Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information for:

Resonance light scattering as a powerful tool for sensitive detection of β-amyloid peptide by gold nanoparticle probes

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Experimental section

Materials and reagents. Peptides (CALNN, CALNN GK(biotin)G, and GK(biotin)G DAEFR HDSGY EVHHQ K ($A\beta_{1-16}$(biotin)) were purchased from Scilight Biotechnology Ltd. (Beijing, China). DAEFR HDSGY EVHHQ KLVFF AEDVG SNKGA IIGLM VGGVV IA ($A\beta_{1-42}$) was purchased from A Peptide Ltd. (Shanghai, China). Hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$), 2-[4-(2-Hydroxyethyl)-1-piperazine]ethanesulfonic acid (HEPES), 2-(4-Morpholino)ethanesulfonic acid (MES) and human serum albumin (HSA) were purchased from Sigma-Aldrich Co. (USA). Streptavidin was purchased from Promega Co. (USA). The human plasma (HP) samples were obtained from healthy persons at No. 208 hospital of Liberation Army (Changchun, China). Other chemicals were analytical grade, and they were used as received. Milli-Q water (18.2 MΩ.cm) was used in all experiments.

Preparation of streptavidin-conjugated nanoparticles. The citrate stabilized 13 nm GNPs were synthesized by traditional Turkevich–Frens method.[1] Peptide-stabilized nanoparticles were prepared by previously reported peptide capping procedure.[2] Generally, an aqueous solution of peptide mixture (CALNN : CALNN GK(biotin)G = 9 : 1) was added to the solution of 1 mL 13 nm GNPs (5 nM) to give a final concentration of total peptides of 1.5 mM. After 1 h incubation, excess peptides were removed by repeated centrifugation at 8800 rpm (~ 7200 g, 3 times) using an Eppendorf centrifuge (Eppendorf, Germany). The purified GNPs were resuspended in 1 mL water. Then a 30 μL streptavidin (2 mg mL^{-1}) aqueous solution was added to the solution. After 1 h incubation, excess streptavidin was removed by repeated centrifugation at 8800 rpm (~ 7200 g, 3 times). The purified streptavidin-conjugated GNPs were resuspended in 1 mL HEPES buffer (pH 7.4 or 6.6, 10 mM HEPES, 0.8 % NaCl (w/w)) or MES buffer (pH 5.8 or 5.0, 10 mM MES, 0.8 % NaCl (w/w)), which were named as SA-GNPs. All experiments were carried out at room temperature except mentioned specially.

Detect $A\beta_{1-42}$ in aqueous solution. Different kinds of $A\beta_{1-16}$(biotin) and Zn^{II} complexes were prepared by adding desired amount of $ZnCl_2$ aqueous solution into 1 mL $A\beta_{1-16}$(biotin) solution in the absence or presence of different amounts of $A\beta_{1-42}$, and then incubated at 37 ^{o}C for 72 h, respectively. Subsequently, 50 μL of the solutions were mixed thoroughly with 200 μL of SA-GNPs (1.5 nM),

respectively. After 30 min incubation, the RLS spectra of these solutions were recorded on a Cary Eclipse Spectrophotometer (Varian Inc., USA). The RLS spectra were conducted by synchronous scanning of excitation wavelength (λ_{ex}) and emission wavelength (λ_{em}), and the used λ_{ex} is as same as λ_{em} during the scanning.

Detect $A\beta_{1-42}$ in diluted HP. The similar detection procedure was used as described in aqueous solution, except that diluted HP was applied as reaction matrix. The diluted HP was prepared by adding fresh 10 μl human plasma into the 990 μl HEPES buffer samples, and mixed well. The $A\beta_{1-42}$ spiked diluted HP samples were prepared by adding different amounts of $A\beta_{1-42}$ into the as-prepared diluted HP, and mixed well.

UV-visible spectra measurement. UV-visible spectra of all solutions were recorded on a UV-visible spectrophotometer (UV mini 1240, Shimadzu Instruments, Japan).

Dynamic light scattering (DLS) measurement. The hydrodynamic diameters of GNPs were recorded by a Zetasizer Nano ZS DLS system (Malvern Instruments Ltd., England).

TEM measurement. TEM micrographs of all samples were obtained by a JEOL 2000FX (JEOL, Japan) 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. The grid was then incubated with phosphotungstic acid (1 % (w/v), 10 μL) for 1 min, washed with water and dried at room temperature in the air.

