

## Supporting Information

### Probing the Molecular Mechanism of Cerium Oxide Nanoparticles in Protecting Against the Neuronal Cytotoxicity of A $\beta$ <sub>1-42</sub> with Copper Ions

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#### Materials and Methods

##### *Materials*

The SH-SY5Y human neuroblastoma cell strain was offered by National Neurobiology Laboratory of Fudan University. Complex [Ru(phen)<sub>2</sub>dppz](PF<sub>6</sub>)<sub>2</sub> was obtained from Dr. Shuo Shi of Tongji University, which was synthesized as previous

described<sup>1,2</sup>. Cerium Oxide Nanoparticles (CeONP), 4',6-diamidino-2-phenylindole (DAPI) and 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes), uranyl acetate, and PBS buffer (NaCl, 137 mM; KCl, 2.7 mM; Na<sub>2</sub>HPO<sub>4</sub>, 4.3 mM; KH<sub>2</sub>PO<sub>4</sub>, 1.4mM) were purchased from Aladdin. Ultrapure N-tert-butyl-a-phenylnitron (PBN) was obtained from Sigma. FITC (fluorescein isothiocyanate isomer) was purchased from Sigma. DMEM (Dulbecco's modified Eagle's medium) was purchased from GIBCO-BRL (Gaithersburg, MD, USA). The ROS assay kit (2',7'-dichlorodihydrofluorescein diacetate, DCFH-DA) was purchased from Applygen Technologies Inc. CCK-8 was from Shanghai R&S Biotechnology Co., Ltd. The other chemical reagents were of analytical grade.

### ***Fluorescence Spectroscopy***

The complex of Ru(phen)<sub>2</sub>dppz](PF<sub>6</sub>)<sub>2</sub> (phen = 1,10-phenanthroline, dppz = dipyrido [3,2-a:2'.3'-c] phenazine) was added into A $\beta$ <sub>1-42</sub> (10  $\mu$ M) to a final concentration of 5  $\mu$ M in Tris buffer (150 mM, pH 7.4). Emission spectra were collected from 550 nm to 800 nm with an excitation at 458 nm using Fluorescence Cary Eclipse (Varian company, USA). An extinction coefficient of 20 000 M<sup>-1</sup> cm<sup>-1</sup> at 440 nm was used to adjust the compound concentration.

### ***Transmission Electron Microscopy***

The TEM assays were conducted according to the methods reported by Reinke with marginal change.<sup>3</sup> In the presence or absence of CeONP (50  $\mu$ M), 5  $\mu$ L of A $\beta$ <sub>1-42</sub>

(50  $\mu$ M) samples were incubated at 37°C for 72 hours. After that, 3  $\mu$ L of amyloid peptides were put on the carbon 300-meshcopper grids, and then kept for 5 min at room temperature. The extra peptides solution was blot up by filter paper. The copper grids were washed three times with double distilled water. The peptides were negatively stained with 5 $\mu$ L 2% uranyl acetate for 1 min at room temperature. The copper grids were kept for 15 min at room temperature so that the copper grids were blot up. The samples were examined with TEM (Nova Nano, SEM 450, American FEI) at an accelerating voltage of 200 kV.

### ***Cell Viability Assay***

The cell viability was quantitatively determined using CCK-8 (Cell Counting Kit-8) scientifically based on the complex of WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)- 2H-tetrazolium, monosodium salt]. CCK8 is a cell counting kit which detects cell viability quickly and sensitively. The kit contains a chemical compound called WST-8 which can be reduced by succinate dehydrogenase in mitochondria of living cells, subsequently forming a highly soluble formazan production with orange color. The absorbance of orange formazan at 450 nm is used to reflect the living cell proliferation, because this reaction cannot happen in dead cells. Thus by measuring formazan production OD value at 450nm in Microplate Reader, the relative amount of living cells was obtained indirectly. SH-SY5Y human neuroblastoma cells were cultured in DMEM medium containing 10% horse serum at 37 °C, 5% CO<sub>2</sub>, 95% O<sub>2</sub>. The SH-SY5Y cells were inoculated into 96-well

plates at a concentration of 1000 cells per well. Then cells were incubated at 37 °C under 5% CO<sub>2</sub> for 24 hours. After that, the cells were treated with fresh prepared A $\beta$ <sub>1-42</sub> (25  $\mu$ M) for 2 h and then CeONP (50  $\mu$ M) was added for 24 hours at 37 °C. Then 10  $\mu$ L CCK-8 was added to each 96 well-plate and incubated for another 2 hours. Each well was detected at 450 nm by ELISA.

### ***Reactive Oxygen Species Assay***

The reactive oxygen species (ROS) assay was measured according to the methods as described previously.<sup>4</sup> The ROS assay kit consisting of 2',7'-dichlorodihydrofluorecein (DCFH) and its diacetate form, DCFH-DA, was applied to detect the production of ROS in SH-SY5Y cells with the addition of A $\beta$ <sub>1-42</sub> or Cu<sup>2+</sup>-A $\beta$ <sub>1-42</sub> (25  $\mu$ M) in the presence or absence of CeONP (50  $\mu$ M). Cells were digested and put in 96-well plates at a concentration of about 1000 cells per well. The cells were incubated at 37°C under 5% CO<sub>2</sub> for 24 hours, and different samples were added and incubated for another 24 hours. The reactive oxygen species assay kit, containing DCFH-DA, were added to the final concentration of 10  $\mu$ M. The cells were incubated for 1 hour and the old medium was discarded. The cells were washed with PBS buffer for three times. Then the cells were examined with a 5 fluorescence microscope (TE2000-U, Nikon, Japan).

