

Supporting Information

Synergistic Effect between KLVFF and Self-Assembly Chaperones on Both Disaggregation of beta-Amyloid Fibrils and Reducing Consequent Toxicity

Aoting Qu, Fan Huang, Hao Zhou, Jiafu Long and Linqi Shi

1. Materials

A β 42 was purchased from GL Biochem Ltd. (Shanghai, China). The purity of the peptide was greater than 98%. ϵ -Caprolactone from Alfa Aesar was distilled under reduced pressure before use. Methoxy poly(ethylene glycol) (CH₃O-PEG114-OH) (Mn=5000 and the polydispersity index (PDI) = 1.05) was purchased from Fluka and used after dried under vacuum. ω -2-Pyridyldithio- α -amino polyethyleneglycol (OPSS-PEG114-NH₂) was purchased from Yarebio Ltd. (Shanghai, China). Hexane-1,6-dioldiacrylate (HDD, 99%), and 4,4'-trimethylene dipiperidine (TDP, 97%) were purchased from J&K and used as received. acryloylchloride (95%), NHS, EDC, TEA, N-acryloyloxysuccinimide (NAS), K-peptide was purchased from GL Biochem Ltd. (Shanghai, China), Stannous octoate (Sn(Oct)₂; 96%, Alfa Aesar), Tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl; 98%, J&K), Thioflavin T (ThT; Sigma-Aldrich), 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP; >99%, Sigma-Aldrich), Dimethyl sulfoxide (DMSO; 99.9%, Alfa Aesar), Proteinase K (from *Tritirachium album*; Genview, U.S.A) were used as received. The cell line PC12 was purchased from cell bank of Shanghai Institutes for Biological Science (SIBS), CAS.

2. Synthesis

Synthesis of PCL-b-PEG and PCL-b-PAE

The experiment details for the synthesis and characterization of the two block polymers have been previously reported by our group.

Synthesis of PCL-b-PEG-KLVFF

PCL-b-PEG-COOH was synthesized through the ROP of ϵ -CL monomer with HOOC-PEG-OH as the macro-initiator and Sn(Oct)₂ as catalyst. HOOC-PEG114-OH (0.3 g, 0.06 mmol) and ϵ -CL (0.6 g, 5.27 mmol) was dissolved in 5 ml of toluene, and then one drop of Sn(Oct)₂ was added into the solution. After freeze-degas-thaw cycles for three times, the reaction mixture was stirred at 110 °C for 12 h. Then the solvent was removed and the crude product was dissolved in an appropriate amount of dichloromethane, followed by precipitation into excess diethyl ether. The precipitate was dried under vacuum. GPC results: Mn=15340, Mw=19593, PDI=1.28.

PCL-b-PEG-COOH (150mg, 0.01mmol), EDC (10mg), NHS (5.6mg) and TEA (7 μ l) were dissolved in 5ml of DMF. After stirred 4h at 5°C, the reaction mixture was added with K-peptide (19mg, 0.03mmol). After stirred again overnight at 25°C, the reaction mixture was dialyzed against pure water for three days. PCL-b-PEG-KLVFF was obtained by lyophilisation.

Synthesis of PCL-b-PAE-KLVFF

PCL-b-PAE (0.15g, 0.01mmol) and NAS (17mg, 0.1mmol) were dissolved in 10mL of CHCl₃. After stirred for 12h at 55 °C, the solution was precipitated into excess diethyl ether to obtain PCL-b-PAE-NAS. PCL-b-PAE-NAS (120mg, 8.2 μ mol) and K-peptide (17mg, 26.1 μ mol) were dissolved in 3mL of DMF and 2mL of CHCl₃, followed by addition of 200 μ L of TEA. After stirred overnight at 30 °C, the reaction mixture was dialyzed against pure water for three days. PCL-b-PAE-KLVFF was obtained by lyophilisation.

The synthesis routes and ¹H NMR results of partial polymers above were shown in Figure S1 and S2.

3. Preparation and characterization of MSPMs

1:1 molecular ratio of PCL-b-PEG (or PCL-b-PEG-KLVFF) and PCL-b-PAE (or PCL-b-PAE-KLVFF) were mixed in DMSO (1mL) with a concentration of 5mg/mL. Subsequently, this copolymer solution was added dropwise into 10mL HNO₃ solution (pH~4.0) under vigorous stirring. After stirred for another 4 hours to stabilize the system, the solution was dialyzed (molecular cut off: 7KD) against PBS (pH=7.4) for 3 days to completely remove DMSO and finally formed MSPMs. In this way, MSPM, K-in-MSPM and K-out-MSPM are prepared. For the degradation experiments, the K-out-PM was prepared only with PCL-b-PEG-KLVFF.

