Supplementary Information

A nanoscale polymolybdate builded by two hexavacant Keggin-type fragments *via* a novel $\{Ca_6P_6O_{38}\}$ cluster with *β*-sheet conformation modulation ability

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1. Supplementary Tables and Figures

Table S1 Crystallographic data and structural refinements for CaPM

Empirical formula	$Ca_3K_5Mo_6NaO_{49}P_4$
Formula weight	1822.25
Crystal system	Triclinic
Space group	*P-1*
a / Å	12.8983(13)
b / Å	13.4856(14)
c / Å	16.2197(17)
α / deg	113.259(2)
β / deg	91.930(2)
γ / deg	115.225(1)
V / Å^3	2274.5(4)
Z	2
D_c / g cm^{-3}	2.661
μ / mm^{-1}	2.679
T / K	296(2)
Limiting indices	–15 ≤ h ≤ 14
	–16≤ *k* ≤ 14
	–14 ≤ *l* ≤ 19
Measured reflections	2274.5(4)
Independent reflections	7980

R_{int}	0.0151
Data / restrains / parameters	7980 / 0 / 613
GOF on F^2	1.062
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0344
	wR_2 = 0.0878
R indices (all data)	R_1 = 0.0397
	wR_2 = 0.0931
Completeness	98.6 %

Table S2 Selected bond length (Å) for CaPM

Mo(1)-O(25)	1.704(4)	Mo(1)-O(21)	1.919(4)	Mo(1)-O(17)	2.182(3)
Mo(1)-O(1)	1.715(4)	Mo(1)-O(14)	1.949(3)	Mo(1)-O(33)	2.343(3)
Mo(2)-O(26)	1.703(4)	Mo(2)-O(16)	1.921(4)	Mo(2)-O(8)	2.200(3)
Mo(2)-O(2)	1.713(4)	Mo(2)-O(21)	1.950(3)	Mo(2)-O(32)	2.346(3)
Mo(3)-O(3)	1.706(4)	Mo(3)-O(7)	1.932(4)	Mo(3)-O(13)	2.191(3)
Mo(3)-O(18)	1.715(4)	Mo(3)-O(28)	1.935(3)	Mo(3)-O(31)	2.356(3)
Mo(4)-O(19)	1.714(4)	Mo(4)-O(20)	1.906(3)	Mo(4)-O(22)	2.202(3)
Mo(4)-O(4)	1.717(4)	Mo(4)-O(28)	1.943(3)	Mo(4)-O(31)	2.308(3)
Mo(5)-O(30)	1.710(4)	Mo(5)-O(14)	1.921(3)	Mo(5)-O(9)	2.217(3)
Mo(5)-O(5)	1.712(4)	Mo(5)-O(7)	1.940(4)	Mo(5)-O(33)	2.294(3)
Mo(6)-O(24)	1.709(4)	Mo(6)-O(16)	1.910(4)	Mo(6)-O(12)	2.205(3)
Mo(6)-O(6)	1.719(4)	Mo(6)-O(20)	1.950(3)	Mo(6)-O(32)	2.298(3)
Ca(1)-O(15)#4	2.350(3)	Ca(1)-O(1W)	2.442(4)	Ca(1)-O(34)	2.547(4)
Ca(1)-O(12)	2.382(4)	Ca(1)-O(23)	2.527(4)	Ca(1)-O(20)	2.729(4)
Ca(1)-O(22)	2.403(4)	Ca(1)-O(10)	2.533(4)	Ca(2)-O(23)	2.558(4)
Ca(2)-O(2W)	2.355(5)	Ca(2)-O(17)	2.422(4)	Ca(2)-O(15)	2.587(4)
Ca(2)-O(10)#4	2.360(3)	Ca(2)-O(34)	2.539(4)	Ca(2)-O(21)	2.648(4)
Ca(2)-O(8)	2.421(4)	Ca(3)-O(13)	2.406(4)	Ca(3)-O(10)	2.532(4)
Ca(3)-O(23)#4	2.353(3)	Ca(3)-O(9)	2.431(4)	Ca(3)-O(34)	2.545(4)
Ca(3)-O(3W)	2.369(4)	Ca(3)-O(15)	2.499(4)	Ca(3)-O(7)	2.751(4)
P(1)-O(34)	1.532(3)	P(1)-O(31)	1.536(4)	P(2)-O(11)	1.510(4)
P(1)-O(33)	1.540(4)	P(1)-O(32)	1.541(4)	P(2)-O(15)	1.550(4)
P(2)-O(17)	1.549(4)	P(3)-O(29)	1.503(4)	P(3)-O(13)	1.550(4)
P(2)-O(9)	1.554(4)	P(3)-O(10)	1.546(3)	P(3)-O(22)	1.551(4)
P(4)-O(27)	1.520(4)	P(4)-O(23)	1.545(3)		
P(4)-O(8)	1.542(4)	P(4)-O(12)	1.548(4)		

