Electronic Supplementary Material (ESI) for Chemical Communications

SUPPORTING INFORMATION

Site-specific cellular delivery of quantum dots with chemoselectively-assembled modular peptides

James B. Delehanty, Juan B. Blanco-Canosa, Christopher E. Bradburne, Kimihiro Susumu, Michael H. Stewart, Duane E. Prasuhn, Philip E. Dawson and Igor L. Medintz

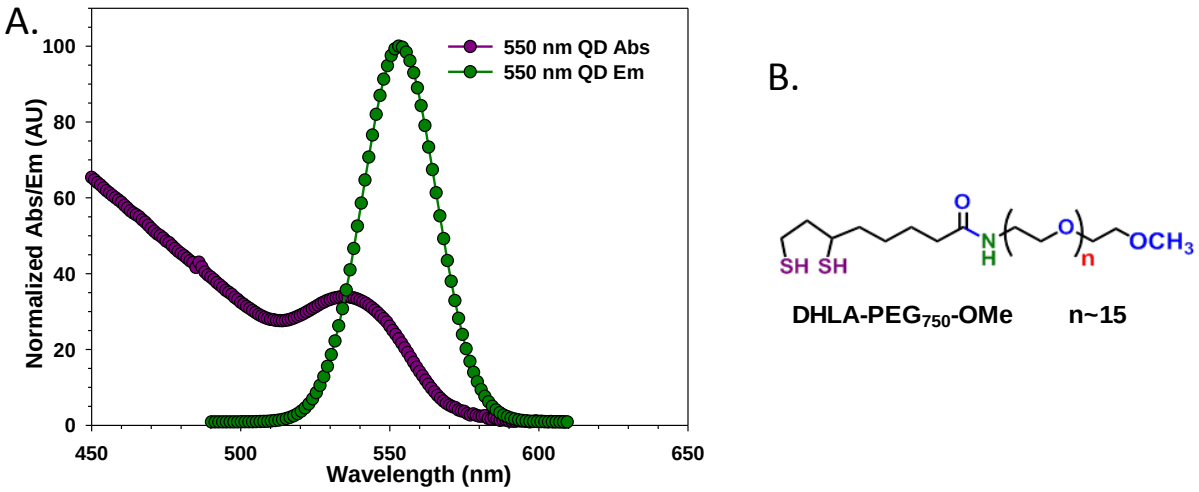

Figure S1. QD spectra and structure of the QD capping ligands. (A) Absorption and emission spectra of the 550 nm emitting QDs. **(B)** Structure of the dihydrolipoic acid appended poly(ethylene glycol) ligand that terminates in a methoxy group (DHLA-PEG_{750}-OMe, PEG MW ~750) which were used for QD surface capping to provide colloidal stability in buffer.

Peptides.

Peptide synthesis: materials and instrumentation. All solvents and chemicals were purchased from commercial sources and used without further purification: DMF (HPLC grade) from OmniSolv, CH_2Cl_2 from Fisher, TFA (trifluoroacetic acid, Biograde) from Halocarbon, CH_3CN from J. T. Baker. Water was purified using a Millipore Milli-Q water purification system. DIEA (*N*,*N*-diisopropylethylamine), piperidine, ß-mercaptoethanol, palmitic acid, were purchased from Sigma-Aldrich. HCTU (*o*-(1-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate), Boc-Dap(Fmoc)-OH (Dap = diaminopropionic), Boc-Dab(Fmoc)-OH (Dab = diaminobutyric) , Boc-Lys(Fmoc)-OH from Peptides International. HATU (*o*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) was from Anaspec. 6-Boc-hydrazinonicotinic acid (6-Boc-HYNIC) and succinimidyl *4*-formylbenzoate (S-4FB) from Solulink Biosciences. Amino acids: Boc-His(Dnp)-OH (Dnp = 2,4-dinitrophenyl), Boc-Gly-OH, Boc-Lys(2-Cl-Z)-OH (2-Cl-Z = 2-chlorobenzyloxy-carbonyl), Boc-Ile-OH, Boc-Val-OH, Boc-Trp(Formyl)-OH were from CS Bio. Boc-Aib-OH (Aib = α-aminoisobutyric), Fmoc-Lys(ivDde)-OH (ivDde = 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl) from Novabiochem.

