Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2023

Supplementary Files

Electrostatic Assembly of Gold Nanoparticle and Metal-Organic Framework Nanoparticles Attenuates Amyloid β Aggregates-Mediated Neurotoxicity

Licong Yang[a,b], Yutong Chen[a], Zhi Jia[a], Xiaoyu Yuan[a], and Jie Liu[a]*

a Department of Chemistry, College of Chemistry and Materials Science, Jinan University, Guangzhou 510632, China;

b College of Biological Science and Engineering, Fuzhou University, Fuzhou, 350108, China.

* Corresponding author. Tel: +86-20-8522-0223; Fax: + 86-20-8522-0223. E-mail: tliuliu@jnu.edu.cn

Experimental Section

Material and cell culture

Amyloid β peptides ($A\beta_{40}$) were purchased from GL Biochem Ltd. (Shanghai, China). $FeCl_3 \cdot 6H_2O$, HF, terephthalic acid, $HAuCl_4 \cdot 3H_2O$ (＞99.9%), PEG-NH_2, $NaBH_4$, thiazolyl blue tetrazolium bromide (MTT), 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), thioflavine T (ThT) and Rhodamine B were from Sigma (St. Louis, MO, U.S.). Terminal transferase UTP nick end labeling (TUNEL) assay kit was obtained from Roche Applied Science (Basel, Switzerland). Lyso-Tracker Red, Tubulin-Trakcer Red and Actin-Tracker Green were purchased from Beyotime Biotechnology. Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS) were obtained from Gibco (Life Technologies AG, Switzerland). PC12 cells (rat pheochromocytoma, American Type Culture Collection) were cultured in DMEM medium supplemented with 10% FBS in a humidified environment containing 5% CO_2 at 37℃.

Preparation of AuNPs@PEG@MIL-101

First, MIL-101 is synthesized by mixing 0.675 g of $FeCl_3 \cdot 6H_2O$ and 0.225 g of terephthalic acid in 15 mL of dimethylformamide (DMF). After 24 h of thermal treatment in a 30 mL stainless steel autoclave at 383 K and cooling to room temperature. The obtained particles were centrifugated and washed with DMF and ethanol. The mixture was filtered by a large-pore fritted glass filter (No. 2, Schott) to remove most of the DMF and carboxylic acid. The MIL-101 powder was further isolated by centrifuging and washed with ethanol and deionized water. Then, the MIL-101 powder was dried overnight under vacuum at 473K.

Second, to improve the biocompatibility of MIL-101, the surface of MIL-101 was modified by PEG-NH_2. PEG@MIL-101 was prepared as follow: 30 mg MIL-101 was dissolved in 2 mL 5mg/mL PEG-NH_2 solution and stirred for 3 h at 37°C. Then, PEG@MIL-101was isolated by centrifuging and washed thoroughly with deionized water.

AuNPs@PEG@MIL-101 was synthesized based on the following process. First, The 10 nm negatively charged AuNPs was prepared as described previously[23]. Second,

to obtain AuNPs@PEG@MIL-101, 100 mg positively charged PEG@MIL-101 was dissolved in deionized water. Then, the excessive of AuNPs was added to the PEG@MIL-101 solution and stirred for another 12 h. Finally, AuNPs@PEG@MIL-101 was isolated by centrifuging and washed thoroughly with deionized water.

Characterization of AuNPs@PEG@MIL-101

The morphology of AuNPs@PEG@MIL-101 on copper grid was observed on a transmission electron microscope (TEM, Hitachi, H-7650). The size distribution and zeta-potential of AuNPs@PEG@MIL-101 was measured by Nano-ZS instrument (Malvern Instruments Limited).

The chemical composition of nanoparticles was conducted by Fourier transform infrared spectroscopy (FT-IR, Equinox 55 IR spectrometer) and UV-vis spectrophotometer (Carry 5000 spectrophotometer), respectively. Nitrogen adsorption desorption isotherms were recorded by NOVA 4200e surface area analyzer (Quantachrome). X-ray diffraction patterns were obtained on Rigaku D/Max 2200 X-ray diffractometer.

TUNEL-DAPI co-staining

Apoptotic DNA fragmentation in the early stage can reflect the cell apoptosis and the DNA fragmentation can be stained by TUNEL. Thus, to measure the Aβ induced cell apoptosis, PC12 cells (5×10^4 per well) were cultured with $A\beta_{40}$ monomers or fibers (30 μM) and nanoparticles (20 μg/mL) for 48 h. After that, cells were fixed with 4% formaldehyde and permeabilized with 0.1% Triton X-100 in PBS. The TUNEL-DAPI co-staining was carried out by TUNEL kit (KeyGen BioTECH, Nanjing, China) according to the manufacturer's instructions. Images were captured on fluorescent microscope (magnification 200×).