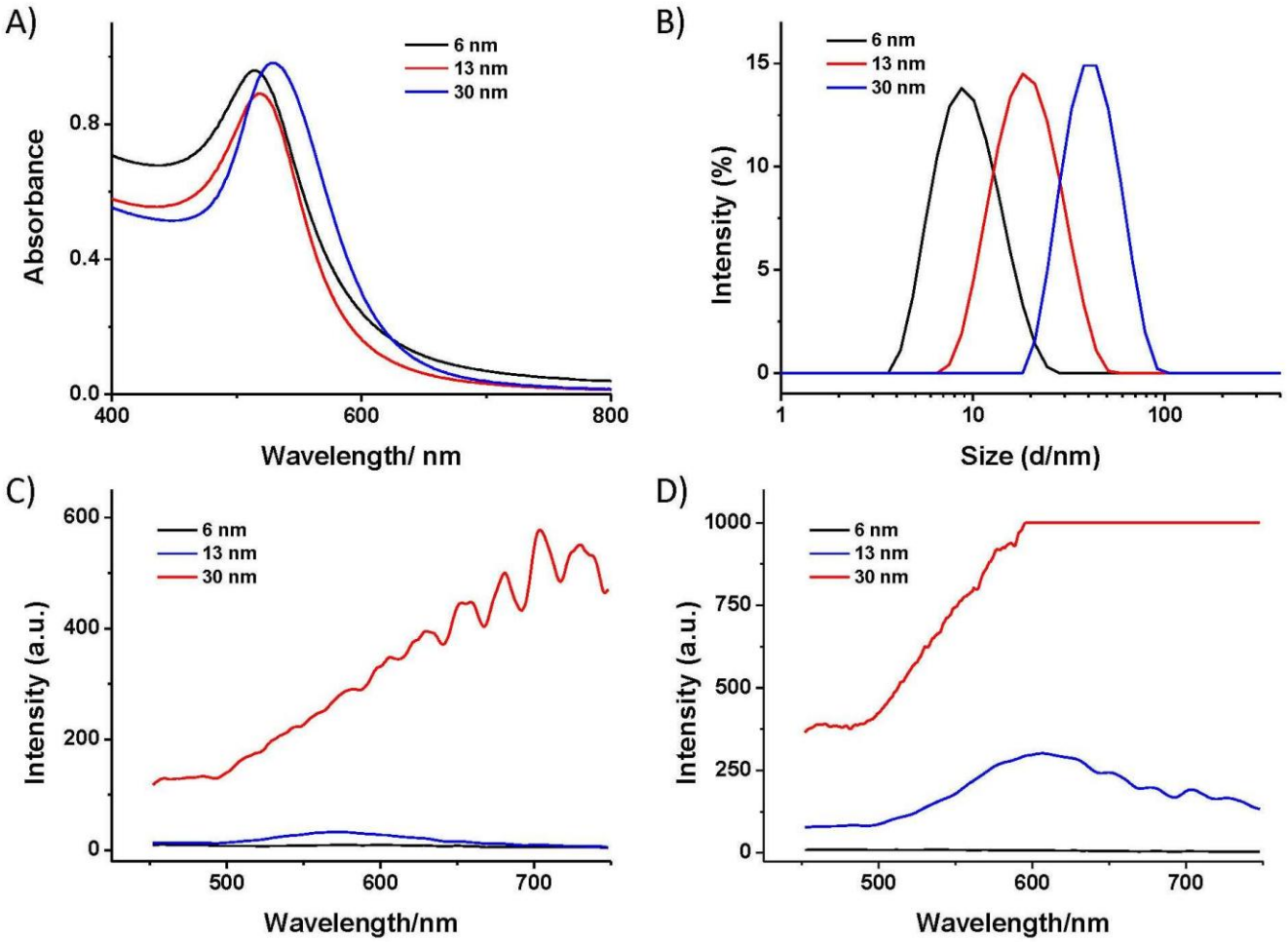

Fig. S1 The UV-visible spectra (A) and hydrodynamic diameter distributions (B) of as-prepared GNPs. Corresponding RLS spectra of SA-GNPs only (C), and the SA-GNPs after incubated with Zn^{II} induced $A\beta_{1\text{-}16}$(biotin) aggregates in HEPES solution (D), respectively. The concentration of $A\beta_{1\text{-}16}$(biotin) is 2.0 μg mL^{-1}, the concentration of Zn^{II} is 4.0 μM. and the concentration of SA-GNPs is 1.2 nM, respectively.

