Supporting Information

Enhanced Uptake of Luminescent Quantum Dots by Live Cells Mediated by a Membrane-Active Peptide

Anshika Kapur,[a] Scott H. Medina,[b,c] Wentao Wang,[a,d] Goutam Palui,[a,e] Xin Ji,[a,f] Joel P. Schneider,[b] and Hedi Mattoussi[a,*]

[a] Florida State University, Department of Chemistry and Biochemistry, 95 Chieftan Way, Tallahassee, FL 32306

[b] Center for Cancer Research, National Cancer Institute, Building 376, Room 104, Frederick, MD 21702-1201

[c] *Present address:* Department of Biomedical Engineering, Pennsylvania State University, University Park, PA 16802

[d] *Present address:* DNA Electronics, Inc, 1891 Rutherford Rd Ste 100, Carlsbad, CA 92008

[e] *Present address:* The Food and Drug Administration, National Center for Toxicological Research, 3900 NCTR Road, Jefferson, AR 7207

[f] *Present address:* Ocean Nanotech, LLC, 7964 Arjons Drive, San Diego, CA 92126

* Email address: mattoussi@chem.fsu.edu

Materials

Poly(ethylene glycol) methyl ether (HO-PEG-OCH_3, average Mw: ~750 Da), poly(ethylene glycol) (HO-PEG-OH, average Mw: ~600 Da), (±)-α-lipoic acid (LA, >98.0%), tetramethylammonium hydroxide (TMAH), tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl, >98%), and triphenyl phosphine (TPP) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from Sigma Aldrich (St Louis, MO). Thioanisole, anisole, 1,8- diazabicyclo[5.4.0]undec-7-ene (DBU), 200 mM glutamine solution, Dulbecco's Modified Eagle's Medium (DMEM), formaldehyde and most of the organic solvents (e.g., $CHCl_3$, CH_3OH, C_2H_5OH, C_6H_{14} and DMF) were also purchased from Sigma Aldrich. 1,2-ethanedithiol and *N*,*N*′-Dicyclohexylcarbodiimide (DCC) were purchased from Fluka/Sigma. Texas Red (TXR)-NHS ester, prolong gold antifade mountant with DAPI, Hoechst 33342 trihydrochloride dye, fetal bovine serum (FBS), and trypsin were purchased from ThermoFisher Scientific (Waltham, MA). Fmoc-protected amino acids were purchased from Novabiochem/Millipore (Billerica, MA). PL-Rink resin was purchased from Polymer Laboratories/Agilent (Santa Clara, CA). 5-Chloro-1-[bis(dimethylamino)methylene]-1H-benzotriazolium (HCTU) was purchased from Peptides International (Louisville, KY). Trifluoroacetic acid was obtained from Acros Organics (Belgium, WI). N-methylpyrrolidone (NMP) and diethyl ether were purchased from Fisher Scientific (Pittsburgh, PA). 2-mercaptophenylacetic acid was purchased from Santa Cruz Biotechnology (Dallas, TX).

Solid phase peptide synthesis

All the peptides utilized in this study were prepared with an amidated C-terminus. Synthesis of the cysteine modified SVS-1 peptide (CGGKVKVKVK^{D}PPTKVKVKVK-NH_2) was carried out via Fmoc-based solid-phase peptide chemistry with HCTU activation on PL-Rink resin using an automated ABI 433A peptide synthesizer. The peptide was cleaved from the resin and simultaneously the side-chain was deprotected using a trifluoroacetic acid/thioanisole/1,2-ethanedithiol/anisole (90:5:3:2) cocktail for 2 hours under argon atmosphere. The crude product was precipitated with cold diethyl ether, lyophilized, then purified using reverse-phase HPLC system equipped with a semi-preparative Vydac C18 column. The solvents used for HPLC purification were A (0.1% TFA in water) and B (0.1% TFA in 9:1 acetonitrile/water). A linear gradient of 0−100% solvent B over 100 min was utilized. The solution was then lyophilized to collect the product and the purity was verified by analytical HPLC-MS.

