Supporting Information

Clathrin- to Lipid Raft-Endocytosis via Controlled Surface Chemistry and Efficient Perinuclear Targeting of Nanoparticle

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Table S1. Property of three control QDs that are used in this study.

No.	Hydrodynamic size (nm)	Functional group	Charge (mV) at pH 4.5, 7.4, 9.0	Uptake mechanism	Subcellular localization
Control I[#]	20-30	NH_2 (640)	+10, +6, +2	clathrin	lysozome
Control II[#]	20-30	NH_2 (335), SO_3^-	+2, -8, -12	clathrin, lipid raft	lysozome, perinuclear
Control III	20-30	NH_2(170), PEG, SO_3^-, octyl (160)	+4, -8, -14	predominate lipid raft	perinuclear

[#] QD is toxic to cell

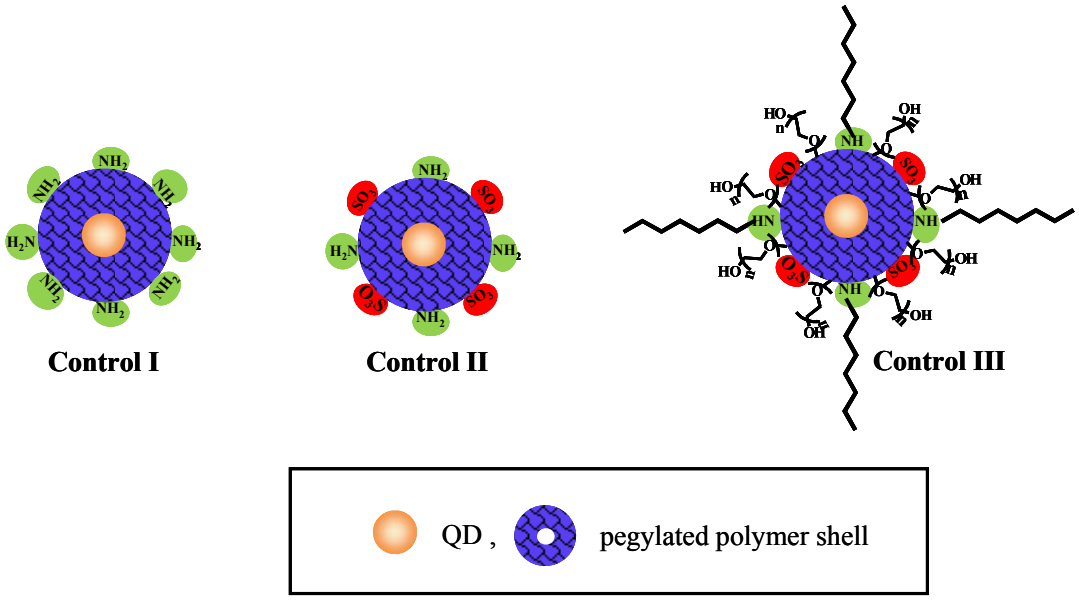

Figure S1. Structure of three control QDs.

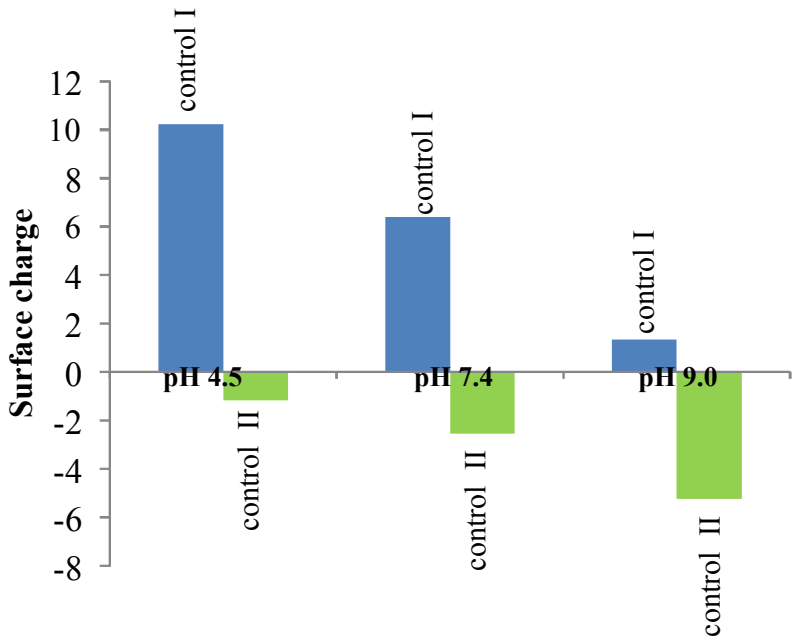

Figure S2. Surface charge of control I and II QDs at different pH.

