Electronic Supplementary Material (ESI) for Molecular BioSystems.

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Supporting Information

Clearance intracellular high level of Tau protein directed by artificial synthetic hydrolase

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Contents:

Materials and Methods:

Materials.

Most of the reagents used in the experiments were bought from Sigma-Aldrich and Acros. The *N*α-Fmoc-protected amino acids were purchased from GL Biochem (Shanghai) Ltd. Aβ42 and Aβ40 were purchased from Yale University. The antibodies were purchased from Sigma-Aldrich and Santa cruz.

General protocol of peptide synthesis.

All the peptides in this work were synthesized according to standard solid phase peptide synthesis method with the *N*α-Fmoc-protected amino acids (GL Biochem (Shanghai) Ltd.). For the coupling step, 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU, 3.6 equiv) plus1-Hydroxy-7-azabenzotriazole (HOAt, 4.0 equiv) (GL Biochem (Shanghai) Ltd.) were used as activator. And N,N-Diisopropylethylamine (DIEA, 8.0 equiv) (GL Biochem (Shang hai) Ltd.) was used as activator base. Peptide was finally cleaved from resin by TFA/water/ thioanisole/phenol/1,2-Ethanethiol =82.5/5/5/5/2.5 for 3 h. Peptide was precipitated by diethyl ether at least three times after removal of TFA. Then the dried peptides were purified by HPLC and identified by MALDI-TOF/MS. The apocyclen building-block was synthesized according to the reported method.[1] Coupling apocyclen to peptides was performed as the coupling of natural amino acid.

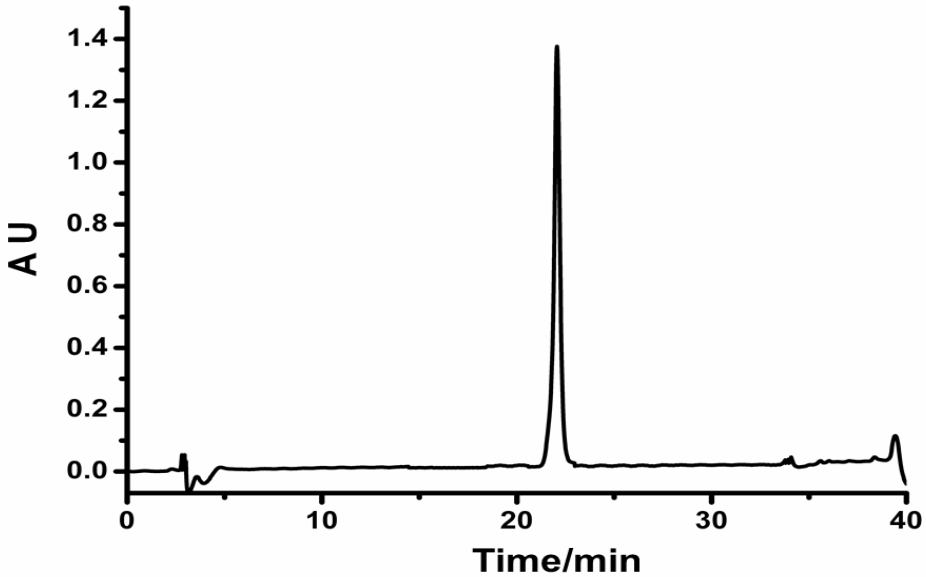
General protocol of RP-HPLC purification.

Purification of peptides was performed with a preparative reversed-phase C18 column (YMC, Japan, 5 μm, 20×250 mm; flow 6.0 ml/min, solution A = water + 0.06 % TFA, solution B = 80 % acetonitrile + 20 % water + 0.06 % TFA). The pure peptide products were lyophilized and analyzed with a reversed-phase analytical C18 column (YMC, Japan, 10 μm, 4.6×150 mm; flow 0.8 ml/min).

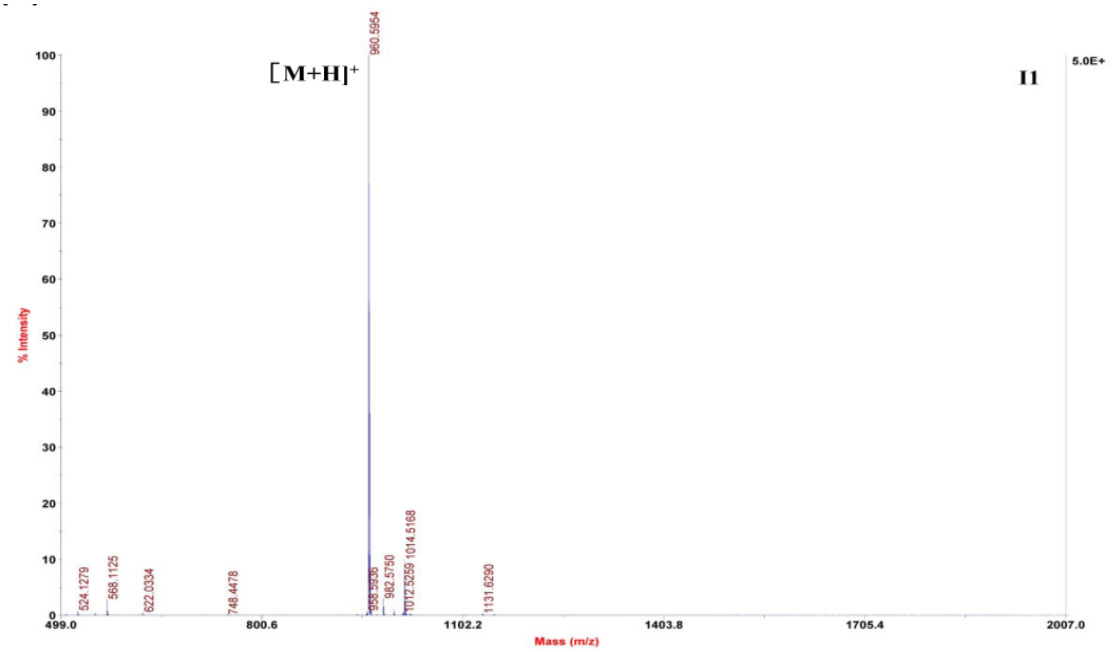
Analytical data

I1: Cyclen-VQIVYK, MS (MALDI/TOF): Calcd. for $C_{46}H_{80}N_{12}O_{10}$ 959.7 $[M+H]^+$; found 960.5 $[M+H]^+$.

