Electronic supplementary information (ESI) for:

Gold nanoparticle based dot-blot immunoassay for sensitively detecting Alzheimer's disease related β-amyloid peptide

Chengke Wang,[a,b] Dianjun Liu[a] and Zhenxin Wang*[,a]

[a] *State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, China,130022;*

[b] *Graduate School of the Chinese Academy of Sciences, Beijing, China,100039.*

E-mail: wangzx@ciac.jl.cn

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Experimental section

Materials and reagents. Peptides (CALNN, CALNN GK(biotin)G and DAEFR HDSGY EVHHQ K ($A\beta_{1-16}$)) were purchased from Scilight Biotechnology Ltd. (Beijing, China). DAEFR HDSGY EVHHQ KLVFF AEDVG SNKGA IIGLM VGGVV ($A\beta_{1-40}$) was purchased from Sigma-Aldrich Ltd. (USA). DAEFR HDSGY EVHHQ KLVFF AEDVG SNKGA IIGLM VGGVV IA ($A\beta_{1-42}$) was purchased from A Peptide Ltd. (Shanghai, China). Hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$), gelatine, PEG NHS ester disulfide (4,7,10,13,16,19,22,25,32,35,38,41,44,47,50,53-hexadecaoxa-28,29-dithiahexapentac ontanedioic acid di-N-succinimidyl ester, NHS-PEG-S-S-PEG-NHS, n=7) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich Ltd. (USA). Streptavidin was purchased from Promega Ltd. (USA). Nitrocellulose (NC) membrane was acquired from PALL Ltd. (USA). Berberine was obtained from Aladdin. Ltd. (Shanghai, China). Polyclonal antibody (pAb) specific to N-terminal of monomeric human $A\beta_{1-42}$ (bs-0104M) and pAb specific to C-terminal of monomeric human $A\beta_{1-42}$ (bs-0076M) were obtained from Bioss. Ltd. (Beijing, China). Second antibody (peroxidase-conjugated goat anti-mouse antibody) was obtained from Dingguo Ltd. (Beijing, China). The human cervical cancer cell line (HeLa), human glioma cell line (SHG-44), human astrocytoma cell line (U-251) and rat pheochromocytoma cell line (PC-12) were obtained from the Shanghai cell bank of Chinese Academy of Sciences (Shanghai, China). TMB solution and cell lysate kit was obtained from Beyotime Ltd. (Haimen, China) and

used according to the manufacturer's instruction. Other chemicals were analytical grade, and they were used as received. Milli-Q water (18.2 MΩ.cm) was used in all experiments. All experiments were carried out at room temperature except mentioned specially.

Synthesis of Ab16-GNPs. The citrate stabilized 30 nm GNPs were synthesized by Turkevich–Frens method.[S1] Ab16-GNPs were prepared by previously reported procedure with slightly modification.[S2] Firstly, 10 μL N-terminal antibody of human $A\beta_{1\text{-}42}$ (Ab16) solution (1 mg/mL) was reacted with 1.5 μL NHS-PEG-S-S-PEG-NHS (25 μg/mL in desiccative dimethyl sulfoxide (DMSO)) overnight. Then, 1 mL 30 nm GNPs (0.6 nM) was mixed with the decorated antibody in 1.8 mM K_2CO_3 solution for at least 4 h. Subsequently, 14 μL CALNN GK(biotin)G aqueous solution (1 mg/mL) was added into the GNPs solution. After 1 h incubation, excess antibodies and peptides were removed by centrifugation at 8000 rpm (~ 6000 g, 3 times) using an Eppendorf centrifuge (Eppendorf, Germany). The purified GNPs (named as Ab16-GNPs) were resuspended in phosphate-buffered saline (PBS, 10 mM NaH_2PO4/Na_2HPO_4, 138 mM NaCl, 2.7 mM KCl, pH 7.4).