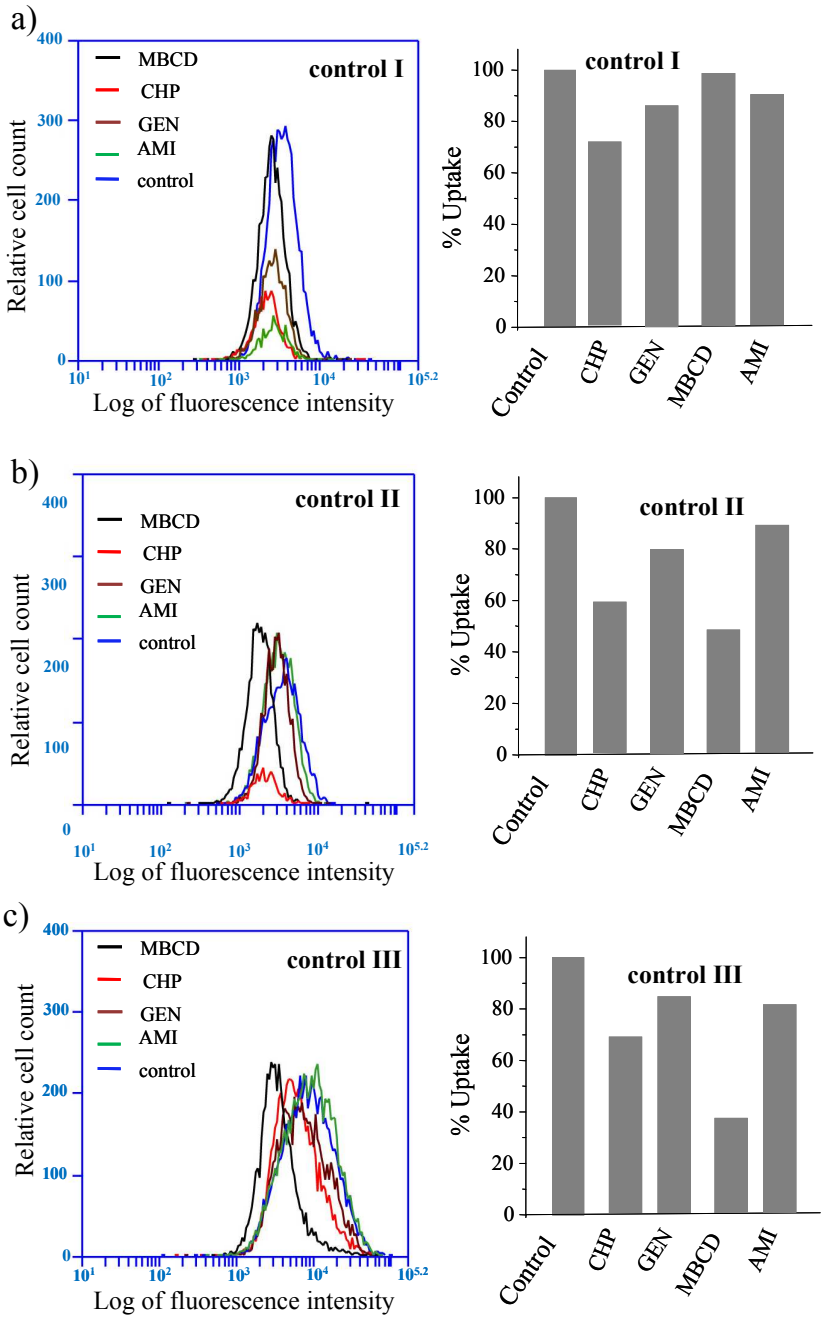

Figure S3. Flow cytometry based quantitative cellular uptake of three control QDs in HeLa cells in presence of different endocytosis inhibitors. About 20,000 events (QD labeled cells) are used for this quantification study and area under the fluorescence intensity is used for uptake quantification. Results show that uptake of control 1 is mainly blocked by CHP, uptake of control 2 QDs is significantly blocked by both CHP and MBCD and uptake of control 3 QDs is blocked mainly by MBCD.

