Electronic Supplementary Information

Electrochemical sensing for caspase 3 activity and inhibition using quantum dot functionalized carbon nanotube labels

Jing-Jing Zhang, Ting-Ting Zheng, Fang-Fang Cheng, and Jun-Jie Zhu*

Key Lab of Analytical Chemistry for Life Science (MOE), School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, 210093, P.R. China

Experimental

Materials. The biotinylated DEVD-peptide (biotin-Gly-Asp-Gly-Asp-Glu-Val-Asp-Gly-Cys) was synthesized and purified by Shanghai GL Biochem, Ltd. (purity 95.98 %, molecular weight 1092.13, Shanghai, China). Multi-walled carbon nanotubes (CNTs, CVD method, purity >95%, diameter 10–20 nm, length 0.5–2.0 μm) were purchased from Nanoport. Co. Ltd. (Shenzhen, China). Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) was purchased from Bio. Basic Inc. (Markham Ontario, Canada). Tris(hydroxyl-methyl)amino-methane (Tris) was bought from Shanghai Chemical Reagent Company (Shanghai, China). Poly(diallyldimethylammonium chloride) (PDDA, 20%, w/w, in water, MW= 200,000–350,000), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 2-(N-morpholino)ethanesulfonic acid (MES), 3-mercaptopropionic acid (MPA), and 6-mercapto-1-hexanol (MCH) were from Sigma-Aldrich (St. Louis, USA). Streptavidin (SA) was obtained from Beijing Biosynthesis Biotechnology Co. Ltd. (Beijing, China). Apoptosis Inducers Kit (C0005), Tissue and Cell lysis solution (P0013), and Caspase 3 Inhibitor (Ac-DEVD-CHO) were purchased from Beyotime

Institute of Biotechnology (Haimen, China). Acute promyelocytic leukemia cells HL-60, etoposide and Caspase 3 cellular activity assay kit were purchased from Nanjing Keygen Biotechnology Co. Ltd. (Nanjing, China). Phosphate buffer saline (PBS, 0.01 M, pH 7.4) contained 136.7 mM NaCl, 2.7 mM KCl, 8.7 mM Na_2HPO_4 and 1.4 mM KH_2PO_4. All other reagents were of analytical grade. All aqueous solutions were prepared using ultra-pure water (Milli-Q, Millipore).

Synthesis of MPA-capped CdTe QDs. The MPA-capped CdTe QDs were prepared as reported previously.[1] Briefly, 0.1142 g of $CdCl_2$•$2.5H_2O$ and 0.0849 g of MPA were successively dissolved in 198 mL of ultra-pure water under N_2 atmosphere, and the pH was adjusted to 8.0-8.5 by the addition of a 5% NaOH solution. Subsequently, 2.0 mL of freshly prepared NaHTe solution produced by the reaction of KBH_4 (0.0480 g, 0.89 mM) with tellurium powder (0.0480 g, 0.375 mM) in a 2.0 mL aqueous solution was mixed with above $CdCl_2$-MPA solution. The reactant mixture was then refluxed under N_2 atmosphere at about 96 ºC for 3h to obtain a red CdTe QDs solution.

Preparation of CNTs-QDs-SA bioconjugates. The CNTs-QDs-SA bioconjugates were prepared via a layer-by-layer (LbL) assembly approach. Briefly, 50.0 mg of CNTs was dispersed in a mixture of sulfuric acid and nitric acid (3:1) and sonicated for 3 h to obtain carboxylic group-functionalized CNTs. After centrifugation from the mixture, the sediment was washed repeatedly with water until the pH reached 7.0. Then, 10.0 mg of oxidized CNTs was dispersed into a 0.5 wt% PDDA salt solution (0.5M NaCl, 10 mL) and the resulting dispersion was sonicated for 60 min to give a homogeneous black suspension. Residual PDDA polymer was removed by high-speed centrifugation (20, 000 rpm, 15 min) at room temperature and the complex was rinsed with water for at least three times. Subsequently, 3.0 mL of CdTe QDs solution was added into a 2 mL dispersion of PDDA functionalized CNTs (2.5 mg mL^{-1}) and the reaction mixture was sonicated for 60 min. Excess particles were removed by subsequent centrifugation and redispersion in water. This procedure

