Supporting Information

A general carbon dots-based platform for intracellular delivery of proteins

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Experimental Section

Materials. Gallic acid (GA) was purchased from Aladdin Reagent Co., Ltd. L-lysine was purchased from Dalian Meilun Biotechnology Co., Ltd. Horseradish peroxidase and bovine serum albumin (BSA) were purchased from Shanghai Yuanye Biotechnology Co., Ltd. Enhanced green fluorescent protein (EGFP) was acquired following the protocol has been reported[1]. O-dianisidine (98%) and glucose oxidase (GOx) were purchased from Saen Chemical Technology Co., Ltd. Titanium sulfate was purchased from Adamas Reagent Co., Ltd. The BCA protein concentration assay kit was purchased from Beijing Solibao Technology Co., Ltd. Living dead cell staining kit and reactive oxygen ROS analysis kit were purchased from Jiangsu KeyGEN Biotechnology Co., Ltd.

Characterizations and instruments. The morphology of carbon dots (CDs) was measured by transmission electron microscopy (TEM) performed on a JEOL JEM-1011 electron microscope operating at an acceleration voltage of 100 kV. Ultraviolet-visible (UV-vis) absorption spectra were obtained by using a Shimadzu UV-2450 PC UV-vis spectrophotometer. Fluorescence spectra were obtained on a PerkinElmer LS-55 spectrofluorophotometer. Fourier transform infrared (FT-IR) spectra of CDs were recorded on a Bruker Vertex 70 spectrometer from 4000 to 500 cm^{-1}. X-ray diffraction spectrum were obtained on a Rigaku Smartlab X-ray diffractometer. Zeta potential was determined by using a Malvern Zeta-sizer Nano. MTT assays were measured at 490 nm by a microplate reader (BioTek, EXL808). Confocal laser scanning microscopy (CLSM) images were taken using a Zeiss LSM 700 (Zurich,

Switzerland). Calcein-AM/PI staining tests were measured by a laser scanning confocal microscopy (NikonC1si, Japan).

Preparation of CDs. First, dissolve GA (1 mmol) and L-lysine (3 mmol) in 10 mL of water. Second, the solution was transferred to the Teflon-lined autoclave and heated at 180 ^{o}C for 6 h. After cooling to room temperature, the crude product was dialyzed against water in the dialysis bag (Mw:3500 Da). Finally, CDs were freeze-dried to obtain a dark brown solid.

Preparation of CDs-BSA. CDs aqueous solution (2 mg/mL, 1 mL) and BSA aqueous solution (1 mg/mL, 1 mL) were added into a beaker. The reaction mixture was stirred for 0.5 h, and centrifuged in an ultrafiltration centrifuge tube (100 kDa) for 5 min. Finally, the samples were stored in the cold storage (4 °C). The synthesis of CDs-EGFP and CDs-GOx was similar with that of CDs-BSA. The molecular weights intercepted by ultrafiltration centrifuge tubes of CDs-EGFP and CDs-GOx were 30 kDa and 100 kDa.

Preparation of working solution for detecting the activity of GOx. A mixture of glucose solution (0.3 mL, 18%), O-dianisidine solution (2.5 mL, 0.33 mM) and horseradish peroxidase (HRP) solution (0.1 mL, 0.02%) was used to detect the enzyme activity of GOx. The solutions mentioned above were prepared with PBS (pH 6.0, 0.1 M) buffer solution.

Working solution for H_2O_2 detection. Ti(IV)OSO_4 solution (30 mM, 50 μL), acetone solution (500 μL), $NH_3 \cdot H_2O$ (100 μL) and H_2SO_4 solution (1 M, 500 μL).

Evaluation of the intracellular H_2O_2 production. Firstly, HeLa cells were seeded in

a 6-well culture plate, which was pre-filled with sterile coverslips. After 24 h, the medium containing CDs (20 μg/mL), GOx (1 μg/mL) and CDs-GOx (GOx: 1 μg/mL) was then added into 6-well culture plates and incubated for 2 h at 37 °C. Subsequently, the cells were washed three times with PBS, and a reactive oxygen detection reagent (DCFH-DA) was added to each well and incubated for 30 min. Cells did not need to be fixed with paraformaldehyde (4%), they were directly sliced with 50% glycerol, and quickly subjected to CLSM imaging.