Analytic HPLC trace of I1. Analytic gradient is 10% to 50% of solution B in 30 min. Retention time is 22 min.

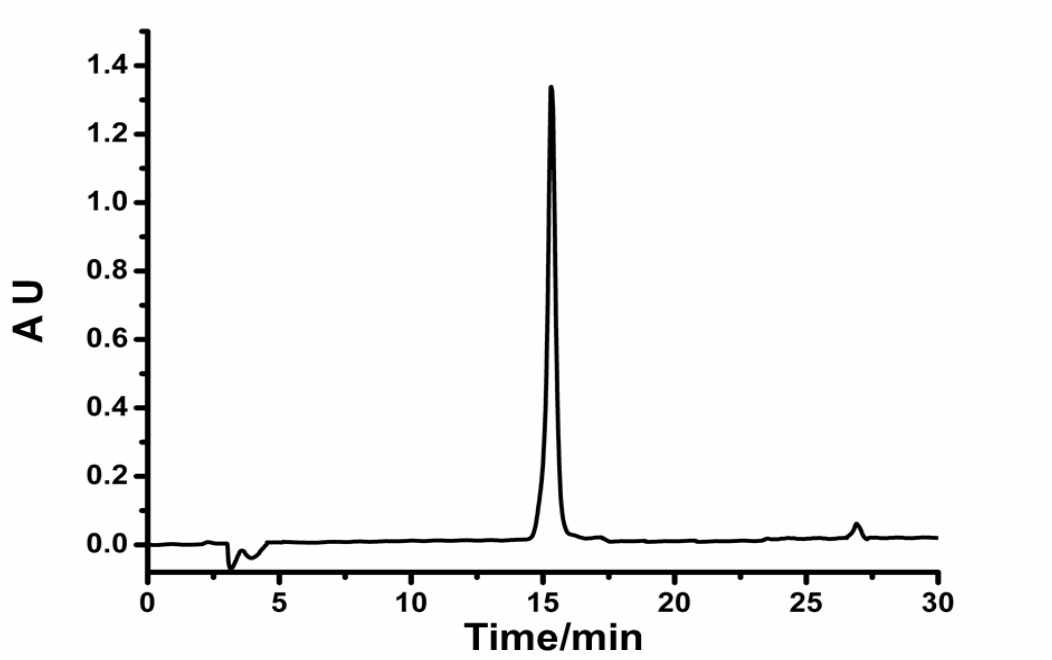


MALDI-TOF/MS of I1

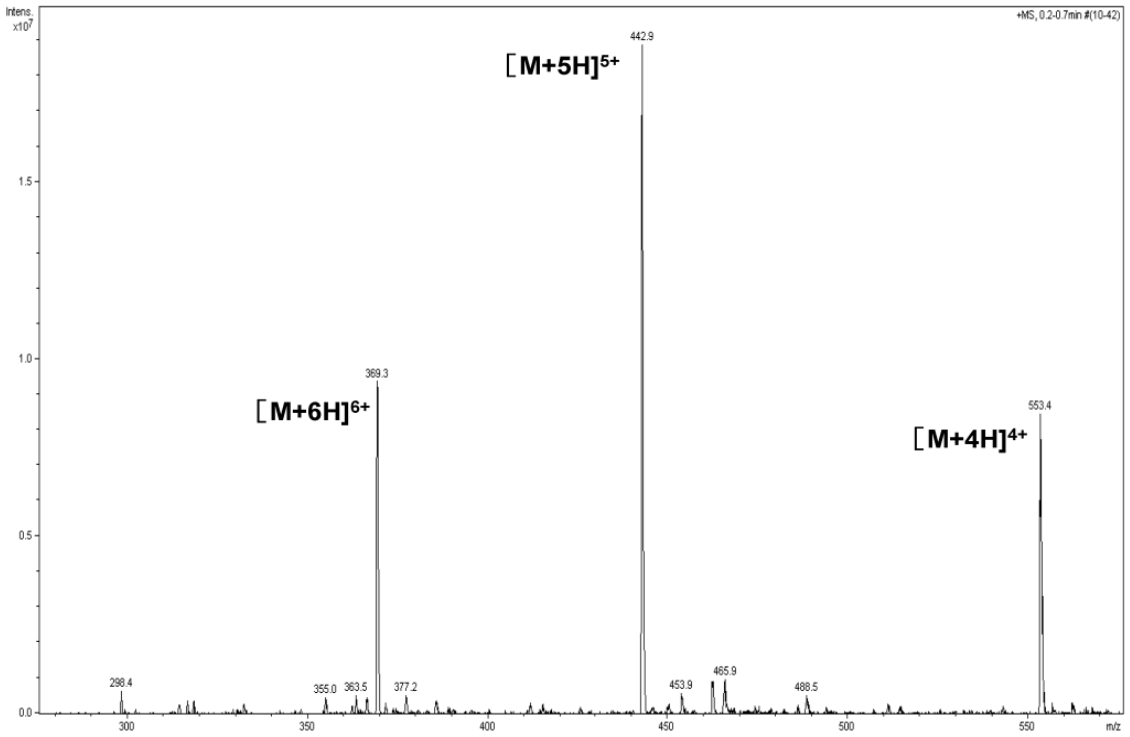


I2: Cyclen-VQIVYK-RRRRRRRR, ESI-MS: Calcd. for $C_{94}H_{176}N_{44}O_{18}$ 2208.7 $[M+H]^+$; found 553.4 $[M+4H]^{4+}$.