Symmetry codes: #4 -x,-y+2,-z+1

Table S3 Bond valence and Σs of Mo, P, and Ca in CaPM

Bond	Valence	Bond	Valence	Bond	Valence	Atom	Σs
Mo(1)-O(25)	1.764	Mo(1)-O(21)	0.958	Mo(1)-O(17)	0.452		
Mo(1)-O(1)	1.709	Mo(1)-O(14)	0.877	Mo(1)-O(33)	0.282	Mo(1)	6.041
Mo(2)-O(26)	1.789	Mo(2)-O(16)	0.944	Mo(2)-O(8)	0.428		
Mo(2)-O(2)	1.719	Mo(2)-O(21)	0.877	Mo(2)-O(32)	0.281	Mo(2)	6.038
Mo(3)-O(3)	1.769	Mo(3)-O(7)	0.923	Mo(3)-O(13)	0.439		
Mo(3)-O(18)	1.709	Mo(3)-O(28)	0.912	Mo(3)-O(31)	0.275	Mo(3)	6.028
Mo(4)-O(19)	1.719	Mo(4)-O(20)	0.991	Mo(4)-O(22)	0.426		
Mo(4)-O(4)	1.709	Mo(4)-O(28)	0.894	Mo(4)-O(31)	0.312	Mo(4)	6.051
Mo(5)-O(30)	1.738	Mo(5)-O(14)	0.952	Mo(5)-O(9)	0.409		
Mo(5)-O(5)	1.729	Mo(5)-O(7)	0.899	Mo(5)-O(33)	0.324	Mo(5)	6.052
Mo(6)-O(24)	1.738	Mo(6)-O(16)	0.980	Mo(6)-O(12)	0.422		
Mo6)-O(6)	1.689	Mo(6)-O(20)	0.877	Mo(6)-O(32)	0.322	Mo(6)	6.029
Ca(1)-O(15)	0.339	Ca(1)-O(22)	0.298	Ca(1)-O(23)	0.220		
Ca(1)-O(12)	0.313	Ca(1)-O(1W)	0.271	Ca(1)-O(10)	0218		
Ca(1)-O(34)	0.209	Ca(1)-O(20)	0.134			Ca(1)	2.001
Ca(2)-O(2W)	0.334	Ca(2)-O(8)	0.285	Ca(2)-O(34)	0.214		
Ca(2)-O(10)	0.330	Ca(2)-O(17)	0.285	Ca(2)-O(23)	0.204		
Ca(2)-O(15)	0.189	Ca(2)-O(21)	0.162			Ca(2)	2.003
Ca(3)-O(23)	0.335	Ca(3)-O(13)	0.295	Ca(3)-O(15)	0.236		
Ca(3)-O(3W)	0.325	Ca(3)-O(9)	0.277	Ca(3)-O(10)	0.218		
Ca(3)-O(34)	0.210	Ca(3)-O(7)	0.127			Ca(3)	2.023
P(1)-O(34)	1.256	P(1)-O(31)	1.244				
P(1)-O(33)	1.244	P(1)-O(32)	1.228			P(1)	4.972
P(2)-O(11)	1.327	P(2)-O(17)	1.204				
P(2)-O(15)	1.207	P(2)-O(19)	1.191			P(2)	4.930
P(3)-O(29)	1.361	P(3)-O(13)	1.201				
P(3)-O(10)	1.210	P(3)-O(22)	1.198			P(3)	4.969
P(4)-O(27)	1.304	P(4)-O(23)	1.219				

