

Supporting Information

Antibodies and Cell cultures

Monoclonal antibody (mAb) Tau-1 against tau non-phosphorylated at Ser198/199/202 was from Chemicon International, Inc. (Temecula, CA). mAb Tau-5 against total tau was from NeoMarkers, Inc. (Fremont, CA). pAb pT231 against tau phosphorylated at Thr 231, pAb pS262 against tau phosphorylated at Ser 262 and pAb pT404 against tau phosphorylated at Thr 404 were purchased from Signalway Antibody Co., Ltd (Pearland, TX). pS396 against tau phosphorylated at Ser 396 was purchased from BioSource International, Inc. (Camarillo, CA). pAb against total GSK-3 β and pS9- GSK-3 β against GSK-3 β phosphorylated at serine 9 were from Cell Signaling Technology, Inc. (Beverly, MA). The bicinchoninic acid protein detection kit and chemiluminescent substrate kit were from Pierce Chemical Company (Rockford, IL). DL-Hcy was from Sigma Chemical CO (St. Louis, MO). Other reagents were of the highest quality available and obtained from commercial sources. mAb DM1A to α -tubulin was purchased from Sigma (St. Louis, MO).

HEK2993/tau cells were cultured in 90% DMEM supplemented with 10% FBS with 100 U/ml penicillin and 100 mg/ml streptomycin in a humidified atmosphere of 5% CO₂ in air at 37°C. The cells were plated onto six-well plates overnight and [Fe₂L₃]⁴⁺ or [Ni₂L₃]⁴⁺ was administered the next day.

Animals and Drug Administration

Male Sprague-Dawley rats (3 to 4 months old, 280±20 g) supplied by the Experimental Animal Central of Tongji Medical College were housed with accessible food and water ad libitum^{1,2}. All animal experiments were performed according to the

“Policies on the Use of Animals and Humans in Neuroscience Research” revised and approved by the Society for Neuroscience in 1995. Rats were kept in cages under a 12:12 light-dark cycle with the light on from 7:00 AM to 7:00 pm. For the time course study, we injected the rats by vena caudalis with Hcy (400 µg/kg/day) or saline with the same volume for 14 days, respectively^{1,2}. while Hcy was continued to inject for 7 days, 1 ml [Fe₂L₃]⁴⁺ or [Ni₂L₃]⁴⁺ (100 µM/day) was administrated by intraperitoneal injection injected for 7 days. The injection was performed each day from 9:00 AM to 2:00 PM and was the animals were sacrificed 24 hours after the final injection following morris water maze test.

Tg2576 mice (12 month old) were treated with 0.14 ml [Fe₂L₃]⁴⁺ or [Ni₂L₃]⁴⁺ (100 µM/day), or saline with the same volumn by intraperitoneal injecton for 7 days then were sacrificed.

Morris Water Maze Test

Spatial memory was measured by Morris water maze test.^{2,3} The temperature of the room and the water was kept at 24±2 °C. Before each experiment (2 hours), the rats were brought to the site to allow them to be acclimated. For spatial learning, rats were trained in water maze to find a hidden platform for six consecutive days, four trials per day with a 30-s interval from 14:00 to 20:00 pm. On each trial, the rat started from one of the middle of the four quadrants facing the wall of the pool and ended when the animal climbed on the platform. The rats were not allowed to search for the platform more than 60 s, after which they were guided to the platform. Through these training sessions, rats acquired spatial memory about location of the safe platform.

The swimming pathways and latencies of the rats to find the hidden platform were recorded each day. The pathway and the length that the rats passed through the previous platform quadrant were recorded by a video camera fixed to the ceiling of the room, 1.5 m from the water surface. The camera was connected to a digital-tracking device attached to an IBM computer loaded with the water maze software. The longer a rat stayed in the previous platform-located quadrant, the better it scored the spatial memory.

