Electronic Supplementary Material (ESI) for Nanoscale.

SUPPLEMENTARY INFORMATION FOR:

Stable, small, specific, low-valency quantum dots for single-molecule imaging

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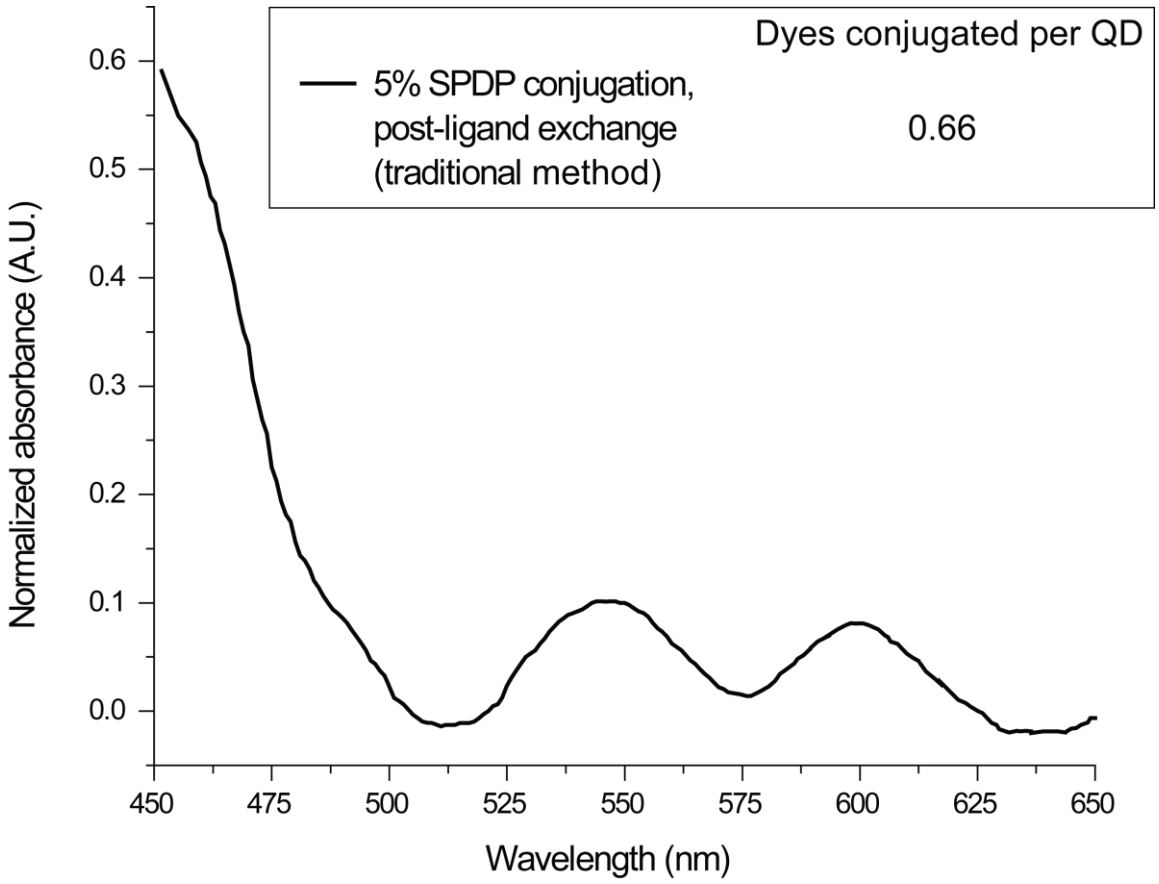
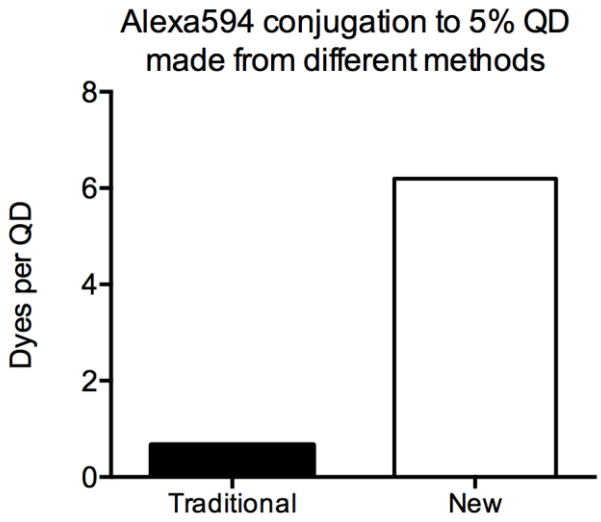
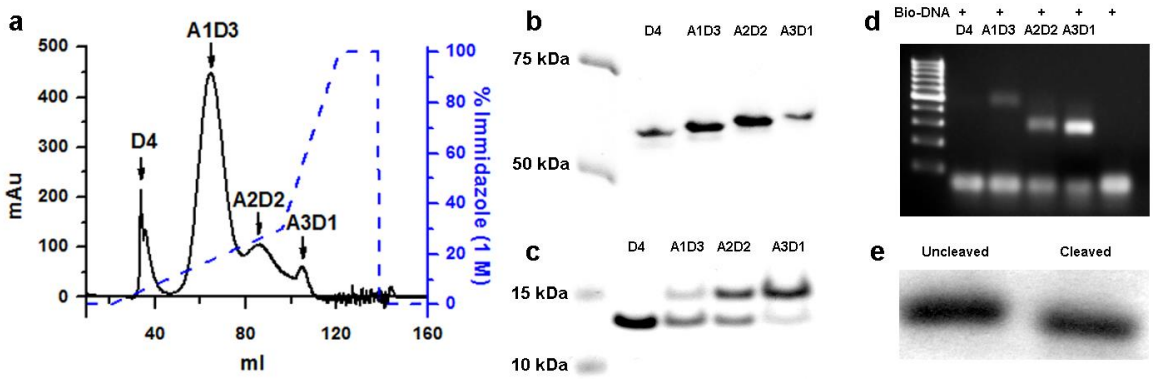
Supplementary Figures



