Supporting Information

Methods

Surface modification of quantum rods. . CdSe/CdS/ZnS quantum rods were synthesized following the published procedure [1, 2]. A 1ml aliquot of CdSe/CdS/ZnS core/shell rods was precipitated using methanol, followed by addition of 200∫l MPS. The sample was vortexed and then 1ml TMAOH was added. The resulting solution was sonicated at 65ºC for 2h. Afterwards, a dialysis solution of 450∫l methanol and 1400∫l of TMAOH was prepared, and 6ml of it was directly mixed with the former rods solution. The mixture was dialyzed for 1h, inside a Spectra/Por membrane (MWCO 25,000) tube (Spectrum Laboratories Inc). Next, 2∫l MPS, 36∫l H2O, 900∫l PEG-silane was added and the sample was sonicated at 65ºC for 1.5h. The sample was then transferred into a 50ml flask under Ar_2. With vigorous stirring, 0.1ml CTS, 2ml methanol and 0.32g of solid TMAOH was added, followed by immediate heating of the sample to 60ºC for 30min. The sample was kept stirring at room temperature overnight, and concentrated with Microcon YM-100 filters. The concentrated sample was dialyzed in 1L of 10mM PB (pH 7.3) overnight. Afterwards, the sample was filtered through MILLEX®-GV 0.22µm filter unit (Millipore), and stored at 4ºC in a refrigerator.

Quantum rods or quantum dots antibody conjugation. 2mg of Sulfo-SMCC was added into 0.15ml of $F(ab')_2$ fragment (0.2mg) goat anti-mouse IgG (H+L) (Jackson ImmunoResearch), or whole goat anti-mouse IgG (H+L) (0.3mg) (Jackson ImmunoResearch), and reacted on a vortexer foam rack for 1h. The sample was then run through a NAP 5 column to remove unreacted sulfo-SMCC, with 50mM PB (pH 7.3) as

an elution buffer. Subsequently 51∫l of quantum rods (OD 488nm 0.11753) or 20μl quantum dots (OD 488nm 0.30016) was mixed with 260μl of sulfo-SMCC labeled antibody and 49∫l 1M NaCl. Then, 31∫l 10mM PB (pH 7.3) was added into the dot-antibody solution to render the solution volume the same as that of rod-antibody solution. Conjugating reaction solution was left on a vortexer foam rack for 2h at room temperature. Next, conjugates were washed using Microcon YM-100 filter. Afterwards, an aliquot of conjugates were analyzed by 3% agarose gel electrophoresis under a voltage of 10 V/cm (Bio-Rad). The remainder of conjugates was stored at 4ºC in a refrigerator overnight before immunolabeling.

Cell culture. MDA-MB-231 and SK-BR-3 cells were obtained from ATCC. Cells were cultured in the appropriate media as following: MDA-MB-231 cells in Leibovitz's L-15 (ATCC) supplemented with 10% fetal bovine serum (Gibco); SK-BR-3 cells in McCoy's 5A medium (ATCC) plus 10% fetal bovine serum.

Cellular updake of nanocrystals. For Chariot™ mediated quantum rods uptake, the cells were subcultured in 8-well chambered cover glass slides (LabTEK) pre-coated with collagen (Vitrogen) at a density of 10,000 cells per well. 80ng/ml Chariot and 2nM silanized Quantum Rods (in PBS) were incubated at room temperature for 30 min. Cell medium was aspirated. Immediately after washing the cells with warm PBS, 50 μL of Chariot-quantum rods mixture was added to each well, followed by 100 μL serum free medium. The cells were incubated at 37°C in the tissue culture incubator for 1h and then 250 μL of medium supplemented with 16% serum was added to each well. The cells were either imaged right away or left in the incubator for later observation. For Streptolysin O (SLO, sigma) mediated quantum rod uptake, the procedure was modified

according to literature [3]. In general, cells were trypsinized and washed twice with serum free medium. About 4 millions cells were then incubated with 0.5nM quantum rods and 40U/ml SLO at 37°C for 20 min. The transfection was stopped by adding complete growth medium and incubated for another 20 min. Cells were washed in complete growth medium twice and put on cover glass for imaging, or sub-cultured in 8-well chambered cover glass slides for later observation.

