

# Supplementary Information: Modulation of Intracellular Quantum Dot to Fluorescent Protein Förster Resonance Energy Transfer via Customized Ligands and Spatial Control of Donor-Acceptor Assembly

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## 1. Supporting Information Materials and Methods

**Chemicals.** *N,N'*-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) were purchased from Acros Organics (Fisher Scientific, Pittsburgh, PA, USA). Tris(2-carboxyethyl) phosphine hydrochloride (TCEP-HCl) was purchased from Carbocore (The Woodlands, TX, USA). H<sub>2</sub>N-NTA(OMe)(OEt)<sub>2</sub> and TA-PEG<sub>600</sub>-COOH were synthesized as previously reported [1–3]. All the other chemicals including solvents were purchased from Sigma Aldrich (St. Louis, MO, USA) or Acros Organics and used as received.

**Instrumentation.** <sup>1</sup>H NMR spectra were recorded on a Bruker SpectroSpin 400 MHz spectrometer. Chemical shifts for <sup>1</sup>H NMR spectra are reported relative to tetramethylsilane (TMS) signal in deuterated solvent (TMS, δ = 0.00 ppm). All *J* values are reported in hertz. Finnigan LCQ Classic electrospray ionization/ion trap mass spectrometer was used for mass spectral analysis. Each sample was dissolved in methanol, and introduced by direct infusion using a syringe pump. Electronic absorption spectra were recorded using an HP 8453 diode array spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) or Shimadzu UV-1800 UV-vis spectrophotometer. Fluorescence spectra were collected using a Spex Fluorolog-3 spectrophotometer (Jobin Yvon Inc., Edison, NJ, USA) equipped with a red-sensitive R2658 Hamamatsu PMT detector.

**Ligand Synthesis.** DHLA-PEG<sub>600</sub>-NTA(OMe)(OEt)<sub>2</sub> was synthesized as described below. The final form (DHLA-PEG<sub>600</sub>-NTA) was prepared via ester hydrolysis after the QDs were ligand exchanged with DHLA-PEG<sub>600</sub>-NTA(OMe)(OEt)<sub>2</sub>.

**TA-PEG<sub>600</sub>-NTA(OMe)(OEt)<sub>2</sub>.** H<sub>2</sub>N-NTA(OMe)(OEt)<sub>2</sub> (0.203 g, 6.11 × 10<sup>-4</sup> mol), DCC (0.151 g, 7.32 × 10<sup>-4</sup> mol), DMAP (18.3 mg, 1.50 × 10<sup>-4</sup> mol) and CH<sub>2</sub>Cl<sub>2</sub> (10 mL) were added to a 100-mL round-bottom flask equipped with an addition funnel, and the reaction vessel was purged with N<sub>2</sub>. TA-PEG<sub>600</sub>-COOH (0.506 mL, ~5.77 × 10<sup>-4</sup> mol) dissolved in 10 mL of CH<sub>2</sub>Cl<sub>2</sub> was added dropwise at room temperature. After 9 h, H<sub>2</sub>N-NTA(OMe)(OEt)<sub>2</sub> (53.6 mg, 1.61 × 10<sup>-4</sup> mol) and DCC (32.4 mg, 1.57 × 10<sup>-4</sup> mol) dissolved in 5.0 mL of CH<sub>2</sub>Cl<sub>2</sub> was further added dropwise. The reaction mixture was stirred overnight under N<sub>2</sub>. The white precipitate was filtered off and the solvent was evaporated. The residue was chromatographed on silica gel with CHCl<sub>3</sub>:MeOH (10:1). Yield = 0.477 g (~69% based on 0.506 g of TA-PEG<sub>600</sub>-COOH). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 6.65 (br s, 1H, NH), 6.45 (br s, 1H, NH), 6.29 (br s, 1H, NH), 4.14 (q, 4H, *J* = 9.6 Hz, -OCH<sub>2</sub>CH<sub>3</sub>), 3.6–3.75 (m), 3.51–3.59 (m, 5H), 3.37–3.51 (m, 5H), 3.15–3.3 (m, 2H), 3.07–3.22 (m, 2H), 2.52 (s, 4H), 2.41–2.50 (m, 1H), 2.20 (t, 2H, *J* = 9.8 Hz, -CH<sub>2</sub>CO-), 1.85–1.95 (m, 1H), 1.60–1.77 (m, 6H), 1.38–1.58 (m, 6H), 1.26 (t, 6H, *J* = 9.6 Hz, -OCH<sub>2</sub>CH<sub>3</sub>).