results in homogeneous coating of the CNTs surface with QDs, and the CNTs-CdTe QDs composite solution was obtained. Finally, 1.0 mL of CNTs-QDs (2.0 mg mL^{-1}) was mixed with 3.2 mg of EDC and 0.1 mg of streptavidin in 50 mM pH 5.2 MES buffer, and incubated 2 h at room temperature under shaking and kept overnight at 4 °C. The reaction mixture was washed with PBS and centrifuged at 13 000 rpm for 5 min three times, and the supernatant was discarded. The obtained CNTs-QDs-SA bioconjugates were redispersed in 1.0 mL of pH 7.4 PBS containing 3% BSA and stored at 4 °C.

Cell Cultured and Induction of Apoptosis. HL-60 cells were cultured in a flask in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (100 μg mL^{-1}) and streptomycin (100 μg mL^{-1}) in an incubator (5% CO_2, 37 °C). At the logarithmic growth phase, 4 mL of HL-60 cells (5×10^5 cells mL^{-1}) was treated with Apoptosis Inducers Kit (1 μL/mL) for 0, 2, 4, 6, 12, and 24 h, respectively. Then cells were collected, washed twice with PBS, and lysed in 200 μL of ice-cold Tissue and Cell lysis solution for 30 min. The lysates were centrifuged at 13 000 rpm for 15 min at 4 °C, and the supernatant was used immediately for apoptosis assays.

Construction of the electrochemical sensor. The Au electrode (3 mm diameter) was first pretreated with freshly made piranha solution (98% H_2SO_4 : 30% H_2O_2 = 7 : 3, v/v) for 5 min twice and then rinsed with water (*CAUTION: piranha solution should be handled with great care*). After that, the electrode was scanned in 0.5 M H_2SO_4 between −0.2 and 1.5V at 100 mV s^{-1} until a reproducible cyclic voltammogram (CV) was obtained. Before modification, the biotin-DEVD solution was activated with 1.5 μL of 10 mM TCEP in pH 5.2 acetate buffer for 1 h to prevent terminal cysteine from forming disulphide bonds. Afterwards, 10 μL of 5 mM biotin-DEVD was spread on the pre-cleaned Au electrode surface for 12 h at 4 °C in 100% humidity, followed by immersion in 1

mM MCH for 1 h to remove the nonspecific adsorption. Subsequently, the above electrode was dunked into 100 μL of cell lysates containing active caspase 3 for 1 h and then washed twice with PBS. Finally, 10 μL of diluted CNTs-QDs-SA bioconjugates was dropped onto the electrode surface for 2 h to form the biotin-streptavidin bioaffinity complexes. Before electrochemical measurement, the biosensor was washed with PBS to remove the physical absorption of the bioconjugates.

Electrochemical detection of captured QDs-based bioconjugates. After the binding, the gold substrate was immersed in 200 μL of 0.1 M HNO_3 solution for 2 h to dissolve the captured QDs, and the resulting solution was mixed with 1.8 mL of 0.2 M pH 5.2 HAc-NaAc buffer to perform anodic stripping voltammetric detection with a conventional three-electrode system comprised of platinum wire as auxiliary electrode, saturated calomel electrode (SCE) as reference, and a mercury film modified glass carbon electrode as working electrode. Mercury film was electrodeposited on the surface of glassy carbon electrode by 4 cycles of alternate deposition at -0.1 V for 40 s and scan from -0.9 to -0.2 V in 0.2 M pH 5.2 HAc-NaAc buffer containing 40 μg mL^{-1} Hg^{2+} under N_2 atmosphere. The anodic stripping detection was carried out by electrodepositing cadmium at -1.1 V for 9 min and then stripping from -0.9 V to -0.2 V under N_2 atmosphere using a square-wave voltammetric waveform, with a 4 mV potential step, a 25 Hz frequency, and an amplitude of 25 mV.

