Supporting Information

One-step synthesis of peptide-programmed QDs as ready-to-use nanoprobes

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EXPERIMENTAL SECTION

Chemicals and Materials:

Cadmium chloride hemidihydrate (99.0%), Sodium borohydride (96%), and sodium hydroxide (96%) were all purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China), 1-thioglycerol (99.0%), Tellurium (99.8%, powder), GST protein, streptavidin immobilized agarose beads and glutathione immobilized agarose beads were purchased from Sigma-Aldrich (St. Louis, MO), Dulbecco's Modified Eagle's Medium (DMEM), Phosphate buffer, Trypsin and fetal bovine serum (FBS) were obtained from Thermo-Fisher Biochemical Products (Beijing, China) Co., Ltd. Cell Counting Kit-8 (CCK-8) was purchased from Beyotime institute of biotechnology (Haimen, China). CellLight® Plasma Membrane-CFP (BacMam 2.0) and LysoTracker® Green DND-26 was purchased from Invitrogen (Carlsbad, CA, USA). Hochest 33242 was purchased from fanbobiochemicals (Beijing, China). The peptides used for QDs synthesis were purchased from Apeptide Co., Ltd. (Shanghai, China) with a purity of 97.0%. All reagents and solvents were obtained from commercial suppliers and were used as received. Milli-Q grade (R > 18MΩ cm) water was used throughout.

Synthesis of peptidic QDs:

A typical synthesis of CdTe QDs is described as follows: First, sodium hydrogen telluride (NaHTe) was freshly made before each synthesis by dissolving 0.025 g sodium borohydride ($NaBH_4$) in 1 ml deionized water and then 0.040 g tellurium powder was added into the $NaBH_4$ solution. This reaction was conducted at room temperature overnight in an eppendorf tube. $CdCl_2{\cdot}2.5H_2O$ (14.5mg) and 12μl 1-thioglycerol (1.25g/ml) were dissolved in 50ml ultrapure water and stirred for 10 min. The solution was adjusted to pH 11.0 by dropwise addition of NaOH solution (0.1 M). A solution containing fresh NaHTe (10μl) was added to the above-mentioned solution (1ml) in an Eppendorf tube (1.5 ml), and then a solution containing peptides (1mg/ml) was added. The proportion of peptides to 1-thioglycerol was1:10000. The reaction was conducted at 100 ^{o}C for 1 h and then gradually cooled to room temperature. The obtained QDs were precipitated with ethanol, and the precipitates were separated by centrifugation and were redissolved in PBS buffer solution. For synthesis of CdHgTe QDs,

$CdCl_2 \cdot 2.5H_2O$ was replaced with a mixture of cadmium chloride and Mercuric chloride with different ratio.

Spectral Analysis of QDs:

The absorption spectra of QDs were collected using a PerkinElmer Lambda 25 UV-Vis absorption spectrophotometer over a wavelength range from 400 to 800 nm. The samples were measured against water (or PBS) as the reference. The emission spectra of QDs were collected using an Edinburgh FSP920 fluorescent spectrometer. Under the excitation wavelength of 400 nm, the fluorescence spectra of QDs were recorded.

Transmission Electron Microscopy:

The inorganic size of QDs was determined using a FEI Tecnai G20 TEM operating at 200 kV. One drop of a dilute sample of QDs in water was placed onto a Formvar coated copper grid, allowed to settle for 60 seconds, and wicked away using an absorbent tissue.

Dynamic Light Scattering:

DLS measurements were carried out using a Zetasizer Nano ZS equipped with HeNe laser illumination at 633 nm and a single-photon counting avalanche photodiode for signal detection (Malvern Instruments, Southcorough, MA). The data were analyzed using Dispersion Technology software (DTS) (Malvern Instruments). All QD samples were between 2-3 μM in concentration and filtered through a 0.02 μm filter before analysis. The sample temperature was maintained at 25 ^{o}C. For each sample, the autocorrelation function was the average of three runs of 10 s each. The hydrodynamic diameters were obtained from a number weighted size distribution analysis. For the zeta potential measurement, a phase analysis light scattering module was used.