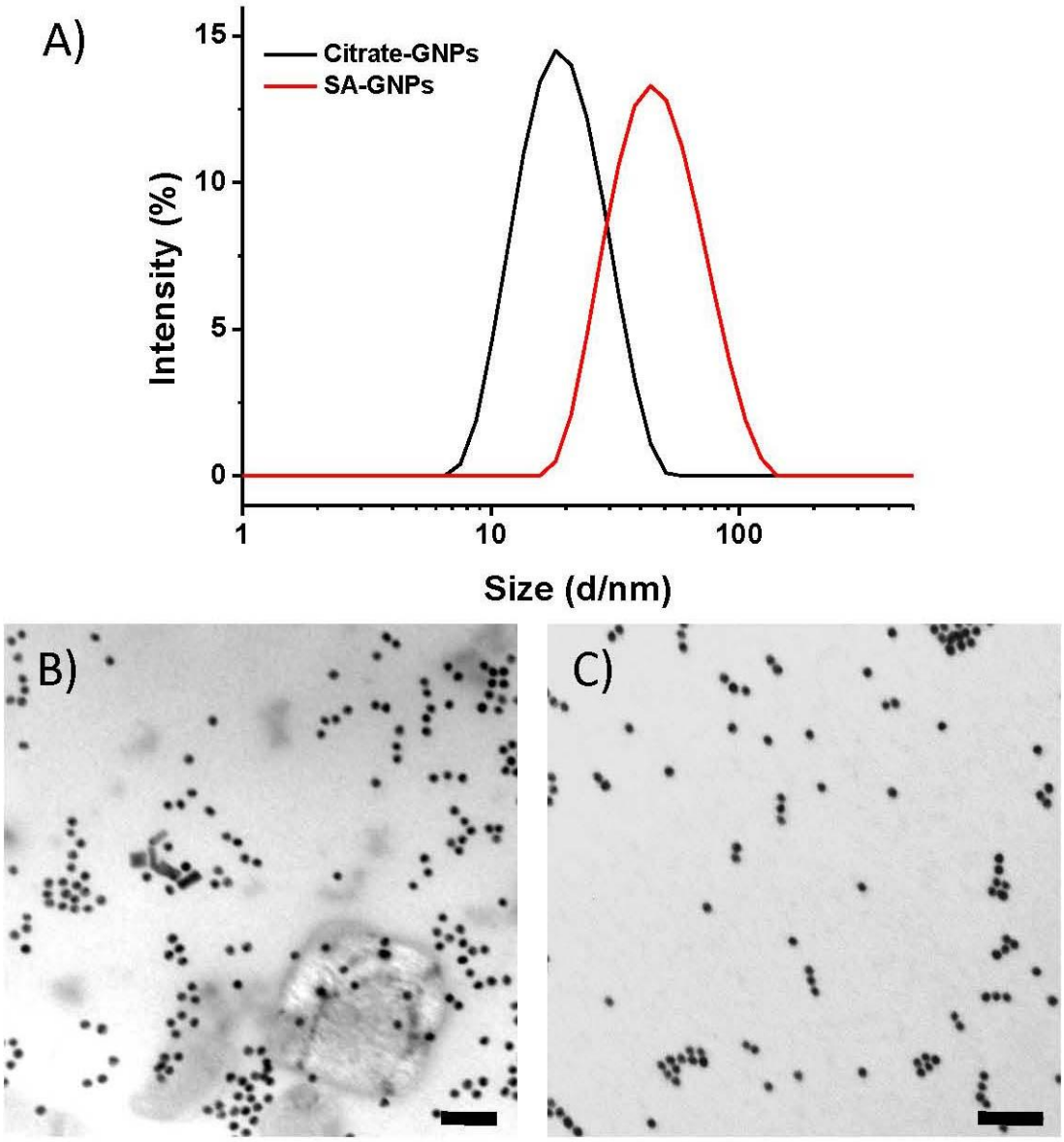

Fig. S2 The hydrodynamic diameter distribution of synthesized and functionalized GNPs acquired from dynamic light scattering (DLS) experiment (A), the polydispersity index (PDI) of these two GNPs are 0.155 and 0.189, respectively; and the corresponding TEM micrographs of as-prepared citrate capping GNPs (B) and SA-GNPs (C). The scale bars indicate 100 nm.