Colorimetric analysis of caspase 3 activity. The activity of caspase 3 was determined by caspase 3 cellular activity colorimetric assay kit according to the manufacturer's instructions (Keygen Biotech., Nanjing, China).[2] Treated HL-60 cells (2–3 × 10^6 per sample) were collected, washed twice with PBS, and resuspended in 50 μL lysis buffer (with 0.5 μL DTT). After incubating on ice for 30 min, the supernatants were collected by centrifugation (13,000 rpm for 5 min, 4 °C) and immediately measured for caspase-3 activity. For the caspase-3 activity assay, cell lysate was placed in a 96-well plate containing 2× reaction buffer and caspase-3 substrate. Plate was incubated at 37 °C and in the

dark for 4 h and the enzyme activity was detected at 405 nm by an automated plate reader (model 680, Bio-RAD).

Apparatus. Transmission electron micrographs (TEM) were measured on a JEOLJEM 200CX transmission electron microscope, using an accelerating voltage of 200 kV. The morphologies of the modified surfaces were studied using atomic force microscopy (AFM, Agilent 5500 model, USA) in tapping mode. Electrochemical measurements were performed on a CHI 660c workstation (Shanghai Chenhua Apparatus Corporation, China) with a conventional three-electrode system comprised of a platinum wire as the auxiliary, a saturated calomel electrode as the reference, and the modified Au as the working electrode. Electrochemical impedance spectroscopy (EIS) was performed with an Autolab electrochemical analyzer (Eco Chemie, The Netherlands) in a 10 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) mixture with 1.0 M KCl as the supporting electrolyte, using an alternating current voltage of 5.0 mV, within the frequency range of 0.1 Hz–10 kHz.

Characterization of MPA-capped CdTe QDs.

The formation of MPA-capped CdTe QDs was investigated by UV-vis and PL spectra. The first UV-vis absorption peak occurred at 595 nm (curve A, Fig. S1), while the PL spectrum (λ_{ex} = 380 nm) showed a relatively narrow emission with a maximum intensity at 651 nm (curve B, Fig. S2). According to the literature,[3] the particle size could be calculated according to the following expression:

$$D = (9.8127 \times 10^{-7})\lambda^3 - (1.7147 \times 10^{-3})\lambda^2 + (1.0064)\lambda - (194.84)$$

λ is the first UV-vis absorption peak (595 nm). The particle diameter was estimated to be 3.62 nm.

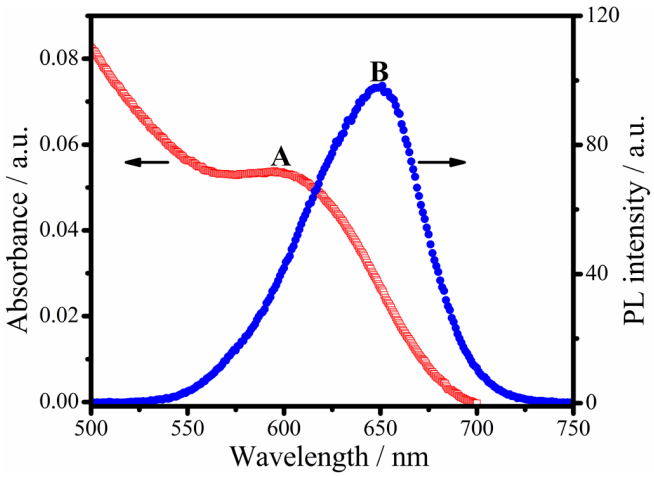

Fig. S1. UV-vis (A) and PL (B) spectra of CdTe QDs in water. Excitation wavelength: 380 nm.

Characterization of the CNTs-QDs composite.

