Supporting Information for

Ni-Nitrilotriacetic Acid-Modified Quantum Dots as a Site-Specific Labeling Agent of Histidine-Tagged Proteins in Live Cells

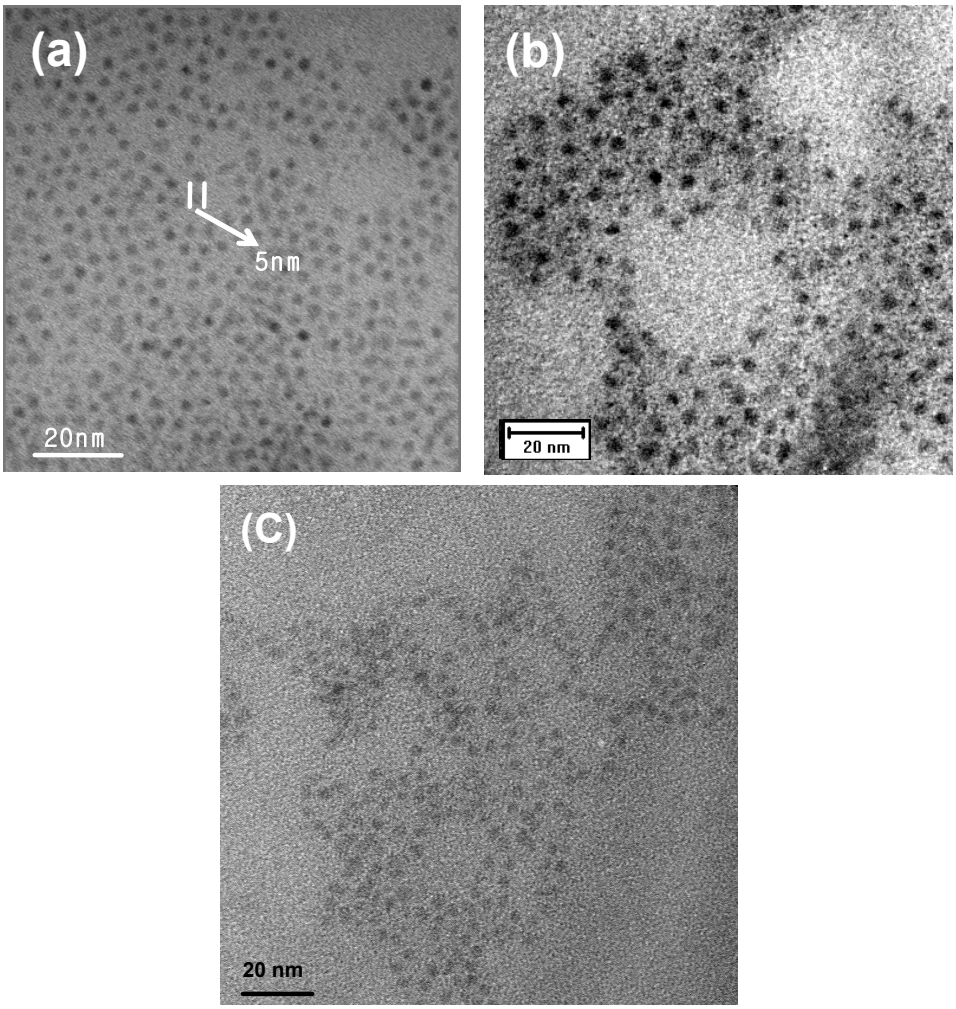
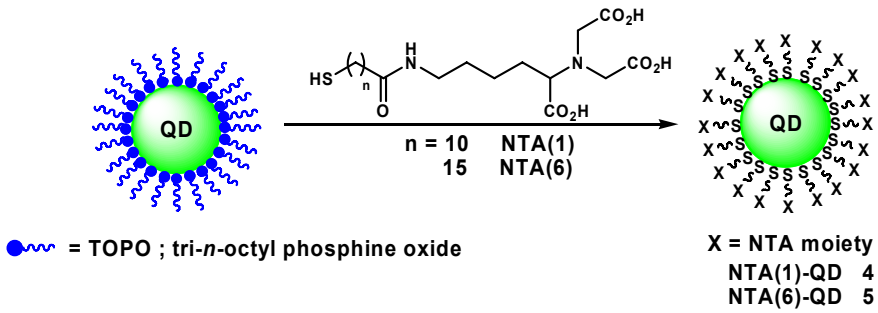


Figure S1. Typical TEM images of CdSe/ZnS quantum dots in organic phase (a), amino-PEG coated CdSe/ZnS QDs in water (b), and Ni-NTA conjugated QD (c)