Analytic HPLC trace of I2. Analytic gradient is 10% to 50% of solution B in 20 min. Retention time is 15.5 min.

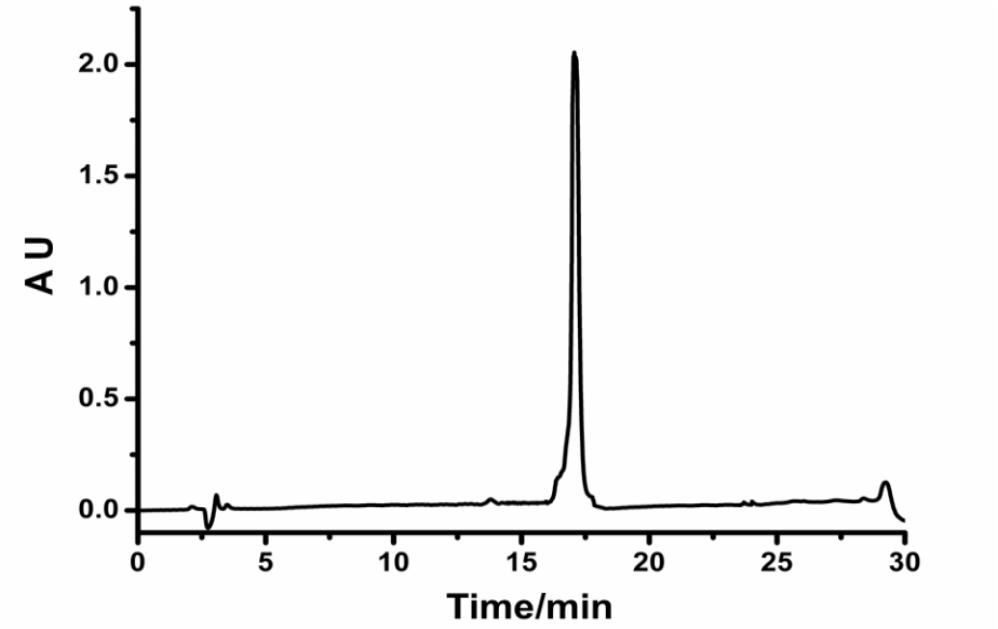


ESI-MS of I2

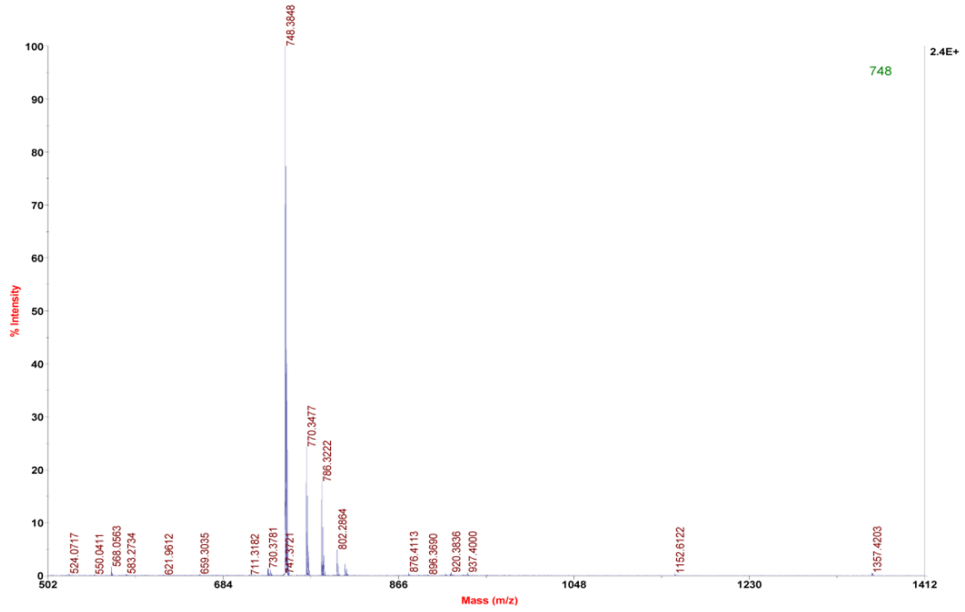


MALDI-TOF/MS of VQIVYK, Calcd. for $C_{36}H_{60}N_8O_9$ 748.4481 $[M+H]^+$; found 748.3848 $[M+H]^+$.

Analytic HPLC trace of VQIVYK. Analytic gradient is from 10 % to 55 % of solution B in 20 min. Retention time is 17 min.