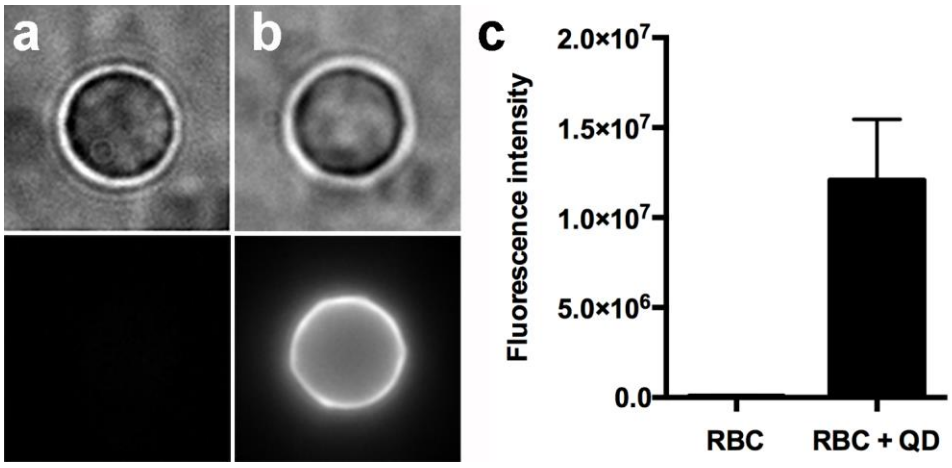
Supplementary Figure 1. Reactivity of 5% SPDP-QDs synthesized with traditional procedure. QDs are water-solubilized by ligand exchange and then conjugated to molecules via amines on the ligand.[1] Because of the low amine reaction yield (the QD population has an average of 0.66 conjugation handles available per QD, meaning most of the QDs cannot be conjugated to anything), the number of amines on the ligand cannot be minimized to 5% for effective conjugation. Dye-per-QD ratio was calculated by reacting with an excess of maleimide-Alexa594 (Alexa594 coefficient at 588 nm = 96,000 $M^{-1}cm^{-1}$, QD absorption coefficient at 350 nm = 1.53×10^6 $M^{-1}cm^{-1}$).



