Controlling the reactivity of ampiphilic quantum dots in biological assays through hydrophobic assembly of custom PEG derivatives

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Supporting Information

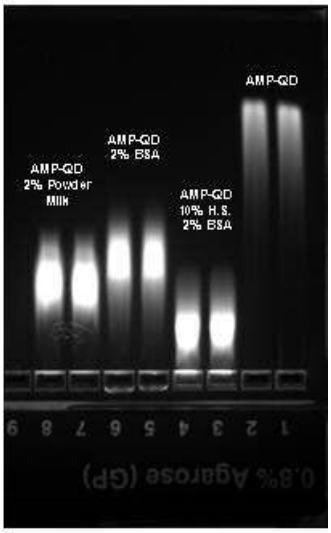

Figure S1. Gel electrophoresis demonstration of protein adsorption to the reactive AMP-QD surface. Protein adsorption to the AMP-QD surface is evident in the presence of standard blocking agents, widely used in biological assays, such as powdered milk, BSA and horse serum (H.S.). The relative electrophoretic mobility of AMP-QDs is reduced upon dilution in different blocking solutions, as compared to unconjugated AMP-QDs, indicating surface modification.

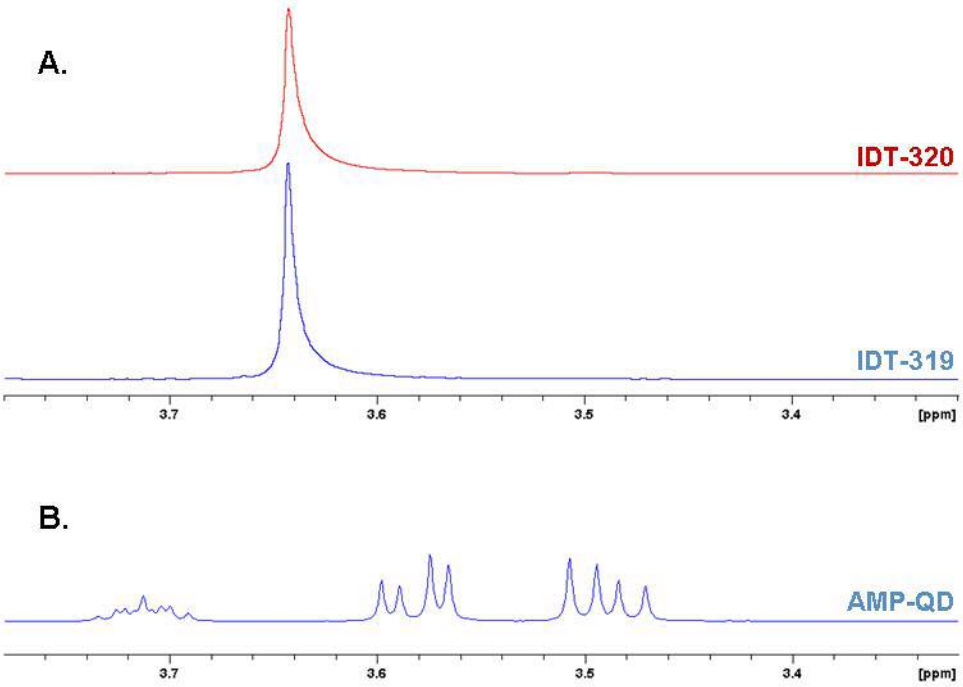

Figure S2. Control ^{1}H NMR experiments illustrating characteristic spectra of both free ligands and unconjugated AMP-QDs to demonstrate a lack of spectral overlap. (A) The spectra of both IDT-319 and IDT-320 are dominated by a single peak at 3.64 ppm resulting from the chemically equivalent protons in their long PEG chains. (B) Unconjugated AMP-QDs present no spectral feature at this chemical shift, but instead have characteristic spectral features presumably due to protons present in their polymeric coating. Consequently, this strong PEG signal may serve as a useful feature in providing spectroscopic characterization of ligand interactions with the QD. Furthermore, the additional AMP-QD features can serve as an internal standard to normalize each spectra to the amount of QDs present.