4. A β preparation

A β 42 solution was prepared by following the literature method. Firstly, the lyophilized A β 42 peptide was dissolved in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) to a concentration of 1mg/mL. Then, the solution was bath-sonicated for 10 min and incubated with shaking at 4°C for 2h in a sealed vials for further dissolution. Subsequently, the obtained sample was divided into aliquots in microcentrifuge tubes and then evaporated under a gentle stream of nitrogen to remove HFIP solvent and stored at -20°C. Before use, the dry aliquot was re-dissolved in anhydrous dimethyl sulfoxide (DMSO) to 1mM followed by a bath-sonication for 5min and then diluted with PBS (10mM, pH=7.4) buffer to a final concentration of 40 μ M. This solution was used immediately for the following experiments.

5. ThT Fluorescence Assay

20 μ L of the incubated sample was withdrawn and injected into 180 μ L of 10 μ M ThT buffer solution as the test sample. ThT fluorescence was measured with excitation and emission at 440nm (slit width=5nm) and 485nm (slit width=10nm).

Effects of degradation were characterized by ThT fluorescence assay as described. For the degradation of initial phase of fibrils, test sample was prepared by mixing fibrils incubated 7 days and MSPMs as a ratio of 1:1. For the degradation of mature fibrils, fibrils incubated 30 days, MSPMs and protease K (1mg/ml) were mixed up as a ratio of 1:1:1.

For experiments evaluate the effects of inhibition of A β aggregation by three kinds of MSPMs, the original sample were prepared by mixing A β solution (40 μ M) and MSPMs by ratio of 1:1. And for experiments monitoring the effects of degradation of fibrils by

different MSPMs, A β monomers solution were first incubated for 7 or 30 days to form initial phase of fibrils or mature fibrils at 37°C, followed by adding the same volume of different MSPMs.

6. Transmission Electron Microscopy (TEM)

TEM measurements were performed with a commercial Philips T20ST electron microscope at an acceleration voltage of 100 kV and Talos F200c electron microscope (acceleration voltage of 200 kV). To prepare the TEM sample, 10 μ L sample solution was dropped onto a carbon-coated copper grid for 10min and blotted with filter paper to remove excess liquid. Then the sample was negatively stained with 2% uranyl acetate (10 μ L) for 1min, blotted again and air-dried before analysis on TEM.

7. ANS Fluorescence Assay

10mg ANS was first dissolved in 5ml PB. 1ml ANS solution after filtration was added to 11ml PB to form ANS solution used in the following experiments. Freshly prepared ANS solutions were always used. For the procedure, 0.75 mL ANS solution were added in different MSPMs. After the mixture was incubated for half an hour at 25°C, 20ul A β solution (20uM) was added into the mixture solution every 10 minutes. The fluorescent spectra of ANS were recorded 30 seconds after A β was added.

8. QCM-D Measurements

OPSS-PEG-PCL were prepared as described previously by replacing the HOOC-PEG-OH with OPSS-PEG-OH. Then the OPSS linked MSPMs were prepared by replacing 20% PEG-PCL (or KLVFF-PEG-PCL) with OPSS-PEG-PCL. The Au sensor chip was immersed in a solution of 0.5mg/mL OPSS-MSPMs which is reduced by TCEP for 24h. Then the chip was rinsed with deionized water, dried with nitrogen gas and put into the standard flow module before measurements. Each sensor chip surface with attached MSPMs was washed with PBS buffer (10mM, pH=7.4) for 1h at a flow rate of 30 μ L/min and then equilibrated at 10 μ L/min until the baseline was stable. Then, A β solution (20uM) in the flow buffer were injected for 30min at 10 μ L/min followed by continuous flow of the same buffer. The experiments were operated at 37°C.

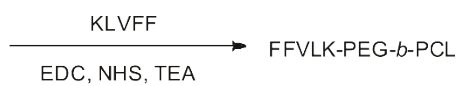
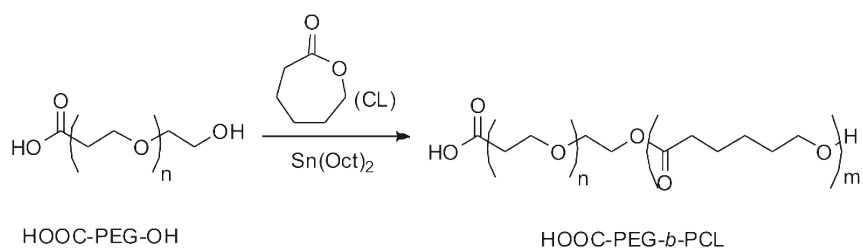
9. Cell viability

PC12 cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum. For the MTT assay, cells were plated at a density of 5000 cells per well on 96-well plates in fresh medium. After incubation of 24h, A β samples that had been preaggregated alone or with or without different MSPMs for 24h at 37°C were added to the cells. A total of 24 h later, 10 μ L of MTT solution and 90ul of culture medium were used to replace the mixture in each well and the cells were further incubated for another 4h. Absorbance values of formazan was measured at 486 nm using a Varioskan Flash (Thermo Scientific Company, USA). Cells without micelles were used as the control.