SDS-Polyacrylamide Gel Electrophoresis. The successful synthesis of CDs-BSA, CDs-EGFP and CDs-GOx was characterized by SDS-polyacrylamide gel electrophoresis. First, the solutions of BSA/CDs-BSA, EGFP/CDs-EGFP or GOx/CDs-GOx were mixed with SDS, β-mercaptoethanol and bromophenol blue, respectively. Then, we use 10% electrophoresis separation gel (lower layer) and 5% concentrated gel (upper layer) for separation, with a current parameter of 80 mV. After 2 h, the electrophoresis gel was cut and stained with Coomassie brilliant blue R-250, and then decolorization in the decolorizing solution until the bands were clear.

Protein stability against enzymatic digestion. The role of CDs in protecting proteins from enzymatic cleavage was probed by trypsin digestion analysis. Briefly, trypsin aqueous solution (0.25%) was introduced into each free BSA/GOx or CDs-BSA/CDs-GOx samples with the same protein concentration, and the enzyme reaction was performed at 37^{o}C for 2 h. All samples were mixed with loading buffer and heated to 100^{o}C for 10 min, and then all samples were analyzed by SDS-PAGE to detect the amount of remainder proteins.

Cell lines and cell culture. HeLa (human cervical carcinoma) cell line was cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO) with 10% heat-inactivated fetal bovine serum (FBS, GIBCO). Cells were cultured in a humidified incubator at 37 °C with 5% CO_2, and the culture medium was replaced once every day.

In vitro cell uptake. Confocal laser scanning microscopy (CLSM) was used to detect cell uptake. The cells harvested in the logarithmic growth phase were seeded in a 6-well plate at a density of 5×10^4 cells/well and cultured in DMEM for 24 h. Then the medium was replaced with 1 mL of DMEM containing free EGFP or CDs-EGFP at the EGFP concentration of 20 μg/mL, and incubated at 37 °C for different time periods, and then washed 3 times with PBS. The cells were fixed with 4% paraformaldehyde solution for 10 min, and then washed with PBS and observed with a confocal laser scanning microscope.

In Vitro Cytotoxicity Assay. MTT assay was applied to evaluate the cytotoxicity of CDs, GOx and CDs-GOx. Firstly, HeLa cells harvested in the logarithmic growth phase were seeded in a 96-well plate at an initial density of 2×10^4 cells/well, and incubated overnight in 100 μL of DMEM at 37 °C in a 5% CO_2 atmosphere. After removing the medium, CDs, GOx or CDs-GOx (100 μL) dispersion diluted with the cell culture medium to the required concentration was added into the cell wells. After incubation for 24 h, the PBS solution of MTT (5 mg/mL, 20 μL) was added, and incubated for another 4 h at 37 °C. After carefully removing the medium supernatant, 150 μL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formed formazan crystals. Finally, the plate was shaken for 5 min, and the absorbance of the

violet product was measured with a microplate reader at 490 nm.

Live/Dead cells staining experiment. We used Calcein-AM/PI live/dead cell double staining kit to stain and detect cells treated under different conditions. The cells harvested in the logarithmic growth phase were seeded in a 6-well plate at a density of 5×10^4 cells/well and cultured in DMEM at 37 °C for 24 h. We cultivated HeLa cells in a medium to make them adherent. Then remove the original medium and add medium containing different concentrations of CDs, GOx and CDs-GOx. After the HeLa cells were incubated for 24 h, the medium was taken out and incubated with Calcein-AM/PI for 30 min at room temperature. Finally, a fluorescence microscope was used to observe and photograph the live/dead cells.

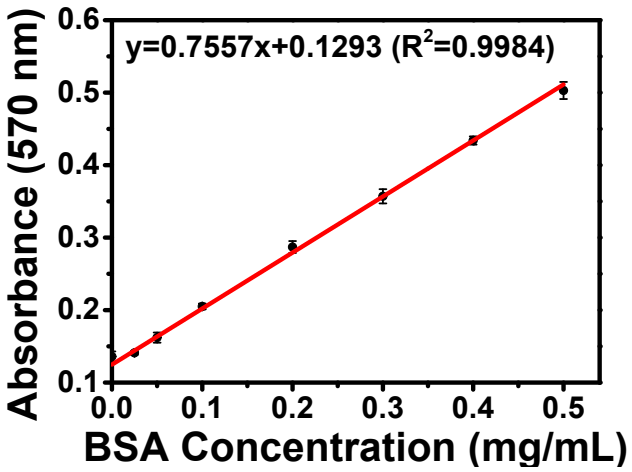

Figure S1. The standard curve of BSA for BCA Protein Assay Kit.