Quantum yield (QY) of peptidic QDs:

The QY of QD was measured relative to Rhodamine 6G (QY 95% in ethanol) with excitation at 490 nm. Solutions of QDs in PBS and dye in ethanol were optically matched at the excitation wavelength. Fluorescence spectra of QD and dye were taken under identical spectrometer conditions in triplicate and averaged. The optical density was kept below 0.1 at the λ_{max}, and the integrated intensities of the emission spectra, corrected for differences in index of refraction and concentration, were used to calculate the quantum yields using the expression $QY_{QD} = (Absorbance)_{dye}/(Absorbance)_{QD} \times (Peak\ Area)_{QD} /(Peak\ Area)_{Dye} \times (n_{QD\ solvent})^2/(n_{Dye\ solvent})^2 \times QY_{Dye}$.

Evaluation of QD Stabilities:

For storage stability test the QDs samples were dissolved in PBS buffer (pH 7.4), respectively. Afterwards, the two samples were stored at room temperature. The corresponding hydrodynamic diameter and PL intensity of the samples were recorded by Malvern Zetasizer Nano ZS instrument and Edinburgh F900 fluorescent spectrometer at different interval times, respectively.

Measuring the Number of peptides on a QD:

The dye labeled peptide (Peptide 2) was used to evaluate the efficiency of the functionalization of QDs. Coupling to a dye offers the advantage of easily monitoring the coupling yield by

following the prominent dye absorption feature. The number of peptides conjugated onto a QD can be calculated as follows. Figure 2B shows the absorption spectra of the peptidic QDs and the peptide 2, with the dye absorption peak clearly visible. The absorbance of the purified QD-dye conjugates were compared to the absorbance of QD only and dye only solutions of known concentrations, and a ratio of dye per QD was calculated. This ratio provides an estimate for the number of peptides per QD. A fit of the spectrum as a sum of QD and dye contributions revealed a peptide/QD molar ratio of nearly 1.

Specificity tests of peptidic QDs:

The biotin labeled peptide (Peptide 3) was used to evaluate the specificity of peptidic QDs. biotin labeled peptidic QDs was added into PBS buffer containing streptavidin immobilized agarose beads and cultured with dry bath incubator at room temperature and the mixing speed was set at 1000 rpm. After incubation for 1h, the beads were separated by simple centrifugation. The supernatant was discarded and the precipitated microbeads were dispersed in PBS buffer. The suspension was dropped on a slide and imaged with a Leica DMI6000 inverted microscope. The QDs without peptides were also incubated with streptavidin immobilized agarose beads as a control. For further evaluating the specificity of peptidic QDs, the streptavidin immobilized agarose beads were replaced with with GST protein functionalized agarose beads (the glutathione-loaded agarose beads functionalized with GST protein though the specific interaction between GST protein and glutathione).

Cell Cultures:

A549 cells (human lung adenocarcinoma epithelial cell line), H460 cells (human large cell lung cancer cell line), and LO2 cells (human normal liver cell line) were originally obtained from American Type Culture Collection (ATCC). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) medium supplemented with 10% fetal bovine serum (Thermo-Fisher) and 100 UI/mL penicillin and 100 μg/mL streptomycin (Lonza). Cell cultures were incubated in a 5% CO2 humidified incubator at 37°C.

Cytotoxicity Assay:

Cell Counting Kit-8 based Cell Viability Assay was performed to assess the metabolic activity of cells. Cytotoxicity Assay was acquired on a multifunctional microplate reader (BioTek Inc., Winooski, VT). Cells were seeded in 96-well plates (Costar, IL, USA) at an intensity of 6×10^4 cells/mL. After 24 h incubation, the old medium was replaced by the QDs/peptidic QDs solution in medium at different QDs/peptidic QDs concentrations, and the cells were then incubated for 24h. After the designated time intervals, 10 μL of CCK-8 Reagent was added into each well. After 3 h incubation at 37°C, The absorbance of CCK-8 at 450 nm was monitored by the microplate reader. Cell viability was expressed by the ratio of absorbance of the cells incubated with QDs/peptidic QDs solution to that of the cells incubated with culture medium only.