Ligand synthesis

The precursors including H_2N-PEG_{750}-OCH_3 and H_2N-PEG_{600}-N_3 were synthesized in our laboratory following the procedures described in previous publications.[1,2] Two sets of molecular scale ligands were prepared and used in the present study, LA-PEG_{750}-OCH_3 (inert terminated) and LA-PEG_{600}-NH_2 (reactive terminated). They were synthesized following our previously reported protocols.[1,2] For LA-PEG-OCH_3, lipoic acid was coupled to H_2N-PEG_{750}-OCH_3 via DCC **(***N*,*N*′-Dicyclohexylcarbodiimide) coupling. The LA-PEG-NH_2 was synthesized in two steps. First, LA was reacted with H_2N-PEG_{600}-N_3 using DCC to provide LA-PEG_{600}-azide. The terminal azide was then converted to amine in the presence of triphenyl phosphine (TPP).

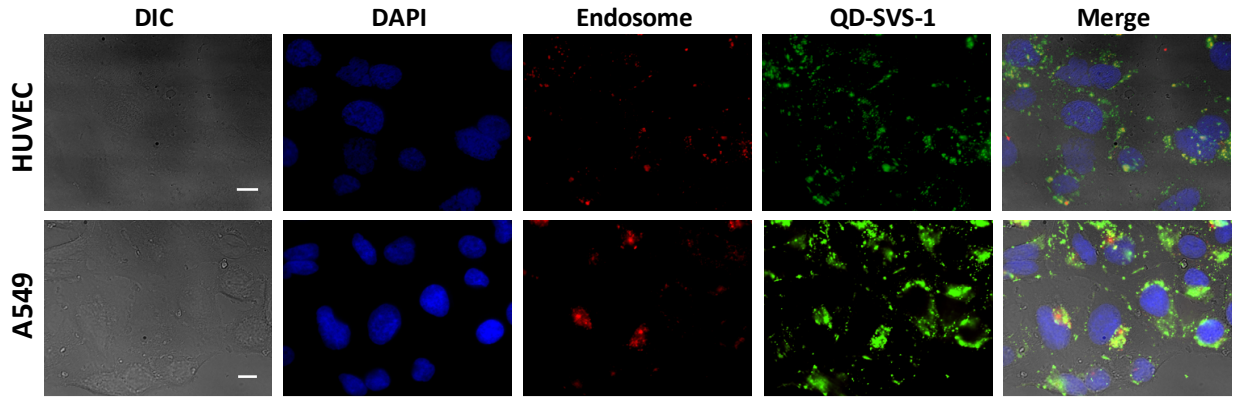

Figure S1. Representative images collected from HUVEC and A549 cells, incubated with 50 nM green QD-SVS-1 conjugates for 1 hour at 37 °C. The cells were co-incubated with Texas-Red labelled transferrin marker, washed, fixed and stained with DAPI to visualize the nuclei. The differential interference contrast (DIC), DAPI, Endosome (TR dye), QDs and the merged images are shown in each row for both the cell lines. Scale bar ~ 10 μm.