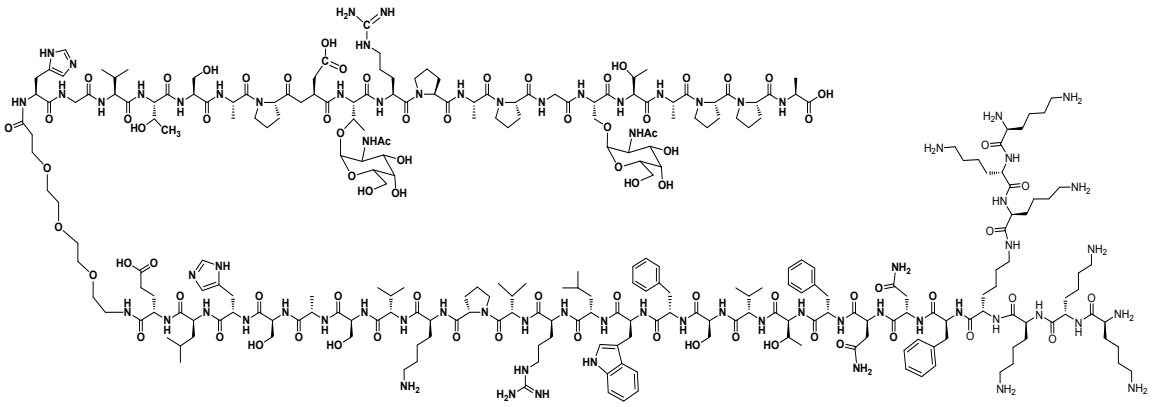
MALDI-TOF/MS of VQIVYK



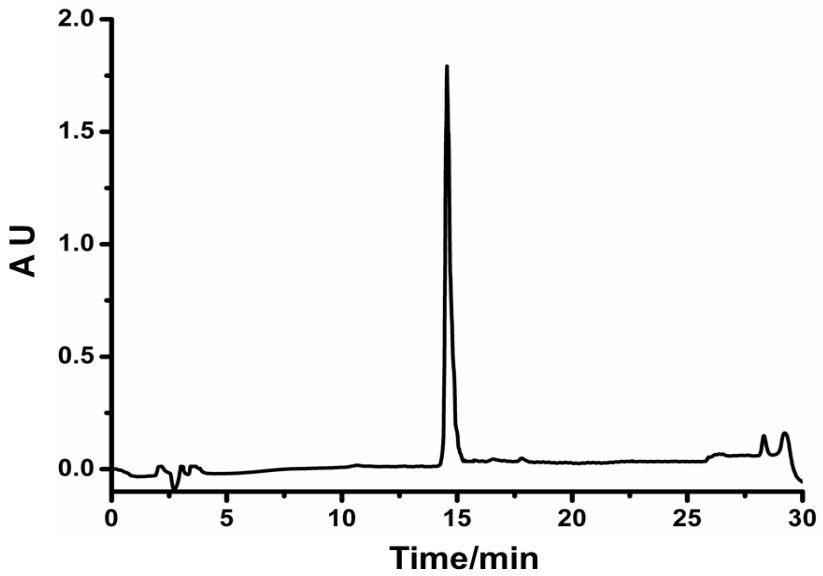
Random control peptide 1 (RCP1):

The RCP1 was synthesized according to the previous reported methods.[2]

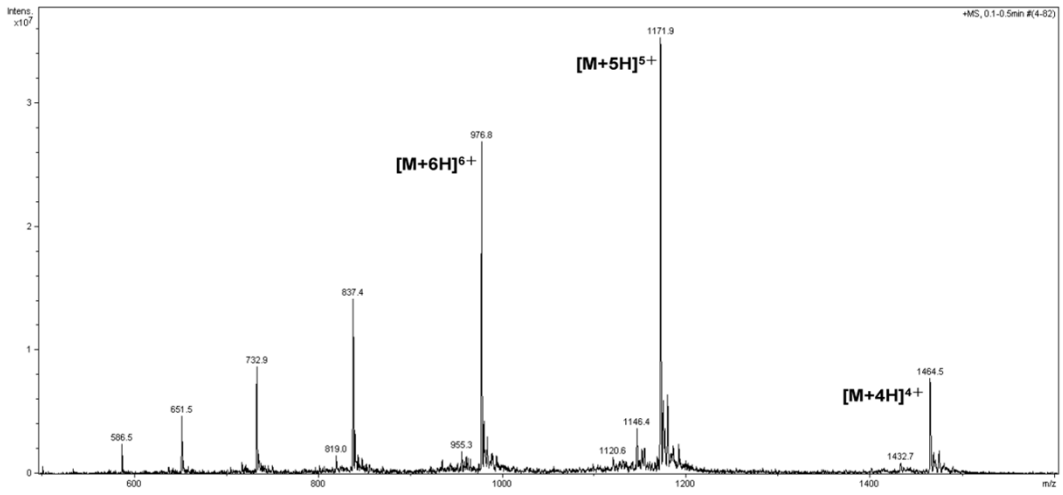
Structure of RCP1



Analytic HPLC trace of RCP1. Analytic gradient is 5 % to 50 % of solution B in 20 min. Retention time is 14.5 min.



ESI-MS result of RCP1: Calcd. for $C_{265}H_{425}N_{71}O_{78}$ 5850.15 $[M+H]^+$; found 1464.5 $[M+4H]^{4+}$.



Plasmids construct

To express the recombinant tau K18 and K19 proteins, the cDNA sequences were inserted into Nde Ⅰ and Xho Ⅰ restriction sites of pET-28a(+) vector. To construct the stable mouse N2a neuroblastoma cell line that expressed Tau-EGFP, the coding sequence of tau, was inserted into Xho Ⅰ and BamH Ⅰ restriction sites of the pEGFP-N3 plasmid.

Cell culture and gene transduction

Mouse N2a neuroblastoma cells were grown in the medium containing 45 % α-MEM (Minimum Essential Medium; Gibco), 45 % DMEM (Dulbecco's Modified Eagle's Medium; Gibco), 10 % FBS (Fetal Bovine Serum; Gibco), Penicillin (100 U/ml) and Streptomycin (100 μg/ml) at 37 °C in 5 % CO_2. To construct the stable cell line that expressed tau-EGFP protein, N2a cells were transfected with pEGFP-N3-Tau plasmid using Lipofectamine™ 2000 transfection reagent (Invitrogen). After transduction, the cells were cultured in the medium containing 600 μg/ml antibiotic G418 (Amerisco) for screening the stable cell line.

Thioflavin T fluorescence Assay [3]

10 μM K19 protein was incubated with 2.5 μM heparin in the buffer (Tris-HCl 20 mM, NaCl 150 mM, pH 7.4), at 37 ℃. The fibril formation kinetics was monitored with 10 μM ThT (Thioflavin T) in a 96-well black plate on a Biotek Synergy 4 microplate reader with excitation at 440 nm and emission at 485 nm wavelength. Each experiment was repeated at least 3 times.

Transmission electron microscopy

A droplet (10 μl) of the samples was placed on the carbon-coated grids for 90 seconds, and then stained with 10 mg/ml phosphotungstic acid (pH 7.4) for 90 seconds. After the grids were dehydrated, they were observed by transmission electron microscopy (H-7650B, Hitachi High-Tech). Each experiment was repeated at least 3 times.