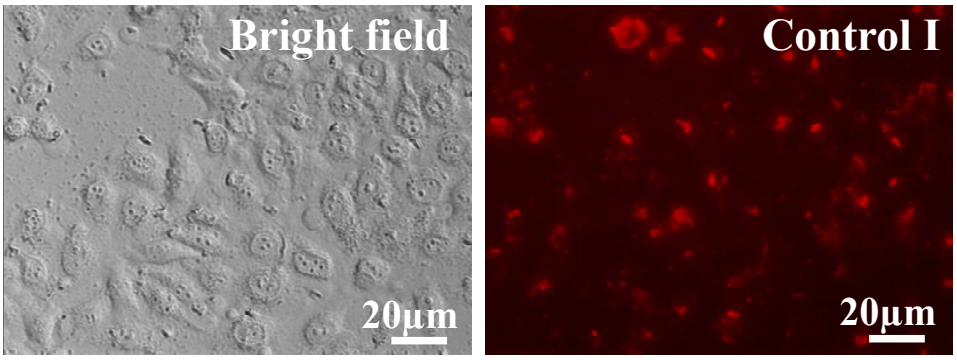

Figure S4. Typical bright field and fluorescence image of HeLa cells labelled with type I QD.

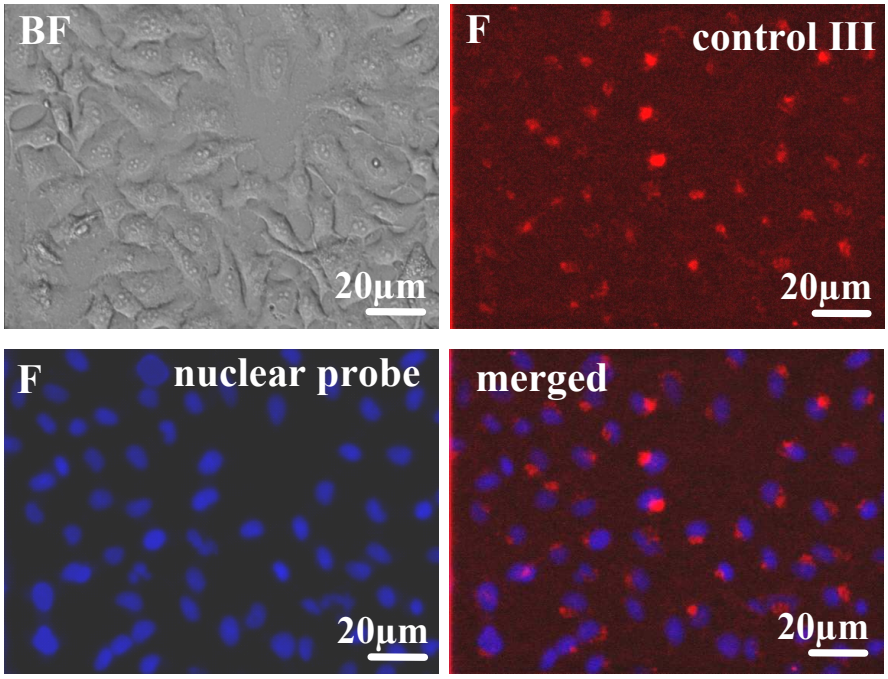

Figure S5. Subcellular localization study of control III QD, entering via predominant lipid raft endocytosis, is trafficked to perinuclear region. QD solution is incubated with cells for one hour and then washed cells are further incubated with fresh media for next 28 hrs. (BF correspond to bright field mode, F corresponds to fluorescence mode, blue color corresponds to nuclear probe and red color corresponds to QD