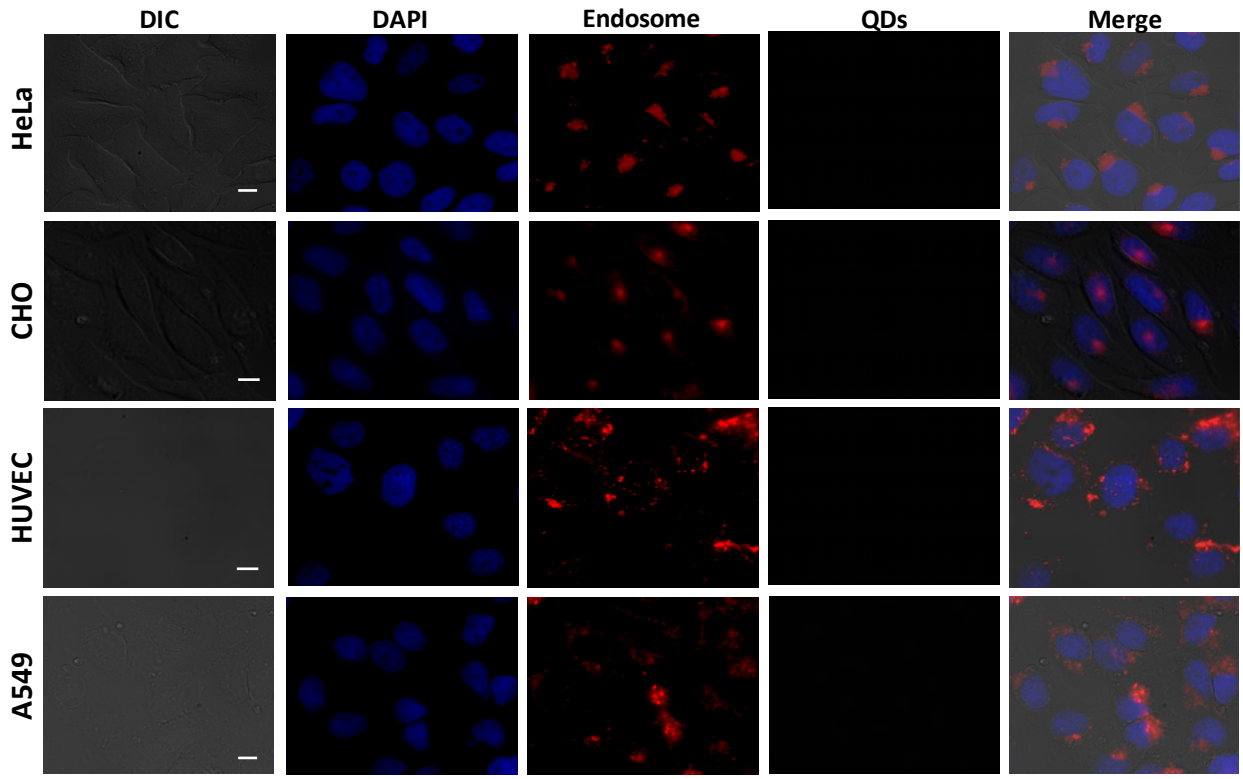

Figure S2. Control experiments carried out using all four different mammalian cell lines, HeLa, CHO, HUVEC and A549 incubated with unconjugated green-emitting QDs (75 nM) for 1 hour at 37 °C. The cells were co-incubated with Texas-Red labelled transferrin marker, washed, fixed and stained with the nuclei stain, DAPI. The differential interference contrast (DIC), DAPI, Endosome (TR dye), QDs and the merged images are shown in each row for all the cell lines. Fluorescence and DIC images collected from all four-cell lines show no QD internalization. Scale bar ~ 10 μm.