Western blotting

Western blotting was performed according to methods established in our laboratory. Briefly, the brain extracts or cell lines lysates were mixed with sample buffer containing 50 mmol/L Tris HCl (pH 7.6), 2% SDS, 10% glycerol, 10 mmol/L DTT, and 0.2% bromophenol blue and boiled for 5 min. The proteins were separated by 10% SDS/PAGE and transferred to PVDF membrane. Immunostaining was visualized with a chemiluminescent substrate kit and CL-XPosure Film and quantitatively analyzed by digital science 1D software (Eastman Kodak, Rochester, NY). Band intensity was measured as the sum optical density and expressed as a level relative to each control. Tau phosphorylation levels were normalized to the total tau level.

ELISA assay

The levels of A β 1-42 were analyzed by ELISA from freshly frozen hippocampal samples. For detection of A β levels, the homogenized hippocampi were centrifuged for 1 h at 48,000 g at 4°C, and supernatant was taken for the analysis of soluble A β species, whereas the remaining pellet was suspended in 5 mol/L guanidine-HCl/50

mM Tris·HCl, pH 8.0. The levels of A β 1-42 were quantified by using Signal Select Beta Amyloid ELISA kits⁴ according to manufacturer protocol (Biosource International). Standard A β peptide was purchased from Sigma-Aldrich[®] Co., SL, USA.

Statistic Analysis

Data were expressed as mean \pm SD and analyzed using SPSS 12.0 statistical software (SPSS Inc., Chicago, Illinois). The one-way analysis of variance procedure, followed by least significant difference post hoc tests, was used to determine the statistical significance of differences of the means.

1. C. E. Zhang, Q. Tian, W. Wei, J. H. Peng, G. P. Liu, X. W. Zhou, Q. Wang, D. W. Wang, J. Z. Wang, *Neurobiol Aging*, 2008, **29**, 1654.
2. S. J. Liu, A. H. Zhang, H. L. Li, Q. Wang, H. M. Deng, W. J. Netzer, H. Xu, J. Z. Wang, *J. Neurochem.*, 2003, **87**, 1333.
3. R. Morris, *J. Neurosci. Methods*, 1984, **11**, 47.
4. K. Kanninen, R. Heikkinen, T. Malm, T. Rolova, S. Kuhmonen, H. Leinonen, S. Ylä-Herttua, H. Tanila, A. L. Levonen, M. Koistinaho, J. Koistinaho, *Proc Natl Acad Sci USA*, 2009, **106**, 16505.
5. T. Tomiyama, A. Shoji, K. Kataoka, Y. Suwa, S. Asano, H. Kaneko, N. Endo, *J. Biol. Chem.* 1996, **271**, 6839-6844.

Table S1 Stern-Volmer quenching constants of $[\text{Ni}_2\text{L}_3]^{4+}$ and $[\text{Fe}_2\text{L}_3]^{4+}$ complexes for Bis-ANS fluorescence displacement.

Complex	A β 1-40	A β 12-28
$[\text{Ni}_2\text{L}_3]^{4+}$	$1.0 \times 10^6 \text{ M}^{-1}$	$1.6 \times 10^6 \text{ M}^{-1}$
$[\text{Fe}_2\text{L}_3]^{4+}$	$2.7 \times 10^6 \text{ M}^{-1}$	$3.2 \times 10^6 \text{ M}^{-1}$