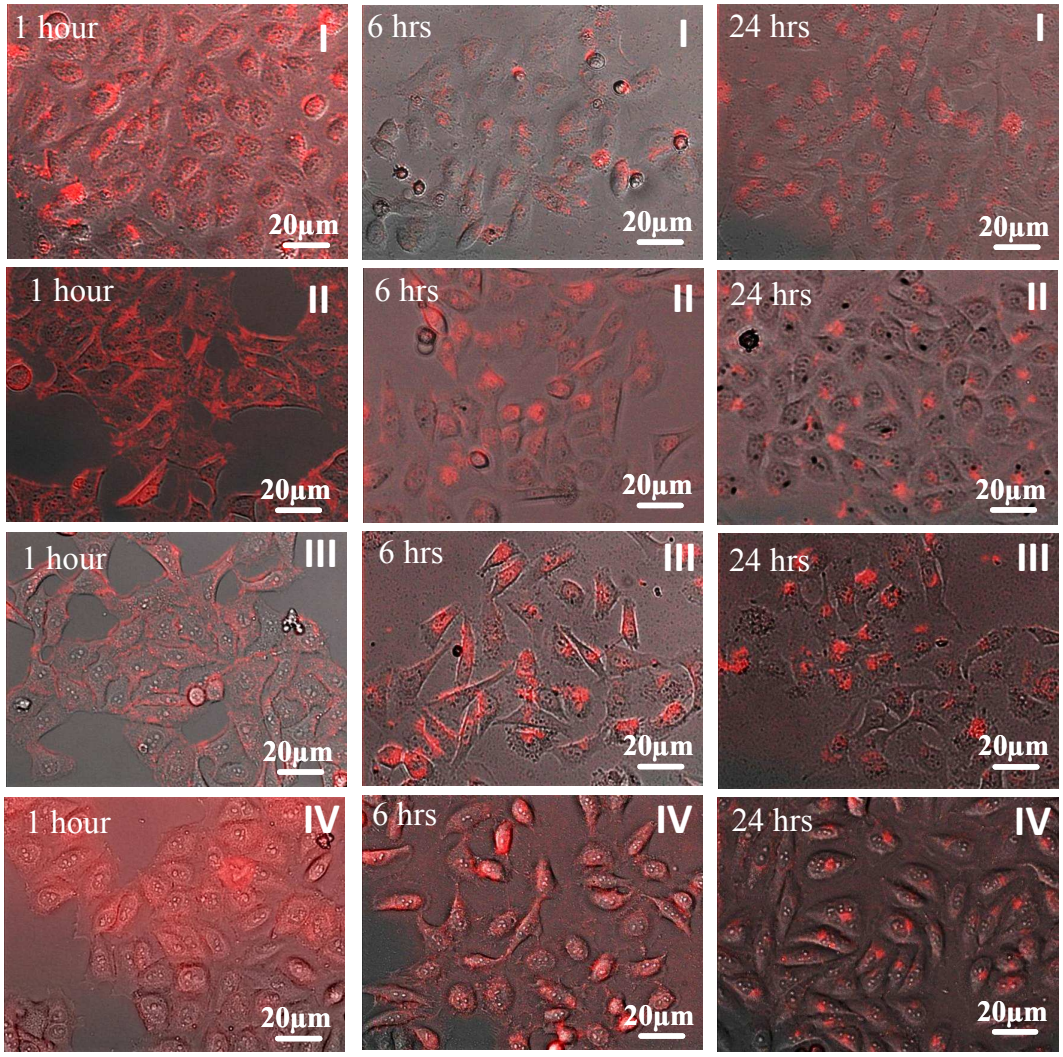

Figure S6. Kinetics of uptake of four different QD in HeLa cells. Cells are incubated with QD solution for one hour and washed cells are further incubated with fresh media between 1-24 hrs. Next, washed cell are imaged under bright field and fluorescence mode and merged images are shown here. Results show that type I and II QDs have high binding with cell membrane, enter into cell within one hour and then starts localizing inside cell in next 1-6 hrs. In contrast other two QDs have low binding, localize in cell membrane in first one hour and take 6-24 hrs for entry and sub-cellular localization.