**DHLA-PEG<sub>600</sub>-NTA(OMe)(OEt)<sub>2</sub>.** TA-PEG<sub>600</sub>-NTA(OMe)(OEt)<sub>2</sub> (0.247 g, ~2.07 × 10<sup>-4</sup> mol), ethanol (2.5 mL) and deionized water (1.0 mL) were mixed in a 50-mL round-bottom flask. TCEP-HCl (0.100 g, 3.49 × 10<sup>-4</sup> mol) was added to the reaction mixture, which was stirred at room temperature for 3.5 h under N<sub>2</sub>. The reaction mixture was poured into 50 mL of 0.2 M NaHCO<sub>3</sub> solution. The product was extracted with CHCl<sub>3</sub> (4 times). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>. After the inorganic solid was filtered off, the solvent was evaporated to obtain the product as transparent oil. Yield = 0.168 g (~68% based on 0.247 g of TA-PEG<sub>600</sub>-NTA(OMe)(OEt)<sub>2</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 6.65 (br s, 1H, NH), 6.43 (br s, 1H, NH), 6.28 (br s, 1H, NH), 4.1–4.2 (m, 4H, -OCH<sub>2</sub>CH<sub>3</sub>), 3.59–3.76 (m), 3.51–3.59 (m, 4H), 3.38–3.51 (m, 5H), 3.16–3.31 (m, 2H), 2.86–2.99 (m, 1H),

2.60–2.81 (m, 2H), 2.51 (s, 4H), 2.20 (t, 2H,  $J = 9.8$  Hz,  $-CH_2CO-$ ), 1.84–1.97 (m, 1H), 1.4–1.8 (m, 13H), 1.36 (t, 1H,  $J = 10.7$  Hz,  $-SH$ ), 1.31 (d, 1H,  $J = 10.1$  Hz,  $-SH$ ), 1.26 (t, 6H,  $J = 9.5$  Hz,  $-OCH_2CH_3$ ).

**Ligand Exchange onto Quantum Dots.** The hydrophobic QDs were made hydrophilic by exchanging the native ligands with the customized ligands containing dihydrolipoic acid (DHLA) using either a biphasic mixture method for CL4 or a premetallation method for DHLA-PEG<sub>750</sub>-OMe and DHLA-PEG<sub>600</sub>-NTA(OMe)(OEt)<sub>2</sub> as previously reported. The terminal methyl and ethyl ester groups of DHLA-PEG<sub>600</sub>-NTA(OMe)(OEt)<sub>2</sub> were hydrolyzed in 0.1 M NaOH solution after the ligand exchange to expose carboxyl groups.

## 2. Supporting Information Tables

**Table S1.** Fluorescence microscopy imaging filter sets used in this study.

<b>Imaging Filter Set</b>	<b>Excitation Filter</b>	<b>Dichroic Filter</b>	<b>Emission Filter</b>
DIC <sup>a</sup>	Open	400 nm	Open
QD <sup>b</sup>	410 ± 30 nm	510 nm longpass	535 ± 50 nm
MCherry <sup>b</sup>	576 ± 23 nm	595 nm longpass	620 ± 40 nm
QD-mCherry FRET <sup>b</sup>	410 ± 30 nm	595 nm longpass	620 ± 40 nm

<sup>a</sup> DIC (differential interference contrast) imaging was performed using a white light illumination source, a polarizer and a Wollaston prism. Images were false-colored grey for visual clarity; <sup>b</sup> UV excitation was performed using a metal halide short-arc lamp.