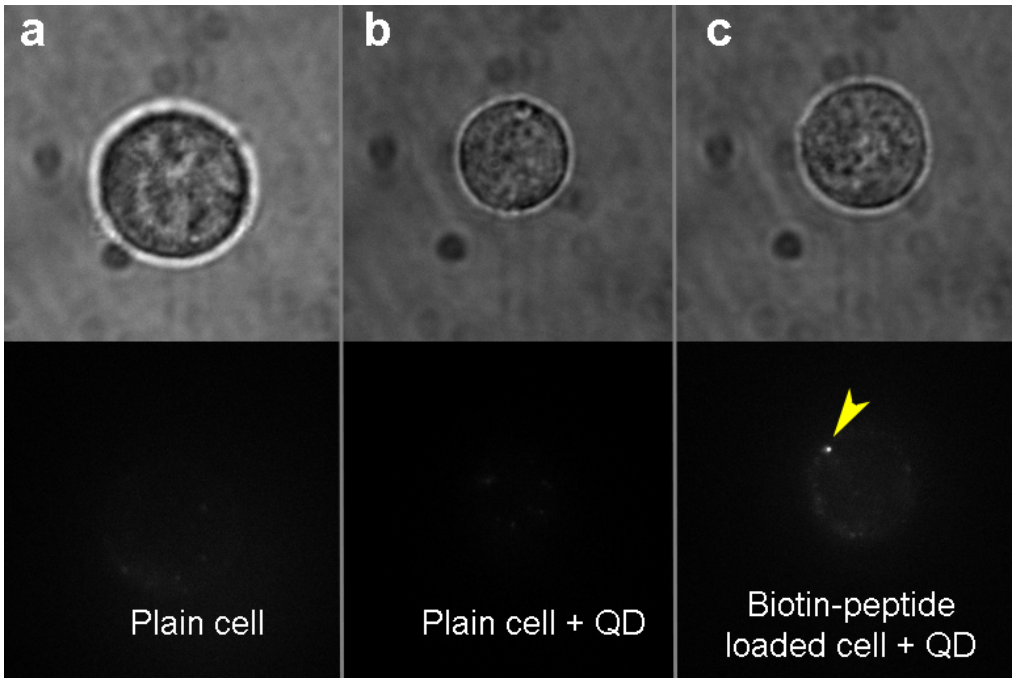
Supplementary Figure 2. Conjugation efficiency comparison between traditional method and new protocol. The traditional method and our new method generate 0.66 and 6.20 reactive groups per 5% SPDP-QD, respectively.