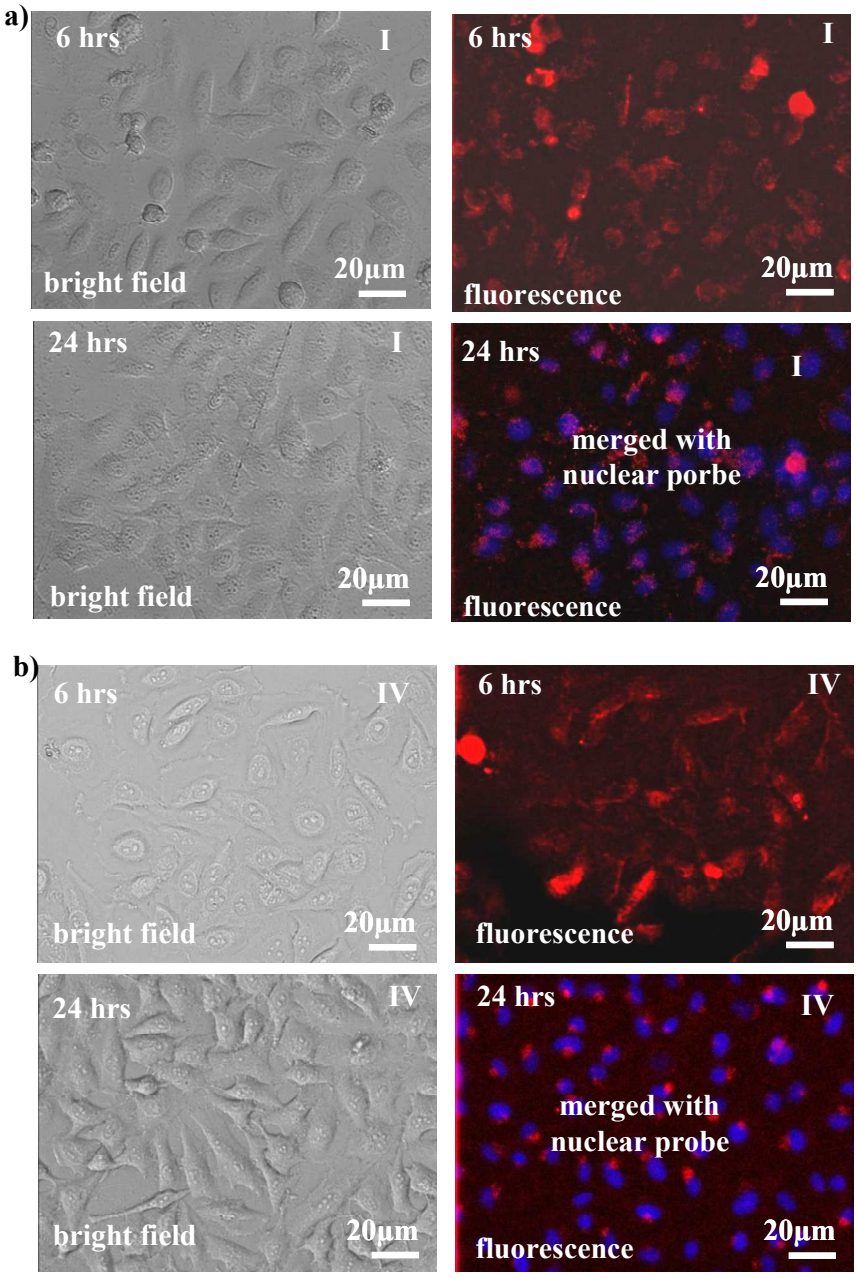

Figure S7. Time dependent cellular localization of type I QD (a) and type IV QD (b). Type I QD enters into cell within hour and localize in next 24 hrs outside nucleus. In contrast type IV enters into cell in 6 hrs and in next 24 hrs localizes at the perinuclear regions.