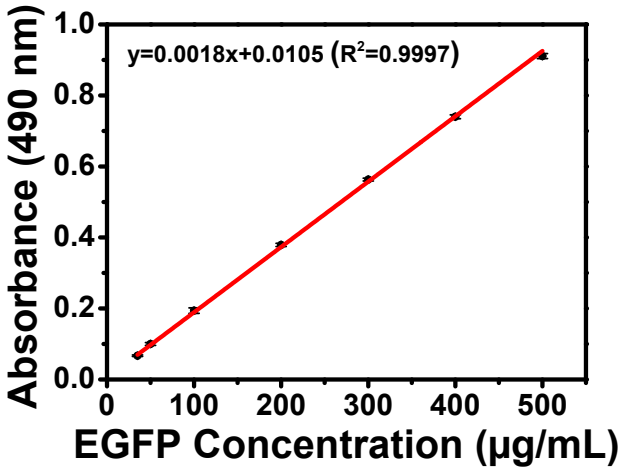

Figure S2. The UV absorbance standard curve of different concentrations of EGFP at 490 nm.

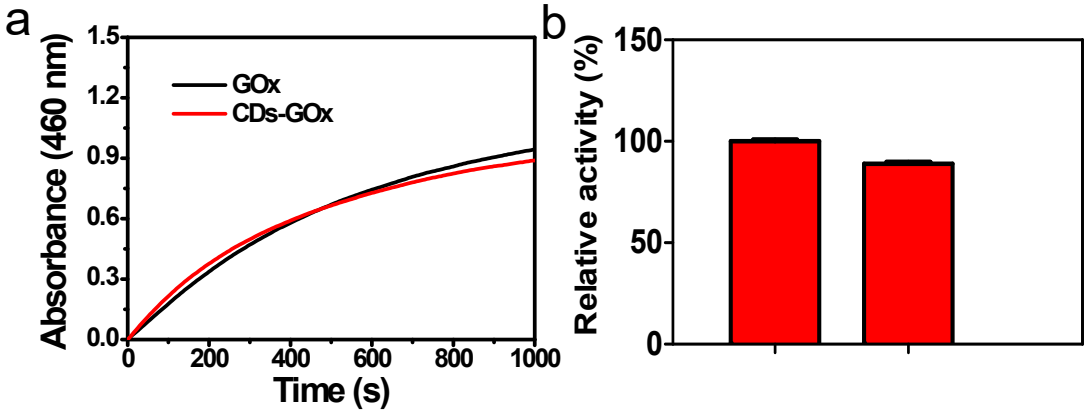

Figure S3. (a) The time-dependent absorption spectra of O-dianisidine at 460 nm after the GOx/CDs-GOx cascade reaction. (b) Relative enzyme activity of GOx/CDs-GOx (GOx: 2 μg/mL).

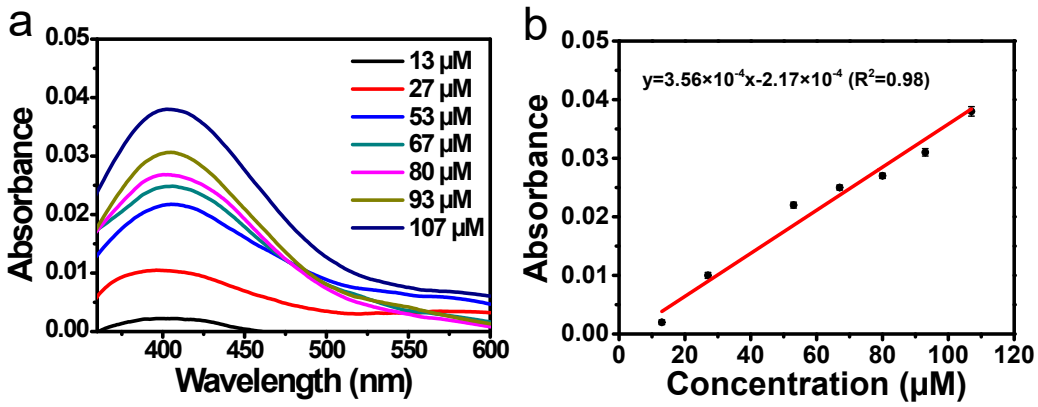

Figure S4. (a) UV-vis absorption spectra of formed Ti(IV)O_2SO_4 at different H_2O_2 concentrations. (b) The standard curve of oxidation state Ti(IV)O_2SO_4 at 404 nm in the presence of different concentrations of H_2O_2.

