Supporting Information

Screening a specific Zn(II)-binding peptide for improving the cognitive decline of Alzheimer's disease in APP/PS1 transgenic mice by inhibiting Zn^{2+}-mediated amyloid protein aggregation and neurotoxicity

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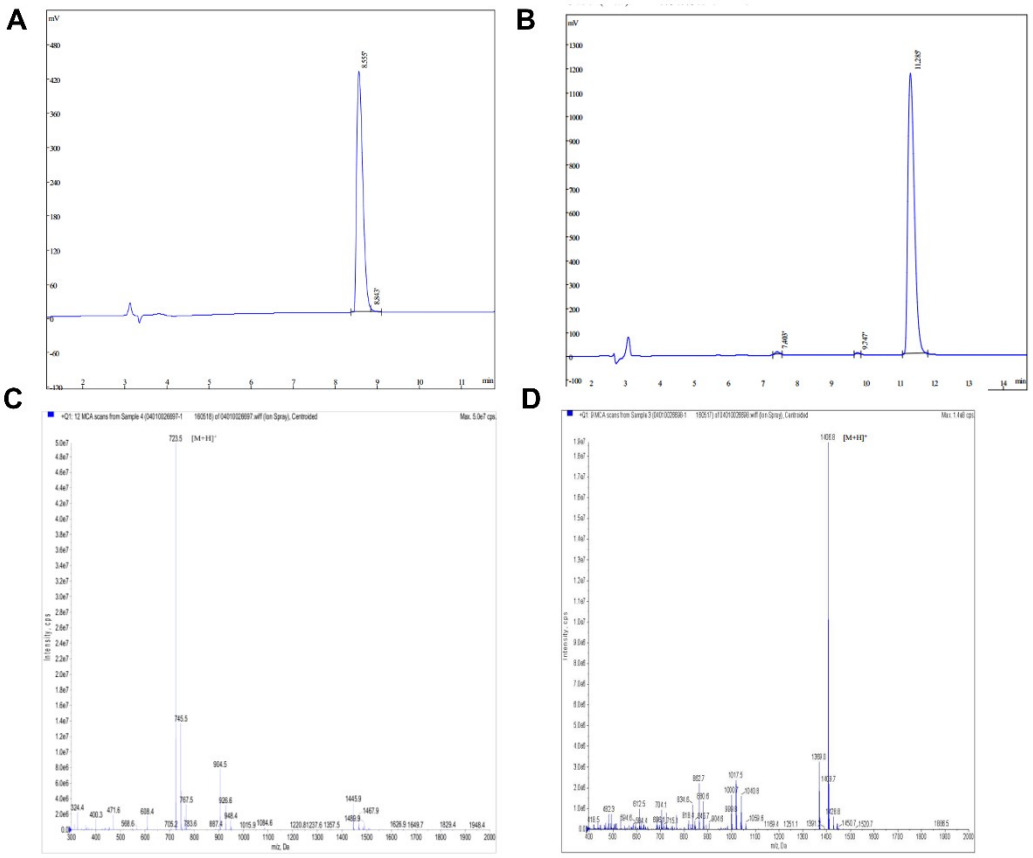

Figure S1. Analysis of the synthetic PZn and Fitc-PZn. (A-B) HPLC chromatogram. **(C-D)** Electrospray ionization mass spectrum.

The result of HPLC analysis showed that the purity of PZn and Fitc-PZn were greater than 95%. The results of mass spectrometry analysis showed that the molecular weights of synthesized PZn and Fitc-PZn were consistent with the theoretical values.

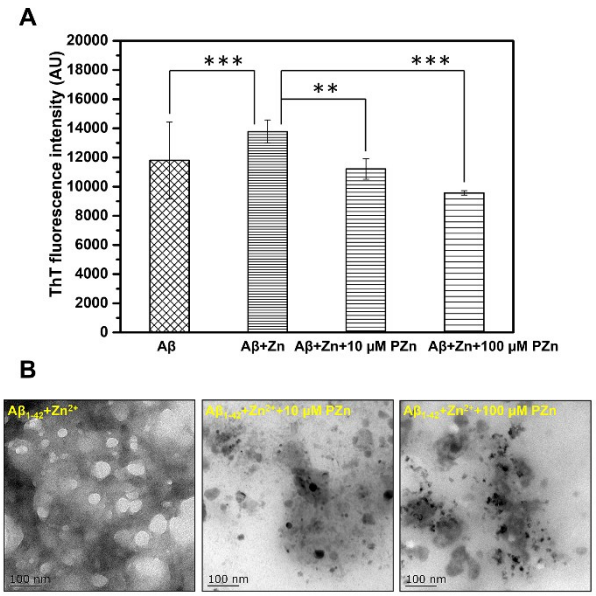

Figure S2. PZn inhibited Zn^{2+}-induced aggregation of Aβ1-42 in vitro. $A\beta_{1\text{-}42}$ were incubated with 70 μM Zn^{2+}, 10 μM PZn + 70 μM Zn^{2+} and 100 μM PZn + 70 μM Zn^{2+}. **(A)** ThT fluorescence assay. **(B)** TEM images of $A\beta_{1\text{-}42}$ aggregation under different solution conditions. Scale bar : 100 nm. The data represent the mean ± S.E. of 3 independent experiments. **, $p < 0.01$; ***, $p < 0.001$ compared to the Zn^{2+}-induced group.