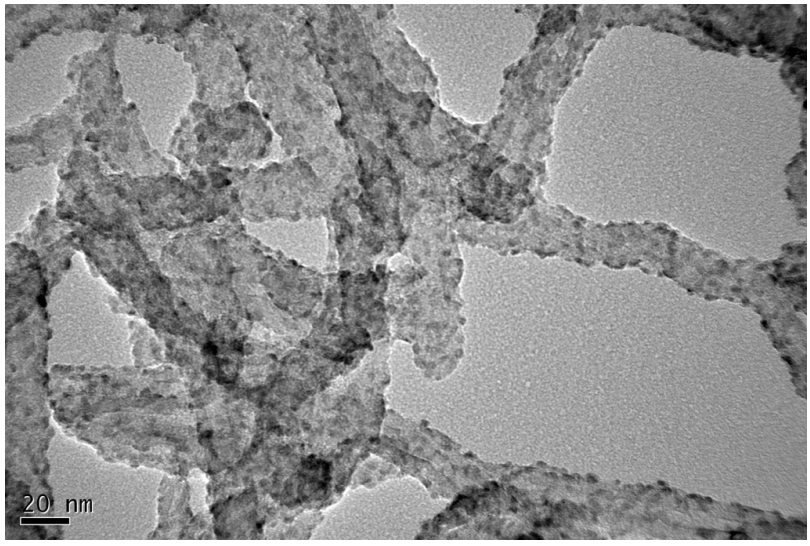

Fig. S2. TEM image of the CNTs-QDs composite.

Time-dependent cleavage of biotin-DEVD by active caspase 3.

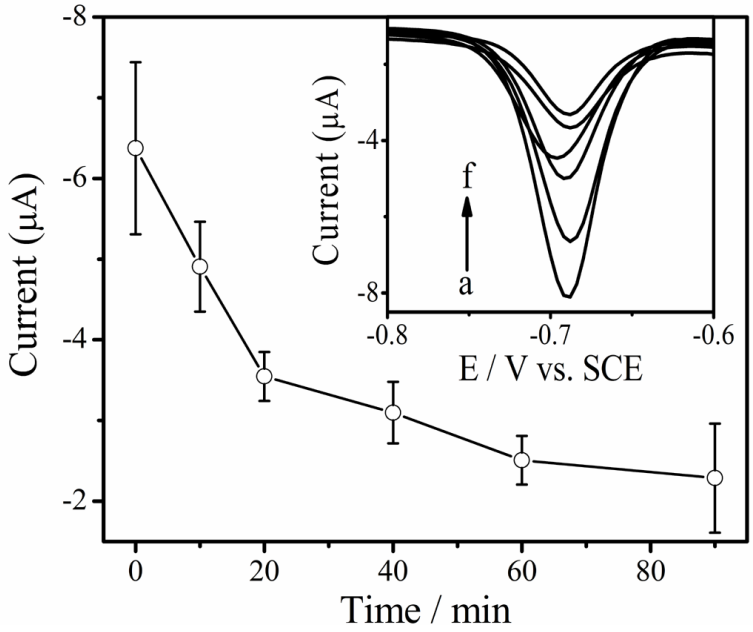

Fig. S3. Relationship between the peak currents and the time that the MCH/biotin-DEVD/Au immersing in the HL-60 apoptotic cell lysates. Inset: Anodic stripping voltammetry measurements for captured CdTe QDs on the MCH/biotin-DEVD/Au electrode after being immersed in apoptotic cell lysates with (a) 0 min, (b) 10 min, (c) 20 min, (d) 40 min, (e) 60 min, and (f) 90 min. Cells were incubated with apoptosis inducer for 12 h. Error bars represent one standard deviation for three independent measurements.

Time course of caspase 3 activation by apoptosis inducer.

Caspase 3 activity was quantified by colorimetric detection of the chromophore *p*-nitroanilide (*p*NA) after cleavage from the peptide substrate Ac-DEVD-*p*NA (K_m = 9.7 μM). Figure S4 shows the change of caspase 3 activity during treatment with apoptosis inducer by comparing the absorbance of *p*NA from apoptotic samples with untreated controls. With the increasing time of treatment, the absorbance increased gradually and trended to a constant value, indicative of the time course of caspase 3 activation by apoptosis inducer.