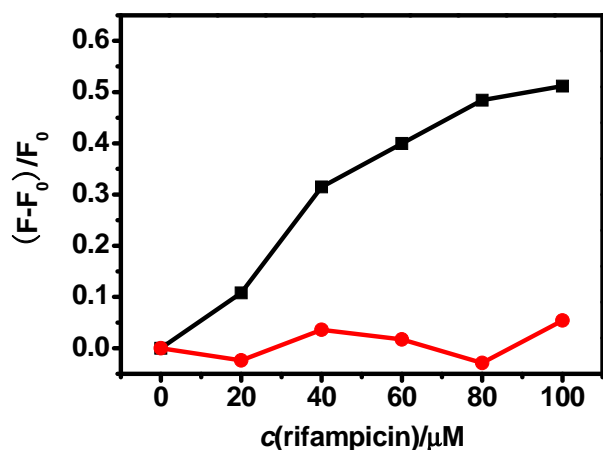


Fig. S1 Validation of the A β -ECFP screen system using a well known A β amyloid inhibitor, rifampicin⁵, as reference. Rifampicin enhanced the fluorescence intensity of A β -ECFP (black square) with the increase of the concentration while hardly changed the fluorescence intensity of ECFP (red circle). F, the fluorescence intensity in the presence of rifampicin; F₀, the fluorescence intensity in the absence of rifampicin.

5. T. Tomiyama, A. Shoji, K. Kataoka, Y. Suwa, S. Asano, H. Kaneko, N. Endo, *J. Biol. Chem.* **1996**, 271, 6839-6844.

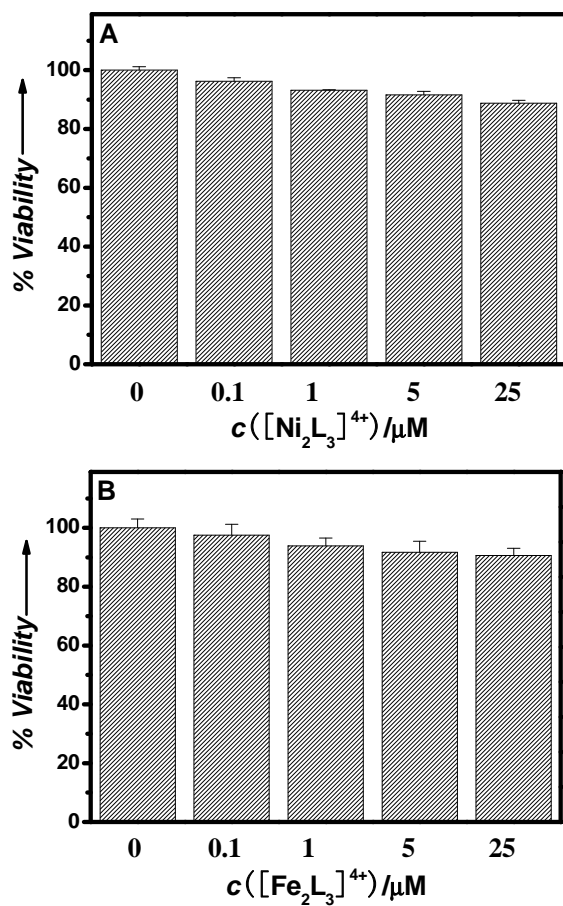


Fig. S2 Effect of Complex $[\text{Ni}_2\text{L}_3]^{4+}$ and $[\text{Fe}_2\text{L}_3]^{4+}$ on PC12 cell viability determined by MTT method.

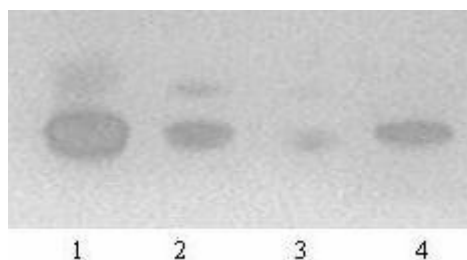


Fig. S3 SDS Gel electrophoresis of the resuspended A β peptide. 50 μ M A β 1-40 peptide alone (Lane 1) or with equimolar [Ni₂L₃]⁴⁺ (Lane 2), [Fe₂L₃]⁴⁺ (Lane 3). These samples were incubated at 37°C for 7 days and separated by centrifugation. The pellets were resuspended and boiled after the addition of sample buffer. Samples were run on a 12% Tris-tricine SDS gel at 100 V for 1 hour, followed by silver staining. A non-incubated fresh A β 40 sample was also prepared in parallel and shown in Lane 4.