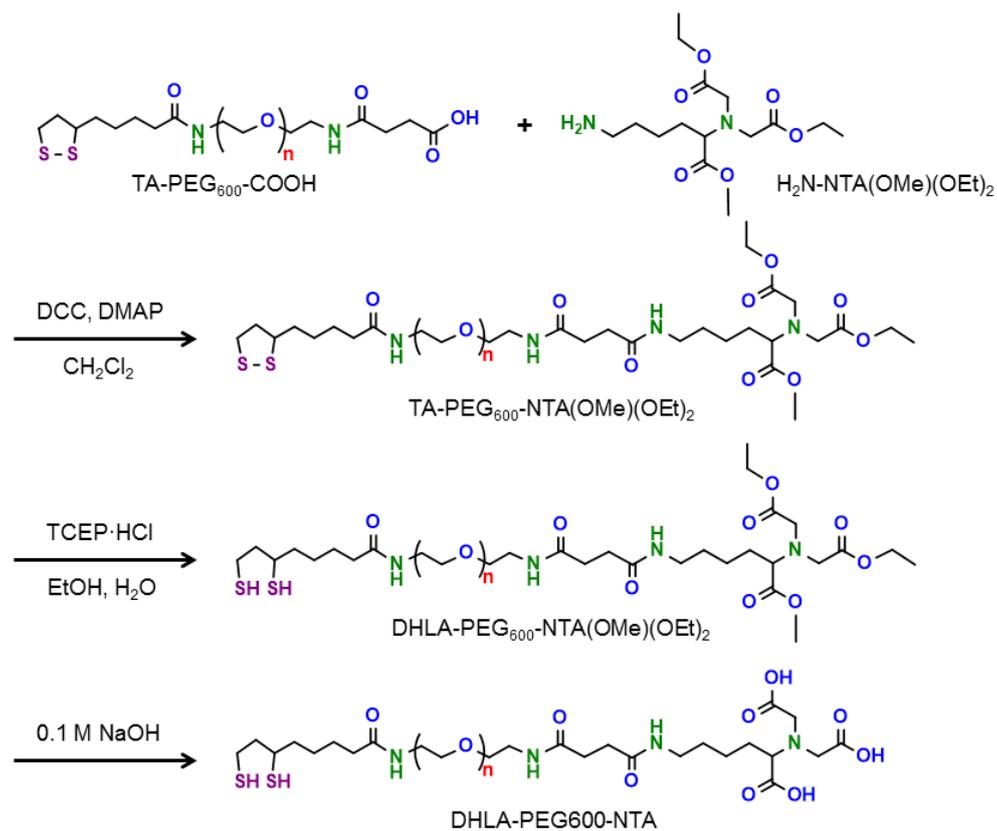
## 3. Supporting Information Figures

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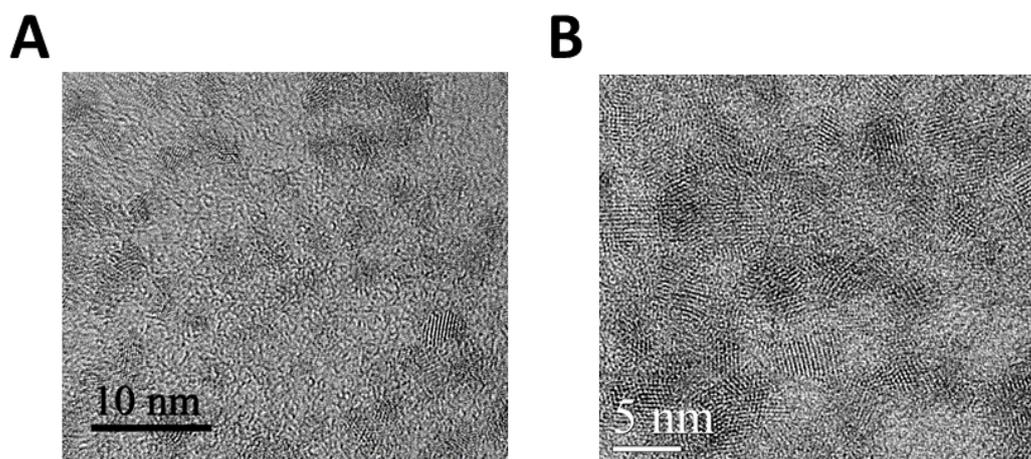
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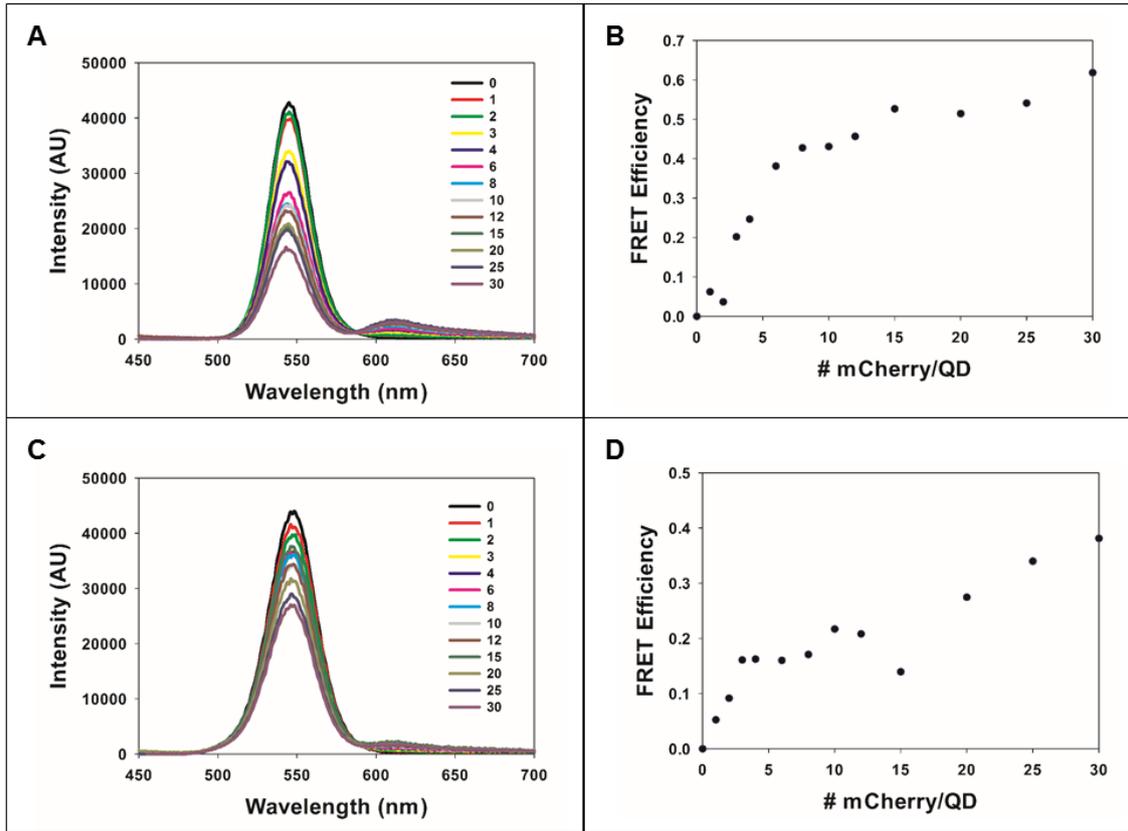
**Figure S1.** Nucleotide and translated amino acid sequence of the CD1b-mCherry construct. Shown are the nucleotide and translated amino acid sequence of the membrane-localized CD1b-mCherry construct used in these studies. The various domains are color-coded as indicated. The *KpnI* and *NotI* restriction sites used to clone the as-synthesized gene sequence