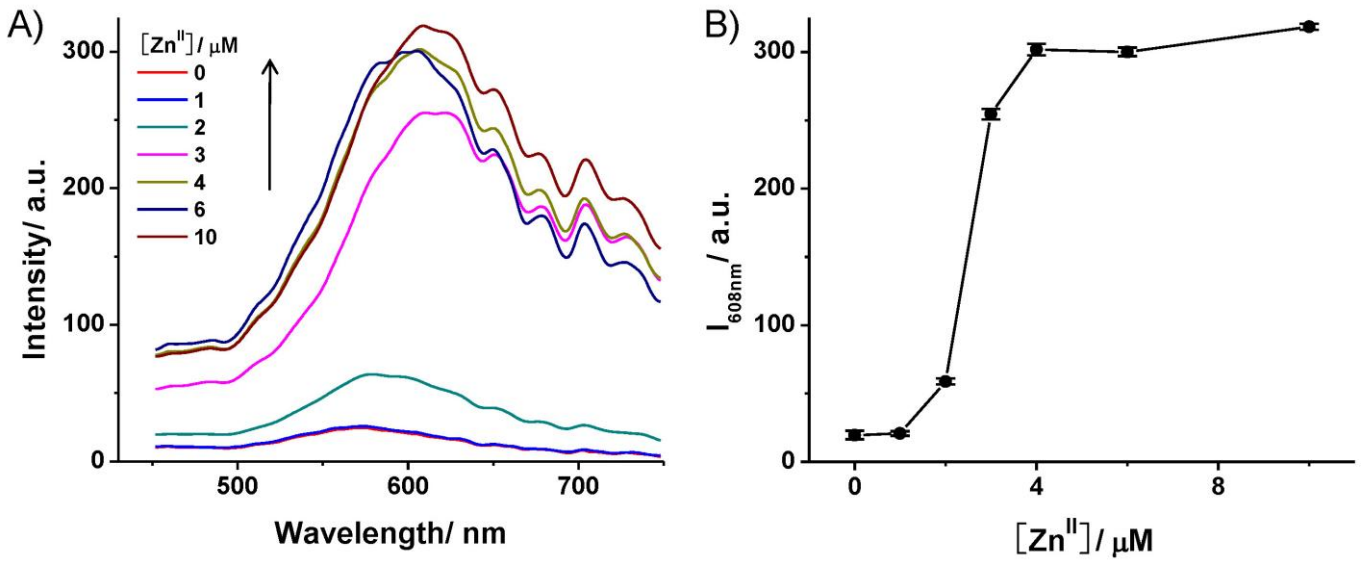

Fig. S3 The RLS spectra of SA-GNPs after incubated with Zn^{II} induced $A\beta_{1\text{-}16}$(biotin) aggregates in HEPES solution in the presence of 100 μg mL^{-1} HSA (A); and the RLS intensity at 608 nm as a function of the concentration of Zn^{II} (B), respectively. The concentration of $A\beta_{1\text{-}16}$(biotin) is 2.0 μg mL^{-1}, and the concentration of SA-GNPs is 1.2 nM, respectively.