Synthesis of SA-GNPs. SA-GNPs were also prepared by previously reported procedure with slightly modification.[S3] Firstly, 100 μL peptide mixture (the molar ratio of CALNN to CALNN GK(biotin)G is 9) was mixed with 1 mL 30 nm GNPs (0.6 nM) to give a final concentration of total peptides of 1.5 mM. After 1 h incubation, excess peptides were removed by centrifugation at 8000 rpm (~ 6000 g, 3 times). The peptide capped GNPs were resuspended in 1 mL water and mixed with 7

μL streptavidin aqueous solution (1 mg/mL). After 1 h incubation, excess streptavidin was removed by centrifugation at 8000 rpm (~ 6000 g, 3 times). The purified GNPs (named as SA-GNPs) were resuspended in PBS.

Preparation of Aβ solution. The $A\beta_{1-42}$ stock solution (2 mg/mL) was prepared daily according to the previous reports.[S4,S5] Briefly, 2 mg lyophilized $A\beta_{1-42}$ (purity: 96 %, measured by HPLC; molecule weight: 4514.1, measured by mass spectrometry) were dissolved in freshly prepared 1 mL NaOH (10 mM) solution and ultrasonic agitated for 1 min. Then, the solution was centrifuged at 13,000 rpm for 30 min to remove any insoluble particles. Subsequently, desired amount of basic $A\beta_{1-42}$ stock solution was diluted by precooled PBS (4 °C) and applied immediately to the dot-blot immunoassay. Under this experimental condition, the aggregation of $A\beta_{1-42}$ is effectively inhibited. The $A\beta_{1-16}$ and $A\beta_{1-40}$ solutions were prepare using the same procedure. The $A\beta_{1-42}$ protofibrils and fibrils were prepared according to the previous reports.[S4,S5] Briefly, desired amount of $A\beta_{1-42}$ stock solution was diluted by PBS and incubated at 37 ^{o}C for 4 h (for protofibrils) and 72 h (for fibrils), respectively, and applied for the dot-blot immunoassay. For comparison, the concentrations of Aβ are also determined by the UV-visible spectral method using maximum wavelength at 276 nm (ε=1410 M^{-1} cm^{-1}).[S4,S5]

The CSF samples were obtained from No. 3 hospital of Jilin University and stored at -80 ^{o}C until thawed for the dot-blot immunoassay. After thawed, the CSF sample was centrifuged at 13,000 rpm for 30 min to remove the potential aggregates. Then, the supernatant was pipetted out and applied for the dot-blot immunoassay.

Characterization of GNPs. The UV-visible spectra of GNPs solutions were recorded by the microtiter plate reader (BioTek® Powerwave XS2, BioTek Instruments Inc., USA). The hydrodynamic diameters of GNPs were measured by a Zetasizer Nano ZS DLS system (Malvern Instruments Ltd., England). The UV-visible spectra and dynamic light scattering (DLS) spectra were shown in Fig. S1.

Fabrication of Dot-blot immunoassay. Firstly, different amounts (1 mg/mL, 100 μg/mL and 10 μg/mL) of C-terminal antibody of $A\beta_{1-42}$ were carefully immobilized on the NC membrane using micropipette (1 μL/spot). Then the membrane was blocked by 30 mL 2 % (w/w) gelatin for 30 min. Subsequently, samples containing various concentrations of $A\beta_{1-42}$ were incubated with the C-terminal antibody spots (1 μL/spot) on the membrane for different time (1 h, 2 h, 4 h and 8 h), respectively. The membrane was then washed with 30 mL washing buffer (10 mM NaH_2PO4/Na_2HPO_4, 138 mM NaCl, 2.7 mM KCl, 0.05 % (v/v) Tween-20, pH 7.4) for 3 min. The membrane was then incubated with 0.25 nM Ab16-GNPs (300 μL) in an incubator at 37 ^oC under a humidity of 60 % for 30 min. Then the membrane was washed with 30 mL washing buffer for 3 min to remove any nonspecific binding of Ab16-GNPs. Subsequently, the membrane was then reacting with 0.25 nM SA-GNPs (300 μL) at 37 ^oC under a humidity of 60 % for 30 min. Finally, the membrane was washed with 30 mL washing buffer for 3 min to remove any nonspecific binding of SA-GNPs.

Quantitative analysis. The light scattering images of spots on the NC membrane was scanned by an ArrayIt SpotWare colorimetric scanner (TeleChem. International Ltd., USA). According to the manufacturer's preset parameters, all images were

collected with a broad spectrum of white light source. The light scattering intensity of each spot was calculated using Image J software (National Institutes of Health, USA). The light scattering intensity of the sample spot (I_s) and background (I_b) were measured, and the relative intensity (ΔI) of sample was defined as $\Delta I = I_s - I_b$.