Peptides were purified by reverse phase preparative HPLC in a Waters Delta Prep 4000 equipped with a Gilson UV detector model 116 and a Phenomenex Proteo column (10 µm, 90 Å, 250 × 21.20 mm) at a flow rate of 15 mL/min employing the following gradient: 0 –> 5% B in 5 min, then 5 –> 70% B in 85 min. Preparative injections were monitored at 220 nm. Peptide masses were obtained by electrospray ionization on a LC/MS API *I* Plus quadrupole mass spectrometer. Masses were calculated from the experimental mass to charge (*m*/*z*) ratios from all of the observed protonation states of a peptide by using the MacSpec software (Sciex). Peptides were desalted with Oligonucleotide Purification Cartridge (OPC, Applied Biosystems) before conjugation to QDs, dried in a DNA120 speed-vacuum (ThermoSavant), and stored as a pellet at -20 °C (Sapsford, Farrell et al. 2009).

General protocol for peptide synthesis. Peptides were synthesized using Boc-solid phase peptide synthesis (Boc-SPPS) on a 0.1 mmol MBHA resin (4-methylbenzhydrylamine, Peptides International, 0.65 mmol/g). Couplings (unless otherwise stated) were performed using 1.0 mmol of amino acid, 1.0 mmol of HCTU (0.4 M solution in DMF) and 1.2 mmol of DIEA. Boc

deprotection was carried out with neat TFA for 90 sec. For peptides containing polyproline sequences (JB829 and JB873), the resin was briefly washed with DIEA/DMF (10%) and another wash with DMF after Boc removal. In the Boc deprotection steps of the other amino acids, the resin was only washed with DMF. Coupling times were typically 1 h. Boc-Pro-OH was coupled twice. Boc-Aib-OH and the next amino acid were double coupled using HATU as activating agent. Dnp and Formyl/Fmoc groups were removed in ß-mercaptoethanol/DIEA/DMF (2:1:7) and Pip/DMF 20% solutions, respectively, for 1 h. Following chain assembly, peptides were cleaved from the resin with 10% of anisole/HF for 1 h at 0 °C. The HF was removed and the crude peptide precipitated in cold ether, filtered off and washed again with ether, being finally collected by dissolving it in 50% CH_3CN/H_2O with 0.1% TFA.

Synthesis of JB26, JB392, JB829, JB873, JB395

JB26:1 mg of peptide ($7.4x10^{-4}$ mmol) was dissolved in DMSO (10 µL) and diluted with borate buffer. Next, a solution of succinimidyl 4-formylbenzoate (1 mg, $4.05x10^{-3}$ mmol) dissolved in DMSO (10 µL) was added and the reaction mixture (final peptide concentration ~1 mM) periodically vortexed at room temperature during 4 h. Peptide **JB26** was purified by HPLC.

JB392 and JB395: following Boc removal of the N-terminal α-amine, the resin was washed with DIEA/DMF (10%) and DMF. 6-Boc-HYNIC-OH (2 equiv) and HATU (2 equiv) were dissolved in DMF (final HYNIC concentration 0.1 M). DIEA (2.2 equiv) was added and the mixture stirred for 1 min 30 sec. The activated HYNIC solution was mixed with the resin and the resulting suspension slightly shaken for 2 h. Following Boc-HYNIC deprotectionwith TFA, the desired peptides could be obtained after HF cleavage and HPLC purification.