Quantitative real-time PCR (qRT-PCR)

To measure the mRNA expression level of apoptosis related genes, total RNA from various treatment groups were extracted by TRIzol reagent (Invitrogen, Thermo Fisher Scientific). cDNA was prepared by the cDNA reverse transcription kit (Roche, Basel, Switzerland), and β-actin was used as an internal reference. qRT-PCR was performed using a Light Cycler®480 system (Roche, Switzerland). Utilizing the 2-

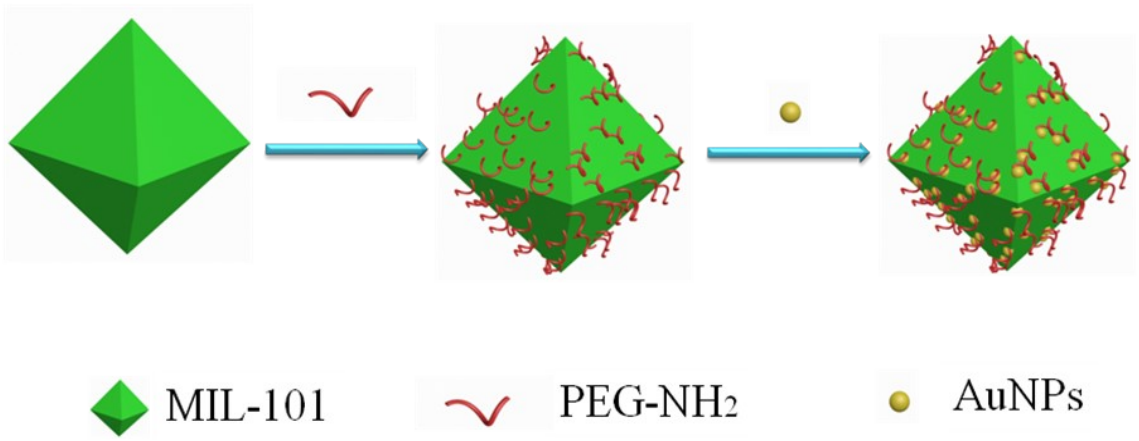
ΔΔCt approach, the relative mRNA expression was determined. The gene primers were shown as follow: Caspase-3 forward primer: ACCGGAATGGCATGTCGATC, reverse primer: CTGTTTCAGCGCTGCACAAA; Caspase-8 forward primer: CCTCAAGTTCCTGAGCCTGG, reverse primer: TCTCCCAGACAGTCCGACA; Bax forward primer: CATGAAGACAGGGGCCCTTT, reverse primer: CTTCCAGATGGTGAGCGAGG.

Reactive oxygen species (ROS)

To investigate the effect of nanoparticles on Aβ induced ROS formation in cells, PC12 cells were treated with $A\beta_{40}$ monomers/fibers and nanoparticles. Then, the cells were washed twice with PBS, and incubated with 10 mM DCF-DA at 37°Cfor 30 min. The intracellular ROS level was examined under confocal laser scanning microscope. The excitation and emission wavelengths were 488 nm and 525 nm, respectively.

Cytoskeleton immunostaining

To verify the protective effect of NPs on cytoskeleton structure of cells treated with Aβ, PC12 cells were treated in the same way as above and fixed with Karnovsky's fixative (2% glutaraldehyde and 2% paraformaldehyde, pH 7.2) for 15 min. Then, the cells were permeabilized with 0.25% Triton X-100. Finally, the neurites of PC12 cells were incubated with Tubulin-Trakcer Red according to the manufacturer's instructions. The nucleus was stained by Hoechst 33342. After rinsing cells with PBS, fluorescent images were taken using Zeiss LSM meta 510 multiphoton laser scanning confocal microscope.



Scheme 1. Plan for the Synthesis of AuNPs@PEG@MIL-101. MIL-101 can absorb PEG-NH_2, therefore, to improve the biocompatibility of MIL-101, the surface of MIL-101 was modified by PEG-NH_2. The positively charged amine groups can enhance the ability of MIL-101 binding with negatively charged AuNPs. Thus, the positively charged PEG@ MIL-101 can be decorated with negatively charged AuNPs to form AuNPs@PEG@MIL-101.

