Research Findings

In summary we are describing our research findings as mentioned here. In this work, emphasis is to utilize FRET method to analyze GOx and glucose using fluorescent ZnO QDs as the probes. Fluorescent ZnO and ZnOext QDs were synthesized using sol–gel technique and green synthesis method as donor and acceptor nanoprobes. Structural, optical and morphological characterization of QDS were carried out using UV-visible absorption, fluorescence, FTIR, XRD and field emission scanning electron microscopy.

We synthesized ZnO QDs without surfactant and ZnOext QDs using methanolic extract of *Butea monosperma* (palash) which work as surfactant. Bandgap of 3.49 eV and 3.35 eV was estimated from Tauc's plot for ZnO and ZnOext. FTIR fingerprint region in range 600–1000 cm^{-1} confirms the synthesis of metal oxide. FE-SEM examination reveals the spherical shaped particles of size ∼8 nm and ∼10 nm for ZnO and ZnOext, respectively and XRD reveal wurtzite ZnO crystals for both ZnO and ZnOext QDs. Systematic absorption study if wavelength for ZnO–GOx and ZnOext–GOx association constant of −8.3361 M−1 and −2.57646 M−1 for ZnO and ZnOext using Benesi-Hildebrand plot. The binding constant obtained from Stern-Volmer equation is 1.0466 μM^{-1} and 1.97 μM^{-1} for ZnO/ZnOext–GOx conjugate and suggest static quenching in the system. CD spectroscopy reveal native state of protein in conjugate system. Overall there is no change in structure and conformation of protein which is correlated to the natural states of GO_x in buffer after conjugating with ZnO_{ext} QDs as observed by CD spectroscopy as mentioned above.

Constant Förster radius (Ro) in ZnOext–GOx system suggest the average (k^2) as 2/3 and is independent of donor–acceptor distance as normally assumed in FRET system exhibit varies from ∼4.5% in ZnO–GOx and ∼5% in ZnOext–GOx system indicate better energy transfer in earlier system than formal. An increased efficiency is correlated to the accumulative effect of the increase of refractive index and the alignment of donor and acceptor dipoles with increasing glucose concentration. The physiological range of glucose from 60 mg–440 mg/dl using this technique in which ZnOext–GOx as donor probe exhibit better response towards glucose than ZnO–GOx system and correlated to the presence of phytochemicals on the QD surface.

From the current docking, MD simulations, MM/GBSA study we found that the $(ZnO)_{12}$ quantum cluster is capable of tightly binding to GO_x protein. Further, to analyze effect of absence or presence of glucose on GO_x Protein-FAD-$(ZnO)_{12}$ interactions; MM/GBSA analysis was also performed on GO_x Protein-FAD-$(ZnO)_{12}$ complex. For the current analysis GO_x -FAD complex was taken as the receptor and $(ZnO)_{12}$ was taken as ligand. The ΔG binding of $(ZnO)_{12}$ Quantum Cluster–GO_x FAD complex which is **-33.43 Kcalmol^{-1}** while for glucose-$(ZnO)_{12}$ Quantum Cluster–GO_x-FAD complex is **-39.64 Kcalmol^{-1}**. Thus we can say that $(ZnO)_{12}$ quantum cluster can be used as an excellent nano probe to monitor the interaction of nano-bio system.

So overall from our wet lab experiment and *Insilco* study we found that ZnO quantum dots or ZnO quantum cluster can be used as an excellent nano probe for qualitative and quantitative analysis of nano-bioconjugate interaction and biomolecular interaction using FRET.