Similarly, the cytotoxicity of materials was measure by MTT assay as described above.

For the cell viability assay, A β used was 40 μ M and the materials used were all 0.5 mg/ml. The ratio in Fig. 4d represented for the volume ratio between the two solutions in the final mixed samples.

a)



b)

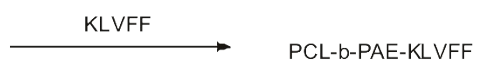
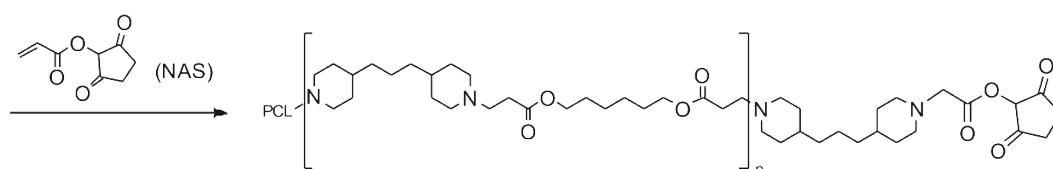
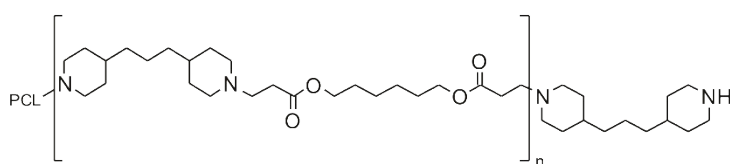


Fig. S1 synthesis routes of PCL-b-PEG-KLVFF (a) and PCL-b-PAE-KLVFF (b).