Preparation of NTA modified QD by ligand exchange

The quantum dots in the organic phase (in chloroform) were treated with ligand 6 with vigorous stirring to form NTA modified water soluble QDs. The ratio of ligand (500 equiv.) to QDs was



Scheme S2. NTA modification on QD by ligand exchange using NTA-functionalized thiol ligand

quite critical for maintaining the water solubility of the final Ni-NTA modified QDs. When all the NTA ligands on the QDs were saturated with Ni^{2+}, water solubility was significantly decreased as indicated by the formation of aggregates. Therefore, a small amount of Ni^{2+} (14 and 30 equiv.) was reacted to form the Ni-NTA complex, which allows the water solubility of QDs to be conserved. The partial Ni complexation was unambiguously confirmed by ICP-MS analysis (11 and 22 Ni^{2+}). The ligand 6 bearing longer spacer exhibited higher efficiency in the ligand exchange process, probably due to the enhanced hydrophobic accessibility of the ligand, facilitating the contact of ligand with the ZnS shell.

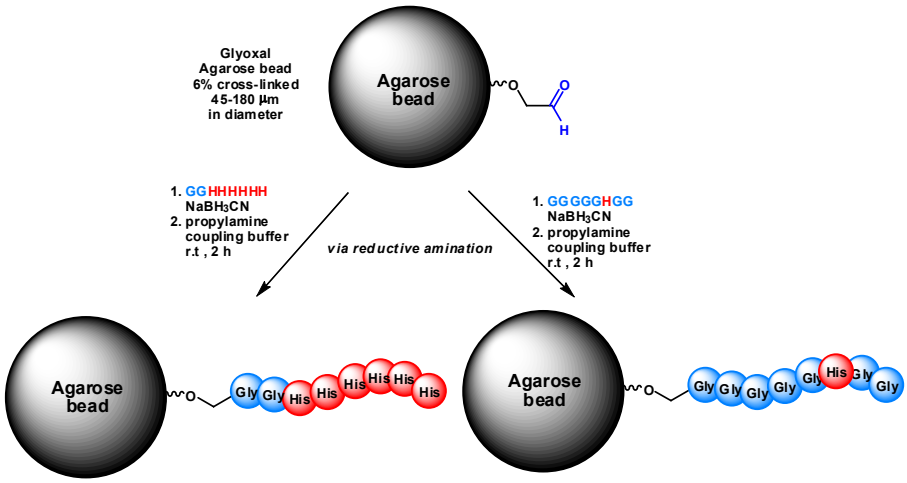
Next, the stability of QD 5 was examined under various conditions results of which are summarized in Table S2. QD 6 showed no significant aggregation or loss of photoluminescence in 24 hr within the pH range 5 to 9 at room temperature. Corresponding solutions were checked with a confocal microscope to conform the homogeneity and dispersity (supporting information). These NTA modified QDs are expected to be used not only in Ni complexation reactions but also as new water soluble QD precursors for conjugation with various biomolecules in biological applications

Table S3. NTA modification on QD by ligand exchange using NTA-functionalized thiol ligand

Entry	Condition	1 h at 25 °C	24 h at 25 °C	7 days at 25 °C
1	pH = 4.0	+++	+++*	+++*
2	pH = 5.0	+++	+++	+++*
3	pH = 6.0	+++	+++	+++
4	1 M NaCl	+++	++	+
5	5 M NaCl	++	++	+
6	pH = 7.4	+++	+++	+++
7	pH = 9.0	+++	+++	+++
8	Deionized buffer	+++	+++	+++

+++ Very stable
++ Considerably stable
+ Stable

Oligopeptide immobilization. The immobilization of oligopeptides on glyoxal agarose bead was performed as follows. To a 500 µL slurry of 6% cross-linked glyoxal agarose beads (ABT, Tampa, FL, 6BCL-GL0-50) was added 1.6 mL of cyanoborohydride coupling buffer (20 mM sodium phosphate at pH=8.0 and 3 g/L sodium cyanoborohydride). After 15 min, the beads was filtered and resuspended in fresh coupling buffer (1.0 mL). To a suspension of agarose beads was added a solution of oligopeptide (2G6H, 4 mg, 4.19 µmol) in water (1.0 mL). This reaction mixture was stirred at room temperature for 17 h and the beads were then drained and subsequently washed with the coupling buffer. To block the free aldehyde sites, the resins were resuspended in coupling buffer (1.0 mL) and treated with a solution of propylamine (32 µL, 400 µmol) in water (500 µL). After another 3 h of incubation, the resins were purified with a membrane separation filter (Pall, M_w cutoff ~ 100,000) with water. The oligopeptide functionalized agarose beads were stored in water for further use.



Scheme S4. Conjugation of G2H6 and G5HG2 peptides on glyoxal agarose bead.