JB829 and JB873: following chain assembly, the N-terminal Trp was acetylated using Ac_2O. Then, Formyl and Fmoc groups (Fmoc side chain temporal protecting group of the modified palmitate amino acids Dap and Dab) were removed. Palmitic acid (1.0 mmol, 10 equiv.) and HCTU (1.0 mmol) were dissolved in a CH_2Cl_2/DMF mixture (4 mL, 1:1). DIEA (0.174 mL, 1 mmol) was added and the solution stirred for 1 min 30 sec and added to the resin. The resulting mixture was shaken for 30 min, after what the resin was washed off with DMF. Next, ivDde group was removed from the side chain amine of the C-terminal Lys by treatment with hydrazine in DMF (2%, 2 x 30 min), and 6-Boc-HYNIC-OH was coupled using the conditions reported

above for **JB392** and **JB395**. Boc group was removed and peptides could be obtained following HF cleavage and HPLC purification.

Chemoselective peptide ligation. Conjugates were produced through chemoselective ligation using the following procedure. Stocks of peptides (JB26 - 2 mM / JB392, JB829, JB873, JB395 - 4 mM) were prepared in 10% DMSO/0.1 M ammonium acetate (NH_4OAc), pH 5.5. These were reacted at final concentrations 1 and 2 mM, respectively, with 100 mM aniline catalyst at room temperature overnight in the dark. Resulting ligated conjugates were purified using mini Ni-NTA resin columns, desalted on an Oligonucleotide Purification Cartridge and quantitated using absorption of the conjugated hydrazone bond (ε_{354}=29000 $M^{-1}cm^{-1}$). Ligated peptides were then dried in a speed-vac, and stored as a pellet at -20 °C as described in detail in reference (Sapsford, 2009) until utilized with QDs.

Quantum dot synthesis. CdSe/ZnS core/shell QDs with emission maxima centered at 550 nm were synthesized and made hydrophilic by exchanging the native hydrophobic capping shell with polyethylene glycol-(PEG) appended dihydrolipoic acid (DHLA) ligands that terminated in a methoxyl group as described previously.(Mei, 2008)

Cell Culture. African green monkey kidney (COS-1) cell lines (ATCC, Manassas, VA) were cultured in complete growth medium (Dulbecco's Modified Eagle's Medium (DMEM; purchased from ATCC)) supplemented with 1% (v/v) antibiotic/antimycotic and 10% (v/v) heat inactivated fetal bovine serum (ATCC). Cells were cultured in T25 flasks and incubated at 37ºC under 5% CO_2 atmosphere and a subculture was performed every 3-4 days as described.(Delehanty, 2010)

Cellular delivery of quantum dot-peptide complexes and fluorophores. For delivery experiments, cells were seeded in complete growth medium (typically ~1 x 10^4 cells/well) into the wells of Lab-Tek 8-well chambered #1 borosilicate coverglass (Nalge Nunc, Rochester, NY) and cultured overnight. Prior to the addition of materials for cellular delivery, the cells were washed once with PBS. QD-peptide bioconjugates were formed by diluting the peptide into DMEM-HEPES followed by the addition of a stock solution of QDs to achieve the ratios

indicated. The peptide was self-assembled onto the QD surface for 20 min prior to addition to the cells.(Delehanty, 2010) The final assembled complexes were diluted into DMEM-HEPES then added to the cells. All QD-peptide complexes were incubated on cell monolayers for 1 h. AlexaFluor647-transferrin (100 nM) or MitoTracker™ Red (10 nM) were included for counterstaining (as noted in main text). Following delivery, cells were washed with DMEM-HEPES, fixed with 4% paraformaldehyde/PBS and nuclei were stained with DAPI prior to imaging. Imaging of QDs delivered to mitochondria was performed on live cells as described in the text. The final QD concentrations and peptide/QD ratios used for each QD-peptide assembly were as follows: JB392-JB26 (endosomal delivery): 100 nM QD assembled with 25 peptides/QD; JB829-JB26 (cytosolic delivery): 100 nM QD with ~75 peptides/QD; JB873-JB26 (plasma membrane): 100 nM QD with ~75 peptides/QD; JB395-JB26 (mitochondria): 100 nM QD appended with 25 peptides/QD.