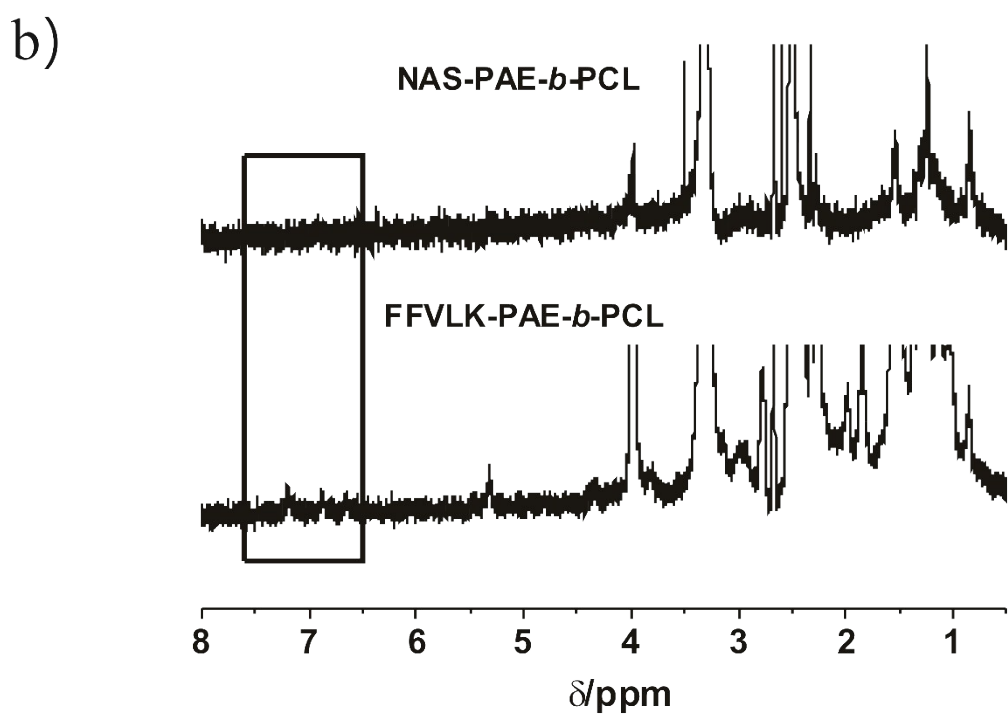
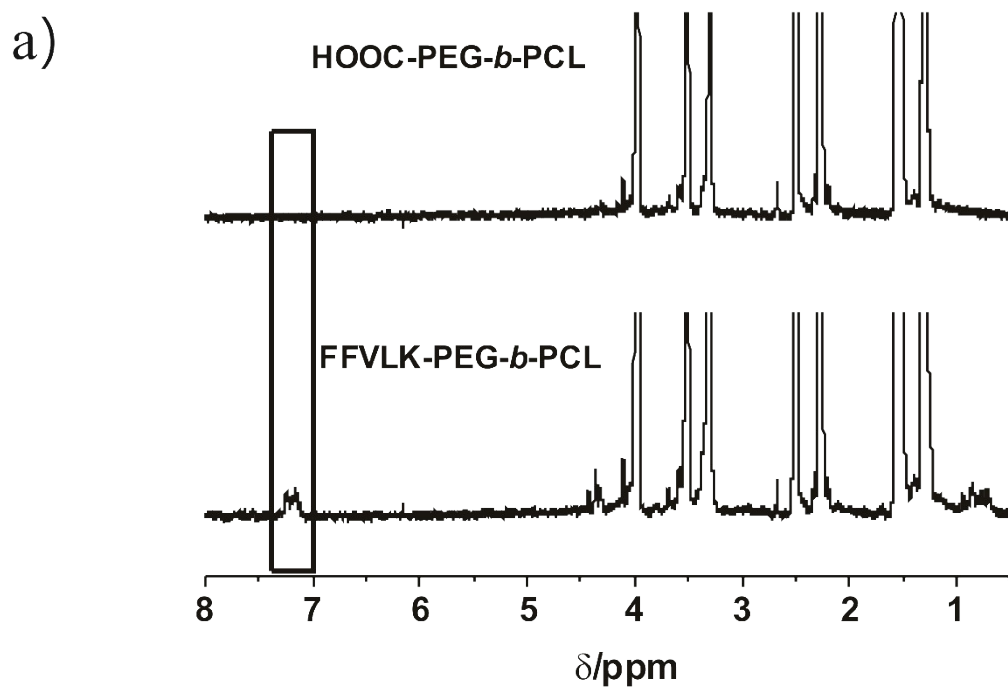


Fig. S2 ^1H NMR results of HOOC-PEG-*b*-PCL, FFVLK-PEG-*b*-PCL (a), NAS-PAE-*b*-PCL and FFVLK-PAE-*b*-PCL (b).

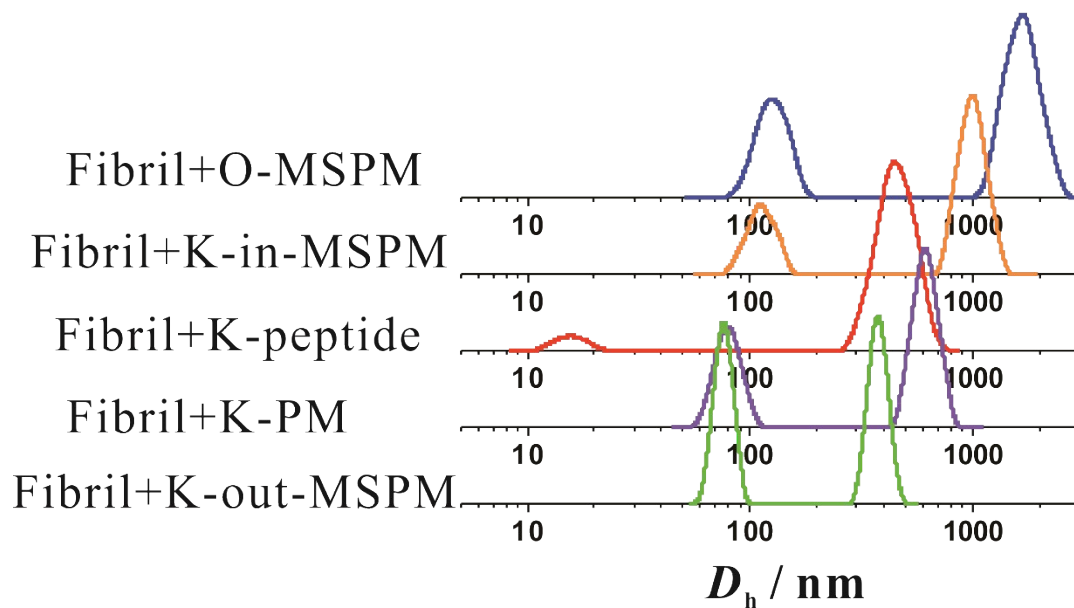


Fig. S3 Diameter distribution measured of fibrils incubated with or without the five materials separately after 7 days.