P(4)-O(8)	1.222	P(4)-O(12)	1.210		P(4)	4.995

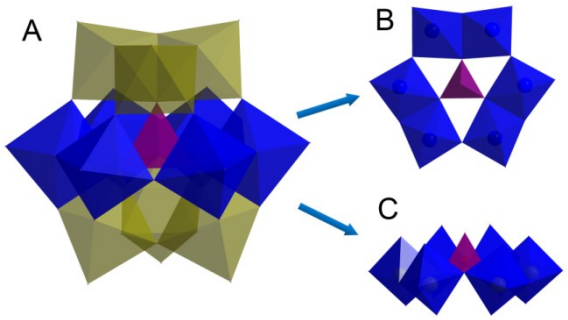

Fig. S1 The anatomical view of the hexavacant building block of $[PMo_6O_{28}]^{15-}$ (B and C) derived from B-α-Keggin-type polyanion (A).

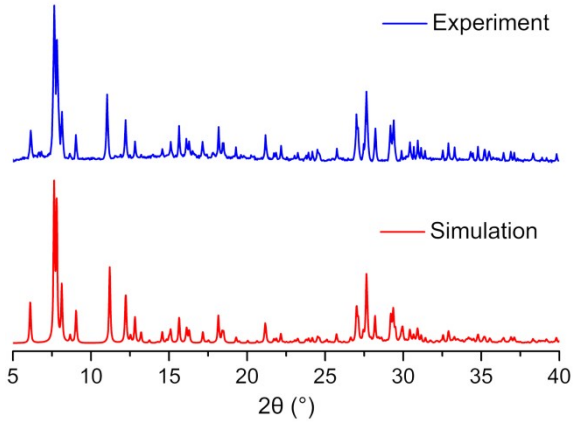

Fig. S2 Comparison of the experimental and simulated PXRD patterns of CaPM.

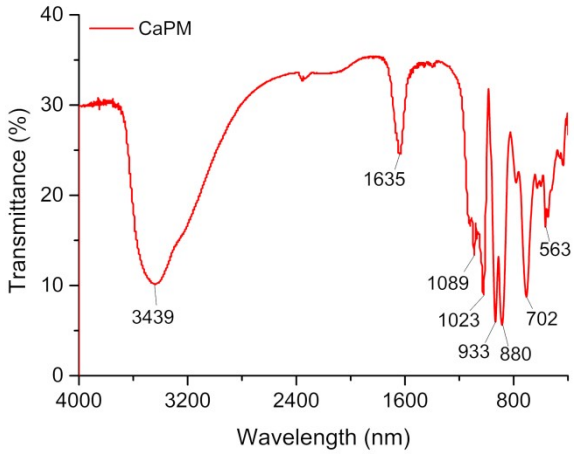

Fig. S3 IR spectrum for CaPM.

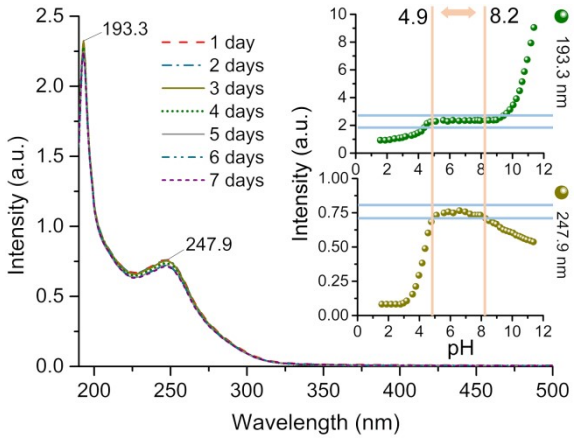

Fig. S4 UV-Vis spectra of CaPM in deionized water for 1–7 days; and pH stability of CaPM (inset).