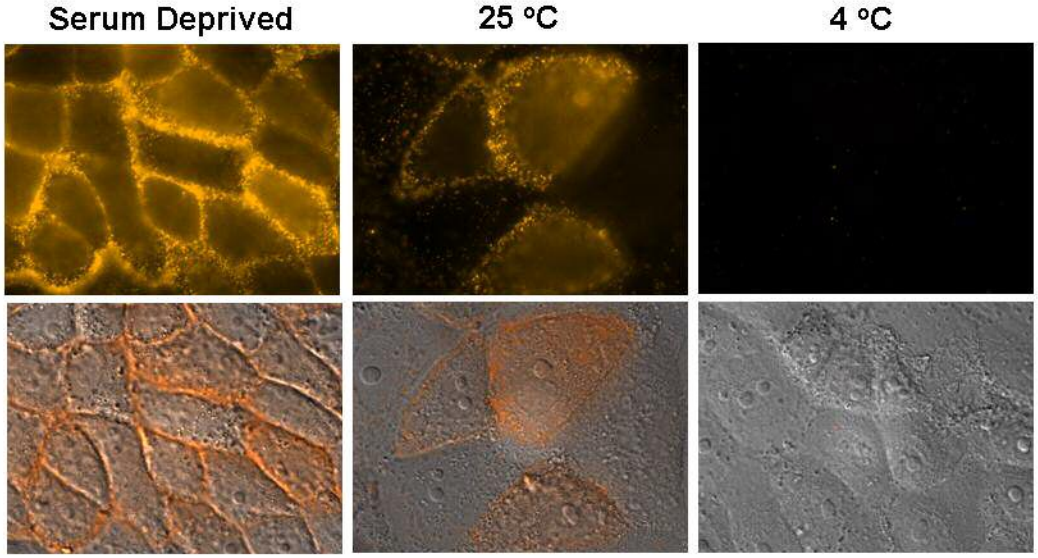

Figure S3. Characterizing the cellular response of IDT-319 QD conjugates in the presence of serum proteins. Cellular labeling conditions were varied in order to better understand the nature of the observed artifactual labeling with IDT-319 QD in a high serum protein blocking solution (2% BSA with 10% horse serum). Cells initially serum deprived overnight prior to QD labeling demonstrated a significant increase in QD fluorescence (Left, Serum Deprived)), an indication that this artifactual response is cell cycle dependent. This cell cycle dependence also explains the non-uniform nature of fluorescent staining for protein-QD conjugates in the absence of serum deprivation. Fluorescent staining caused by serum protein adsorption also exhibits a distinct temperature dependence. A non-uniform, endosomal fluorescent staining pattern is observed when the QD labeling is carried out at room temperature (Middle, 25 ^{o}C) while all fluorescent labeling is blocked upon QD incubation at 4 ^{o}C (Right). This temperature dependence suggests active cellular transport is involved in the observed fluorescent labeling, consistent with endosomal association.

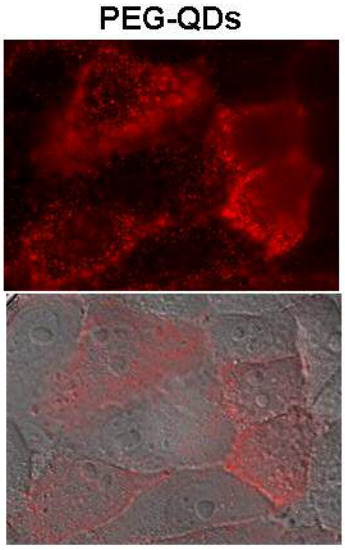

Figure S4. Red emitting AMP-QDs (655 nm emission), covalently modified with PEG2000 as previously reported (*20*), result in the same non-uniform fluorescent staining pattern as IDT-319 derivatized PEG conjugates upon cellular incubation in serum blocking conditions. This artifactual fluorescent staining is observed despite the improved passivation of PEGylated QD conjugates.