Supporting Information for

High-intensity near-IR fluorescence in semiconducting polymer dots achieved by cascade FRET strategy

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Measurements

^{1}HNMR and ^{13}CNMR spectra were recorded on a Bruker AV500 and AV300 spectrometers. Elemental analysis was performed using flash 2000 series CHN analyzer. MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) analysis was performed on Bruker Autoflex II spectrometer. The molecular weight of polymers was measured by the GPC method (Viscotek TDA305 GPC), and polystyrene was used as the standard (THF as eluent). The particle size was characterized by dynamic light scattering (DLS) (Malvern Zetasizer NanoS). For the TEM measurements, one drop of the Pdot dispersion was placed on a carbon-coated copper grid. After evaporation of the water, the nanoparticles were imaged with a transmission electron microscope (FEI Tecnai F20). UV-Vis absorption spectra were collected on a UV–2450 UV-Vis-NIR Spectrophotometer. Fluorescence measurements were performed with a Fluoromax-4 Spectrofluorometer. Fluorescence quantum yields were measured using a Hamamatsu photonic multichannel analyzer C10027 equipped with CCD integrating sphere. The measurement was calibrated with Coumarin-102. Fluorescence quantum yields were measured using different batches of samples and repeated at least four times and the results were reproducible. Fluorescence lifetime measurements were carried out using a PicoQuant Fluorescence lifetime system (PicoQuant Photonics North America Inc. Westfield, MA USA) equipped with a FluoTime 100(Compact Fluorescence Lifetime spectrameter), a PicoHarp 300 (Time-correlated Single Photon Counting (TCSPC) system), and a PDL800-D (Picosecond pulsed Diode laser: LDH-P-375 laser (laser wavelength: 371nm) for measuring lifetimes of P1 Pdots, 4-NIR Pdots and P1 in 4-NIR Pdots; LDH-P-C470 laser (laser wavelength: 470 nm). A long pass filter RG665 was selected for measuring the lifetime of 4-NIR Pdots and 3-NIR Pdots. A long pass filter UVB390 was selected for measuring the lifetime of P1 Pdots. A long pass filter GG475 was selected for measuring the lifetime of P2 Pdots. A long pass filter UVB390 was selected and a 445/45-nm band pass filter (FF01-445/45-25; Semrock Inc. Rochester, NY USA) was added to measure the lifetime of P1 in 4-NIR Pdots. A long pass filter GG475 was selected and a 535/40-nm band pass filter (NC210224 - D535/40x; Chroma Technology Corp. Bellows Falls, VT USA) was added to measure the lifetime of P2 in 3-NIR Pdots. PicoQuant FluoFit-Global fluorescence Decay Data Analysis software was used and monoexponential function was selected to fit the obtained lifetime. For the measurement of single-particle fluorescence brightness, fluorescent samples were diluted in Milli-Q water, dried on cleaned glass coverslips (previously functionalized with (3-aminopropyl)trimethoxysilane (APTMS)), and imaged on a customized TIRF fluorescence microscope described as follows. The 488-nm laser beam from a sapphire laser (Coherent, Santa Clara, CA USA) was directed into an inverted microscope (Nikon TE2000U, Melville, NY, USA) using home-built steering optics. Laser excitation power was measured at the nosepiece before the objective. The objective used for illumination and light collection was a Nikon CFI Plan Fluor 100XS Oil (with iris) objective with 100 × magnification and 0.5-1.3 N.A (Nikon, Melville, NY, USA). Fluorescence signal was filtered by a 500-nm long pass filter (HQ500LP; Chroma, Rockingham, VT, USA) and a 665-nm long pass (FGL665; Thorlabs-Inc, Newton, New Jersey, USA) and imaged onto an EMCCD camera (Photometrics Cascade: 512B, Tucson, AZ USA). Fluorescence intensity emitted per frame for a given particle was estimated by integrating the CCD signal over the fluorescence spot.

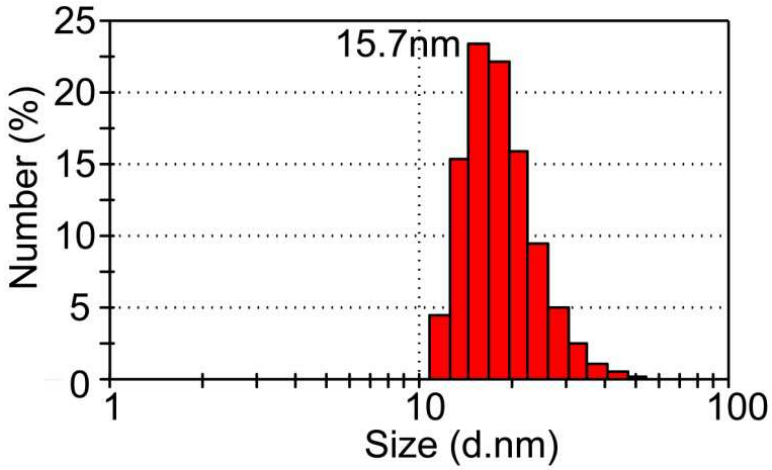

Fig. S1. The size distribution of the commercial product Qdot705-streptavidin (Qdot® 705 ITK™ streptavidin Conjugate Kit) purchased from Life Technologies (Grand Island, NY 14072, USA) as measured by DLS.

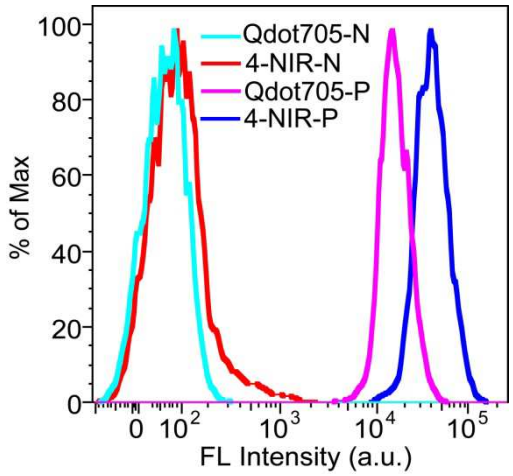

Fig. S2. Flow-cytometry measurements of the intensity distributions of MCF-7 cells labelled with Qdot705-Streptavidin (negative labelling, cyan curve; positive labelling, orange curve) and 4-NIR Pdot-streptavidin (negative labelling, green curve; positive labelling, red curve). All the positive and negative labelling was completed and measured under identical experimental conditions, except in the negative labelling primary biotinylated antibody was absent.