Supporting Information

Counting quantum dots aggregates for the detection of biotinylated proteins

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Experimental

Chemical and Material

Biotin-QD (Qd655-biotin ITK) and biotin-XX microscale protein labelling kit were purchased from Invitrogen/Molecular Probes (Eugene, OR). Streptavidin (SA) and biotin were obtained from Sigma Aldrich (St. Louis, MO). AT-III was brought from Dongfeng Biotechnology (Shanghai, China). Microscope coverslips (0.17 mm thick, 22 × 22 mm) and glass slides were purchased from Fisher Scientific (Hanover Park, IL) and Shitai Experiment Equipment (Shitai, China). Other chemical reagents were of analytical grade and purchased from local reagent suppliers.

Preparation of biotinylated AT-III

The procedure of the biotinylation of AT-III was followed with manufacturing. Briefly, AT-III was dissolved in sodium bicarbonate solution (1M, pH 8.3) to obtain a 1 mg/mL solution. BiotinXX, sulfosuccinimidyl ester (SSE) was prepared in 10 µL deionized water to obtain 14.93 nmol/µL reactive biotin stock solution. Then, 1µL Biotin-XX, SSE solution was added to 80 µL of an AT-III solution (1 mg/mL). The biotinylating solution was acquired after reacting at room temperature for 15 min. The biotinylating solution (50 µL) were purified with spin filter columns containing 800 µL Bio-Gel P-6 fine resin at 16000 × g for 1 min to remove excessive biotin.

Formation of QD aggregate

To prepare QD-biotin-SA aggregate with different DOA, 13 aliquots of 1 µL, 50 nM biotinQD655 were reacted with streptavidin (10 µg/mL) at different volumes in 100 µL of borate buffer solution (pH, 8.3) for 20 min incubation at 45°C water bath. The molar ratios of streptavidin to biotin-QD655 are 0:1, 0.2:1, 1:1, 5:1, 10:1, 20:1, 30:1, 40:1, 50:1, 60:1, 80:1, 100:1, 200:1, respectively.

Quantifying biotinylated AT-III

Biotin-QD655 (1 µL, 50 nM) and SA (15 µL, 10 µg/mL) were mixed with AT-III-biotin (100nM) at different volumes (0, 1, 3, 5, 6, 7.5, 9, 10, 12, 15, 18, 25, 40, and 60 µL). The mixtures were added into borate buffer solution (pH 8.3) to finally
achieve the total volume of 100 µL. The reaction was incubated in a water bath at 45 °C for 20 min.

**QD spectral imaging**

An aliquot (4µL to 8 µL) of the QDs aggregate solution was deposited on a glass slide and immediately covered with a coverslip. An inverted fluorescent microscope (Olympus IX71) equipped with a 100× oil immersion objective (numerical aperture of 1.45, UPLSAPO, Olympus, Japan) and an electron-multipled charge-coupled device ( EMCCD; Evolve512, Photometrics, USA ) were used to observe the QD aggregate. A transmission grating with 70 lines/mm (Edmund Scientific, Barrington, NJ) was placed between the EMCCD and a long-pass filter (510 nm to 800 nm, Semrock, Rochester, USA) to obtain the zeroth-order spot and the first order streak of QD aggregate. Image J and Origin software were used to process data.

**Dynamic light scattering (DLS) and transmission electron microscope (TEM)**

DLS measurements were performed at a scattering angle of 90° at 258C with a Zeta sizer ZEN3600 (Malvern Instruments, England). TEM images of QDs were taken by a FEI Tecnai G2 T12 (FEI company, USA).

**Supplementary results**

![Figure S1](image)

Figure S1. Typical TEM images of 0.5 nM Biotin-QD655 mixed with SA at different concentrations. A) 0.5 nM; B) 15.0 nM; C) 25.0 nM.

![Figure S2](image)

Figure S2. Hydrodynamic size of 0.5 nM Biotin-QD655 mixed with SA at different concentrations.