Cellular Uptake and Imaging:

A549 cells were cultured in ATCC-modified Eagle's minimum essential medium (EMEM) with 10% fetal bovine serum (FBS) at 37 °C (5% CO_2) and were grown in 8-well LabTek chambers

(Nalgene Nunc) to 20% confluency. Twenty-four hours after seeding, cells were rinsed with the medium, and QD solution (1 or 5 nM) was added. The cells were washed with fresh culture medium after being incubated with Tat-QDs for 1 h. To label cell lipid membranes, CellLight Plasma Membrane-CFP BacMam 2.0 (Invitrogen) based on an insect virus (baculovirus) for efficient transduction and transient expression in mammalian cells was used. For colocalization studies of QDs, two fluorescent dyes LysoTracker Green (Invitrogen) and Hochest 33242 were used to label lysosomes and nuclei, respectively, by incubation with live cells for 15 min. Fluorescent images were acquired on a Leica TCS SP5 laser scanning confocal microscope equipped with Argon, red HeNe, and green HeNe lasers. Images were collected using a Plan-apochromat 63X/1.4 oil immersion objective by sequential line scanning, with excitation at 405 and 488 nm along with a brightfield image. Emission was collected by photomultiplier tubes in the ranges 423-492, 510-570 and 590-630 nm, respectively, obtained by tunable high-reflectance mirrors. Cells were seeded in 8-well plates (Costar, IL, USA) at an intensity of 1 x 105 cells/mL. After 24 h incubation, the old medium was replaced by the QDs/peptidic QDs solution in medium in the same QDs/peptidic QDs concentrations, and the cells were then incubated for 1h. After incubation for 1 h, the medium was removed and the adherent cells were washed twice with PBS buffer.

Table S1. Sequences of the Peptides Used

	Sequence (from N- to C-terminus)
Peptide 1	Methyl-PEG_4-HHHHHH
Peptide 2	TAMRA-KKKKK- PEG_4-HHHHHH
Peptide 3	Biotin- PEG_4-HHHHHH
Peptide 4	RRRQRRKKRGY-PEG_4-HHHHHH

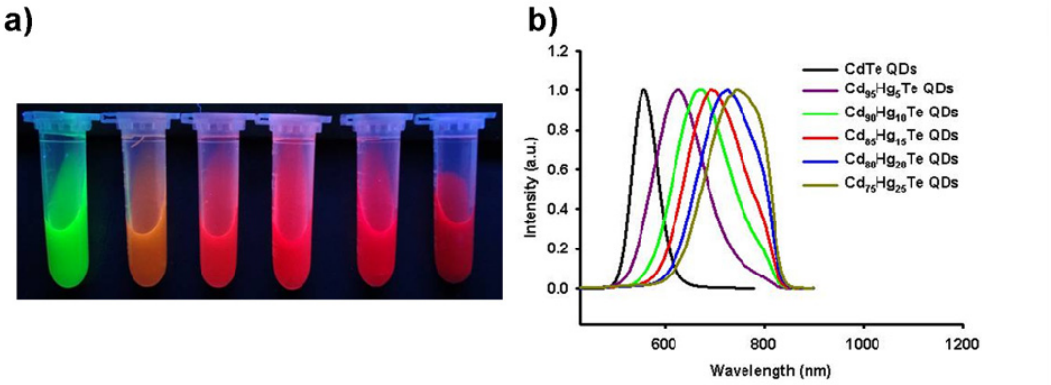

Fig. S1. a) The photos of peptidic CdHgTe QDs under 365 nm UV irradiation and b) normalized PL spectra of the series of CdHgTe QDs with increasing Hg content.

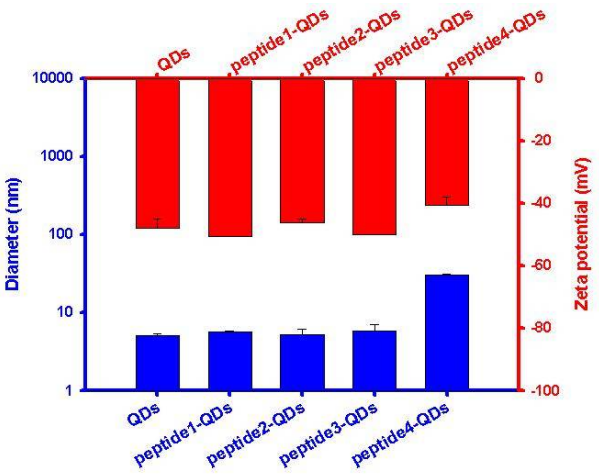

Fig. S2. Hydrodynamic size (blue) and zeta potential (red) measurements of QDs and peptidic QDs. Error bars represent standard deviations from multiple trials.