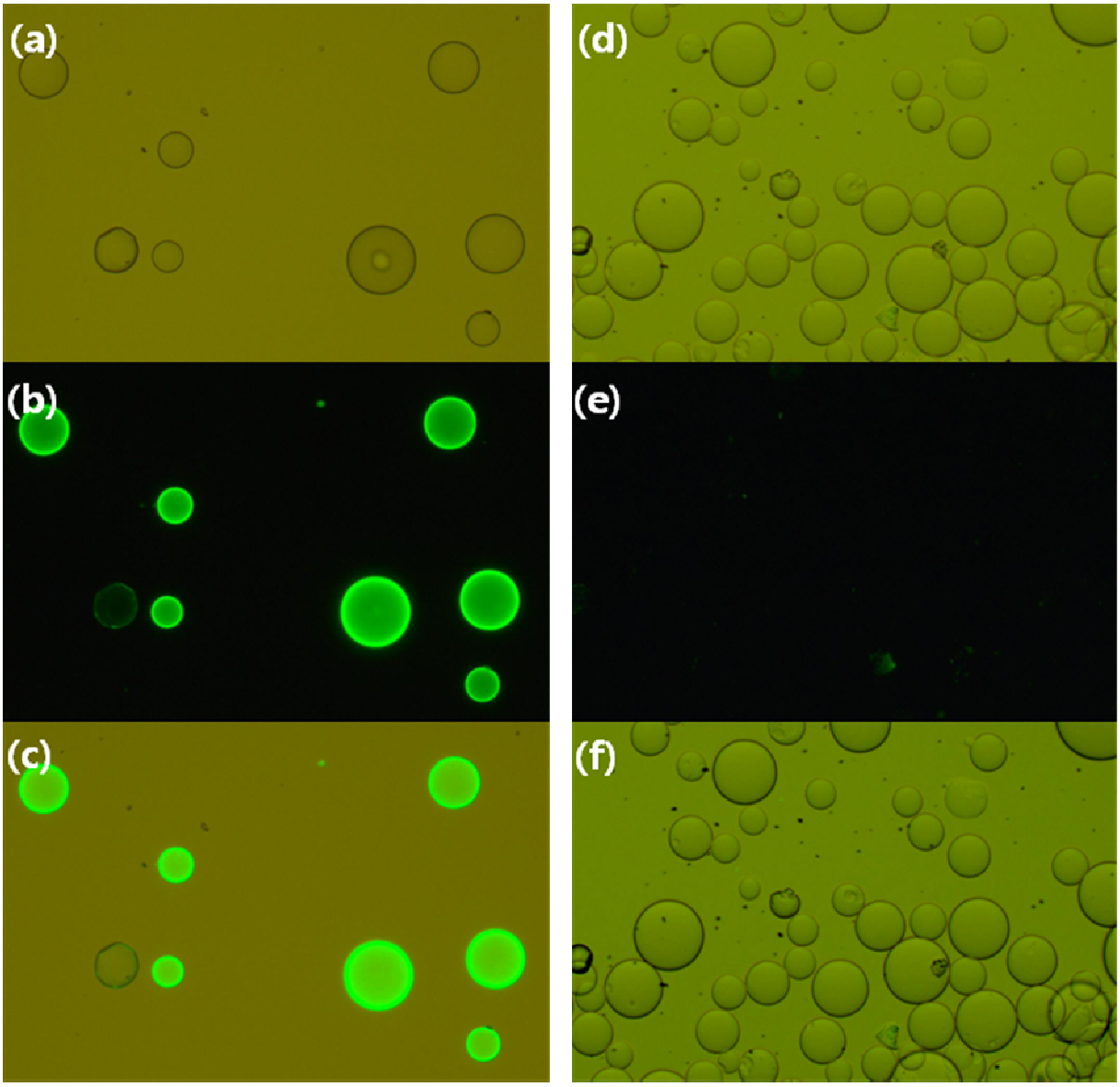


Figure S5. Microscope images of Ni-NTA modified QDs incubated with G2H6-modified AB in (a) bright field, (b) fluorescence, and (c) merged. Ni-NTA modified QDs incubated with G5HG2-modified AB (control) in (d) bright field, (e) fluorescence, and (f) merged.