Cell experiment. Different cell lines were cultured with fresh Dulbecco's modified Eagle's medium (DMEM), supplemented with 10 % fetal bovine serum in 6 wells (for inhibition assay) or 96 wells (for MTT assay) microtiter plate (10^4 cells/mL) under a humidified 5 % CO_2 at 37 °C for 12 h; then different amounts of berberine were added into the cell culturing medium and co-cultured with the cells for desired time. For the inhibition assay, the cell culturing mediums were collected and cells at the bottom of 6 wells microtiter plate were lysated using the cell lysate kit following the manufacturer's instruction, respectively. The cell culturing mediums and lysates were centrifuged at 13,000 rpm for 30 min to remove the insoluble aggregates and used immediately. The cell lysate solution contains high concentration of surfactant, which enables to disassemble $A\beta_{1\text{-}42}$ aggregates. So, after treated with lysate solution, the cell lysates contained mainly Aβ monomers.The amounts of $A\beta_{1\text{-}42}$ in both the cell culturing mediums and lysates were determined by our GNPs based dot-blot immunoassay. For the MTT assay, after interacting with berberine for desired time, the cells in the 96 well microtiter plate were washed with PBS (100 μL, 3 times), then 10 μL MTT (5 mg/mL in PBS) and 100 μL cell culturing solution were added into each well and incubated for another 4 h. Finally, the supernatant was discharged, and DMSO was added into the wells (150 μL per well) and shaken in the dark for 10 min,

respectively. The optical density at 555 nm was measured by a microtiter plate reader. The cell viabilities were calculated by literature reported method.[S6]

ELISA experiment. The $A\beta_{1-42}$ expression level in four cells lines (PC-12, HeLa, U-251 and SHG-44) have been detected by traditional ELISA according to the previously reported method with slightly modification.[S7] Briefly, 100 μL different cell culturing mediums and lysates were added into the ELISA plate. After incubated at 4 ^{o}C overnight, the cell solutions were removed and the plate was washed with PBS, respectively. Then, 100 μL 2 % (w/w) gelatin was used to block the plate well. After incubated at 37 ^{o}C for 1 h, the gelatin solution was removed and the plate was washed with PBS, respectively. Then, 100 μL C-terminal antibody of $A\beta_{1-42}$ (1 μg/mL) was added into each well. After incubated for 1 h at 37 ^{o}C, the antibody solution was removed and the plate was washed with PBS, respectively. Subsequently, 100 μL peroxidase-conjugated second antibody was added into each well. After incubated for 1 h at 37 ^{o}C, The second antibody was removed and the plate was washed with PBS, respectively. Finally, 200 μL TMB solution was added into each well and incubated for 10 min, and 50 μL H_2SO_4 (2 M) was added into each well and incubated for another 1 min, respectively. After treated by H_2SO_4, the samples were subjected to detect.

Transmission electron microscope (TEM) measurement. TEM micrographs of all samples were obtained by a JEOL 2000FX (JEOL, Japan) transmission electron microscope operated at an accelerating voltage of 120 kV. Specimens were prepared by evaporating a droplet of sample solution on a carbon-coated copper mesh grid and

dried in the air.

Scanning electron microscope (SEM) measurement. SEM micrographs of all samples were obtained by a XL30 scanning electron microscope (FEI Ltd., USA) operated at an accelerating voltage of 20 kV. NC membrane was sputter-coated with gold (30 mA, 20 s) and imaged by SEM to study the morphologies of sample spots on the membrane.