into the pcDNA3.1-myc-his(A) vector are underlined. The domains are color-coded as follows: 1-**yellow**: Signal sequence to guide the insertion of the protein into the secretory pathway is (cleaved in the mature protein); 2-**red**: Extracellular domain of CD1b; 3-**magenta**: Transmembrane-spanning domain of CD1b; 4-**blue**: Cytosolic domain of CD1b; 5-**green**: Flexible linker/spacer; 6-**grey**: mCherry. The mature protein contains a C-terminal His<sub>6</sub> domain for assembly to QDs (**bold**).



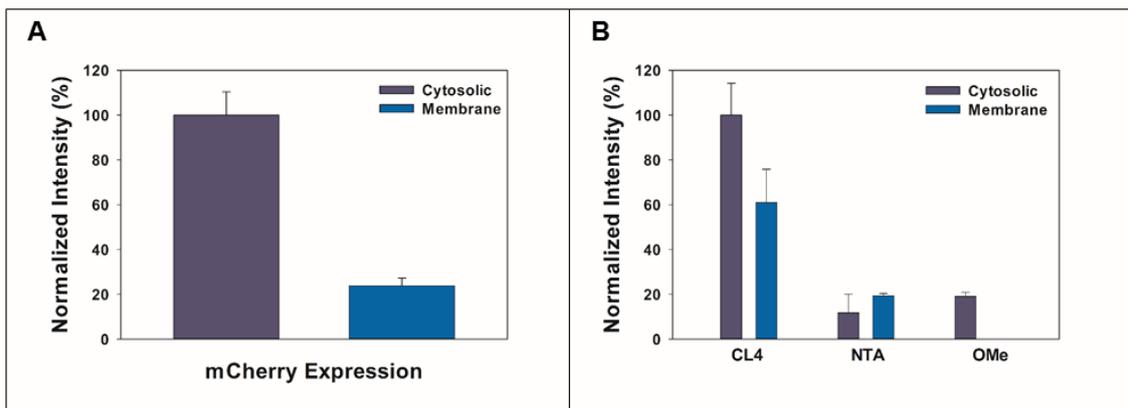
**Figure S2.** Synthetic scheme for ligand DHLA-PEG<sub>600</sub>-NTA.