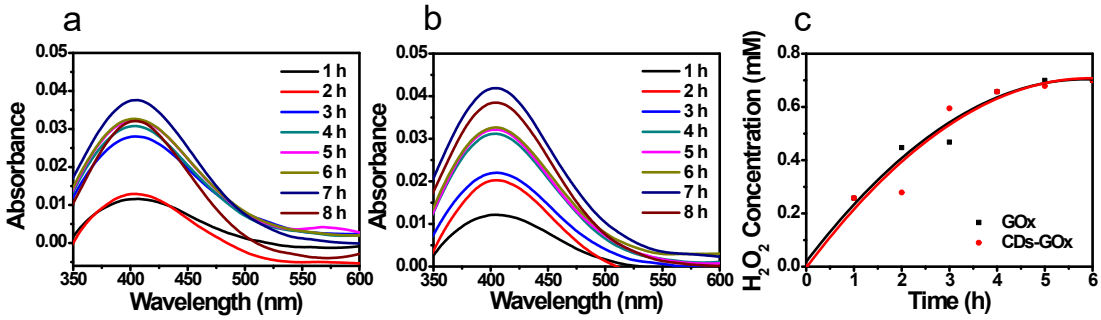

Figure S5. UV-vis absorption spectra of Ti(IV)O_2SO_4 at different time points after adding (a) GOx and (b) CDs-GOx. (c) Fitting curves of H_2O_2 produced by GOx and CDs-GOx at different time points.

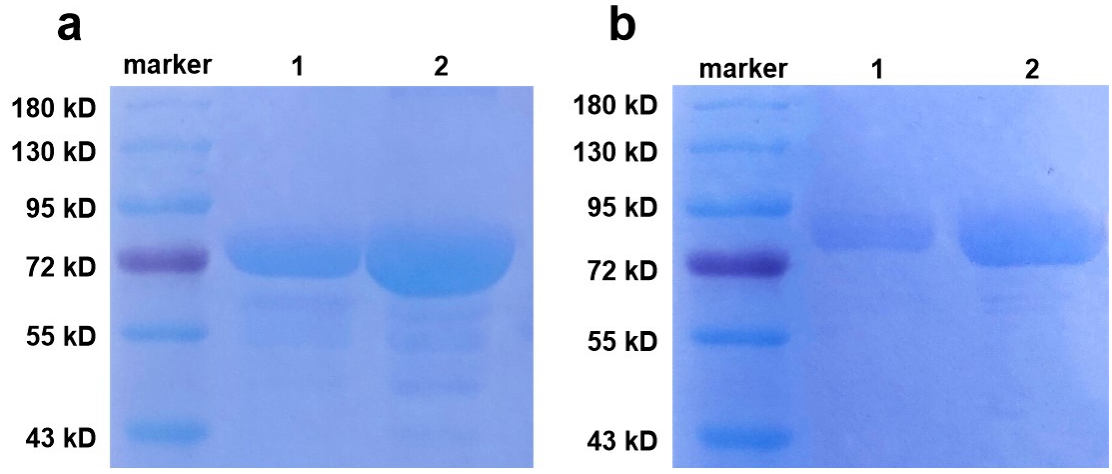

Figure S6. SDS-PAGE analysis of CDs in protection of proteins from enzymatic digestion. (a) lane 1: free BSA with trypsin digestion; lane 2: CDs-BSA with trypsin digestion. (b) lane 1: free GOx with trypsin digestion; lane 2: CDs-GOx with trypsin digestion.