Microscopy methods

Epifluorescence and imaging. Cellular imaging was performed using differential interference contrast (DIC) and epifluorescence microscopy on an Olympus IX-70 inverted microscope equipped with a 60x oil immersion objective. In some cases, a 100x oil immersion objective was used (as noted in text). Samples were excited using a Xe lamp and images were collected using filter sets as described in Table S1. Images were merged and analyzed in Image J (version 1.38).

Table S1. Standard fluorescence microscopy imaging settings used in this study

Imaging configuration	Excitation Filter	Dichroic Filter	Emission Filter
DIC[a]	488 ± 10 nm	525 nm longpass	545 ± 25 nm
DAPI[b]	350 ± 25 nm	400 nm longpass	460 ± 25 nm
550 nm QD	488 ± 10 nm	525 nm longpass	545 ± 25 nm
AF647	620 ± 30 nm	660 nm longpass	700 ± 40 nm

[a]DIC (differential interference contrast) imaging was performed using a white light illumination source, a Wollaston prism and a fluorescein filter cube. Images were false-colored grey for visual clarity. [b]UV excitation utilized a xenon short-arc lamp.

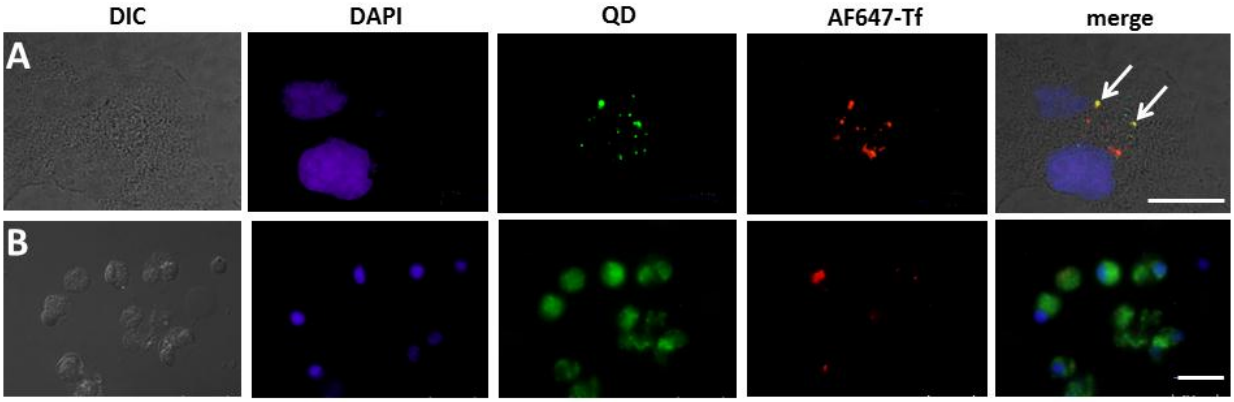

Figure S2. Peptide-mediated QD delivery to endosomes and the cellular cytosol of COS-1 cells. (A) Chemoselectively assembled Arg_{10}-containing CPP (JB392-JB26) delivers QDs to endocytic vesicles. Arrow indicates region of colocalization of CPP-delivered QDs with the AF647-Tf marker within endosomes. (B) Chemoselectively assembled palmitoylated peptide (JB829-JB26) provides for initial endocytosis and subsequent release of QDs to the cytosol after ~48 hrs. Note the diffuse fluorescence of the QD relative to the punctate transferrin endosomal marker. Panels show the corresponding differential interference contrast (DIC), DAPI (~460 nm), QD (~550 nm), AF647-Tf, (~670 nm) and composite merged images. Magnification: (A) 100x, (B) 60x. Scale bar, 50 µm.