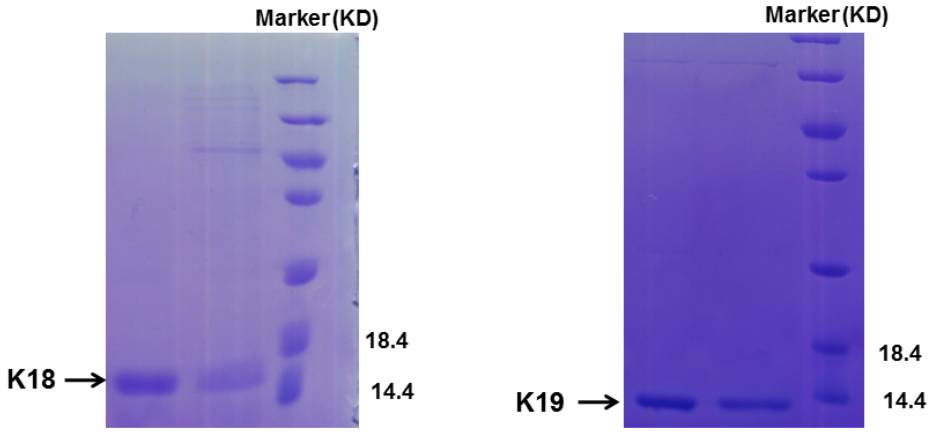
Flow Cytometry Assay

Cells that expressed tau-EGFP were treated with increasing concentration (0 μM, 30 μM, 60 μM, 90 μM, 120 μM, 200 μM) of I2-Cu(Ⅱ) for 24 h or with 60 μM I2-Cu(Ⅱ) for different time (0 h, 6 h, 12 h, 24 h). Then the treated cells were rinsed with PBS buffer (pH 7.4) before harvest. FACS Calibur (BD Biosciences) flow cytometer was applied for detecting the fluorescence intensity of cells. 10,000 cells were detected in each individual experiment. Each experiment was repeated at least 3 times.

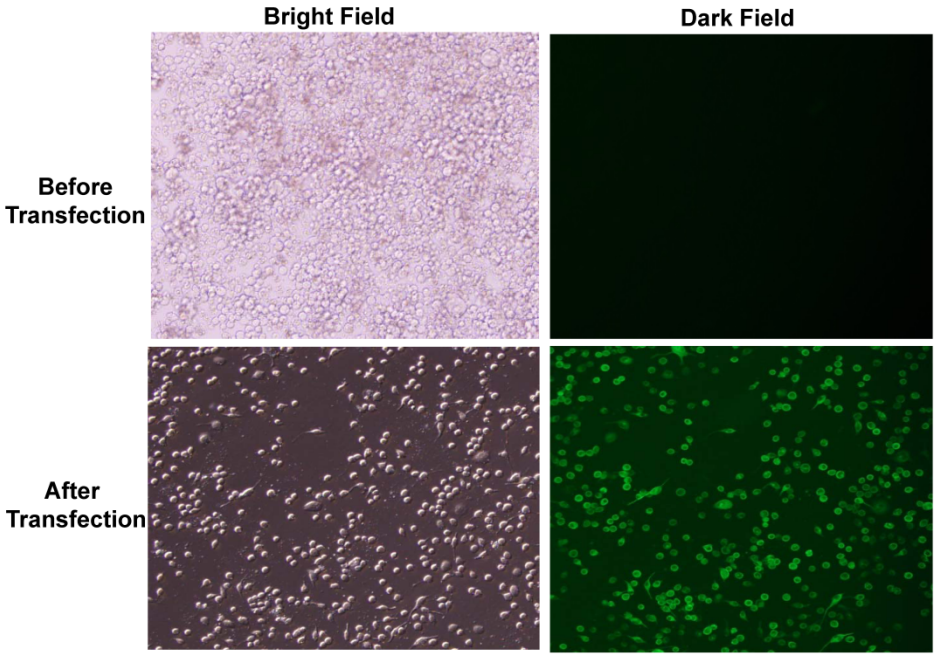
MTT assay [4]

N2a wild cells were grown in a 96-well plate (2×10^5/ml, 100 μl for each well) and treated with different concentration gradients (0 μM, 30 μM, 60 μM, 90 μM, 120 μM) of I2-Cu(Ⅱ) for 24 h with each gradient repeated in five wells. After the incubation, 20 μl MTT reagent (5 mg/ml) was added to each well and incubated for 4 h. Then 150 μl DMSO (dimethyl sulfoxide) was mixed to each well after removal of MTT reagent. The absorbance value at 490 nm wavelength was monitored by a Biotek Synergy 4 microplate reader after the plate was vibrated gently for 10 min. Each experiment was repeated at least three times.

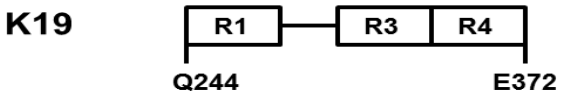
Supplementary information



Supplementary Figure S1. SDS-PAGE results of expressed Tau K18 (*left*) and K19 (*right*) proteins.



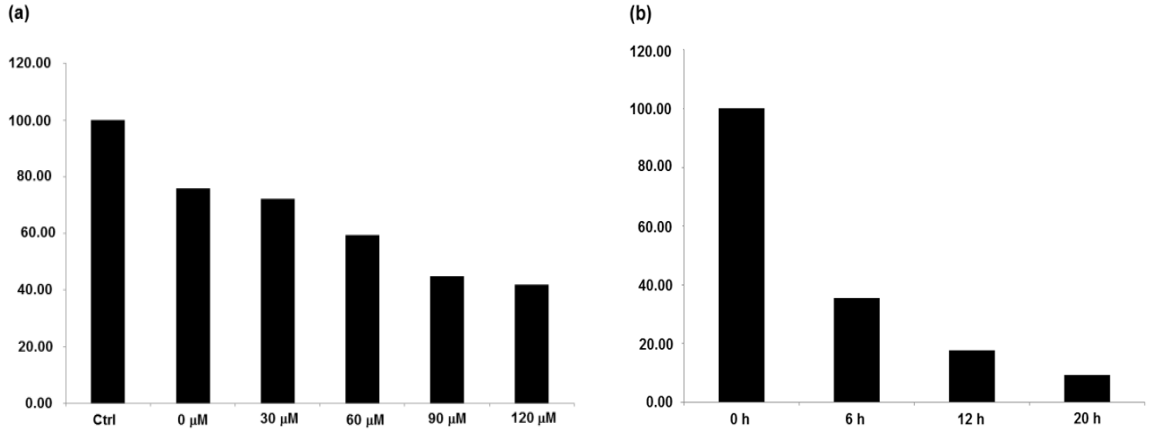
Supplementary Figure S2. The microscopic images of N2a cells expressing Tau-EGFP protein.