Table 1 Physicochemical characterization of the nanoparticles (mean ± S.D., n=3)

Formulation	Size (nm)	Zeta potential (mV)
MIL-101	856.13±16.45	29.7±1.23
PEG@MIL-101	207.04±12.05	40.2±1.25
AuNPs@PEG@MIL-101	229.33±15.78	20.6±1.01

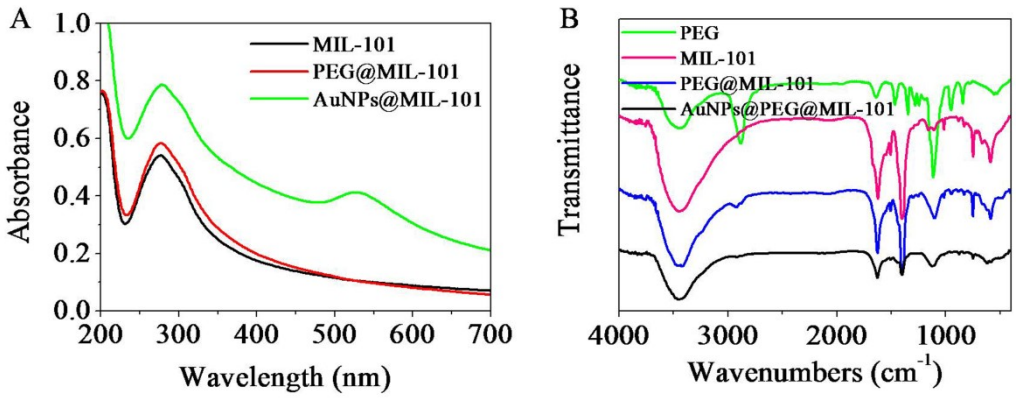


Figure S1. UV-vis spectra of MIL-101, PEG@MIL-101 and AuNPs@PEG@MIL-101 (A); FTIR spectra of MIL-101, PEG@MIL-101 andAuNPs@ PEG@MIL-101 (B).

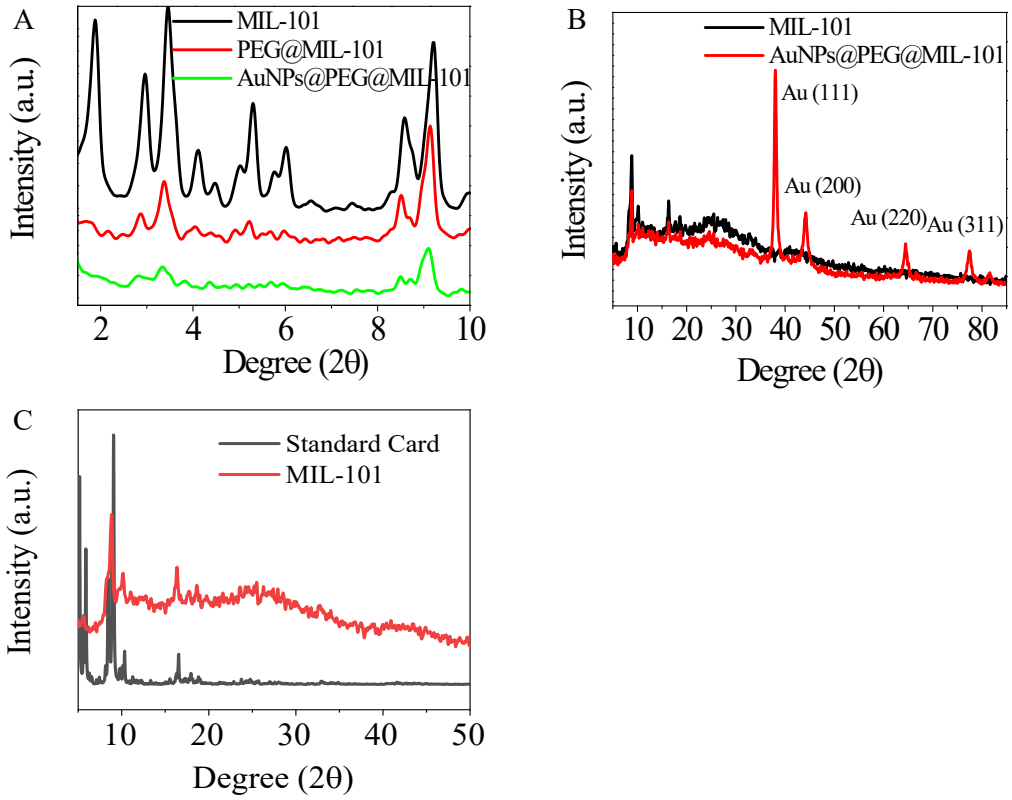
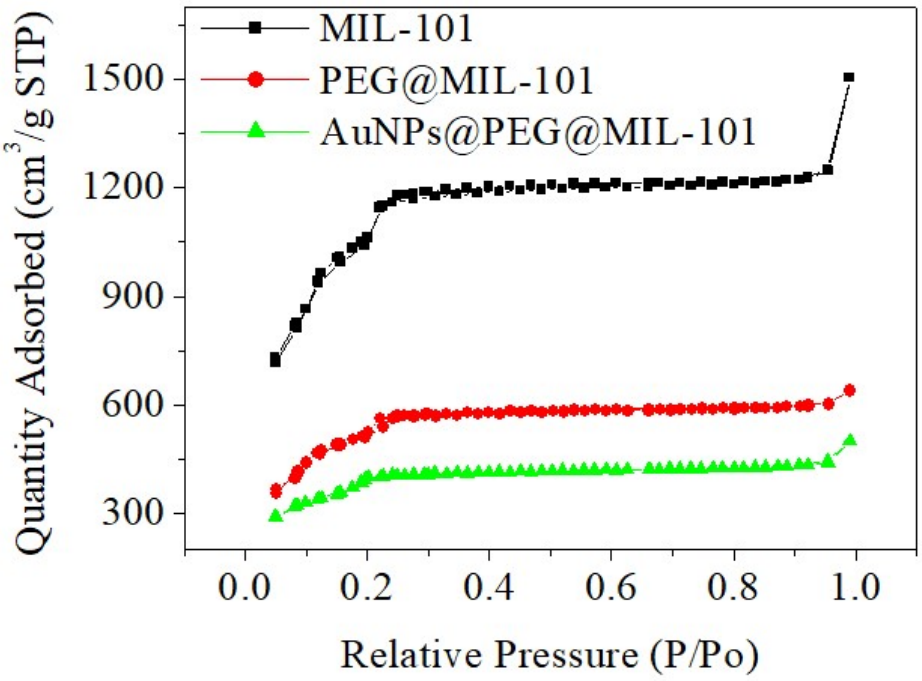