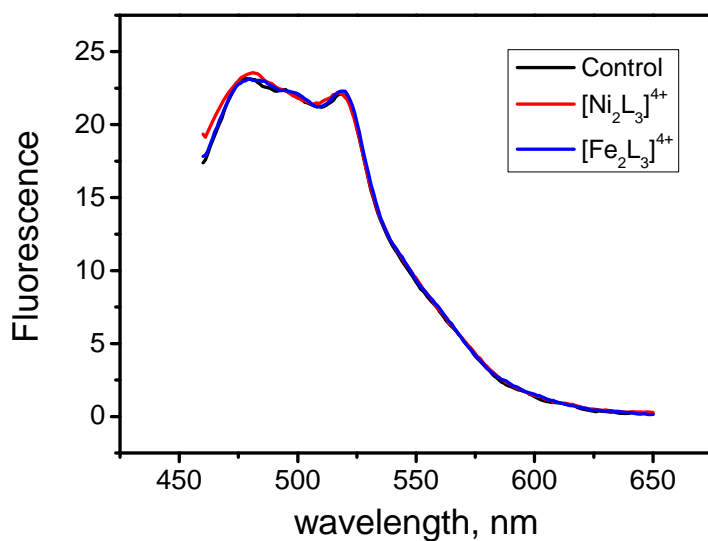


Fig. S4 The fluorescence spectra of 10 μ M Th T in the absence (black) or presence of 1.25 μ M complex: [Ni₂L₃]⁴⁺ (red); [Fe₂L₃]⁴⁺ (blue).

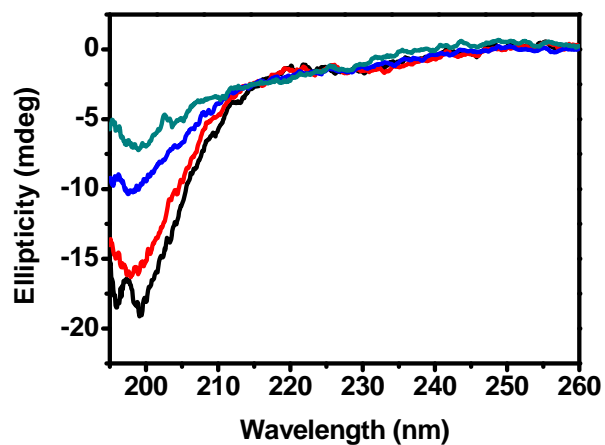


Fig. S5 CD spectra of 50 μM Aβ1-40 (A) in the presence of 25 μM rifampicin as a function of time. Spectra were taken at 0 day (black), 1 day (red), 5 days (blue), 10 days (dark cyan).