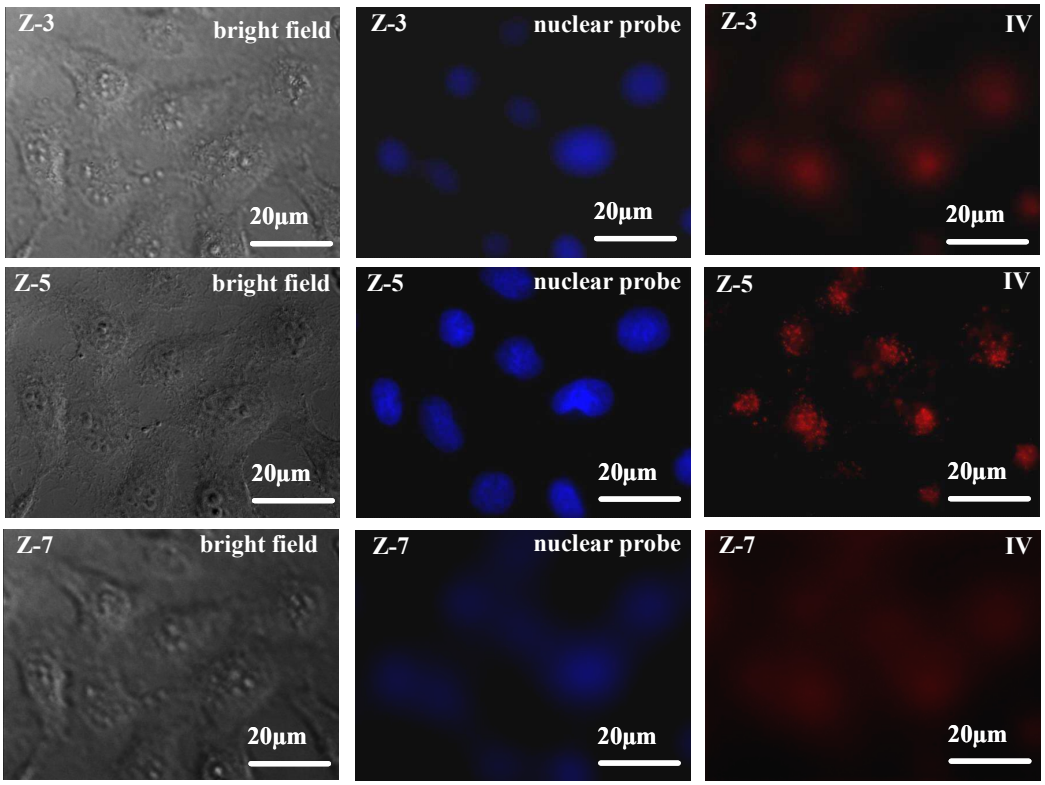

Figure S8. Microscopic imaging of QD and nuclear probe labeled HeLa cells to confirm that QD is inside cell and localized at perinuclear region. Images were taken at different Z planes (top to bottom with consecutive z axis slices of 0.75 µm starting from Z-1 to Z-10) and from the images it is found that the emission from the QD and nuclear probe are coming from the same Z plane, suggesting that QDs and the nucleus are at same plane.