Figure S2. Low-angle (A) and wide-angle (B) XRD patterns of MIL-101, PEG@MIL-101 and AuNPs@PEG@MIL-101. (C) Standard card of MIL-101 in the XRD patterns.



	MIL-101	PEG@MIL-101	AuNPs@PEG@MIL-101
S_{BET} [m^2/g]	3853.07	1846.96	1283.65
Pore volume [cm^3/g]	2.33	0.99	0.77
Pore diameter [nm]	2.41	1.79	-

Figure S3. N_2 adsorption-desorption isothermsa for MIL-101, PEG@MIL-101 and AuNPs@PEG@MIL-101. Table: BET specific surface values, pore volumes, and pore sizes calculated from N_2 adsorption-desorption isothermsa.

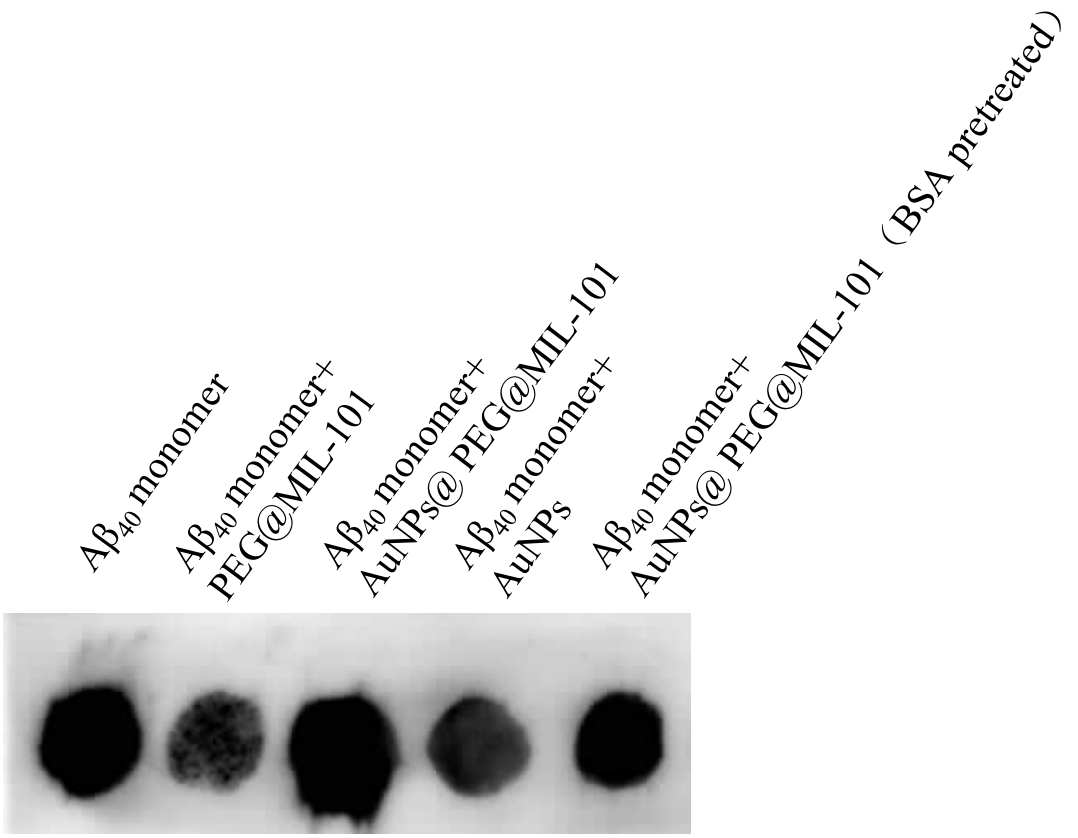
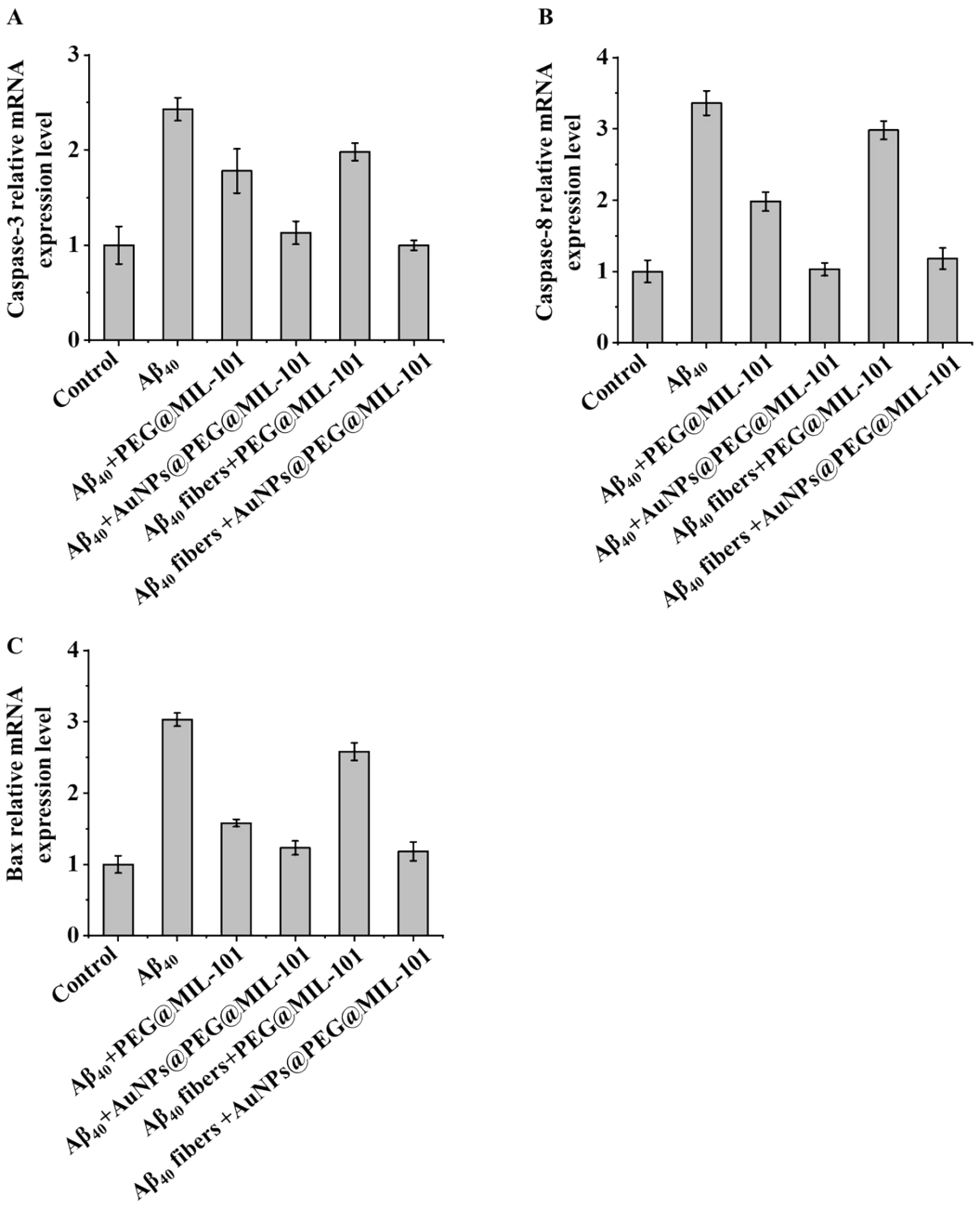
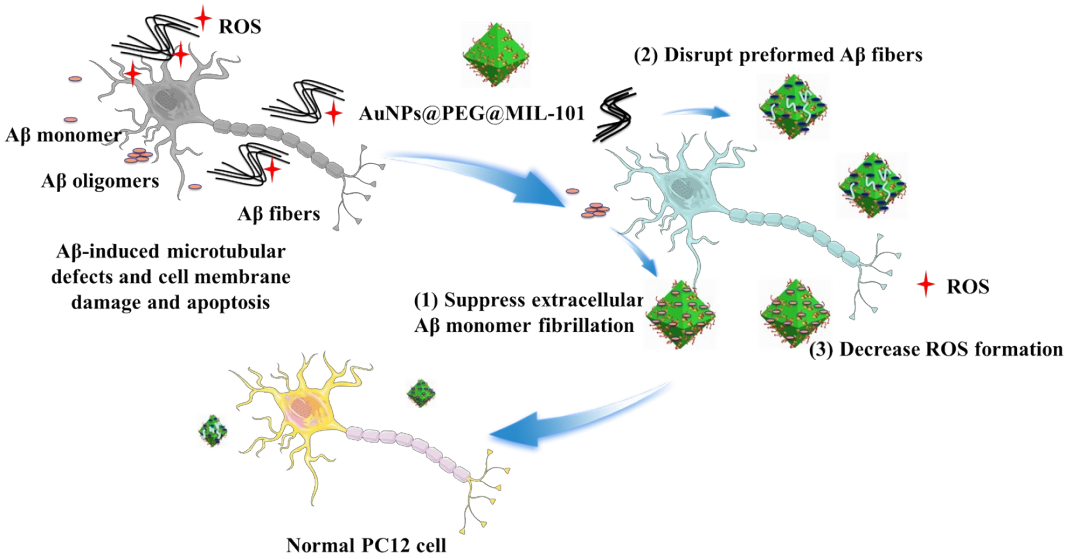