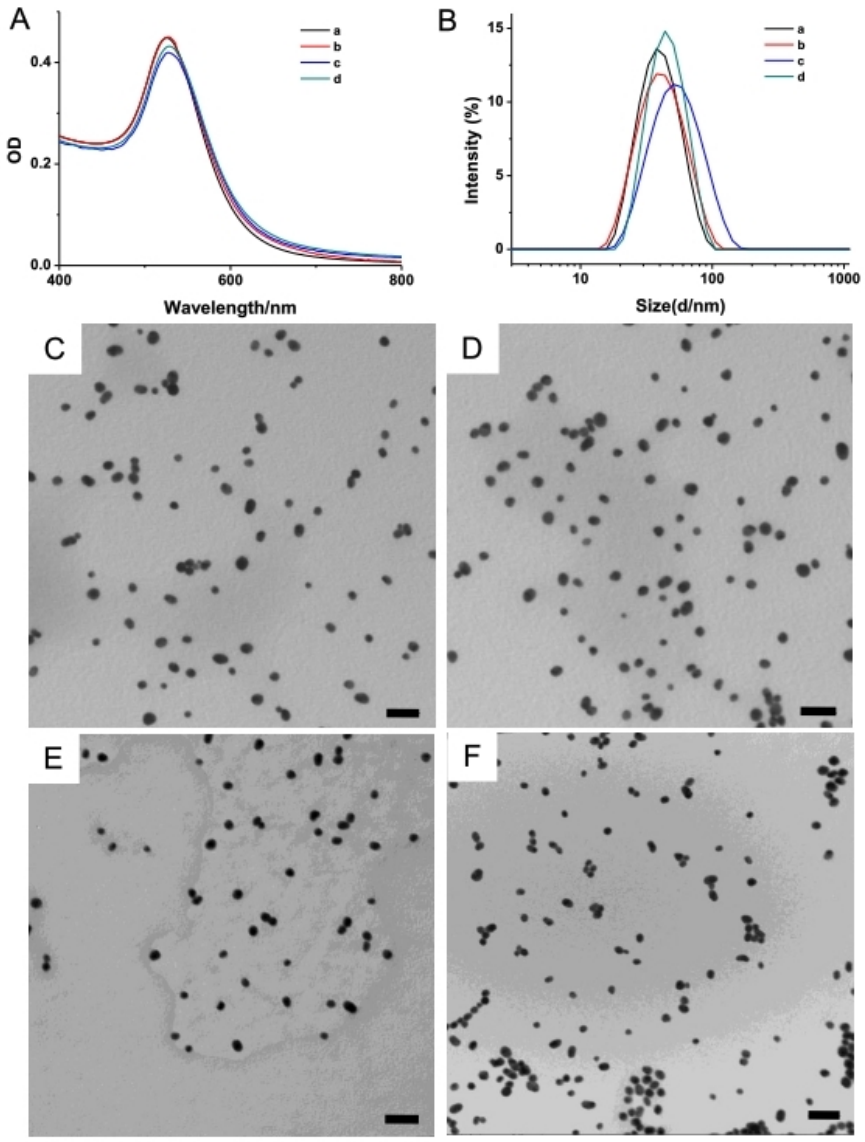

Fig. S1 (A) The UV-visible spectra measurement of GNPs, (B) the hydrodynamic diameter distribution of GNPs acquired from dynamic light scattering (DLS) experiment and TEM measurement of GNPs. (a) and (C) citrate stabilized GNP; (b) and (D) peptide capped GNP; (c) and (E) N-terminal antibody and biotin co-functionalized GNP (Ab16-GNP); and (d) and (F) streptavidin functionalized GNP (SA-GNP), respectively. The scale bars in TEM images indicate 100 nm.

No significant change of the surface plasmon resonance band was observed after the surface modification of GNPs.

The hydrodynamic diameters of citrate stabilized GNPs, peptide capped GNPs, Ab16-GNPs and SA-GNPs were 42 nm, 43 nm, 57 nm and 48 nm, respectively.

The diameters of citrate stabilized GNPs, peptide capped GNPs, Ab16-GNPs and SA-GNPs were 28.2±3.4 nm according to TEM measurement. The experimental result indicates that proteins (streptavidin and antibody) are conjugated to the GNPs.

