

Supporting Information

Experiments: materials and methods

Expression and purification of gCTX

The CTX nucleotide sequence was generated from the natural CTX peptide by an overlapping PCR method. A second PCR reaction was performed using the products of the overlapping PCR as a template. The second PCR was used FP2 and RP2 as primers. After digestion with *Bam*HI and *Xho*I, the PCR products were subcloned into pGEX-6p-1 at the *Bam*HI-*Xho*I-cut site. The recombinant pGEX-6p-1-CTX plasmid confirmed by sequencing was transformed into *Escherichia coli* (*E. coli*) Rosetta (DE3) cells and the transformed cells were cultured at 37 °C in LB medium with ampicillin (100 µg/mL). When the cell density reached an OD of 0.6, 1.0 mM isopropyl thio-β-d-galactoside was added to induce expression at 28 °C. The cells were harvested after 4 h and resuspended in 50 mM Tris-HCl and 10 mM Na₂EDTA (pH 8.0). Supernatant from the bacterial cell lysate was loaded onto a glutathione transferase (GST) binding column. The purified fusion protein was desalted and enriched using centrifugal filtration (Millipore). The purity of gCTX fusion protein was identified by high performance liquid chromatography (HPLC).

Synthesis of Au@gCTX NCs

In a typical experiment, aqueous HAuCl₄ solution (5 mL, 10 mM, 37 °C) was added to the gCTX (1 mL, 10 mg/mL, 37 °C) solution under vigorous stirring. NaOH solution (0.5 mL, 1M) was introduced 2 min later, and the reaction was allowed to proceed under vigorous stirring at 37 °C for approximately 32 h. The resultant aqueous solution of Au@gCTX NCs was purified using centrifugal filtration (Millipore) and freeze-dried for further use. As a control sample, Au@BSA NCs were synthesized with the same method by using BSA to replace gCTX.

Characterization

High-resolution transmission electron microscopy (HRTEM) observations were performed with a JEOL 2010 FET transmission electron microscope operated at 200 kV. Hydrodynamic diameter was measured by Malvern Zetasizer Nano ZS90. X-ray photoelectron spectra (XPS) were taken on a Kratos Ltd. XSAM800 electron energy spectrometer. Fourier transform infrared (FTIR) spectra were recorded on an Avatar-360 spectrometer. Absorption spectra were measured by using a Varian Cary 5000 UV-vis-NIR spectrophotometer. Fluorescence emission spectra were obtained with a Hitachi F-4500 fluorescence spectrophotometer.

Cell lines and cell culture

Rat C6 glioma cells and NIH 3T3 fibroblasts obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) were maintained in DMEM (Gibco BRL, USA) with 1% streptomycin/penicillin and 10% fetal bovine serum (Everygreen, Hangzhou, China) at 37 °C with 95% air, 5% CO₂, and 100% humidity. The cells were digested with 0.5% trypsin when they reached 90% confluence. The cells were prepared for assays with modifications as described below. Cell suspensions were centrifuged at 4 °C and 1,000 g for 10 min and the supernatants were removed carefully and completely. Cell pellets were resuspended in serum and antibiotic-free DMEM, and cell number was counted using a hemocytometer.

Cellular uptake studies by confocal fluorescent microscopy

A total of 2×10^3 cells were seeded into 8-well chambered coverglasses (Lab-Tek, Nunc, USA) in 200 µL of medium, respectively. After 24 h, the medium was replaced by the medium containing the Au@gCTX NCs (500 µg/mL) or Au@BSA NCs (1000 µg/mL). In another set of competitive experiment, the same batch of C6 cells incubated firstly with 2500 µg/mL free gCTX for 2 h, then added with 500 µg/mL Au@gCTX NCs. After 4 h incubation, the cells were washed thrice with PBS and fixed with 4% paraformaldehyde solution for 20 min, then the nuclei were stained by 10 µg/mL Hoechst 33258 for 5 min and washed thrice with PBS. Finally the fixed cells were observed by confocal laser scanning microscope (CLSM, Leica TCS SP5, Germany). The FL was imaged with λ_{ex} of 488 nm for Hoechst and λ_{ex} of 380 nm for the Au NCs.

Gelatin Zymography

C6 cells were grown to a confluent monolayer in DMEM with 1% streptomycin/penicillin and 10% fetal bovine serum. Then, the serum-containing medium in these cultures were exchanged with serum-free DMEM containing NS, 3 µM CTX, 18 µM gCTX, 18 µM Au@gCTX NCs and 18 µM Au@BSA NCs. After 24 h incubation, serum-free conditioned medium was collected to detect the activity of secretory MMP-2. Cell conditioned medium was mixed 1:1 with 2 × SDS sample buffer, and 20 µL of the mixture were loaded into a well of the precast 10% zymogram gel copolymerized with 0.1% gelatin (Sigma). After electrophoresis, the gels were washed in 2.5% Triton X-100 for 30-60 min at room temperature, incubated overnight at 37 °C in 50 mM Tris buffer (pH 8.0), 5 mM CaCl₂, 0.02% Brij-35, 0.2 M NaCl and 1 µM ZnCl₂, and stained with Coomassie Blue to visualize bands of

gelatinolytic activity.

MTT cell viability assay

To assess the cytotoxicity of the Au@gCTX NCs, C6 glioma cells and NIH 3T3 cells were grown in the presence of the Au@gCTX NCs and the cell viability was measured using MTT assay. Cells were cultured in 96-well plates (approximately 1×10^4 cells per well). After 24 h old medium was discarded, followed by replacement with medium containing various concentrations (60, 170, 500, 1500 $\mu\text{g/mL}$) of the Au@gCTX NCs for 24 h. Then, 20.0 μL of MTT solution (5 mg/mL MTT in PBS, pH 7.4) was added to each well and the cells were incubated for 4 h at 37 $^\circ\text{C}$. After removal of the medium, 150 μL DMSO was added to each well to completely dissolve the MTT crystals. The absorbance of the cell lysate at 490 nm was then measured using an enzyme photometer. Cell viability was expressed as a percentage of the viability of control cells. The results presented represent the mean \pm standard deviation (SD) of ten samples.

Measurement of intracellular ROS by flow cytometry

To measure Intracellular accumulation of reactive oxygen species (ROS), a fluorometric assay using intracellular oxidation of dichlorofluorescein-di-acetate (DCFH-DA) was performed. DCFH-DA, a cell permeable, non-fluorescent precursor of 2,7-dichlorofluorescein (DCF) can accumulate within cells upon de-acetylation, and then DCFH reacts with ROS to form fluorescent DCF. C6 cells were trypsinized and seeded at a density of 2×10^5 cells/well into 6-well tissue-culture plates. After 12 h, old medium was discarded followed by replacement with medium containing 1500 $\mu\text{g/mL}$ of Au@BSA NCs and Au@gCTX NCs respectively. Negative controls were replaced with fresh medium containing equivalent volume PBS. After 12 h of incubation at 37 $^\circ\text{C}$, cells were washed with PBS (pH 7.4) three times and incubated with 30 μM of DCFH-DA (dissolved in 100% ethanol, filter sterilized) at 37 $^\circ\text{C}$ for 30 min. The cells were then harvested with trypsin. The intensity of fluorescence was detected by flow cytometry (BD AccuriTMC6) with an excitation filter of 488 nm and a band-pass emission filter of 530 ± 15 nm.