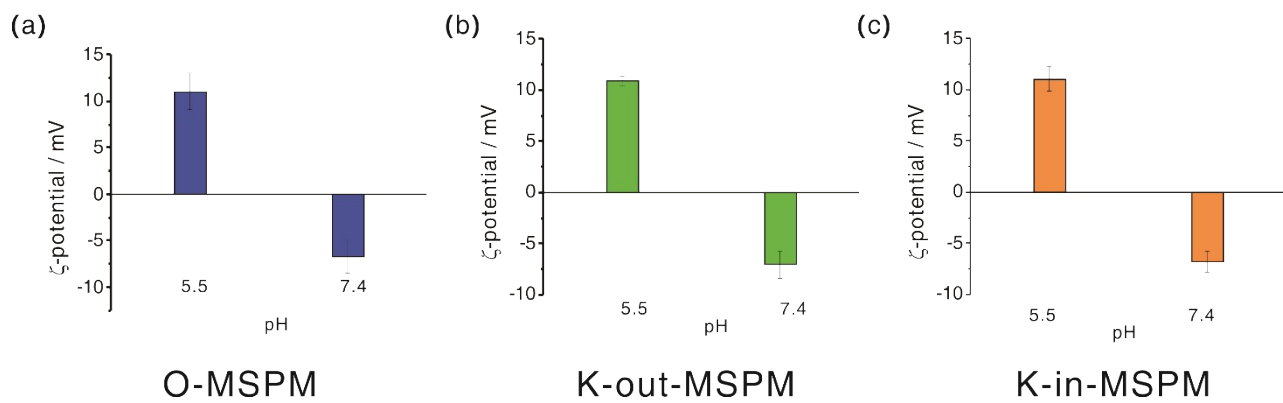


Fig. S4 Zeta-potential of the three MSPMs at two pH values separately.

