Supporting Information

Nanoblade delivery and incorporation of quantum dot conjugates into tubulin networks in live cells

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Materials and methods

Multi-step tubulin QDs conjugation.

- 1) Tubulin polymerization buffer was prepared by mixing 178 μ L PM buffer (100mM PIPES and 10 mM MgCl₂) with 20 μ L anhydrous DMSO and 2 μ L 100 mM GTP (Cytoskeleton Inc).
- 2) QDs were pre-activated by mixing $80~\mu L$ 4 μM QDs (antibody conjugation kit, cat# A10197, Invitrogen) with 2 μL 20 mg/mL fresh Bis[sulfosuccinimidyl] suberate (Pierce) solution. The mixture was incubated for 30 min at room temperature. The size of QDs is around 20 nm in diameter¹.
- 3) During the QDs activation procedure, 1 mg porcine tubulin (Cytoskeleton Inc) was dissolved into 200 μ L fresh polymerization buffer, incubated at 37°C for 10 min. The solution turned to turbid.
- 4) A 0.5 mL zeba spin desalting column (Pierce) was washed by PM buffer 3 times, followed by loading the activated QDs to the column bed and spinning at 1500g for 2 min. The flow through solution was collected.
- 5) The polymerized tubulin solution was mixed with the collected QDs and incubates at 37°C for 1 h.
- 6) The mixture solution was spun at 20817 g for 30 min in a benchtop centrifuge (eppendoff).
- 7) The supernatant was removed and discarded.
- 8) Added 80 μ L ice cold PM buffers to the pellet; re-suspended it by pipette up and down.

9) The solution was remained in the ice bath for 10 min and purified by a superdexTM 200 (GE) filled column (~4.5 x 0.7 cm).

Single-step tubulin QDs conjugation.

QDs were active and purified as above step 2 and 4, then mixed with 5 mg/mL tubulin solution and incubated at room temperature for 2 h. The tubulin-QDs conjugates were purified as above step 9.

Thermal nanoblade.

A Q-switched, frequency doubled Nd: YAG laser (Minilite I, Continum) operated at 532 nm wavelength and 6 ns pulse-width. The laser beam was sent into the fluorescence port of an inverted microscope (Axio Observer, Zeiss) and through the 40 x (0.6 NA) objective, generate a 260 μ m wide laser spot on the sample plane. An electrical switch was built to synchronize the excitation laser pulse with FetomJet injection system (Eppendof).

Microinjection.

The microinjection needles were prepared by pulling (P-97, Sutter Instrument Inc.) a borosilicate glass capillary tubing (Sutter Instrument Inc.). Fresh prepared QDs were centrifuged at 20817 g at 4°C for 5 min to remove the aggregates before loading into the needles. The microinjection was operated by Femotojet injection system (Eppendoff) at room temperature.

Fluorescence imaging.

The cells were incubated at 37°C and 5% CO₂ for 1 hour, and washed by fresh medium 3 times before imaging. The cells were observed with eclipse Ti microscope (Nikon) using a 60 x or 100 x TIRF objective (N.A. 1.47), and a cooled coupled-charge device camera (Andor). Experiments were performed at room temperature

Reference:

 http://www.invitrogen.com/site/us/en/home/References/Molecular-Probes-The-Handbook/Ultrasensitive-Detection-Technology/QDot-Nanocrystal-Technology.html

SI Figures captions

Figure SI-1a: Images of live HeLa cells after photothermal nanoblade delivery of tubulin-QD conjugates prepared with the three-step conjugation strategy (scheme in Figure 1b), scale bar: $16 \mu m$.

Figure SI-2: Images of live HeLa cells after photothermal nanoblade delivery of tubulin-QD conjugates prepared with the single-step conjugation strategy (Figure 1c), scale bar: 16 μm.

Figure SI-3: Images of fixed HeLa cells after photothermal nanoblade delivery of tubulin-QD conjugates (green) and immunocytochemistry labeling of tubulin (alexa 647, red); Tubulin-QD conjugates were prepared based on scheme in Figure 1b; Third column is overlay of QDs and alexa 647 channels, scale bar: 24 μm.

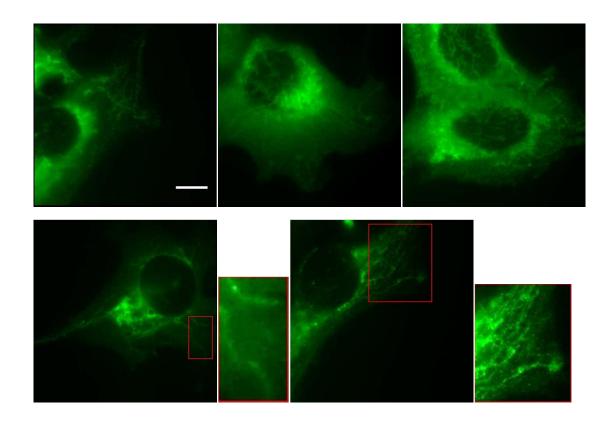


Fig. S1 Xu et al.

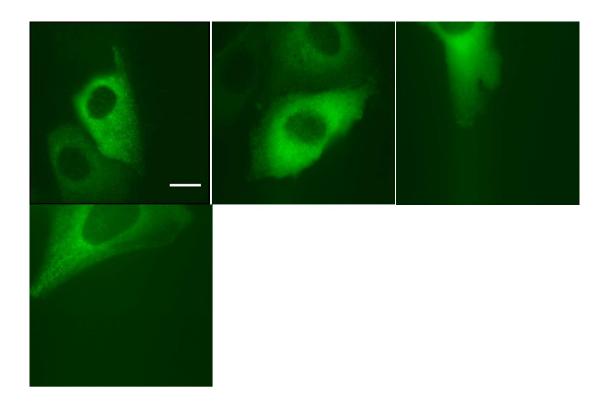


Fig. S2 Xu et al.

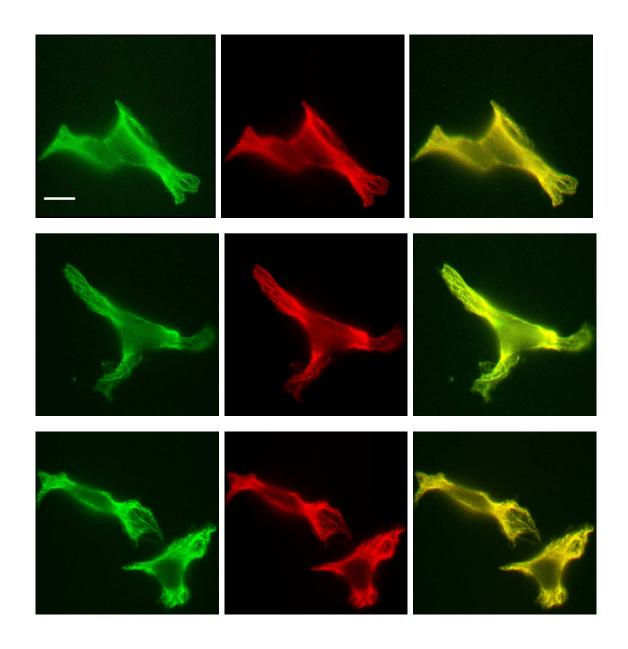


Fig. S3 Xu et al.