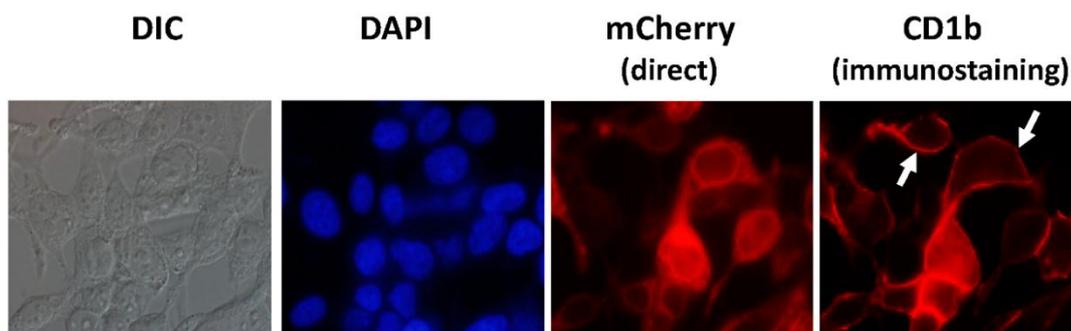
**Figure S3.** Representative transmission electron microscopy analysis of QDs used in this study. (A) QDs capped with CL4 and DHLA-PEG<sub>600</sub>-NTA had average diameter of  $4.6 \pm 0.4$  nm; (B) QDs capped with DHLA-PEG<sub>750</sub>-OMe had average diameter of  $4.7 \pm 0.4$  nm.



**Figure S4.** Förster resonance energy transfer (FRET) analysis for nitrilotriacetic acid (PEG<sub>600</sub>-NTA) and methoxy (PEG<sub>750</sub>-OMe) capped QDs. (A) Emission spectra of PEG<sub>600</sub>-NTA capped 550 nm QDs showing sensitization of QD donor emission with increasing ratio of mCherry acceptor; (B) Plot of QD-mCherry FRET efficiency for PEG<sub>600</sub>-NTA-capped QDs; (C) Emission spectra of PEG<sub>750</sub>-OMe capped 550 nm QDs showing sensitization of QD donor emission with increasing ratio of mCherry acceptor. Data has been corrected for direct excitation of mCherry; (D) Plot of QD-mCherry FRET efficiency for PEG<sub>750</sub>-OMe-capped QDs.



**Figure S5.** Relative quantification of cytosolic *vs.* membrane-localized mCherry expression and ligand-dependent FRET intensity. (A) Normalized fluorescence intensity of mCherry expression level in the cytosol *vs.* localized to the plasma membrane; (B) Normalized comparison of FRET intensities of mCherry expressed in the cytosol *vs.* at the plasma membrane as a function of QD-capping ligand. The FRET intensities for the PEG<sub>600</sub>-NTA and PEG<sub>750</sub>-OMe ligands are normalized to the intensity of CL4. No FRET intensity could be measured for OMe-capped QDs at the plasma membrane. The data are expressed as the average FRET intensity  $\pm$  standard error of the mean.



**Figure S6.** Confirmation/characterization of expression of mCherry as membrane fusion to CD1b. Shown is the expression pattern of mCherry when expressed on the inner (cytofacial) leaflet of the plasma membrane as a C-terminal fusion to the extracellular and transmembrane domains of CD1b in HEK 293T/17 cells. See Figure S1 for nucleotide and amino acid sequence. mCherry fluorescence was visualized via direct excitation using the imaging settings in Table S1. The extracellular domain of CD1b was probed/visualized using an anti-human CD1b antibody conjugated to 660 nm-emitting nanocrystals (eBioscience/Affymetrix) after the cells were fixed with 4% paraformaldehyde. The CD1b staining clearly shows the localization of the CD1b-mCherry fusion to the plasma membrane (arrows).

## References

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3. Susumu, K.; Mei, B.C.; Mattoussi, H. Multifunctional ligands based on dihydrolipoic acid and polyethylene glycol to promote biocompatibility of quantum dots. *Nat. Protoc.* **2009**, *4*, 424–436.