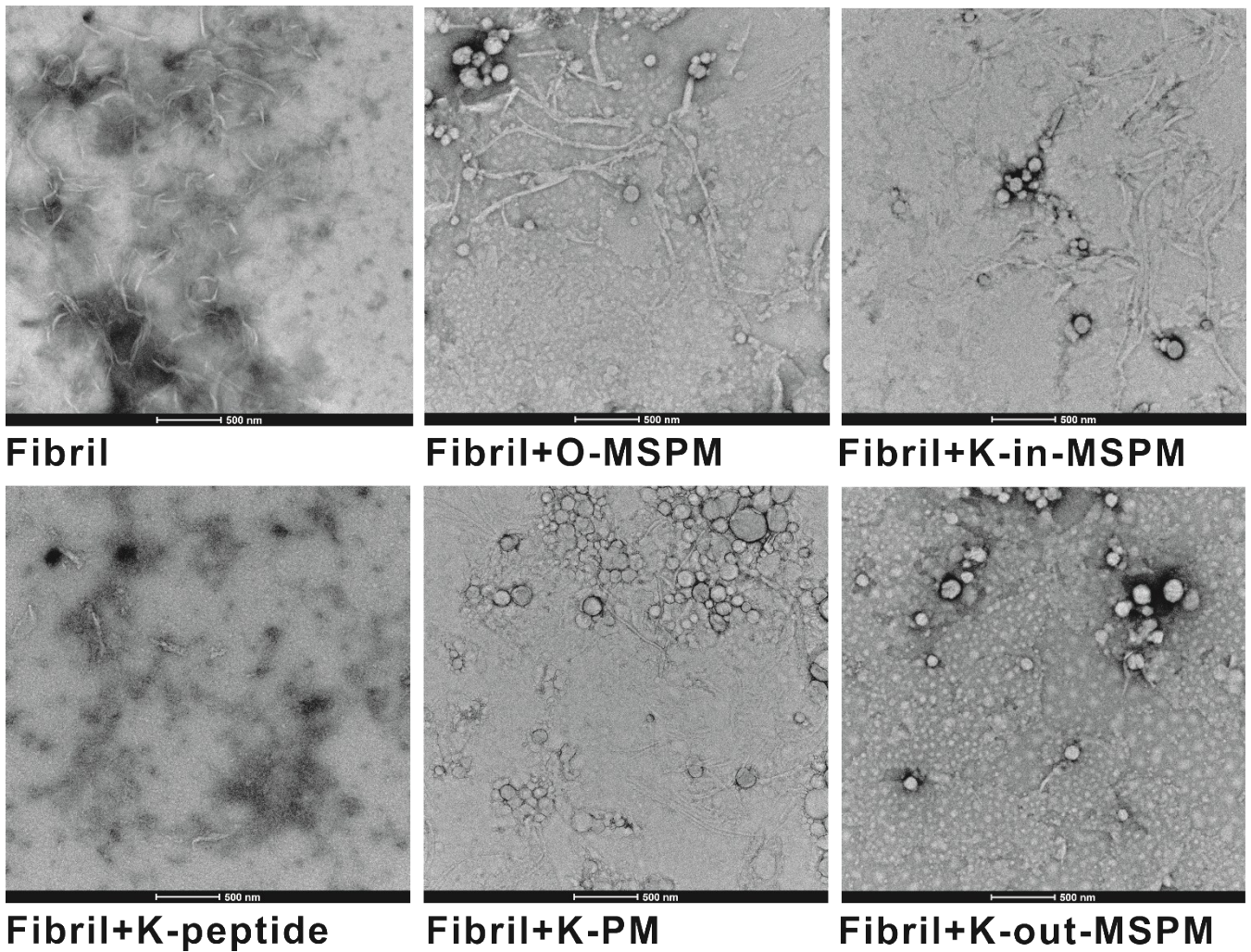


Fig. S5 TEM images of proteinase K mixed tightly tangled fibrils incubated with or without the five materials separately after 7 days.

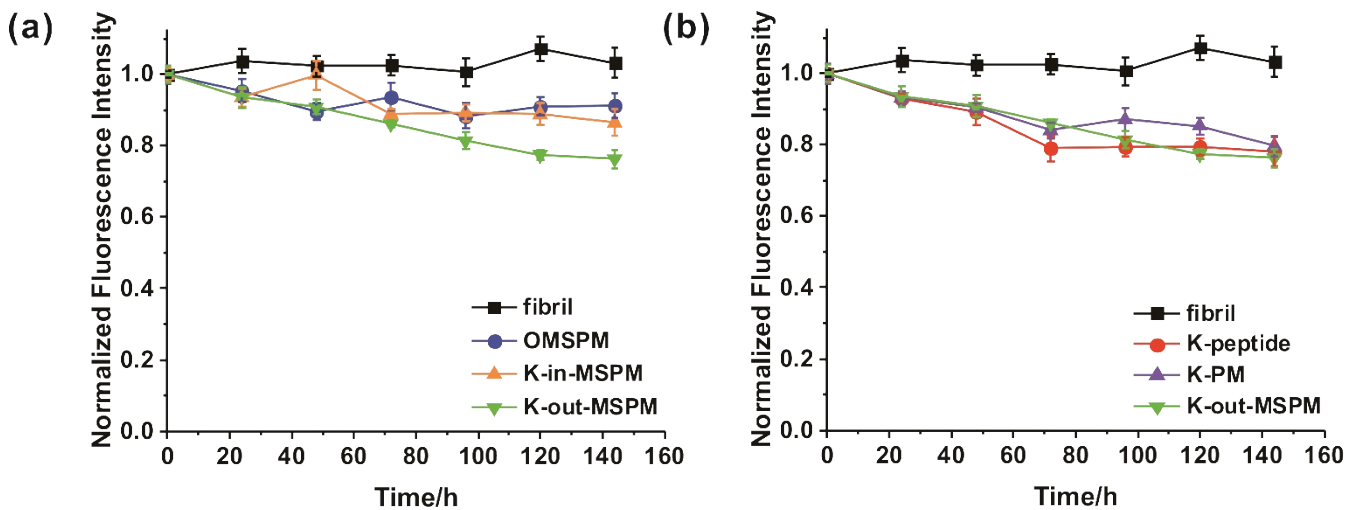


Fig. S6 Disaggregation of tightly tangled fibrils (a, b) by K-peptides, K-PMs, O-MSPMs, K-out-MSPMs or K-out-MSPMs measured by ThT fluorescence assay.