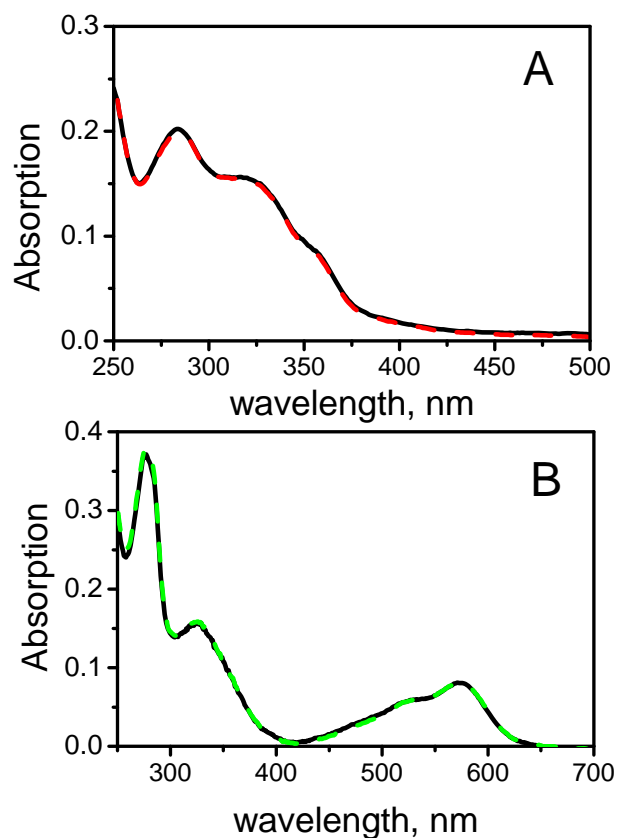


Fig. S6 Absorption spectra of 5 μM $[\text{Ni}_2\text{L}_3]^{4+}$ (A, dashed red line) or $[\text{Fe}_2\text{L}_3]^{4+}$ (B, dashed green line) in the absence (solid line) or presence of equimolar A β 1-40.

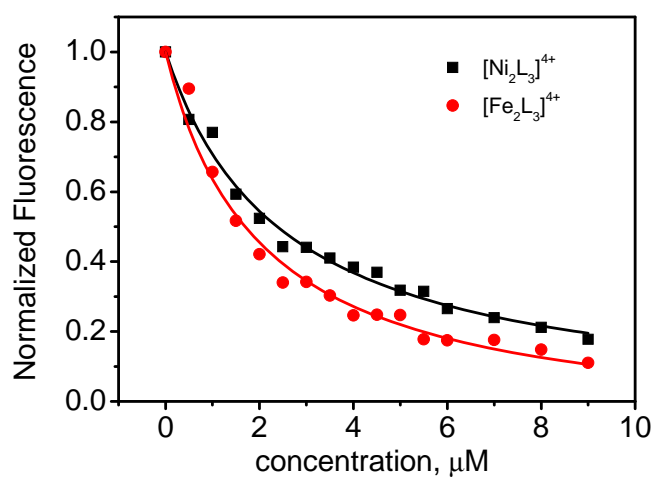


Fig. S7 Fluorescence titration of A β 1-40 (3 μM) with various concentrations of metallo-supramolecular complex in 20 mM Tris buffer. The excitation wavelength was 278 nm and the emission intensity at 306 nm was used for analysis.

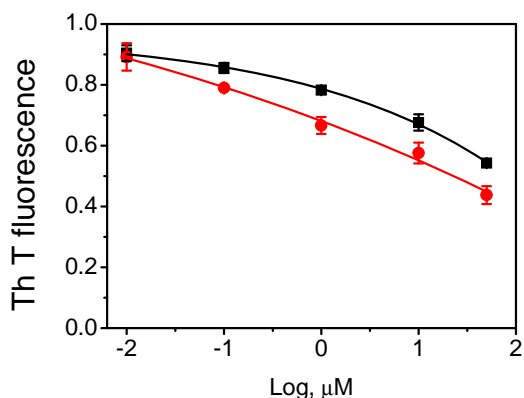


Fig. S8 Concentration-dependent inhibition of A β 25-35 fibrillogenesis by $[\text{Ni}_2\text{L}_3]^{4+}$ (squares) and $[\text{Fe}_2\text{L}_3]^{4+}$ (circles). Details were described in experimental section.

