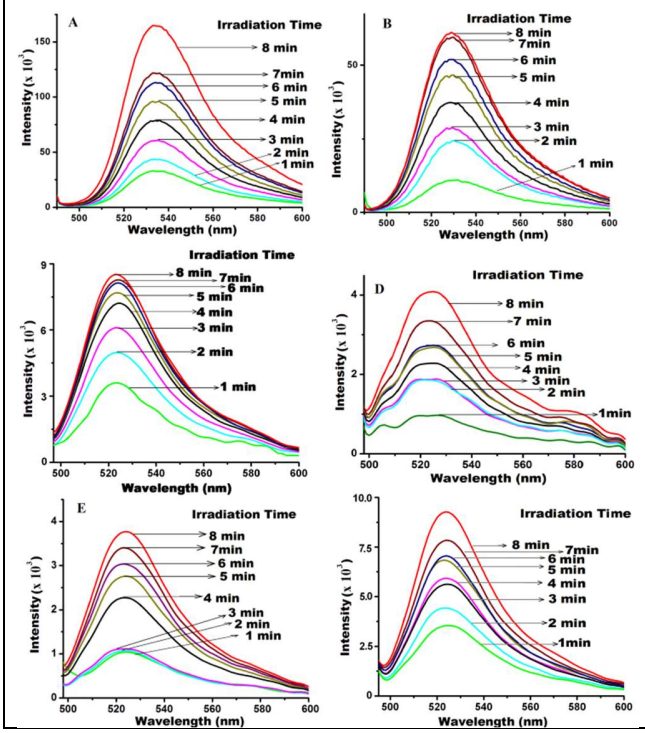


Fig. 4 Rhodamine 123 Fluorescence Emission due to ROS generation in (A) QD-Pp IX direct conjugate (B) QD-DNA-Pp IX assembly (C) QD and Pp IX in free state (D) QD-DNA and Pp IX-DNA in free state (E) QD only and (F) Pp IX only

We postulate that with the careful manipulation of the DNA sequence, the donor and the acceptor, the assembly of QD-DNA-photosensitizer can have general applicability, in particular in PDT with the dual purpose of imaging and targeted, controlled release of ROS for the treatment of relevant cancers.