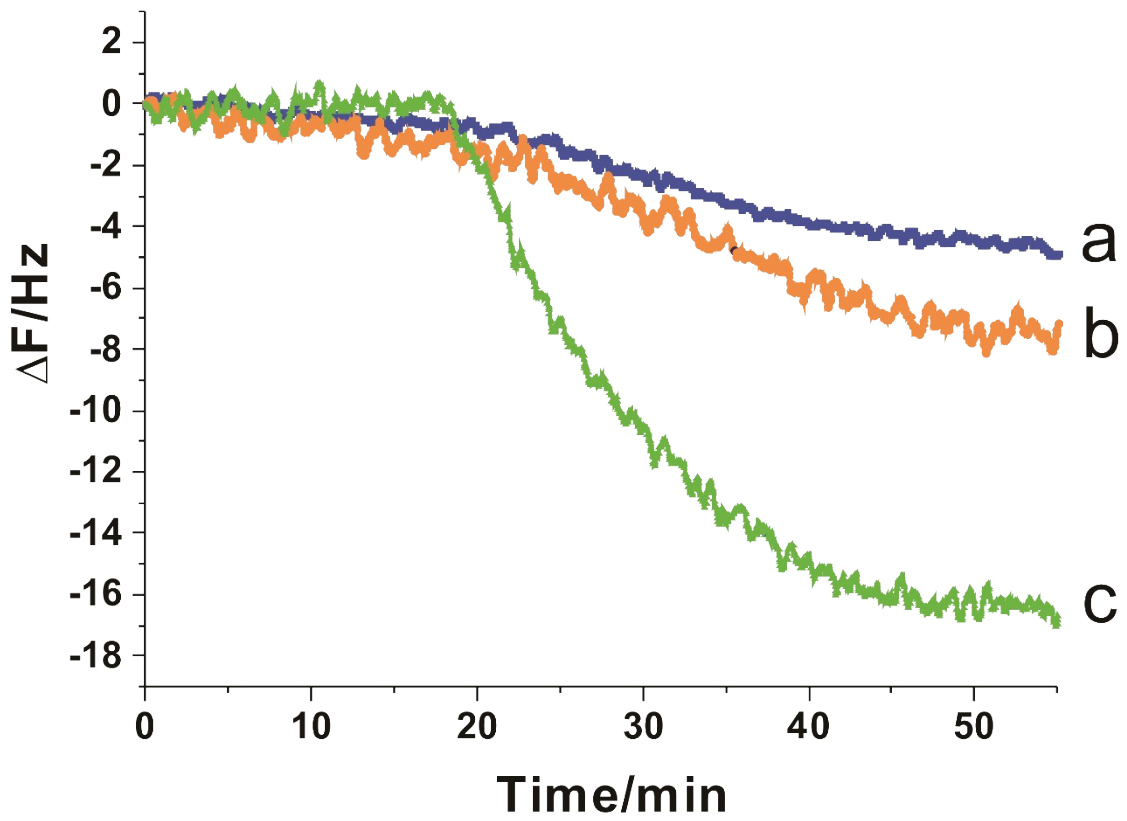


Fig. S7 QCM-D experiment probing the binding of A β species to O-MSPM (a), K-in-MSPM (b) and K-out-MSPM (c).