Immunofluorescence labeling. Cells were fixed in 4% paraformaldehyde (Ted Pella) in cytoskeleton buffer (CSK: 100 mM KCl, 3 mM $MgCl_2$, 5 mM PIPES, 150 mM sucrose, pH 6.8) at room temperature for 30 min. Cells were rinsed in Superblock (SB, Pierce) (5 min x 3), then incubated in a solution of 1:100 anti-human, 1:100 anti-mouse Fab fragments (Jackson ImmunoResearch), and 10% goat serum (GS, Gibco), in SB for 30 minutes to block non-specific labeling. After rinsing, cells were incubated in mouse anti-Her2 antibody (1:30, zymed) in SB + 10% GS for 1h while rocking at room temperature. They were washed (5 min x 3) with phosphate buffer saline (PBS) and incubated in goat anti-mouse conjugated quantum rods or dots (OD 0.0613 at 488nm with 0.2cm pass length) in PBS for 1h, and then washed again (5 min x 3) in PBS and ready to be observed. Control cells were treated with primary antibody ani-Her2, however for the secondary antibody labeling step, unconjugated quantum rods/dots were added.

Fluorescence microscopy. A Zeiss AxioVert 200M fluorescence inverted microscope with a 103-watt mercury lamp and an AxioCam MRm CCD camera was used. Fluorescence signal was detected using either a Cy3.5 filter set (zeiss, exciter: BP 565/30, emitter: BP 620/60) or a QDot 605 filter set (chroma, exciter: E460SPUV, emitter: D655/20m). For detection of single molecules, 2 μL of 0.6 nM quantum rods, or 2 μL of

5 nM quantum dots were deposited on cover glass, dried using a compressed air blower, and then imaged with the microscope using a 60X 1.4 NA oil immersion lens. For detection of single molecules inside the cells, the MDA-MB-231 cells were first loaded with small amounts of quantum rods with the use of SLO (see above part of cellular uptake of nanocrystals), and imaged with a 60X 1.4 NA oil immersion lens. For other fluorescence microscope experiments, a 40X 1.2 NA water immersion lens were used.

Statistical analysis. A Matlab program was written to analyze single particle fluorescence images. Positions of all particles within the images were determined by averaging all images into a single image. The most intense pixels corresponding to the single particle fluorescence are determined by selecting those pixels above an average threshold intensity greater than that of the background. Also, only those bright pixels within a threshold distance of five pixels are selected. The intensities of each particle are then tracked for each subsequent image. After the same background level and threshold intensity being set for both images of quantum rods and quantum dots, the program automatically output maximum intensities of 1064 rod particles and 91 dot particles. The S/N ratios were calculated as the intensity of a particle minus the average intensity of the background divided by the square root of the average intensity of the background. Histograms and mean values of the distributions were generated using Origin 6.0 Plot Statistical Graphs function.

Figures and Movies

Figure S1. Human breast cancer cell MDA-MB-231 and colon cancer cells SW 480 cultured on top of quantum rod layer left fluorescence free area after 24 hours incubation.

The size of the fluorescence free area is related to the invasiveness of the cancer cell, as being reported previously while cells were cultured on quantum dot layer.

Figure S2. (a) TEM images of 2 rods protruding out into the holes of Holy Carbon grids (LC225-Cu grids from Electron Microscopy Science). The different TEM contrasts differentiate the silanization coating from the original CdSe/CdS/ZnS core of QRs. Shell thickness of roughly 2.5 to 3.0nm are observed. Scale bars are 5nm. (b) Sizes of CdSe/ZnS QDs in chloroform (red) and silanized CdSe/ZnS QDs (blue) in PB buffer measured using Malvern Instruments ZetaSizer NanoZS, which only provides size information of spherical particles. A shell thickness of 1.8nm can be directly calculated. Since unsilanized QDs have a surfactant layer of trioctylphosphine oxide (TOPO) on the surface, which contributes to the measured hydrodynamic diameter of rods in chloroform. Consequently, the real thickness of the silanization coating may be a little bit bigger than 1.8nm. As the QRs and QDs went through the same silanization procedure, the silanization shell thickness of QRs should be similar to that of QDs.

Movie S1. Movie of blinking quantum rods(a) and quantum dots(b). Both movies were made from 15 fluorescent images subsequently taken using a Zeiss Axio Vert 200M fluorescent invert microscope. Each image was integrated for 1s.

Movie S2. Quantum rods, at single molecule level retain their brightness inside living cells. The movie was also made from 15 fluorescent images subsequently taken using a Zeiss Axio Vert 200M fluorescent invert microscope. Each image was integrated for 1s.