Supplementary Figure S3. Summary of the cleavage sites of tau K19 when cleaved by I1-Cu(Ⅱ) estimated according to the MALDI-TOF/MS results.

- Supplementary Results

- Appendix



Supplementary Figure S4. Quantification of tau levels according to western blots results in Figure 3c and 3d.

Reference

1 a) W. H. Wu, P. Lei, Q. Liu, J. Hu, A. P. Gunn, M. S. Chen, Y. F. Rui, X. Y. Su, Z. P. Xie, Y. F. Zhao, A. I. Bush and Y. M. Li, *J. Biol. Chem.* **2008**, *283*, 31657-31664; b) J. W. Jeon, S. J. Son, C. E. Yoo, I. S. Hong, J. B. Song and J. Suh, *Org. Lett.* **2002**, *4*, 4155-4158.

2 H. Cai, M. S. Chen, Z. Y. Sun, Y. F. Zhao, H. Kunz and Y. M. Li, *Angew. Chem.-Int. Edit.* **2013**, *52*, 6106-6110.

3 M. Lindgren, K. Sorgjerd and P. Hammarstrom, *Biophys. J.* **2005**, *88*, 4200-4212.

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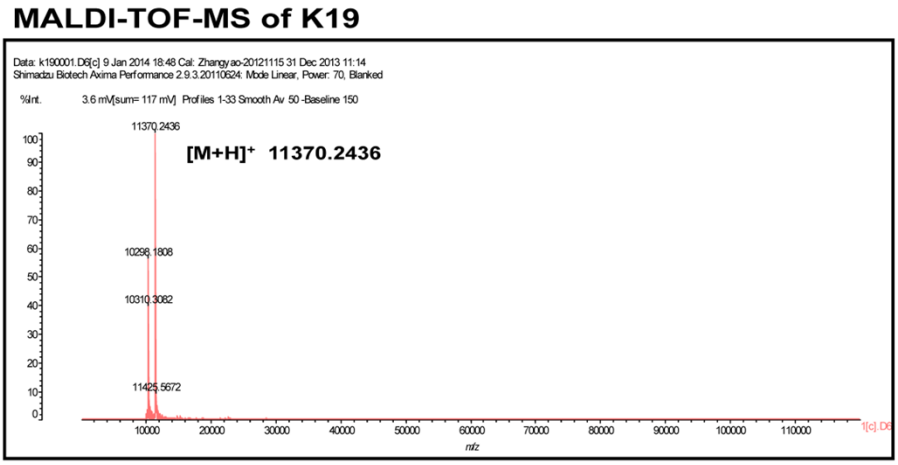
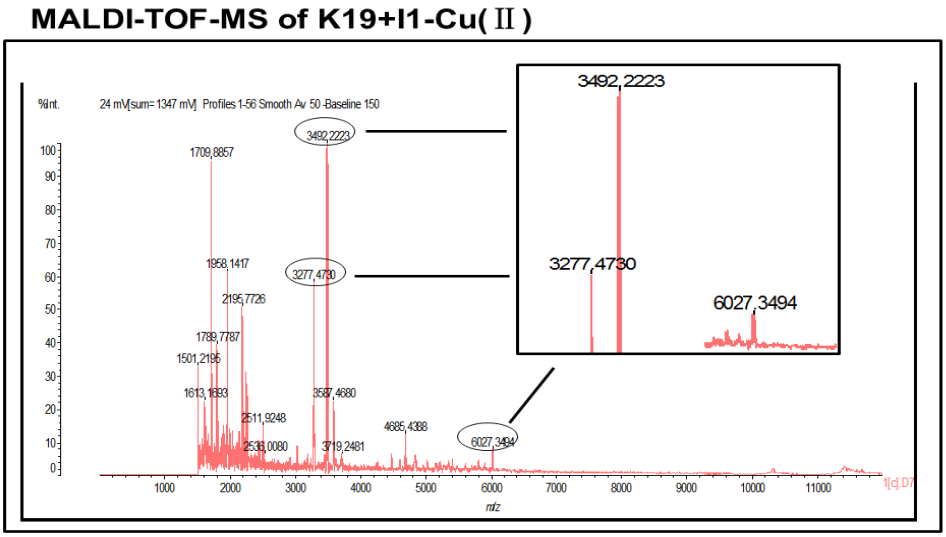
Appendix：