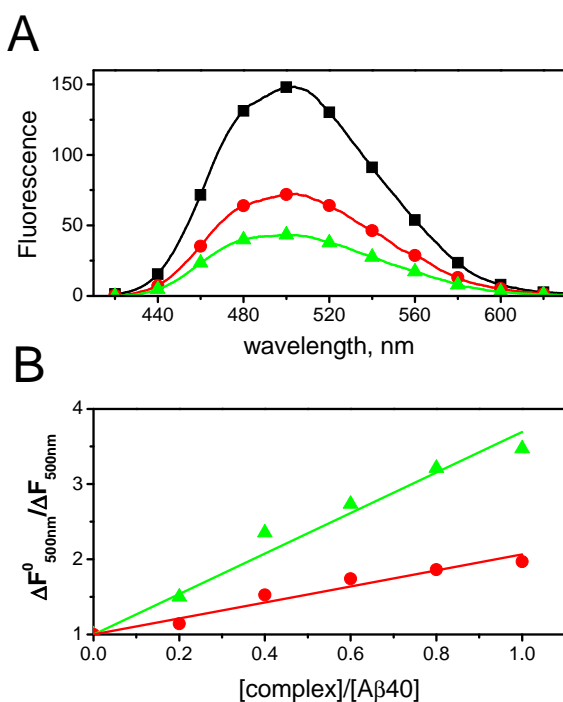


Fig. S9 A, Emission spectra of 20 μM Bis-ANS with 1 μM A β 1-40 in the absence (squares) or presence of 1 μM $[\text{Ni}_2\text{L}_3]^{4+}$ (circles), and 1 μM $[\text{Fe}_2\text{L}_3]^{4+}$ (triangles). B, Stern-Volmer plots of Bis-ANS fluorescence quenched by $[\text{Ni}_2\text{L}_3]^{4+}$ (circles), $[\text{Fe}_2\text{L}_3]^{4+}$ (triangles).

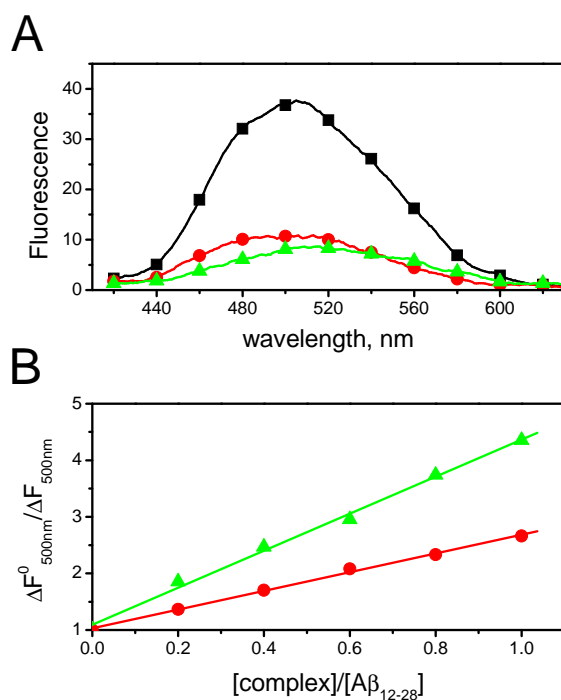


Fig. S10 A, Emission spectra of 20 μM Bis-ANS with 1 μM Aβ₁₂₋₂₈ in the absence (squares) or presence of 1 μM [Ni₂L₃]⁴⁺ (circles), 1 μM [Fe₂L₃]⁴⁺ (triangles). B, Stern-Volmer plots of Bis-ANS fluorescence quenched by [Ni₂L₃]⁴⁺ (circles), [Fe₂L₃]⁴⁺ (triangles).