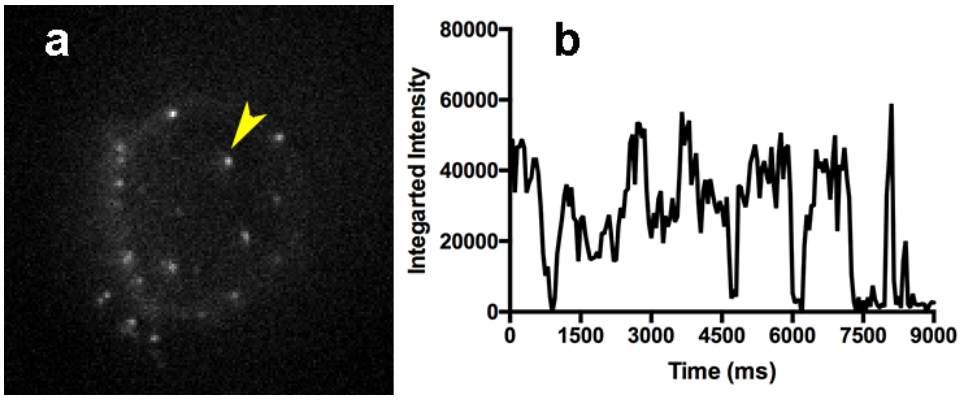
Supplementary Figure 3. Characterization of cleavable monovalent streptavidin (mSA) and divalent streptavidin (dSA). (a) Streptavidin dead subunits (D) and alive subunits with a cleavable 6×His-tag (A) were refolded from denaturant with a ratio of 3:1, generating a mixture of streptavidins with five different binding valencies. Pre-purified streptavidin mixture was further purified using an imidazole gradient FPLC. Four peaks were detected and they were D4, A1D3, A2D2 and A3D1. The imidazole gradient is shown by the dashed blue line and the streptavidin peaks are shown by the black line. (b-c) SDS-PAGE of purified chimeric streptavidins under nondenaturing (b) and denaturing (c) conditions. (d) Streptavidin valency verification using a 58-bp biotin-DNA on a 1% agarose gel. (e) The 6×His-tag of purified mSA (A1D3) was cleaved by the TEV protease and the completion of cleavage was verified by SDS-PAGE.



Supplementary Figure 4. Demonstration of QD labeling specificity by single-cell imaging. (a) The DIC image (top) and fluorescence image (bottom) of a biotinylated RBC. Shown is one representative experiment out of 5 experiments. **(b)** The DIC image (top) and fluorescence image (bottom) of a biotinylated RBC stained by our homemade QDs. Shown is one representative experiment out of 5 experiments. **(c)** The quantitative comparison of fluorescence intensity between unstained and stained biotinylated RBCs. All RBCs in this figure were suspended in a hypotonic solution consisted of 50% PBS and 50% H_2O for imaging.



Supplementary Figure 5. Demonstration of QD labeling specificity by single-molecule imaging. The DIC image (top row) and fluorescence image (bottom row) of a plain CH27 cell (a), a plain CH27 cell stained with our homemade QDs (b), and a CH27 cell loaded with a single biotin-MCC peptide and stained with QDs (c). Single QDs were excited with a 488-nm laser and the emissions passing a single-band emission filter (586/20 nm) were collected after 30 ms of exposure. Shown is one representative experiment out of 6-22 experiments (a-c). The yellow arrow marks the fluorescent single QD (c).



Supplementary Figure 6. Single QD blinking profile at the live cell membrane. **(a)** Imaging single QDs at the live cell membrane. Single QDs (one of which is indicated by an arrow) at the membrane of a CH27 cell were excited with a 488-nm laser and imaged with 50 ms exposure using the stream mode of the EMCCD. **(b)** Representative intensity time trace of the indicated single QD, confirming single QD-labeling.

Supplementary Videos

Movie S1 | Imaging single QDs on coverslip glass surface. Individual QDs were bound onto biotinylated polylysine coated coverslip glass surface. QDs were excited with a 488-nm laser with 10 ms exposure/frame. 30 frames of images were taken using stream-mode and used for demonstration.

Movie S2 | Imaging single pMHC molecules labeled by QDs at the live cell membrane. QD-labeled pMHCs on the live cell membrane were recorded in real time by an EMCCD with 50 ms exposure/frame. The QDs were excited with a 488-nm laser. 180 frames of images were taken using stream-mode and used for demonstration.

Movie S3 | Tracking of a single pMHC molecule labeled by a QD at the live cell membrane in real-time. A single QD-labeled pMHC on the live cell membrane was tracked in real time with 50 ms exposure/frame. The QDs were excited with a 488-nm laser. 71 frames of images were taken using stream-mode and used for demonstration.

Reference

1. W. Liu, A. B. Greytak, J. Lee, C. R. Wong, J. Park, L. F. Marshall, W. Jiang, P. N. Curtin, A. Y. Ting, D. G. Nocera, D. Fukumura, R. K. Jain and M. G. Bawendi, *J Am Chem Soc*, 2010, **132**, 472-483.