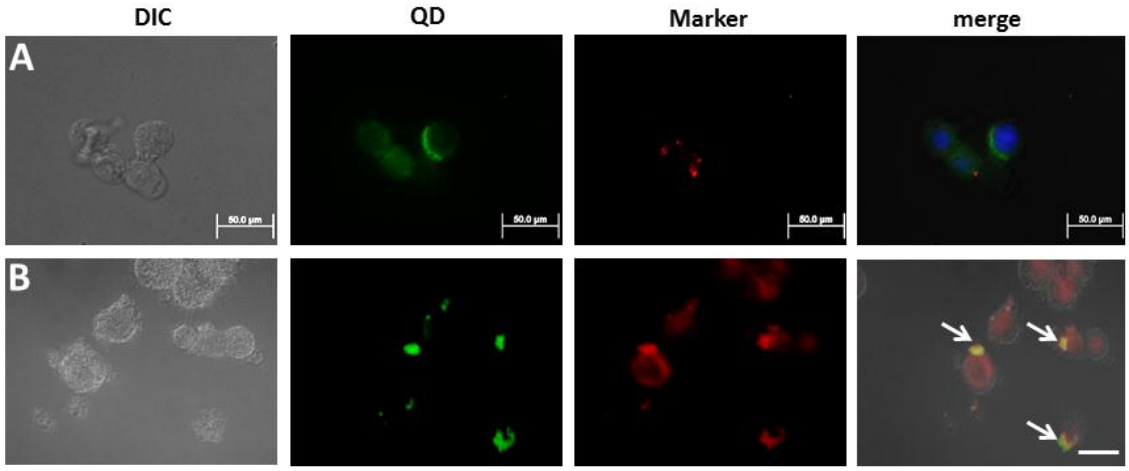

Figure S3. Peptide-mediated QD delivery to mitochondria or plasma membrane. (A) Chemoselectively generated membrane labeling peptide localizes QDs to the plasma membrane in COS-1 cells. Marker corresponds to endosomes labeled with AF647-Tf. Merged panel shows DAPI-stained nuclei merged with QD and transferrin images. (B) A chemoselectively assembled mitochondrial targeting peptide directs QD delivery to the mitochondrial compartment. Marker shows the morphology of mitochondria labeled with Mitotracker Red. Arrows indicate areas of QD-mitochondria colocalization. Imaging was performed on live cells as noted in main text. Scale bar, 50 µm. Panels show the corresponding DIC, QD (~550 nm), MitoTracker Red (~630 nm) or AF647-Tf (~670 nm) marker and composite merged images.

Figure S4. Nonspecific binding control of QD-starter peptide assemblies. QDs decorated with the His_6 starter peptide show no detectable QD uptake or binding to the plasma membrane in the absence of the cognate, functional targeting peptide in these representative cellular micrographs.

References

Sapsford, K.E., Farrell, D. Steven Sun, S., Rasooly, A. Mattoussi, H. and I.L. Medintz. Monitoring of enzymatic proteolysis on a electroluminescent-CCD microchip platform using quantum dot-peptide substrates. *Sensors & Actuators B: Chemical* 139, 13–21 (2009).

Mei, B.C., Susumu, K., Medintz, I.L., Delehanty, J.B., Mountziaris, T.J. and H. Mattoussi. Modular poly(ethylene glycol) ligands for biocompatible semiconductor and gold nanocrystals with extended pH and ionic stability. *Journal of Materials Chemistry* 18, 4949–4958 (2008).

Delehanty, J.B., Bradburne, C.E., Boeneman, K., Susumu, K., Farrell, D. Mei, B.C. Blanco-Canosa, J.B., Dawson, P.E., Mattoussi, H. and Medintz, I.L. Delivering quantum dot-peptide bioconjugates to the cellular cytosol: Escaping from the endolysosomal system. *Integrative Biology* 2, 265-277(2010).