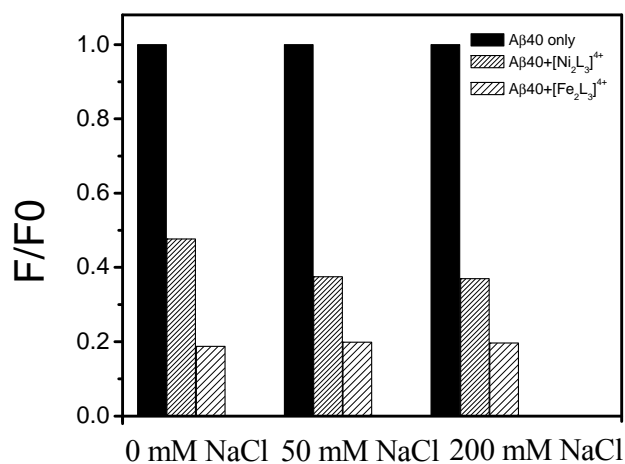


Fig. S11 Ionic strength and effect of the metal complex on the amyloid fibril inhibition. Samples of 50 μM A β 1-40 in the absence and presence of 10 μM $[\text{Ni}_2\text{L}_3]^{4+}$ or $[\text{Fe}_2\text{L}_3]^{4+}$ were incubated under three different ionic strengths: 0 mM NaCl, 50 mM NaCl, 200 mM NaCl, and their Th T fluorescence were measured. The inhibition efficiency of $[\text{Ni}_2\text{L}_3]^{4+}$ or $[\text{Fe}_2\text{L}_3]^{4+}$ shows no difference under different ionic strength.

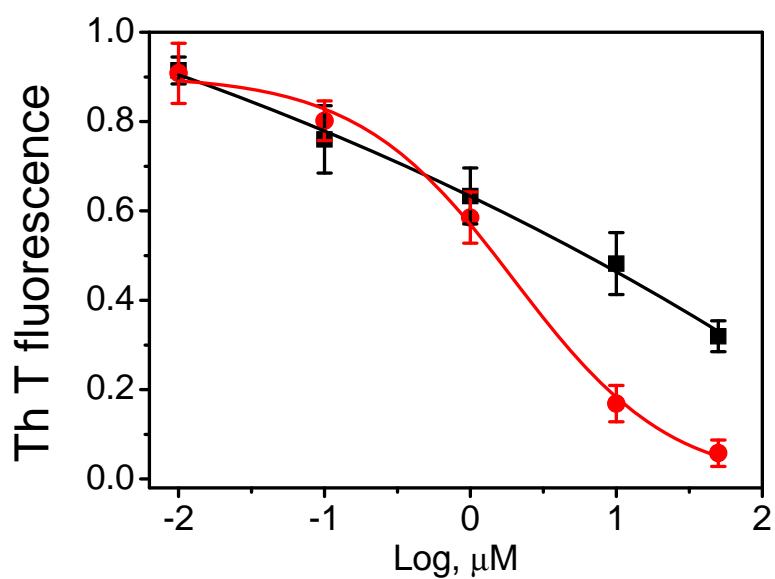


Fig. S12 Dose-dependent destabilization of preformed A β 1-40 fibrils by [Ni₂L₃]⁴⁺ (squares) and [Fe₂L₃]⁴⁺ (circles). 50 μ M fibrillar A β 1-40 was mixed with 0, 0.01, 0.1, 1, 10, and 50 μ M [Ni₂L₃]⁴⁺ or [Fe₂L₃]⁴⁺ complex. Aliquots were kept at 37°C for 5 hours after being mixed. All the reactions were conducted without agitation. Details were described in experimental section.