References:

1. Manna, L.; Scher, E. C.; Alivisatos, A. P. *Journal Of The American Chemical Society* **2000,** 122, (51), 12700-12706.

2. Manna, L.; Scher, E. C.; Li, L. S.; Alivisatos, A. P. *Journal Of The American Chemical Society* **2002,** 124, (24), 7136-7145.

3. Giles, R. V.; Grzybowski, J.; Spiller, D. G.; Tidd, D. M. *Nucleosides & Nucleotides* **1997,** 16, 1155-1163.

Figure S1

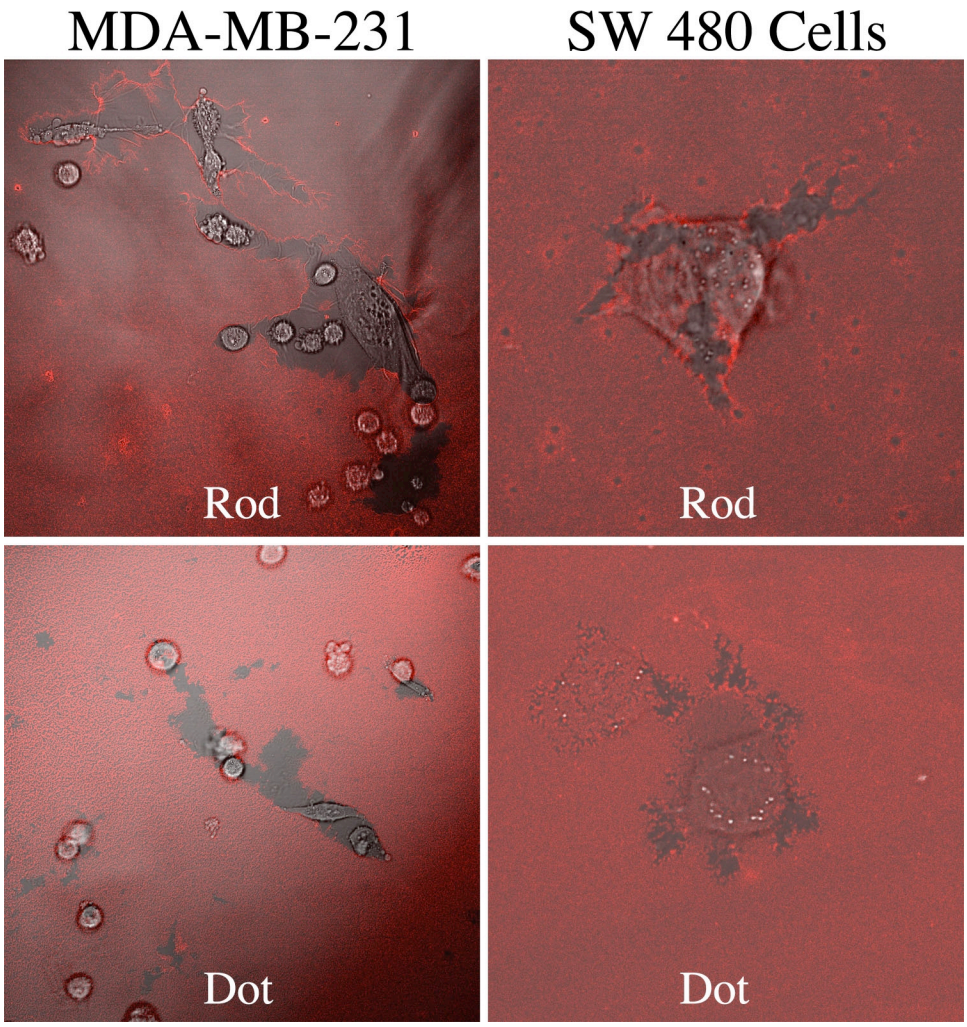
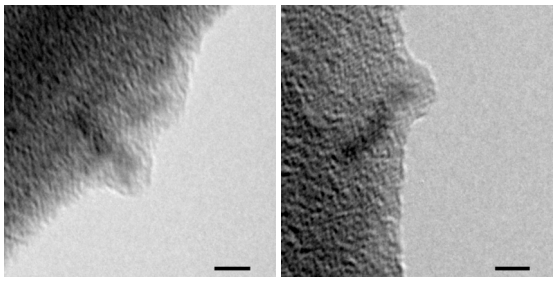


Figure S2

(a)



(b)