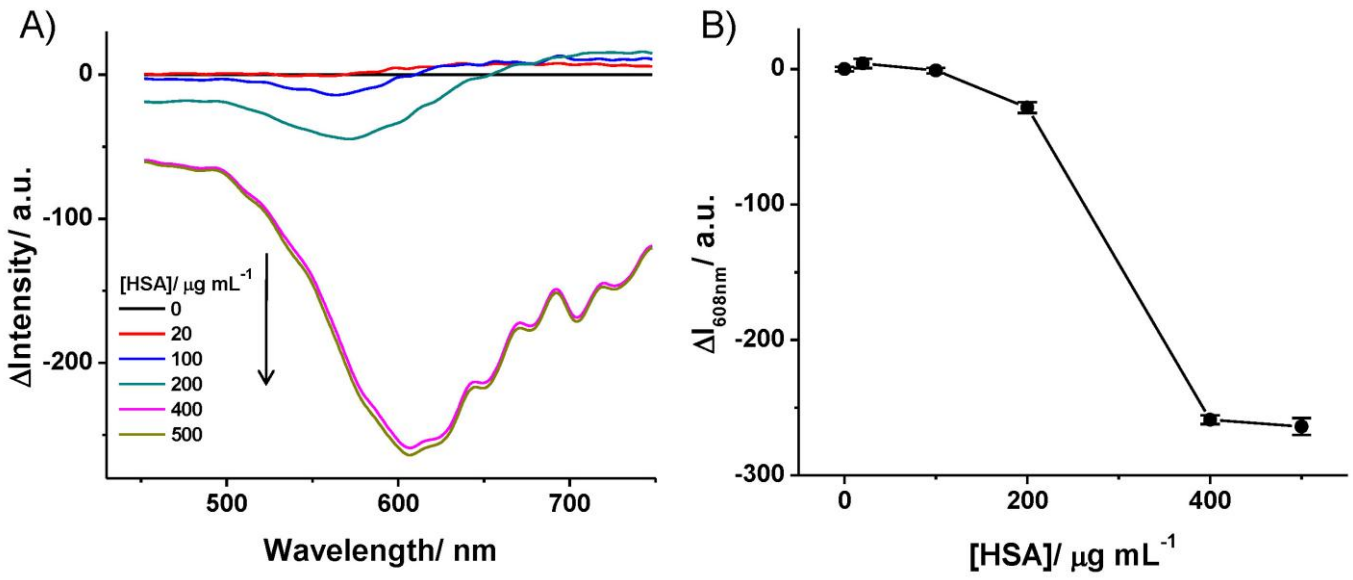

Fig. S4 The RLS spectra changes of SA-GNPs with Zn^{II} induced $A\beta_{1\text{-}16}$(biotin) aggregates by adding different amounts of HSA in aqueous solution (A); and the RLS intensity changes at 608 nm as a function of the concentration of HSA (B), respectively. The concentration of $A\beta_{1\text{-}16}$(biotin) is 2.0 μg mL^{-1}, the concentration of Zn^{II} is 4.0 μM, and the concentration of SA-GNPs is 1.2 nM, respectively. The RLS spectrum of SA-GNPs with Zn^{II} induced $A\beta_{1\text{-}16}$(biotin) aggregates in the absence of HSA was used as blank.