As shown in Fig. S5, the TGA curve of CaPM displays two stages of weight loss, giving a total loss of 14.92 % in the temperature range of 25–300 °C. The first stage from 25 to 150 °C is attributed to the loss of twenty four lattice water molecules, and the observed weight loss 11.87 % is consistent with the calculated value 11.69 %. The second stage with the weight loss of 3.05 % occurs between 210 and 285 °C, which may be assigned to the removal of six structural water molecule (calcd. 2.92 %).

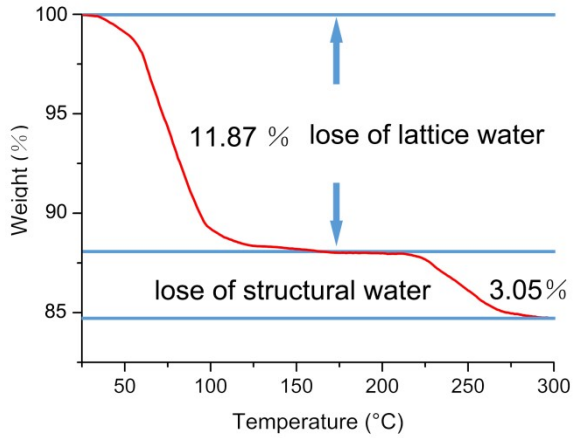

Fig. S5 TGA curve of CaPM.

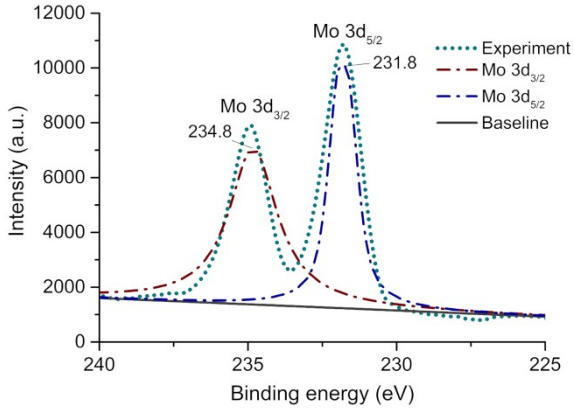

Fig. S6 X-ray photoelectron spectrum (XPS) and the fitted curves of Mo in CaPM.

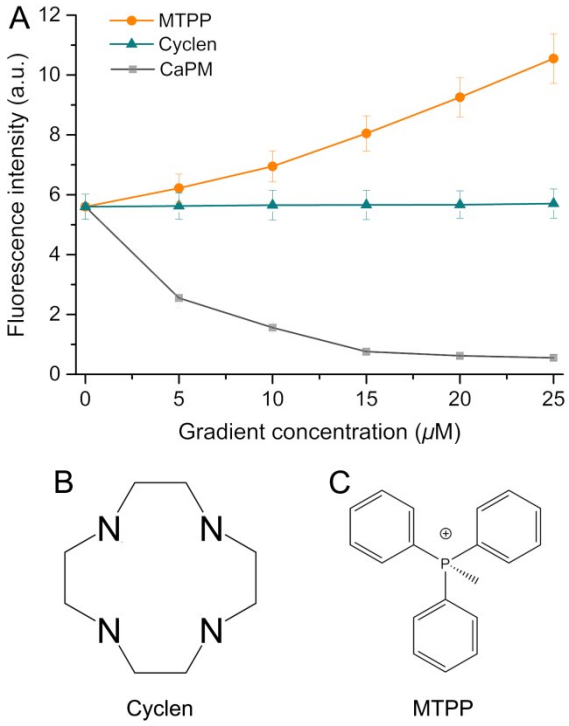

Fig. S7 (A) The effect of CaPM, 1,4,7,10-tetraazacyclododecane (Cyclen) and methyltriphenylphosphonium bromide (MTPP) on the Aβ40 (20 μM) aggregates determined by ThT assay (λ_{ex} = 415 nm, λ_{em} = 480 nm). The structure diagram of Cyclen (B) and MTPP (C).