Figure S4. Dot-blot analysis of soluble $A\beta_{40}$ incubated with nanoparticles. The $A\beta_{40}$ monomer was incubated with nanoparticles for 5 h. Then solutions were centrifuged and the precipitate was dispersed in PBS. The $A\beta_{40}$ monomer bound on nanoparticles was determined by dot-blot using an Aβ-sequence-specific monoclonal antibody (6E10). For bovine serum albumin (BSA) interfering experiment, nanoparticles (20 μg/mL) were incubated with BSA (30 μM) at 37 °C for 1 h, respectively. Then, nanoparticles were centrifuged at 13000 rpm for 10 min, and incubated with $A\beta_{40}$ monomer (30 μM) for 5 h.



Fiugre S5. Expression of mRNA related to apoptosis. mRNA expression level of caspase-3, caspase-6 and Bax.

Graphical Abstract



AuNPs enhanced the disaggregation ability of MIL-101 and the binding affinity between MIL-101 and Aβ. $A\beta_{40}$ monomer and low toxicity aggregates from the disaggregated $A\beta_{40}$ fibrils could be uniformly absorbed on the surface of nanoparticles. Thus, it can efficiently suppress extracellular Aβ fibrillation and disrupt the preformed Aβ firbers. AuNPs@PEG@MIL-101 also decrease intracellular $A\beta_{40}$ aggregation and the amount of $A\beta_{40}$ immobilized on cell membrane, finally protect PC12 cells from the cell membrane damage and microtubular defects induced by $A\beta_{40}$ fibers.