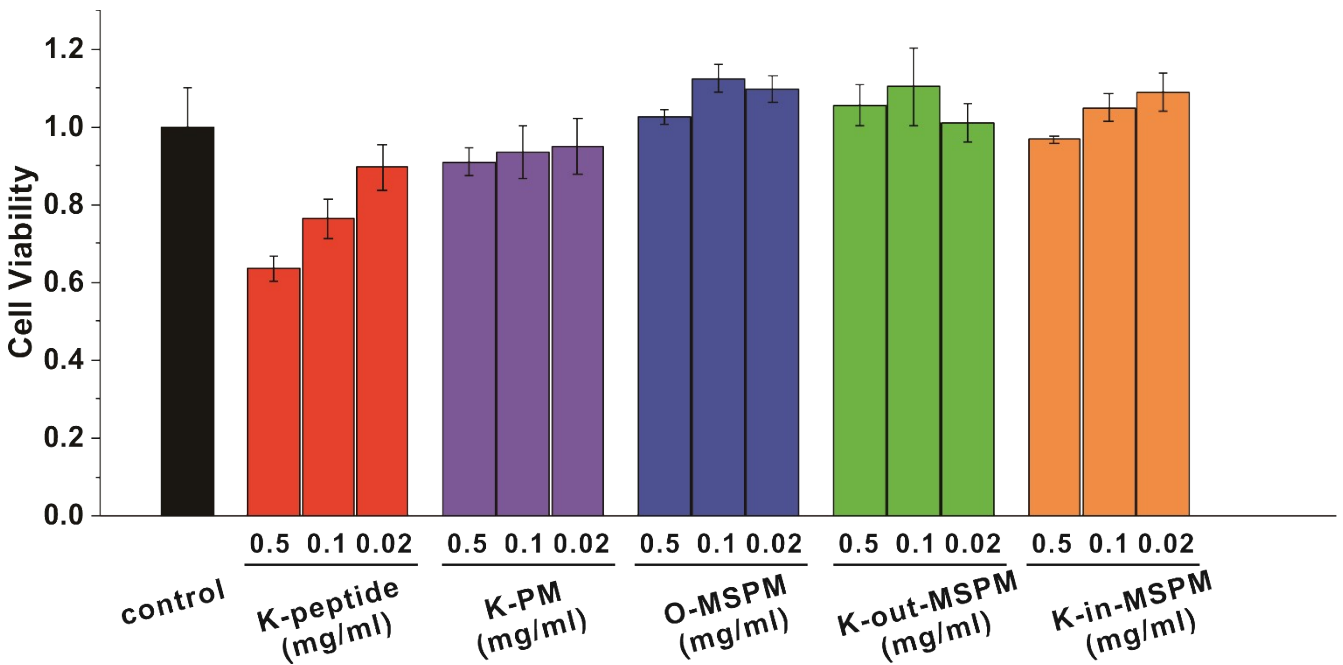


Fig. S8 Cytotoxicity of K-peptides, K-PMs and three MSPMs against PC12 cells.

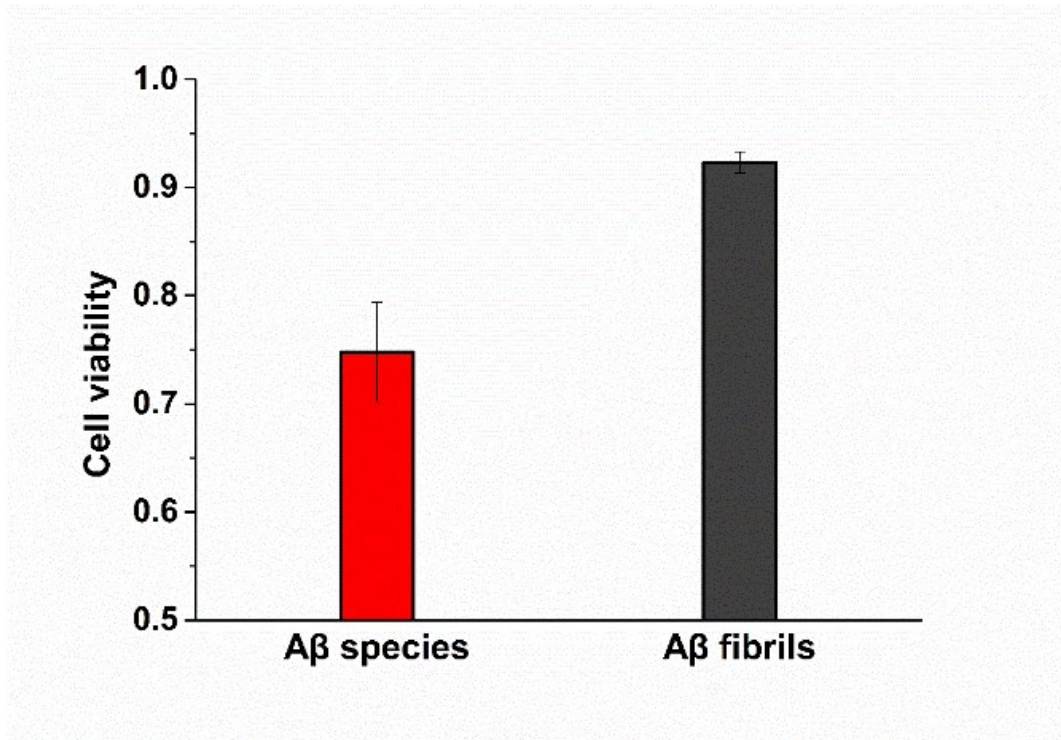


Fig. S9 Cell viability of Aβ species and Aβ fibrils.

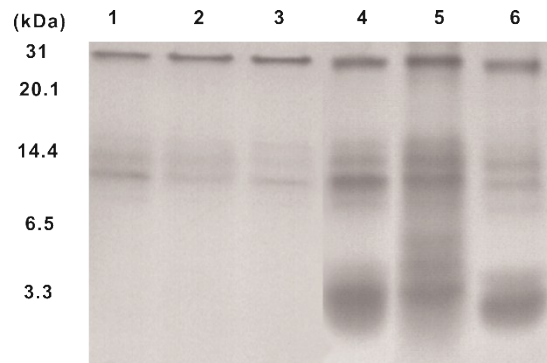


Fig. S10 SDS-PAGE analyzed proteolysis of fibrils by proteinase K incubation with O-MSPMs (2), K-in-MSPMs (3), K-peptides (4), K-PMs (5) and K-out-MSPMs (6) or without materials (1).