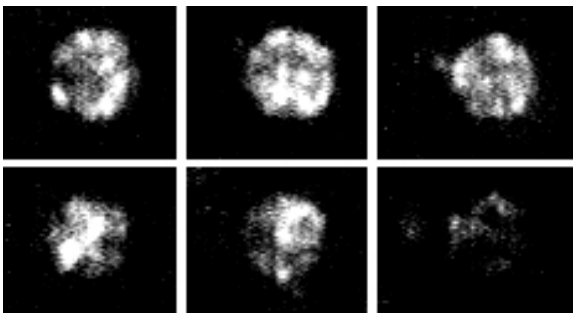

Fig. S2 Light scattering images of C-terminal antibody spots after incubating with 10 μg/mL $A\beta_{1\text{-}42}$ (up) or blank PBS (bottom), identifying with 0.25 nM Ab16-GNPs and reacting with 0.25 nM SA-GNPs, respectively. The concentrations of C-terminal antibody (from left to right) in the spotting solution are 1 mg/mL, 100 μg/mL and 10 μg/mL, respectively. The C-terminal antibody spot from 10 μg/mL C-terminal antibody in spotting solution gives relatively higher signal-to-background noise ratio.

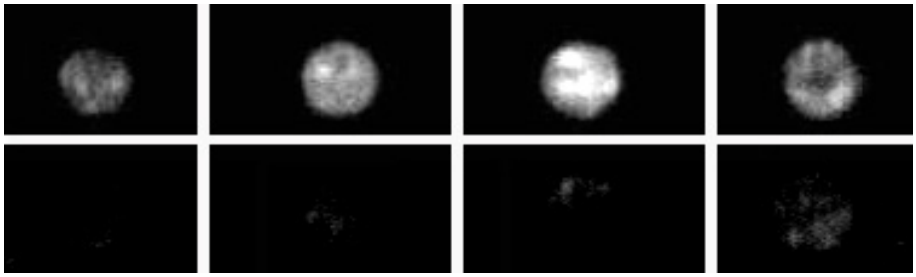

Fig. S3 Light scattering images of C-terminal antibody spots after incubating with 10 μg/mL $A\beta_{1\text{-}42}$ (up) or blank PBS (bottom) for different time, identifying with 0.25 nM Ab16-GNPs and reacting with 0.25 nM SA-GNPs, respectively. The incubating time of C-terminal antibody spots with $A\beta_{1\text{-}42}$ solutions (from left to right) are 1 h, 2 h, 4 h and 8 h, respectively. The highest signal-to-background noise ratio has been obtained after incubation of C-terminal antibody spot with $A\beta_{1\text{-}42}$ solution for 4 h.

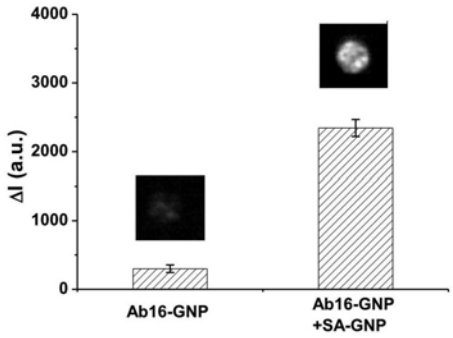

Fig. S4 The light scattering images and corresponding intensities of C-terminal antibody spots after incubating with 10 μg/mL $A\beta_{1\text{-}42,}$ identifying with 0.25 nM Ab16-GNPs, and reacting with or without 0.25 nM SA-GNPs, respectively. The experimental result indicates that the light scattering intensity is greatly increased after reacting with SA-GNPs. The error bars are standard deviations (*n=3*).

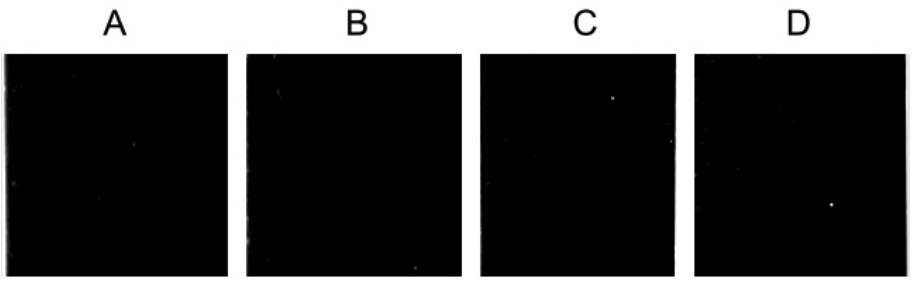

Fig. S5 The light scattering images of C-terminal antibody spots after incubating with 10 μg/mL $A\beta_{1\text{-}16}$ (A),, $A\beta_{1\text{-}40}$ (B), $A\beta_{1\text{-}42}$ profibril (C) and $A\beta_{1\text{-}42}$ fibril (D), identifying with 0.25 nM Ab16-GNPs, and reacting with 0.25 nM SA-GNPs, respectively.