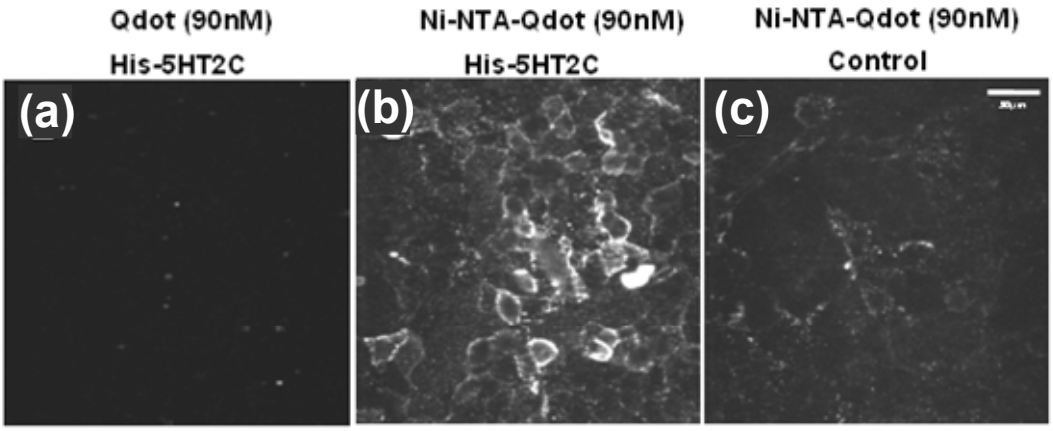


Figure S6. His-5HT2C transfected HEK-293 cells incubated with starting QDs (a) and Ni-NTA QDs (b) and untransfected HEK-293 cells incubated with Ni-NTA QDs (c).

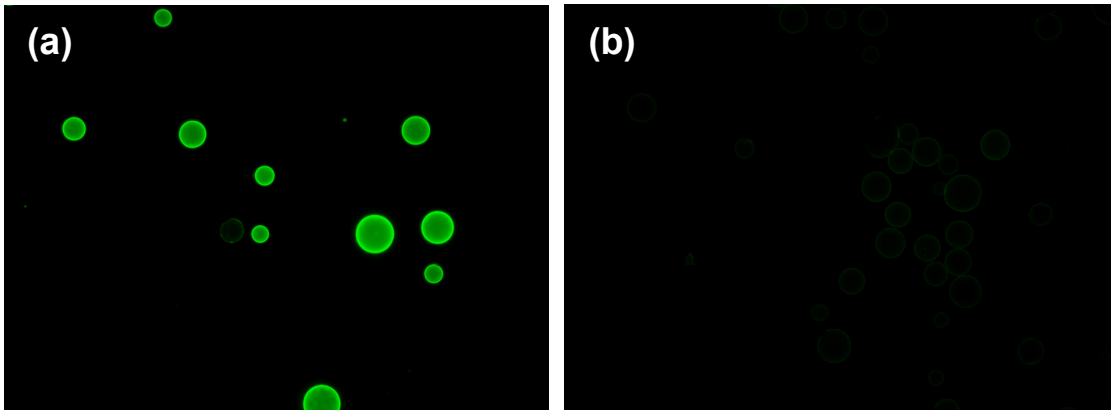


Figure S7. Fluorescence microscope images of Ni-NTA modified QDs incubated with G2H6-modified AB (a) as prepared and (b) after washing x 3 with 1M imidazole solution in PBS.

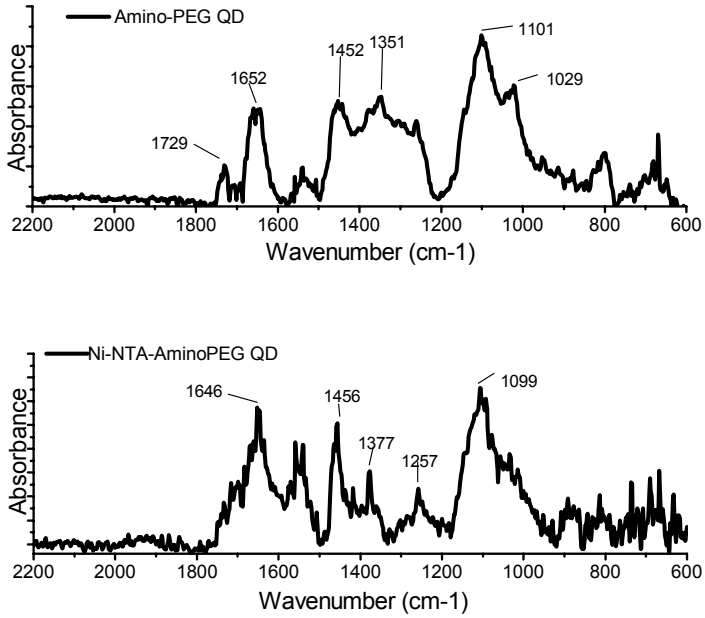


Figure S8. Transmission FT-IR spectra of amino-PEG QD (top) and Ni-NTA-aminoPEG QD (bottom)