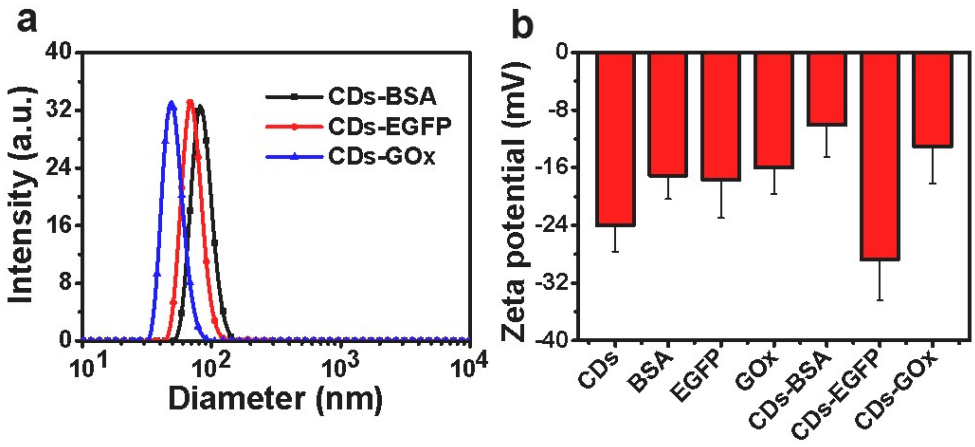

Figure S7. (a) The hydrodynamic diameter of CDs-BSA, CDs-EGFP and CDs-GOx determined by DLS. (b) Zeta potential of CDs, BSA, EGFP, GOx, CDs-BSA, CDs-EGFP and CDs-GOx.

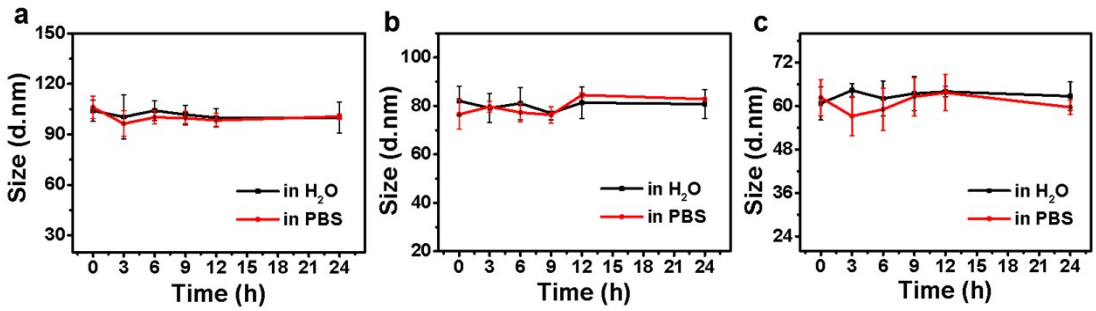

Figure S8. The diameter changes of (a) CDs-BSA, (b) CDs-EGFP and (c) CDs-GOx determined by DLS in water and PBS for 24 h.

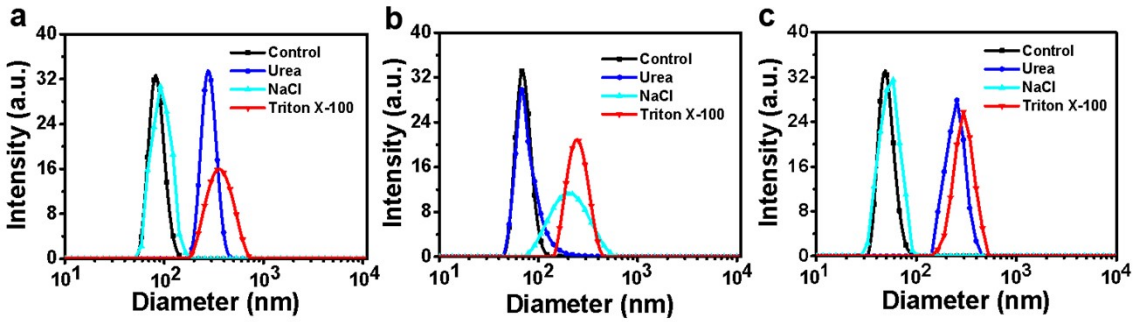

Figure S9. DLS results of the mixtures formed by (a) CDs-BSA, (b) CDs-EGFP and (c) CDs-GOx in the prescence of inhibitors (urea, NaCl and Triton X-100, 25 mM).

[1] X. Guan, C. Li, D. Wang, W. Sun, X. Gai, RSC Adv. 2016, 6 (12), 9461-9464.