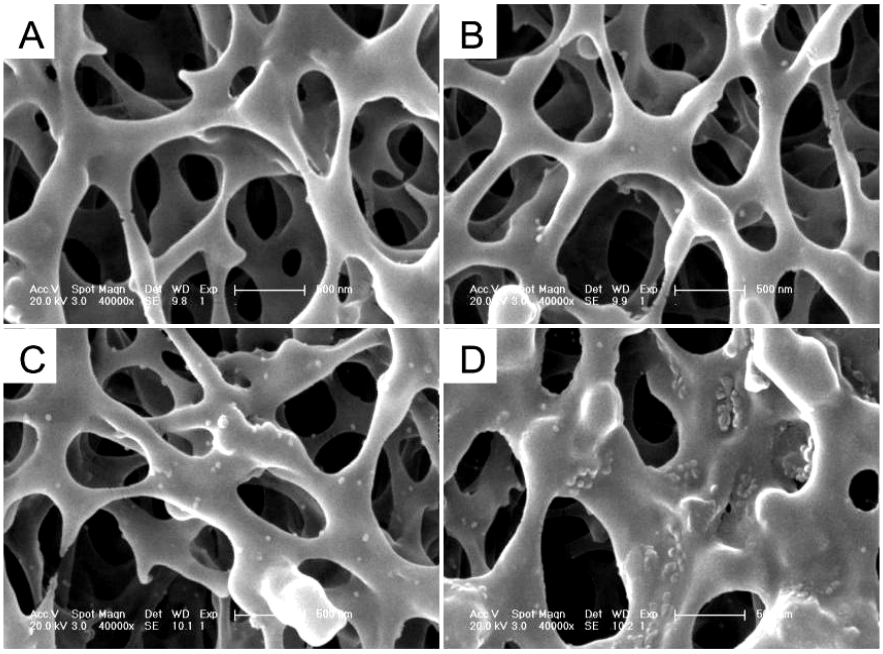

Fig. S6 SEM micrographs of C-terminal antibody spots after incubating with $A\beta_{1\text{-}42}$, identifying with 0.25 nM Ab16-GNPs and reacting with 0.25 nM SA-GNPs, respectively. The concentrations of $A\beta_{1\text{-}42}$ are (A) 0, (B) 100 pg/mL, (C) 100 ng/mL and (D) 10 μg/mL, respectively. The scale bars indicate 500 nm.