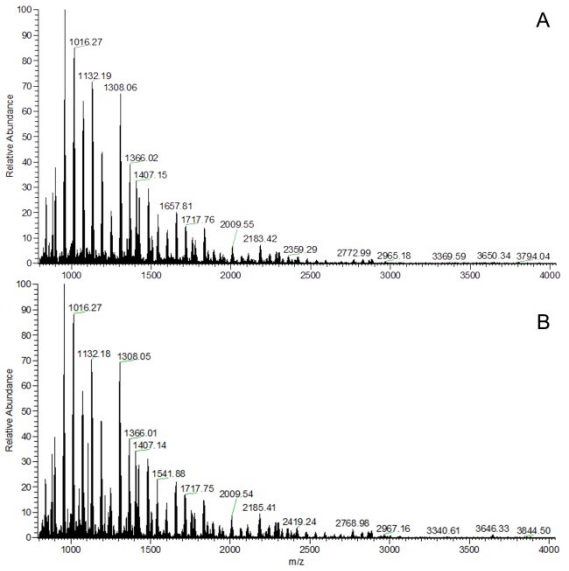

Fig. S8 ESI-MS spectra of A*β*40 in the absence (A) and presence (B) of CaPM after incubation at 37 °C and pH 7.4 for 24 h.

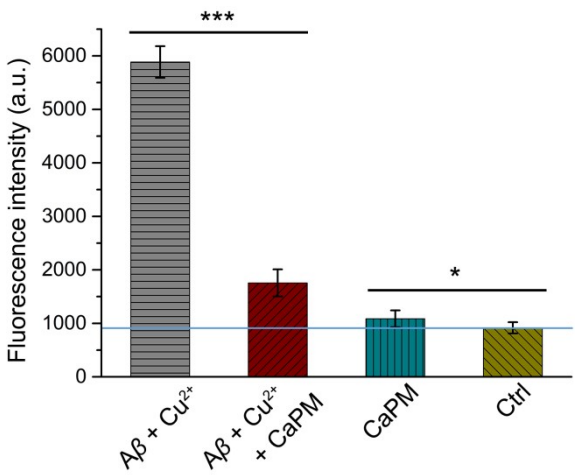

Fig. S9 Fluorescence intensity of DCF (λ_{ex} = 485 nm, λ_{em} = 525 nm) incubated by Cu^{2+} + Aβ40, Cu^{2+} + Aβ40 + CaPM, CaPM alone and control group for 24 h.

2. Materials and Methods

2.1 *Materials*

In this study, reagents used are all of analytical grade, and purchased from commercial suppliers and used as received unless otherwise stated. Human Aβ40 was purchased from Macklin agent Ltd. (China), which were verified by HPLC and electrospray ionization mass spectrometry (ESI-MS). 2′,7′-dichlorofluorescin diacetate (DCFH-DA), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT), nerve growth factor 7S (NGF-7S) and tris(hydromethyl)aminomethane (Tris) were purchased from Sigma-Aldrich Inc. (USA). Calcium chloride ($CaCl_2$), sodium molybdate ($Na_2MoO_4{\cdot}2H_2O$), trisodium phosphate ($Na_3PO_3{\cdot}12H_2O$), potassium chloride (KCl), zinc acetate ($Zn(OAC)_2$), and copper(II) chloride ($CuCl_2$) were purchased from Macklin agent Ltd. (China). Pheochromocytoma cells (PC12 cells) were purchased from American Type Culture Collection (ATCC). Stock solutions of Aβ40, $Zn(OAC)_2$ and $CuCl_2$ were prepared according to the reported procedures.[1] All the solutions were prepared with Milli-Q water and filtered through a 0.22 μm filter (Millipore).