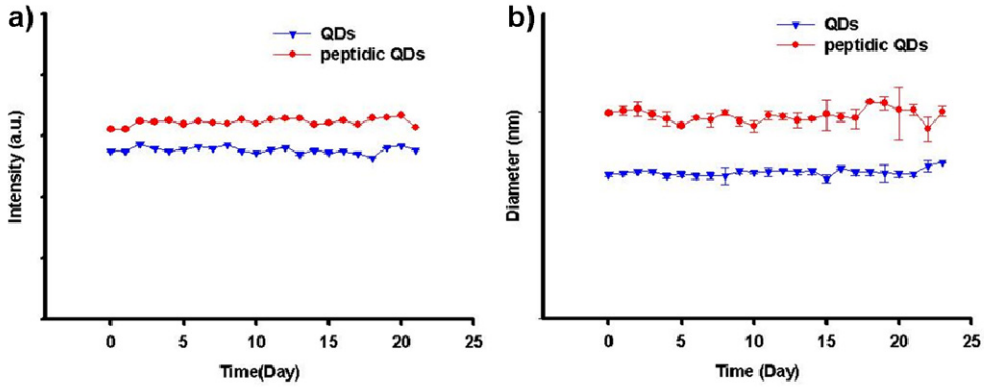

Fig. S3. The stability experiments of peptidic QDs. QDs without peptides were used as control. The concentration of QDs was about 1.7μM. a) The photostability and b) the colloid stability of peptidic QDs. The excitation wavelength was 400 nm. Error bars represent standard deviations from multiple trials.

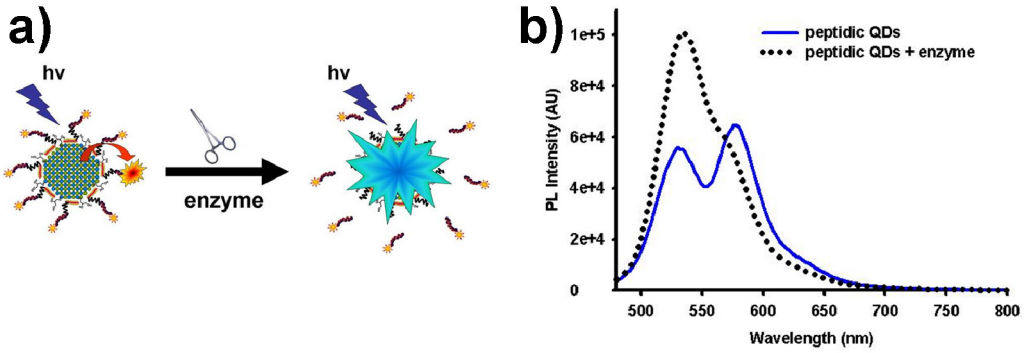

Fig. S4. a) Schematic illustration of the enzymatic cleavage experiments of peptidic QDs. b) The PL spectra of peptidic QDs before (blue, solid line) and after (black, dot line) addition of trypsin.

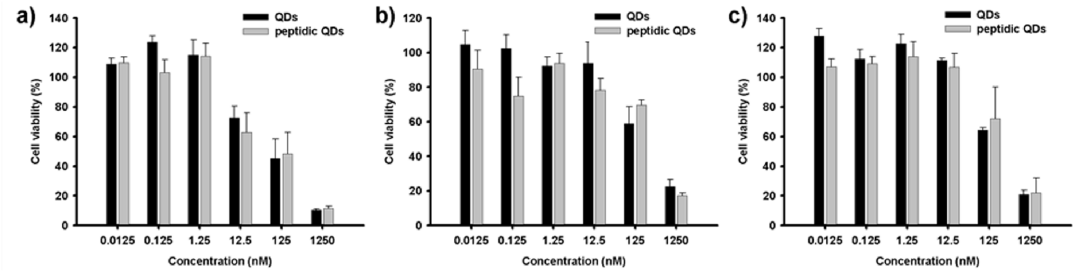

Fig. S5. Cytotoxicity tests of QDs with or without peptides. a) A549, b) H460 and c) LO2 cells were incubated with QDs at different concentrations ranging from 0 to 1250 nM for 24 hours. The cell viabilities were measured with the CCK-8 assay. All conditions were performed in triplicate. Data represent the average of three replicate wells with standard deviation.

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