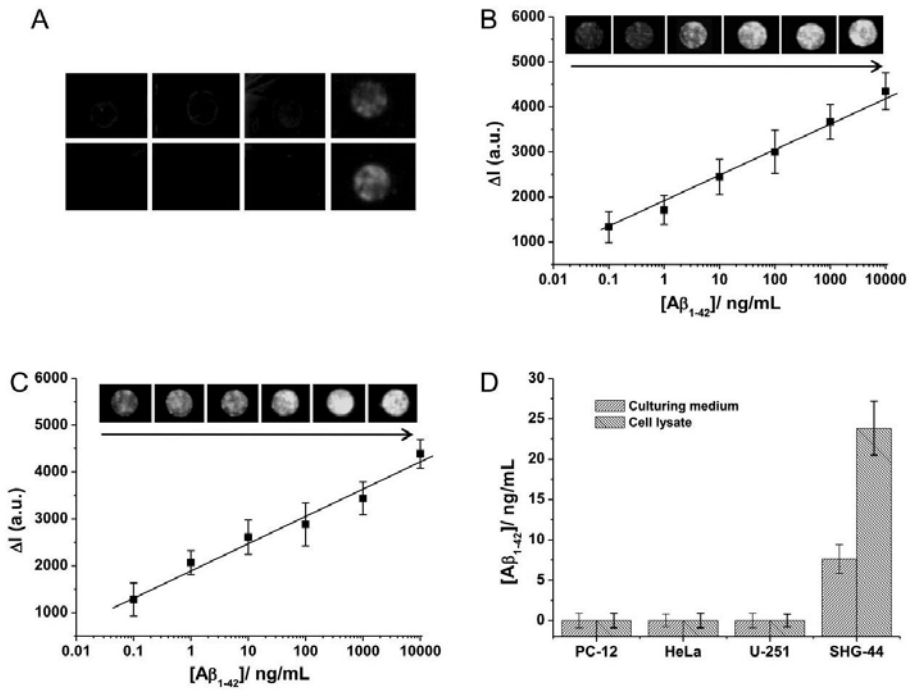

Fig. S7 (A) Light scattering images of C-terminal antibody spots after incubating with $A\beta_{1\text{-}42}$ in culturing mediums (up) and lysates (bottom) of different cell lines, identifying with 0.25 nM Ab16-GNPs and reacting with 0.25 nM SA-GNPs, respectively. From left to right are PC-12, HeLa, U-251 and SHG-44 cells, respectively. Light scattering intensity as a function of the concentration of added $A\beta_{1\text{-}42}$ in the culturing medium (B) and lysate (C) of SHG-44 cells. Insets of (B) and (C) show the corresponding light scattering images of C-terminal antibody spots, respectively. The blank PBS was used as control sample. The concentrations of added $A\beta_{1\text{-}42}$ (from left to right) are 100 pg/mL, 1 ng/mL, 10 ng/mL, 100 ng/mL, 1 μg/mL and 10 μg/mL, respectively. (D) $A\beta_{1\text{-}42}$ expression levels in different cell samples. The error bars are standard deviations (*n=3*)

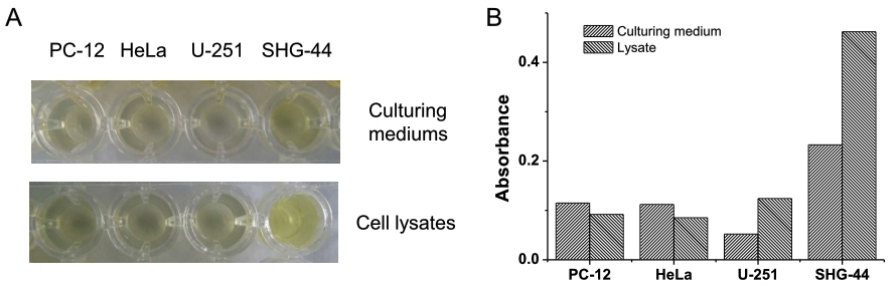

Fig. S8 ELISA experiment. Comparison experiment of $A\beta_{1\text{-}42}$ expression level in culturing mediums (up) and lysates (down) of different cell lines (A) and the corresponding absorbance at 450 nm of solution (B), respectively.

The experimental results indicates that only SHG-44 cells express high level of $A\beta_{1\text{-}42}$ in both of cell culturing medium and cell lysate.

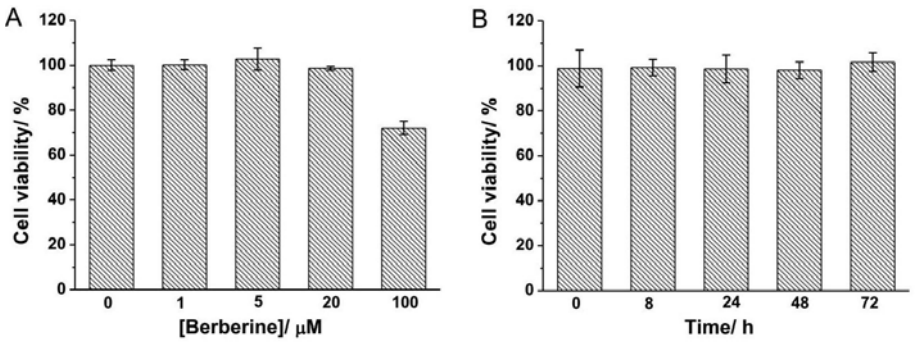

Fig. S9 (A) Effect of berberine concentration on the cytotoxicity of SHG-44 cells for 72 hours of incubation; and (B) effect of incubation time on the cytotoxicity of SHG-44 cells in the presence of 20 μM berberine. The viability of SHG-44 cells cultured under same condition without berberine is defined as 100%. The error bars are standard deviations (*n*=*3*)