ThT fluorescence intensity showed that Zn^{2+} induced a rapid increase of $A\beta_{1\text{-}42}$ aggregation at 2 h **(Figure S2A)**, and the aggregation was inhibited by PZn. As shown in **Figure S2B**, $A\beta_{1\text{-}42}$ mostly aggregated into amorphous aggregates that were stacked together under Zn^{2+} treatment. In the presence of PZn, only a few small amorphous and granular aggregates were observed. This indicated that PZn inhibited Zn^{2+}-induced aggregation of Aβ.

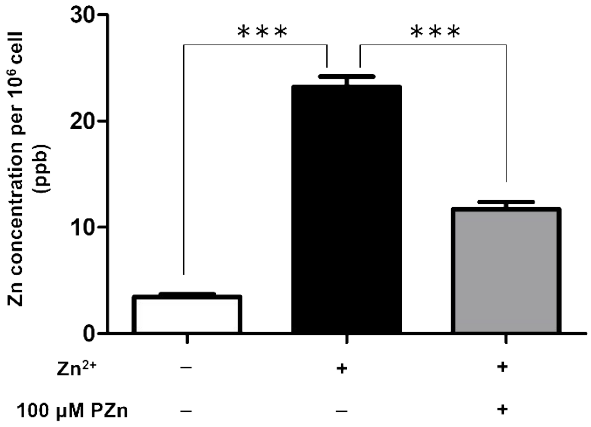

Figure S3. PZn alone reduced zinc concentration in N2a-sw cells. N2a-sw cells were treated with 70 μM Zn^{2+} or co-incubated Zn^{2+} with 100 μM PZn for 12 h. Zinc levels in N2a-sw cells analyzed by ICP-MS. The data represent the mean ± S.E. of 3 independent experiments. ***, $p < 0.001$ compared to the Zn^{2+}-damage group.

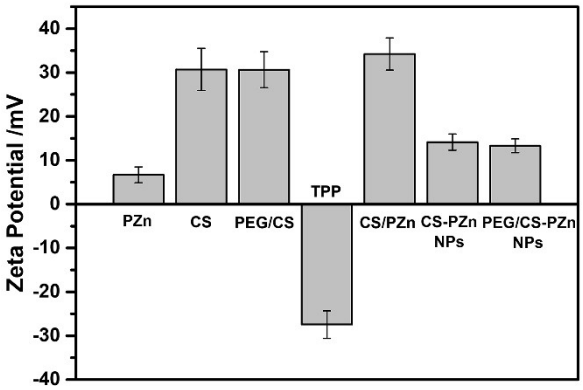

Figure S4. The corresponding zeta potentials at each step of PEG/CS-PZn NPs preparing process.

Zeta potential measurement was conducted by laser light scattering on a Malvern Particle Size Analyzer (Malvern Instruments Ltd., Nano-ZS90, Malvern, Worcestershire, UK).

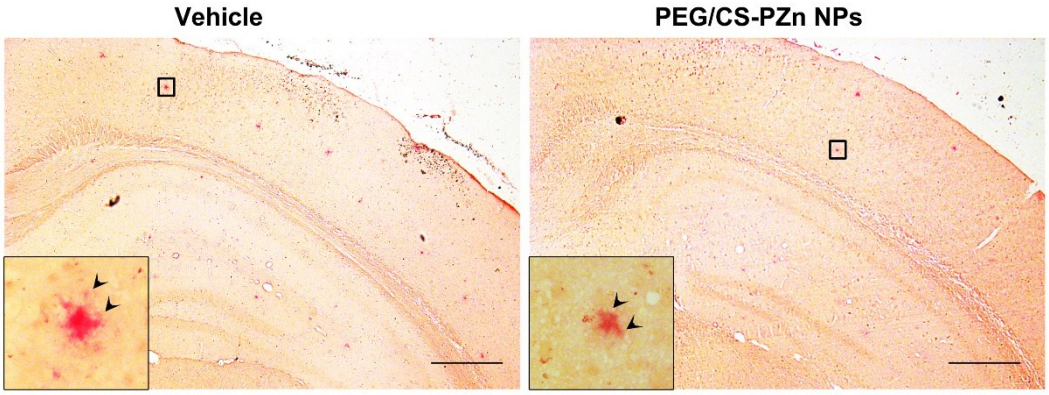

Figure S5. PEG/CS-PZn NPs reduced amyloid plaque deposition in APP/PS1 mouse brain. Six-month-old APP/PS1 mice were treated with PEG/CS-PZn NPs for 3 months. The brains were collected and stained with Congo red. Representative images of Aβ aggregation staining in cortex and hippocampus. Arrows showed the representative morphology at higher magnification. Scale bars: 500 μm.

PEG/CS-PZn NPs treatment decreased the number of plaques in the cortex and the hippocampus of APP/PS1 mice. It was obviously observed that the plaques were reduced at the edge with no changes in the center under high magnification. These morphological results indicated that PEG/CS-PZn NPs could really reduce the aggregation of Aβ consistent with the results of immunohistochemistry.