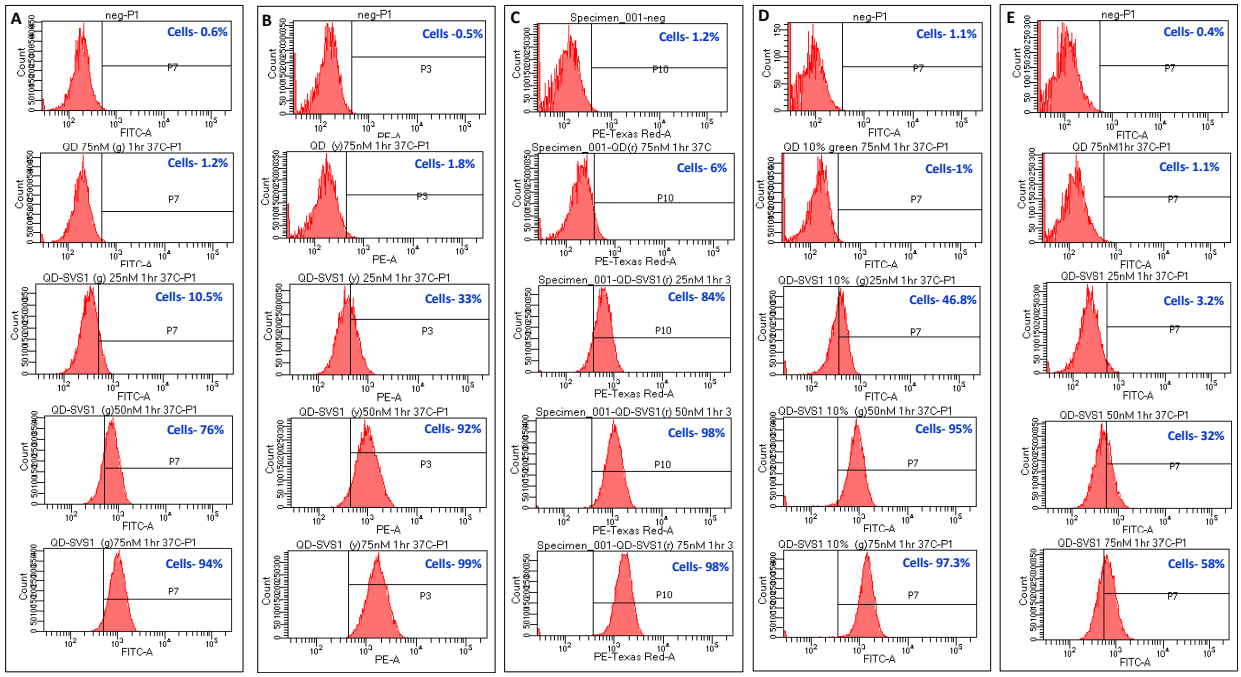

Figure S3. Quantification of the QD-SVS-1 conjugate internalization by cells using flow cytometry measurements. The shown profiles are for: HeLa cells incubated with green- (A), yellow- (B), and red-emitting (C) 5%-QD-SVS-1 conjugates; HeLa cells incubated with green-emitting 10%-QD-SVS-1 conjugates (D); and CHO cells incubated with green-emitting 5%-QD-SVS-1 conjugates (E). The conjugate concentrations are listed in the panels.

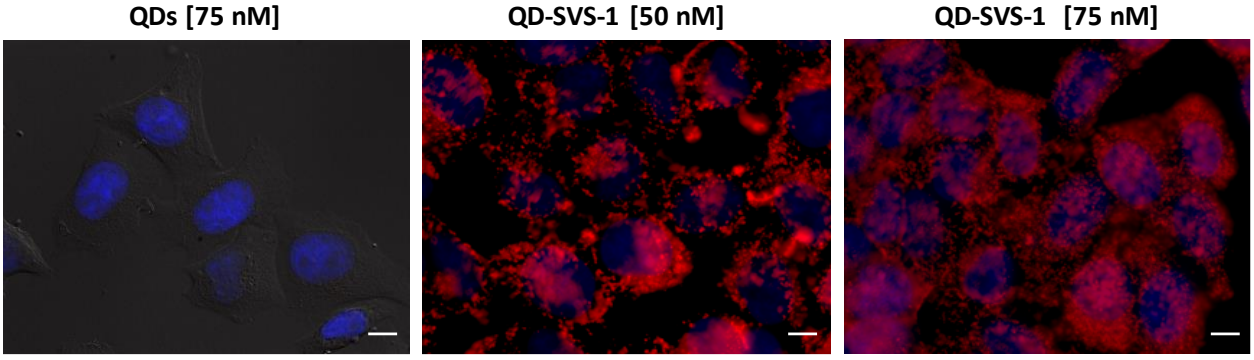

Figure S4. Representative fluorescence images showing the concentration-dependent uptake of red-emitting 5%-QD-SVS-1 conjugates by HeLa cells. The cells were incubated with 50 nM and 75 nM of red-emitting QD-SVS-1 conjugates for 1 hour at 37 °C. Cells incubated with unconjugated QDs at 75 nM show no QD uptake (left, control). Scale bar ~ 10 μm.

Supplementary Movie S1. Video showing live cell images of HeLa cell cultures incubated with red-emitting QD-SVS-1 conjugates. A series of fluorescence frames were collected every 2.5 min, starting immediately after mixing with the conjugates.

References

(1) Mei, B. C.; Susumu, K.; Medintz, I. L.; Mattoussi, H. Polyethylene glycol-based bidentate ligands to enhance quantum dot and gold nanoparticle stability in biological media. *Nature Protocols* **2009**, *4*, 412-423.

(2) Susumu, K.; Mei, B. C.; Mattoussi, H. Multifunctional ligands based on dihydrolipoic acid and polyethylene glycol to promote biocompatibility of quantum dots. *Nature Protocols* **2009**, *4*, 424-436.