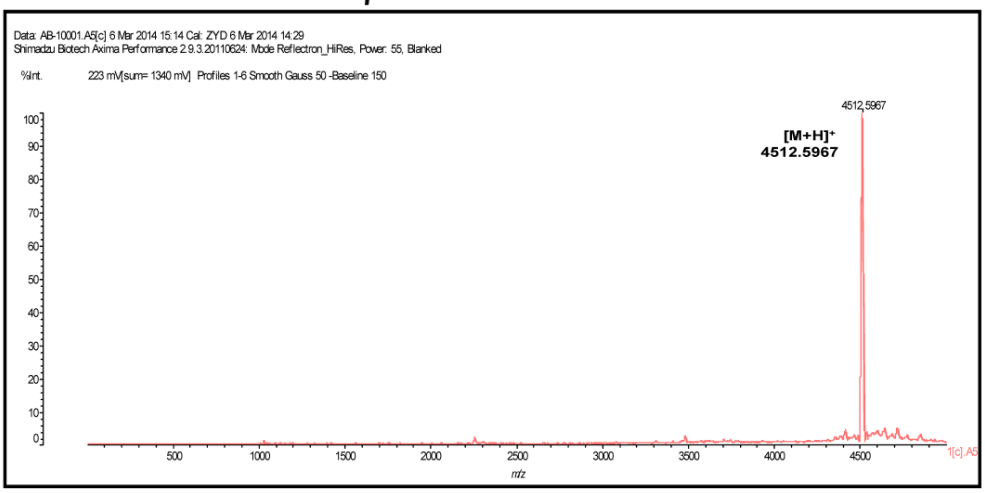


MALDI-TOF/MS of K19 protein, Calcd. for K19 ($C_{497}H_{813}N_{151}O_{147}S_4$) 11376.9664 $[M+H]^+$; found 11370.2436 $[M+H]^+$.



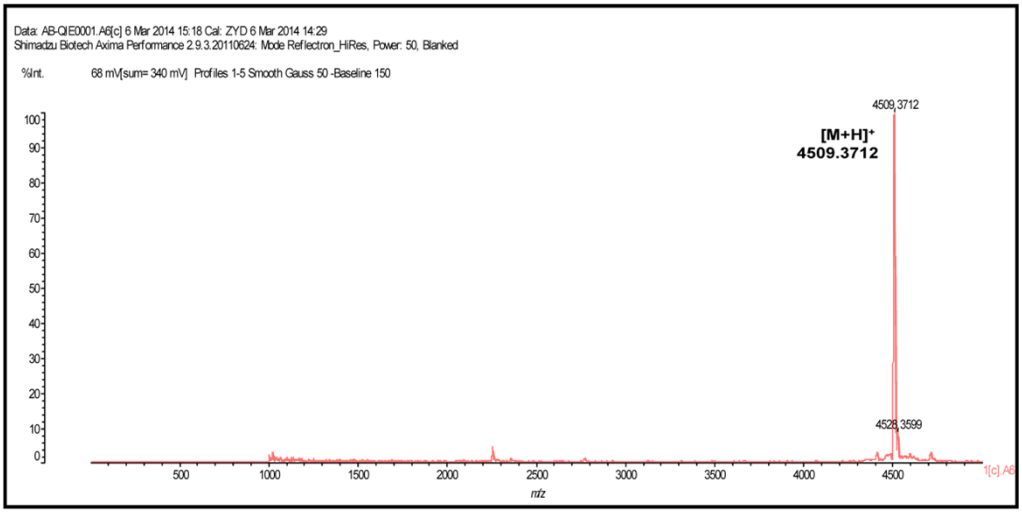
MALDI-TOF/MS of K19 protein incubated with I1-Cu(II) for 24 h.

MALDI-TOF-MS of Aβ42



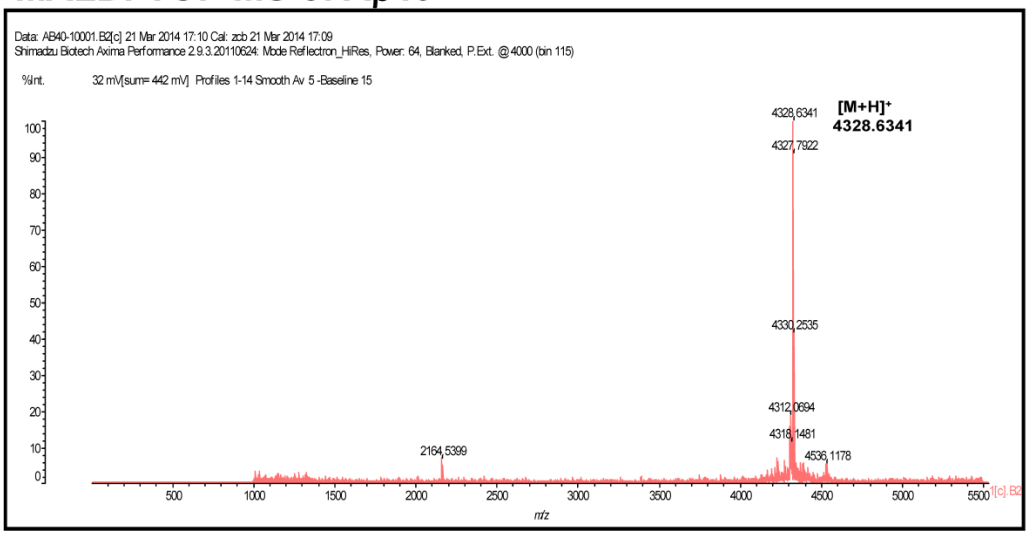
MALDI-TOF/MS of Aβ42 protein, Calcd. for Aβ42 ($C_{203}H_{311}N_{55}O_{60}S_1$) 4514.1 $[M+H]^+$; found 4512.5967 $[M+H]^+$.

MALDI-TOF-MS of Aβ42+I1-Cu(Ⅱ)