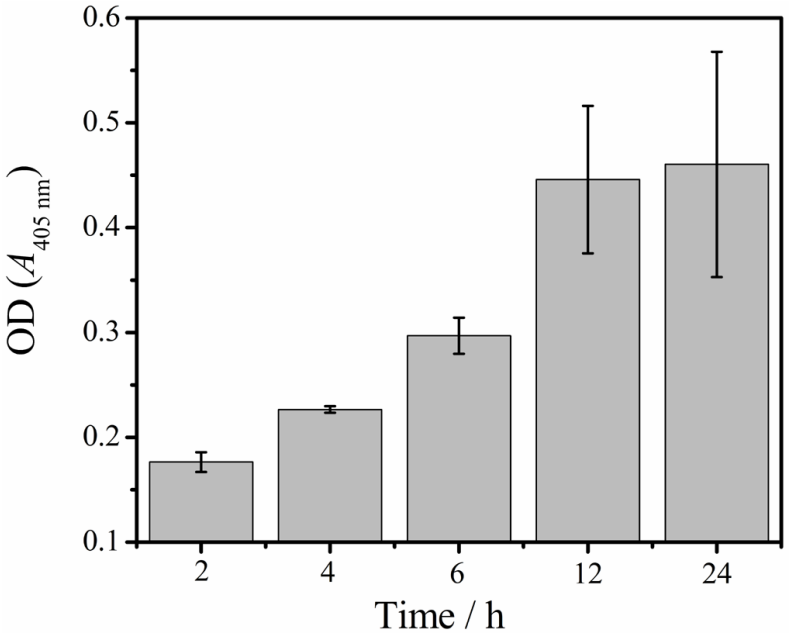

Fig. S4. Time course of caspase 3 activation by apoptosis inducer measured using a caspase-3 cellular activity assay kit plus. Caspase-3 activity was measured according to experimental procedures. Error bars represent one standard deviation for three independent measurements. Other conditions were the same as ***Fig. 3A.***

Colorimetric detection of caspase 3 inhibition by Ac-DEVD-CHO.

In colorimetric analysis of caspase 3 inhibition, HL-60 cells were treated with either apoptosis inducer alone or apoptosis inducer together with Ac-DEVD-CHO (a relatively specific caspase 3 inhibitor). As shown in Fig. S5, in the presence of inhibitor, the absorbance decreased after 12 h and 24 h incubation, indicating that pretreatment with the inhibitor could notably inhibit the activation of caspase 3 during apoptosis.

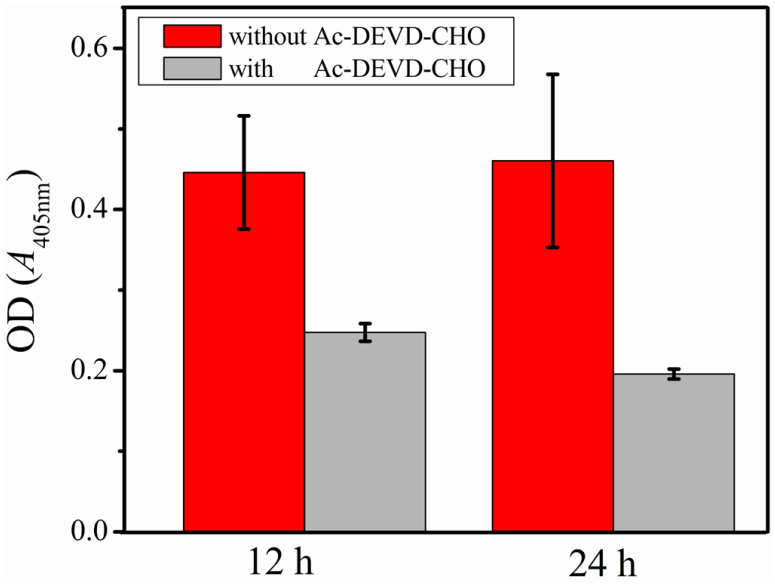

Fig. S5. Colorimetric detection of caspase 3 inhibition by Ac-DEVD-CHO. HL-60 cells were pre-incubated with 5 μM Ac-DEVD-CHO for 30 min, before addition of the apoptosis inducer for 12 h or 24 h. Error bars represent one standard deviation for three independent measurements. Other conditions were the same as ***Fig. 3C.***

References

1 R. J. Cui, H. C. Pan, J. J. Zhu, H. Y. Chen, *Anal. Chem*., 2007, **79**, 8494-8501.

2 X. Q. Kong, H. T. Ge, L. J. Hou, L. M. Shi, Z. L. Liu, *Chem-Biol. Interact.* 2006, **162**, 140-148.

3 W. W. Yu, L. H. Qu, W. Z. Guo, X. G. Peng, *Chem. Mater.,* 2003, **15**, 2854-2860.