2.2 *Synthesis of $K_{11}[Ca_6P_6O_{12}(H_2O)_6][H_{0.5}PMo_6O_{28}]_2 \cdot 22H_2O$ (CaPM)*

A mixture of $Na_2MoO_4 \cdot 2H_2O$ (14.50 g, 60.0 mmol), $Na_3PO_3 \cdot 12H_2O$ (22.80 g, 60.0 mmol), $CaCl_2$ (9.85 g, 60.0 mmol) were dissolved in purified water (200 mL). The pH of above suspension was adjust to 5.60 by 0.1 M HCl. The solution reacts under reflux for 2 hours. After being cooled to room temperature, KCl (2.60 g, 35.0 mmol) was added and the solution was left to evaporate slowly at ambient temperature. After two weeks, colorless crystals of CaPM were obtained in 27 % yield (Based on $Na_2MoO_4 \cdot 2H_2O$).

2.3 *Physical measurements*

DCF fluorescence were conducted on a Thermo Scientific Varioskan Flash microplate reader. The morphological analysis were examined on a JEOL JEM-2100 LaB6 (HR) transmission electron microscope. The CD spectrum of the sample solution was measured on a JASCO J-810 automatic recording spectropolarimeter (Tokyo, Japan). X-ray photoelectron spectrum (XPS) was tested on a PHI5000 VersaProbe instrument. The TGA was tested on a STA449F3 TG-DSC from 25 to 300 °C. The mass spectra were detected on an LCQ Fleet electrospray mass spectrometer from 800 to 4000 M/z. Powder X-Ray diffraction (PXRD) measurements were obtained using a Philips X'pert-MPD instrument with Cu-Kα radiation (λ = 1.54056 Å) at 293 K. UV spectra were recorded on a UV-3600 spectrometer. IR spectra were recorded in the range of 4000–400 cm^{-1} on a Bruker Vector 22 FT-IR spectrophotometer using KBr pellets.

2.4 *X-ray crystallography*

Intensity data of single crystal was collected on a Bruker Apex-2 diffractometer with a CCD detector using graphite monochromatized Mo Kα radiation (λ = 0.71073 Å) at 296 K. Date integration was performed using *SAINT*.[2] Routine Lorentz and polarization corrections were applied. Multiscan absorption corrections were performed using *SADABS*.[3] The structure was solved by direct methods and refined using full-matrix least squares on F^2. The remaining atoms were found from successive full-matrix least-squares refinements on F^2 and Fourier syntheses.

All calculations were performed using the SHELXL-97 program package.[4] No hydrogen atoms associated with the water molecules were located from the difference Fourier map. Positions of the hydrogen atoms attached to the carbon and nitrogen atoms were geometrically placed. All hydrogen atoms were refined isotropically as a riding mode using the default SHELXTL parameters. A summary of crystal data and structure refinements for CaPM is listed in Table S1.

2.5 Comformational modulation of Aβ

ThT fluorescence assay: Aβ40 (20 μM) in Tris buffer solution (20 mM Tris-HCl/150 mM NaCl, 990 μL) was incubated with or without $Zn(OAc)_2$ (4 μL, 10 mM) at 37 °C. Then, CaPM, 1,4,7,10-tetraazacyclododecane (Cyclen) and methyltriphenylphosphonium bromide (MTPP) (with the final concentration of 0–25 μM) were added to each sample respectively and incubated at 37 °C for 24 h. Each sample (300 μL) was injected to a well of a flat-bottomed 96-well black plate (Corning Costar Corp). ThT solution (2 μL, 5 mM) was added to each well simultaneously in the dark and incubated at 37 °C for 1 h. The fluorescence intensity (λ_{ex} = 415 nm, λ_{em} = 485 nm) was recorded by a Varioskan Flash microplate reader (Thermo Scientific). Data were expressed as mean ± standard deviations of at least three independent experiments.