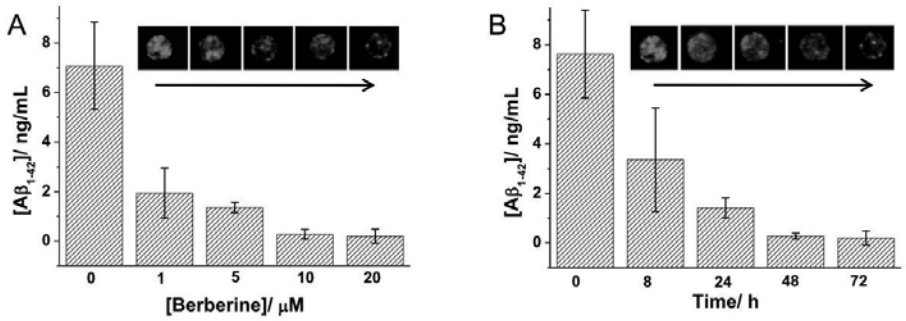

Fig. S10 (A) Effect of berberine concentration on the production of $A\beta_{1\text{-}42}$ in culturing medium of SHG-44 cells by co-culturing with berberine for 72 hours. The concentrations of berberine (from left to right) are 0, 1 μM, 5 μM, 10 μM and 20 μM, respectively. (B) Effect of co-culturing time on the production of $A\beta_{1\text{-}42}$ in culturing medium of SHG-44 cells in the presence of 20 μM berberine. The co-culturing time (from left to right) are 0, 8 h, 24 h, 48 h and 72 h, respectively. The error bars are standard deviations (n=3).

Table. S1 Comparison of the abilities of assays.

Samples	Detected species	Methods	Linear range/ Limit of detection	References
Aqueous solution	Tau protein	Two-photo rayleigh scattering (using antibody)	5-350 ng/mL	*ACS Nano* **2009**, *3*, 2834-2840
Aqueous solution	Tau protein	Fluorescence analysis (designed fluorescent molecule)	0-1250 $\mu g/mL^{-1}$ can be detected	*J. Am. Chem. Soc.* **2009**, *131*, 6543-6548
Aqueous solution	Tau protein	SPR, Immunochip (using antibody)	Limit of detection: 0.01 ng/mL	*Talanta* **2008**, *74*, 1038-1042
Aqueous solution	$A\beta_{1-40}$/ $A\beta_{1-42}$	Electrochemistry (using antibody)	2-40 μg/mL	*Bioelectrochemistry* **2008**, *74*, 118-123
Aqueous solution	$A\beta_{1-28}$/ $A\beta_{1-40}$	Fluorescence analysis	0-5 μg/mL	*Protein Sci*. **1993**, *2*, 404-410
Aqueous solution	$A\beta_{1-40}$/ $A\beta_{1-42}$	Electrochemistry	Limit of detection: 0.7 μg/mL	*J. Am. Chem. Soc.* **2005**, *127*, 11892-11893
Cell derived samples	$A\beta_{1-42}$	Flow cytometry-fluorescence resonance energy transfer (using antibody)	0.04-10 ng/mL	*J. Alzheimer's Dis.* **2007**, *11*, 117-125.
Cell derived samples	$A\beta_{1-40}$/ $A\beta_{1-42}$	Immunoprecipitation-HPLC-mass spectrometry	0.4-12 pg/mL	*FEBS letters* **1998**, *430*, 419-423
Cell derived samples	$A\beta_{1-40}$	Liquid chromatography-capillary electrophoresis-electrospray mass spectrometry	Limit of detection:25 ng/mL	*J. Chromatogr. A.* **2002**, *974*, 135-142
Aqueous solution	$A\beta_{1-42}$	Resonance light scattering	1 ng/mL-1 μg/mL and 5-50 μg/mL	*Chem. Commun*. **2011**, *47*, 9339-9341
Aqueous solution	**$A\beta_{1-42}$**	**GNP based dot-blot immunoassay**	**0.1 ng/mL-10 μg/mL**	**The present work**

Additional references

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