### ***FITC-labeled A $\beta$ <sub>1-42</sub> aggregation in SH-SY5Y cells***

FITC stock solution was prepared in anhydrous DMSO at 2.9 mg/mL due to the

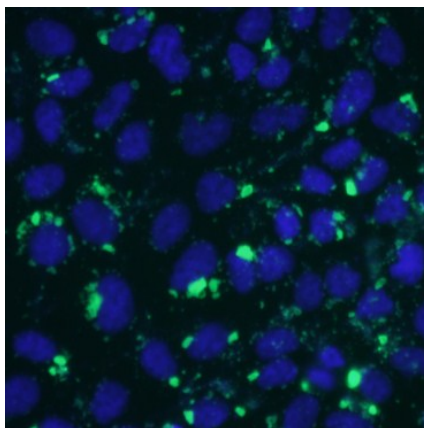
decomposition reaction in water.<sup>5</sup> A $\beta$ <sub>1-42</sub> was labeled covalently as previous described.<sup>6</sup> FITC labeled-A $\beta$ <sub>1-42</sub> (25  $\mu$ M) or Cu<sup>2+</sup>-A $\beta$ <sub>1-42</sub> (25  $\mu$ M) prepared in PBS buffer (pH 7.4, 300 mM NaCl, 100 mM phosphate) was added in SH-SY5Y cells with concentration of about 1000 cells per well at 37°C under 5% CO<sub>2</sub> for 24 hours in the presence and absence of CeONP (50  $\mu$ M), respectively. SH-SY5Y cells were fixed for 30 min using 4% paraformaldehyde and permeabilized on ice with 0.5% triton X-100, and incubated with DAPI for 5min, and then with [Ru(phen)<sub>2</sub>dppz]<sup>2+</sup> for 30 min. After that, the cells were washed three times with PBS buffer. Amyloid beta aggregation in SH-SY5Y cells was analyzed by confocal scanning laser microscopy (CSLM) (TCS-SP5, Leica, England).

### ***EPR spectroscopy***

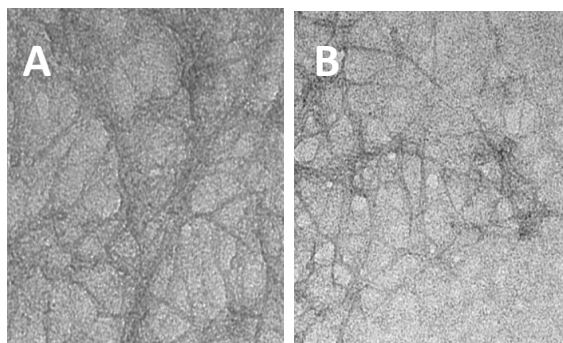
EPR spectra were recorded on a Bruker EMX X-band spectrometer equipped with an Oxford-910 cryostat and ITC-503 temperature controller (Oxford Instruments Ltd.). Experimental conditions: microwave frequency 9.44 GHz, power 2.0 mW, modulation at 100 kHz, modulation amplitude 4 G, and time constant, 163.84 ms. All samples with 50 mM PBN (Ultrapure N-tert-butyl-a-phenylnitron, for radicals trapping), in the absence or presence of CeONP were incubated at 37°C for more than 24 hrs. EPR spectra of radicals-containing samples were recorded at 25°C. EPR signals of Cu<sup>2+</sup>-containing samples in the presence or absence of CeONP were collected at 4 K.

**Figure S1.**

Detection of  $\text{Cu}^{2+}$ - $\text{A}\beta_{1-42}$  entering into SH-SY5Y cells. Fluorescence microscopy images of SH-SY5Y cells stained with FITC-  $\text{Cu}^{2+}$ - $\text{A}\beta_{1-42}$  (50  $\mu\text{M}$ , green) and DAPI (blue).

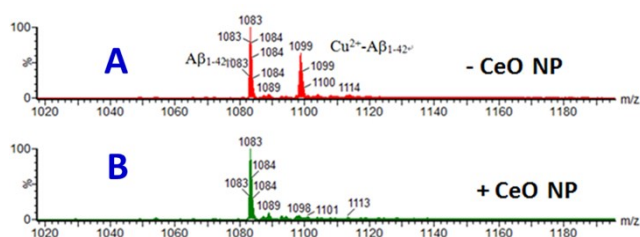


**Figure S2.** Negative stained transmission electron micrographs of  $\text{Cu}^{2+}$  plus  $\text{A}\beta_{1-42}$  (A) and (B)  $\text{Cu}^{2+}$  plus  $\text{A}\beta_{1-42}$  in the presence of CeONP.



**Figure S3.**

**Mass Spectra of  $\text{A}\beta_{1-42}$  plus  $\text{Cu}^{2+}$  in the presence and absence of CeONP**



## References

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