Turbidity assay: Samples were prepared as described above. Each sample was infused to a well of a flat-bottomed 96-well transparent plate. Turbidity of the solutions were recorded using the absorbance at 405 nm. Data were expressed as mean ± standard deviations of three independent experiments.

Morphological analysis: Samples were prepared in the same way as ThT fluorescence assay. A drop of solution (10 μL) was spotted on the 300-mesh carbon-coated copper grids at room temperature. After 2 min, the excess solution was removed. The grids were stained with uranyl

acetate (10 μL, 1%, w/v) for 2 min; Then, they were washed with Milli-Q water (10 μL). The samples were examined on a JEOL JEM-2100 LaB6 (HR) transmission electron microscope.

CD spectra analysis: Aβ40 (20 μM) was dissolved in the Tris buffer solution (20 mM Tris-HCl/150 mM NaCl) and incubated without or with $Zn(OAc)_2$ or $CuCl_2$ (40 μM) at 37 °C, respectively. CaPM (20 μM) was then dropped to each solution and incubated at 37 °C for another 24 h. The CD spectrum of the sample solution was measured on a JASCO J-810 automatic recording spectropolarimeter (Tokyo, Japan) in the range of 190–260 nm. The data acquired in the absence of protein was subtracted from the spectrum.

Mass spectrometry analysis: Aβ40 (20 μM) was dissolved in the buffer solution (20 mM Tris-HCl/150 mM NaCl, pH 7.4, 996 µL) and incubated with CaPM (4 μL, 5 mM) at 37 °C for 24 h. The solution was loaded on the chromatographic column and the saline ions were washed off by Milli-Q water. The products were collected using an acid solution composed of 75% acetonitrile, 20% Milli-Q water and 5% acetic acid. The peptide solution was examined on an LCQ Fleet electrospray mass spectrometer.

2.6 Inhibition of ROS generation and neurotoxicity

Inhibition of ROS generation: DCF stock solution (1 mM) and horseradish peroxidase (HRP) stock solution (4 μM) were prepared with a Tris buffer (20 mM Tris-HCl/150 mM NaCl, pH 7.4) as described in the reported procedures.[5] Samples containing Aβ40 (20 μM) and $CuCl_2$ (40 μM) were incubated without or with CaPM (20 μM) at 37 °C. Then, ascorbate solution (10 μM) was added to each sample and incubated at 37 °C for 10 min. The samples (200 μL) were injected to the wells of a flat-bottomed 96-well black plate. HRP (0.04 μM) and DCF (100 μM) were then injected to each solution and incubated in the dark at 37 °C. Fluorescence intensity (λ_{ex} = 485 nm,

λ_{ex} = 650 nm) was measured by a Varioskan Flash microplate reader (Thermo Scientific) every 10 min from 0 to 2500 min. The other experimental groups were all analyzed according to the above method.

Inhibition of neurotoxicity: The PC12 cells used for the neurotoxicity, and synaptic dysfunction analysis were prepared as described in the previous literature.[6] The effects of CaPM on the inhibition neurotoxicity were evaluated by using the MTT assay. PC12 cells were incubated with Aβ40 (20 μM) alone or with Zn^{2+}- or Cu^{2+}(40 μM)-induced Aβ40 complexes in absence or presence of CaPM (20 μM) for 24 h. Data were expressed as mean ± standard deviations of at least three independent experiments.

Cell morphological analysis: The PC12 cells used for this morphological analysis were prepared as above. After incubation for 24 h, the morphological pictures of those cells were captured by a microscope.

Statistical analysis: The results are obtained from three independent experiments and presented as the mean ± standard deviation of the independent experiments. The results were compared using a twoway ANOVA (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

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