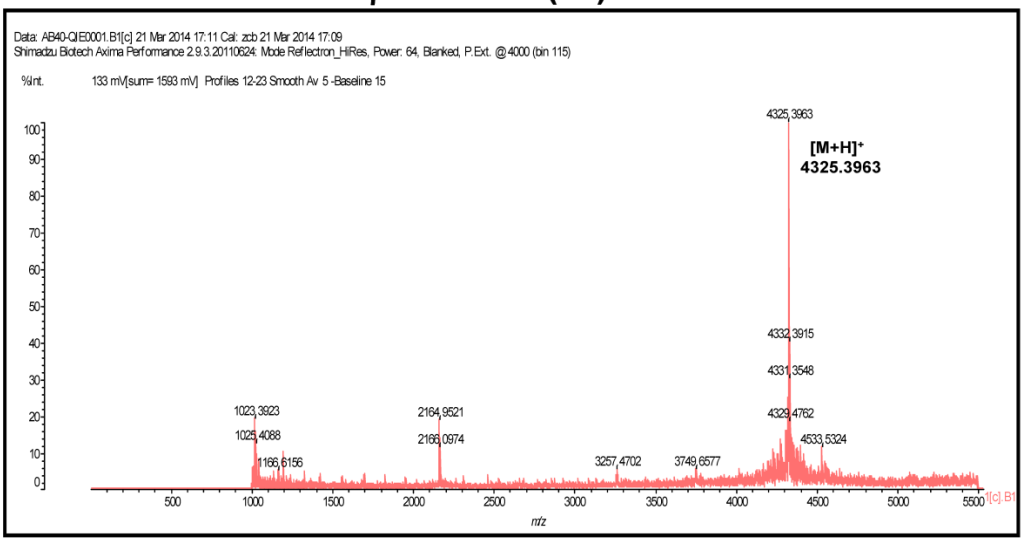
MALDI-TOF/MS of Aβ42 incubated with I1-Cu(II) for 24 h.

MALDI-TOF-MS of Aβ40



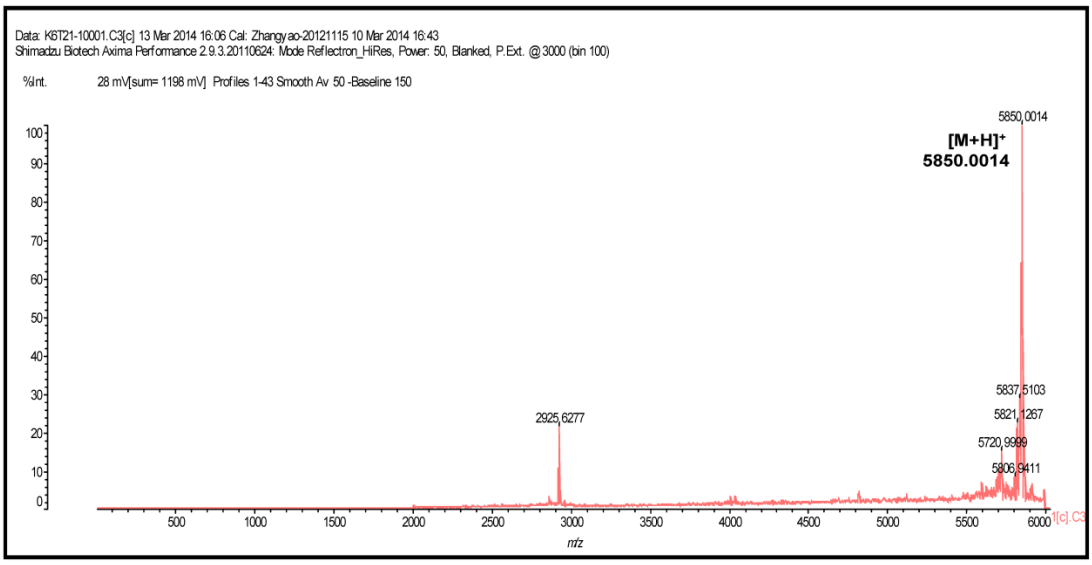
MALDI-TOF/MS of Aβ40 protein, Calcd. for Aβ40 ($C_{194}H_{295}N_{53}O_{58}S_1$) 4329.8 $[M+H]^+$; found 4328.6341 $[M+H]^+$.

MALDI-TOF-MS of Aβ40+I1-Cu(Ⅱ)



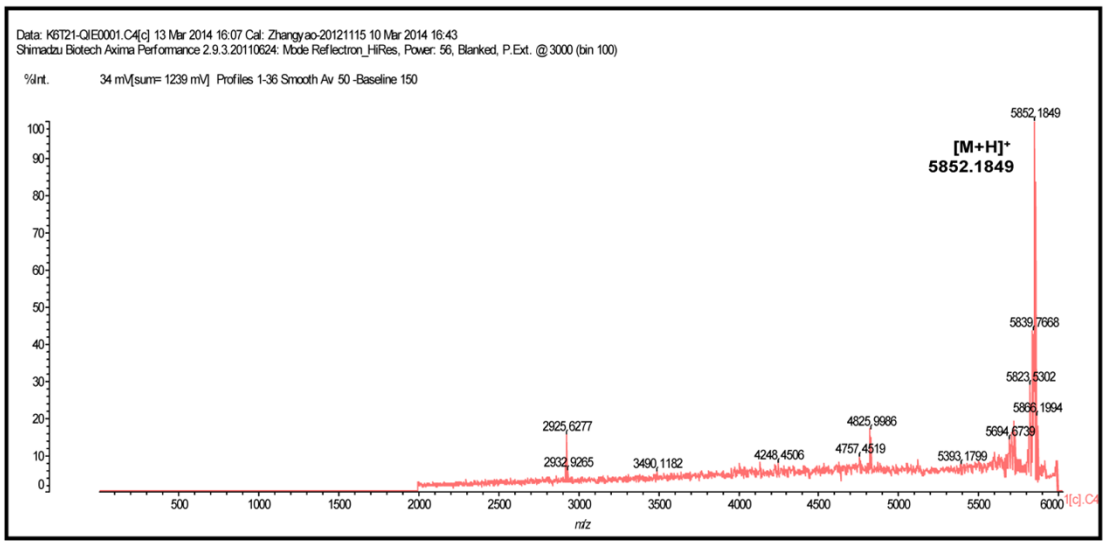
MALDI-TOF/MS of Aβ40 incubated with I1-Cu(II) for 24 h.

MALDI-TOF-MS of RCP1



MALDI-TOF/MS of RCP1, Calcd. for RCP1 ($C_{265}H_{425}N_{71}O_{78}$) 5850.15 $[M+H]^+$, found 5850.0014 $[M+H]^+$.

MALDI-TOF-MS of RCP1+I1-Cu(Ⅱ)



MALDI-TOF/MS of RCP1 incubated with I1-Cu(II) for 24 h.