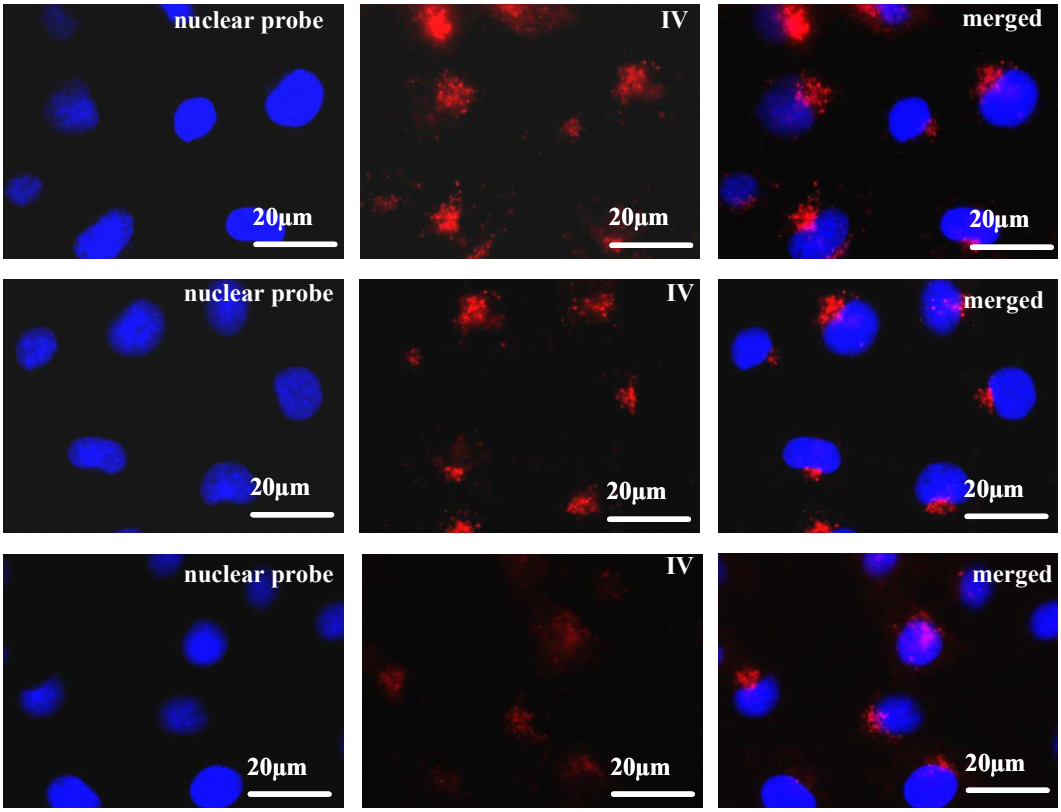

Figure S9. High magnification fluorescence image of nuclear probe (hoechst) and type IV QD labelled HeLa cells. Merged imaged clearly shows that QD localizes at one side of perinuclear regions.