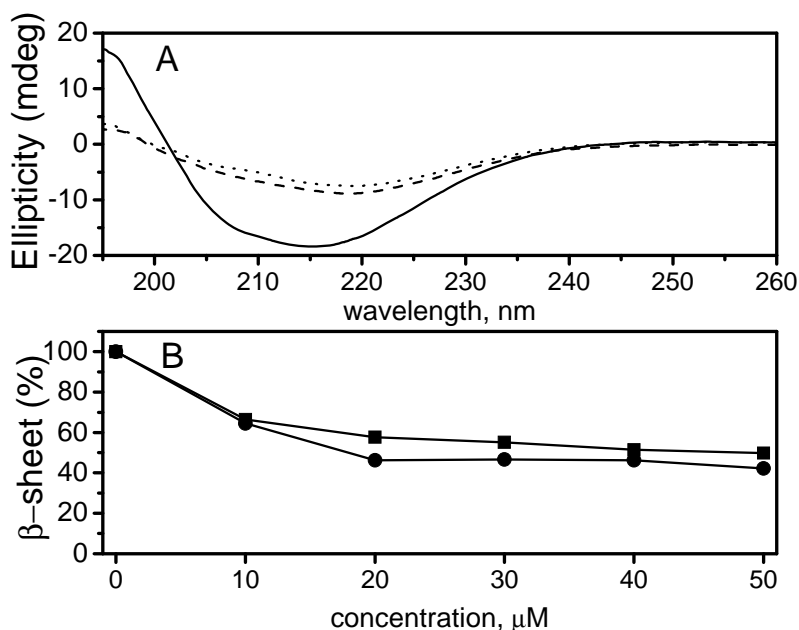


Fig. S13 Influence of metallo-supramolecular complex on the secondary structure of fAβ1-40 studied by CD spectra. All samples were measured after incubation for 5 hours. (A) CD spectra of 50 μM Aβ1-40 alone (solid line), or with 50 μM [Ni₂L₃]⁴⁺ (dashed line), [Fe₂L₃]⁴⁺ (dotted line). (B) Plot of β-sheet percentage of 50 μM Aβ1-40 as a function of the concentration of [Ni₂L₃]⁴⁺ (black square), [Fe₂L₃]⁴⁺ (red circles). All the background was subtracted. For comparison, Aβ1-40 alone after incubation was assumed to be 100% in β-sheet. CD intensity at 218 nm was used for analysis.

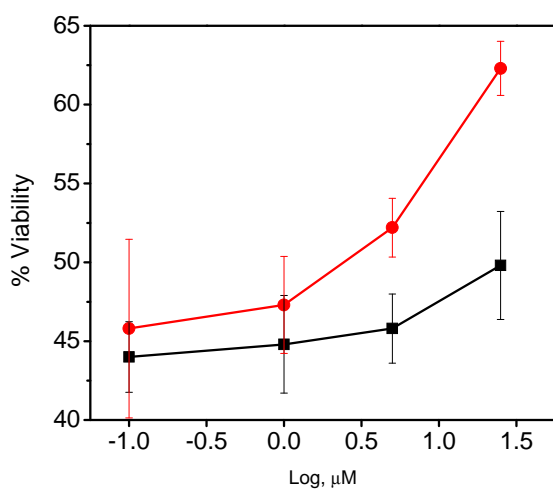


Fig. S14 Effect of the metal complex on aged $\text{fA}\beta_{1-40}$ induced cytotoxicity in PC12 cells determined by MTT and data points (\blacksquare), $[\text{Ni}_2\text{L}_3]^{4+}$; (\bullet), $[\text{Fe}_2\text{L}_3]^{4+}$, are the mean values \pm SEM from three independent experiments.

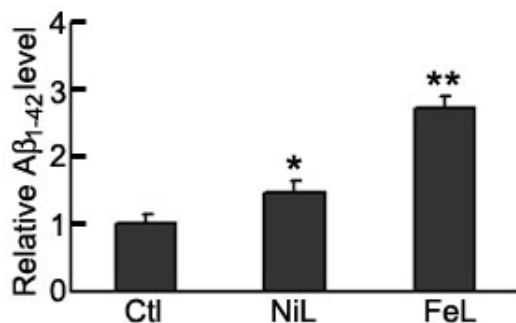


Fig. S15 $[\text{Ni}_2\text{L}_3]^{4+}$ and $[\text{Fe}_2\text{L}_3]^{4+}$ increase soluble $\text{A}\beta_{1-42}$ level in N2a/APP cell medium. The N2a/APP cells were treated with $[\text{Ni}_2\text{L}_3]^{4+}$ or $[\text{Fe}_2\text{L}_3]^{4+}$ ($10 \mu\text{M}$) for 24 h, then $\text{A}\beta_{1-42}$ level in the cell medium was measured by ELISA. *, $p < 0.05$; **, $p < 0.01$ vs Ctl (control).