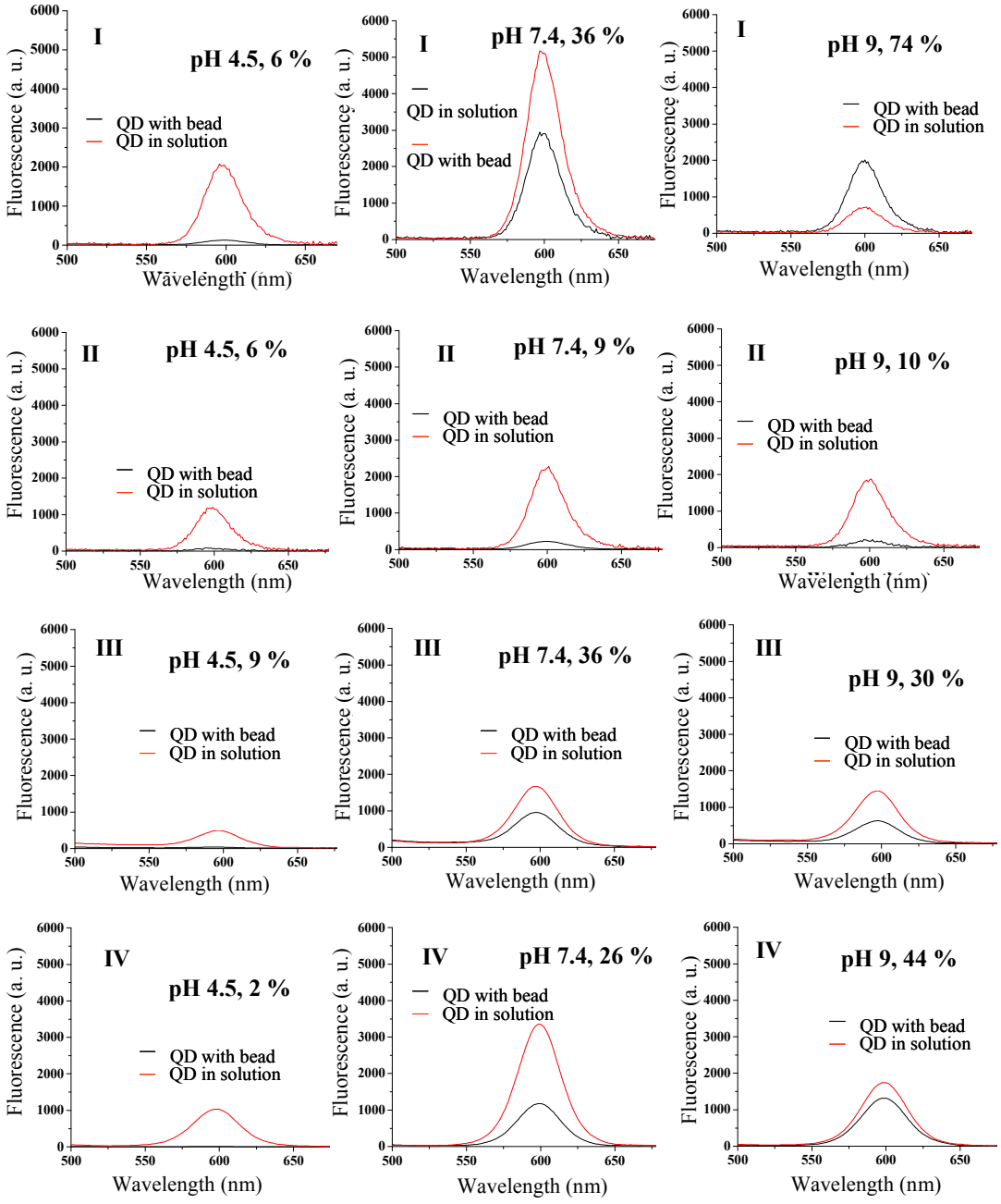

Figure S10. Binding interaction between QD and anionic polybead at pH 4.5, 7.4 and 9.0. Typically, QD is mixed with excess polybeads in 0.6 mL buffer solution and then polybead bound QDs are separated by centrifuge. Next, fluorescence of polybead bound QDs and QDs remaining in solution are measured to determine the % of polybead bound QD. (shown in each graph) Results show that binding of cationic QD (type I QD) is higher than zwitterionic QD (type II) at pH 7.4 and binding of all QD strongly depends on solution pH.

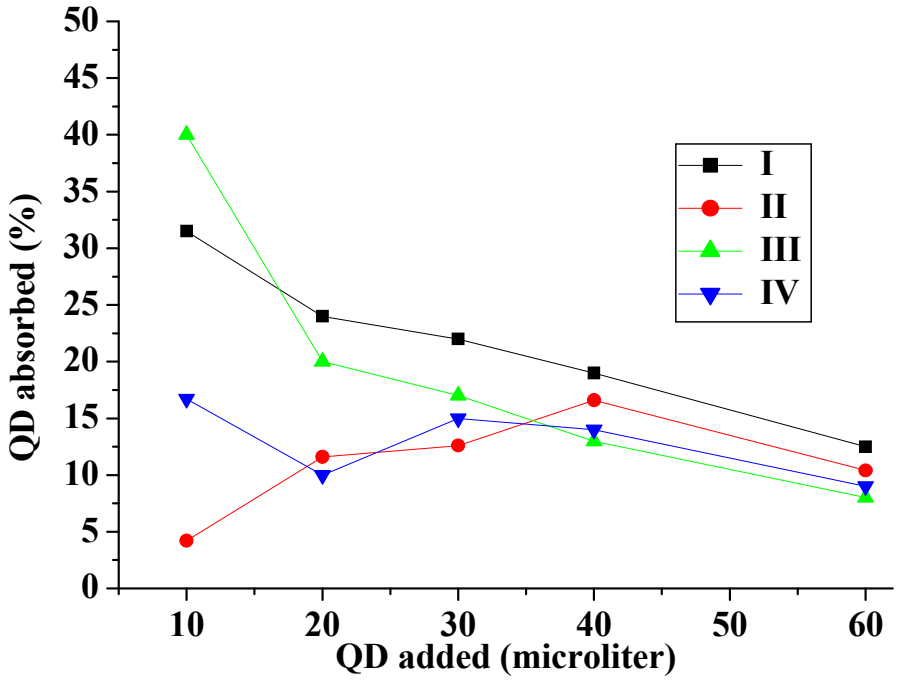

Figure S11. Binding interaction between QD and anionic polybead at pH 7.4 with the increasing concentration of QD. Typically, varying amount of QD is mixed with same concentration of polybeads in 0.6 mL buffer solution and then polybead bound QDs are separated by centrifuge. Next, % of polybead bound QD is determined as described in Figure S10 and plotted against QD concentration. Results show that higher binding of cationic QDs as compared to zwitterionic QDs becomes noticeable at lower QD concentration. However, the exception is also noticed for one of the lipophilic-zwitterionic QD (type III), which may be due to specific interaction between polybead and phenylalanine.