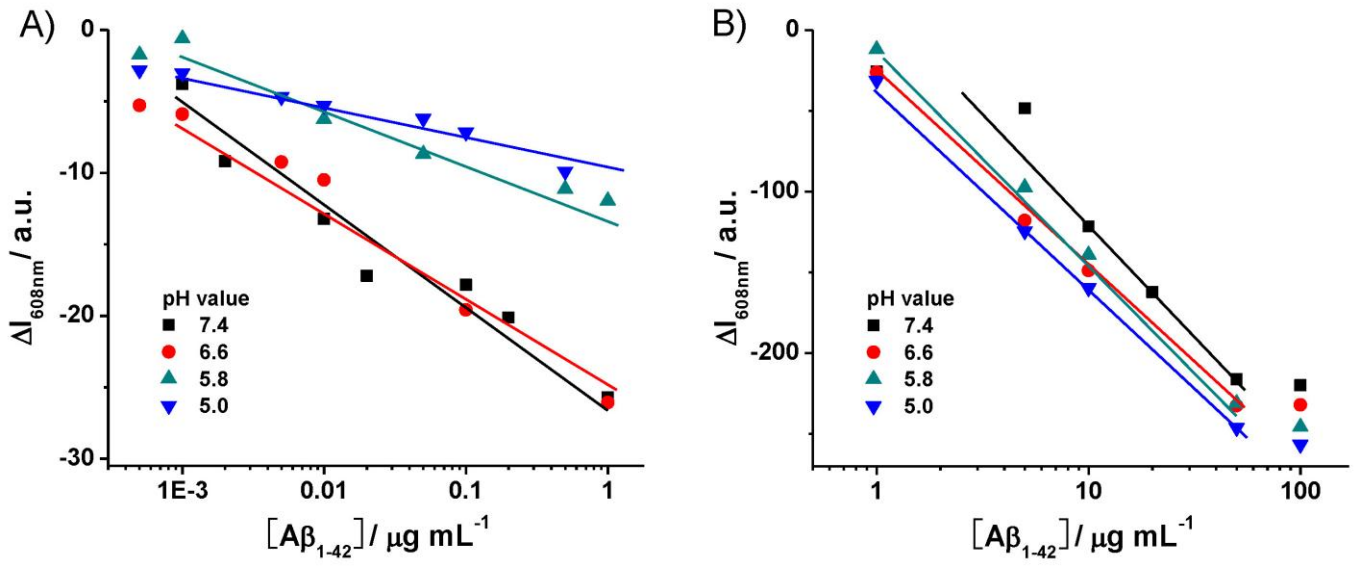

Fig. S5 The effect of solution pH value on the detection of $A\beta_{1-42}$ in aqueous solutions. The RLS intensity changes at 608 nm as a function of the concentration of $A\beta_{1-42}$ below 1.0 μg mL^{-1} (A); and above 1.0 μg mL^{-1} (B), respectively. The concentration of $A\beta_{1-16}$(biotin) is 2.0 μg mL^{-1}, the concentration of Zn^{II} is 4.0 μM. and the concentration of SA-GNPs is 1.2 nM, respectively. The RLS spectra of SA-GNPs with Zn^{II} induced $A\beta_{1-16}$(biotin) aggregates in the absence of $A\beta_{1-42}$ were used as blanks, respectively. The slope of the line indicates the detection sensitivity of the assay.

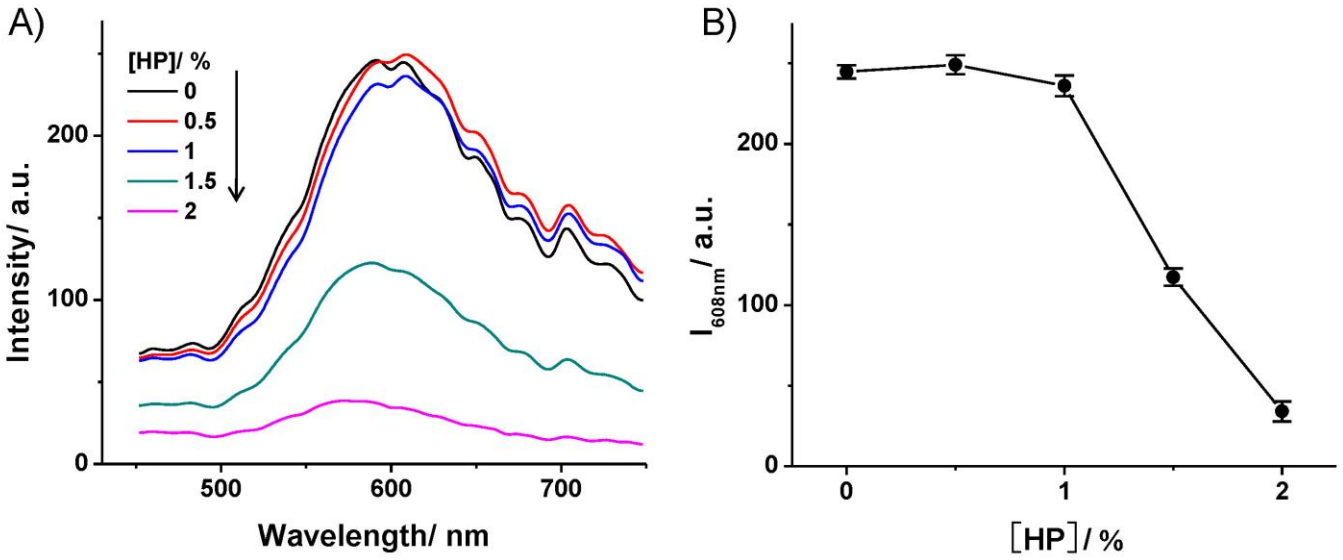

Fig. S6 The RLS spectra of SA-GNPs after incubated with Zn^{II} induced $A\beta_{1\text{-}16}$(biotin) aggregates in different concentrations of HP (A); and the RLS intensity at 608 nm as a function of the concentration of HP (B), respectively. The concentration of $A\beta_{1\text{-}16}$(biotin) is 2.0 μg mL^{-1}, the concentration of Zn^{II} is 25.0 μM, and the concentration of SA-GNPs is 1.2 nM, respectively.

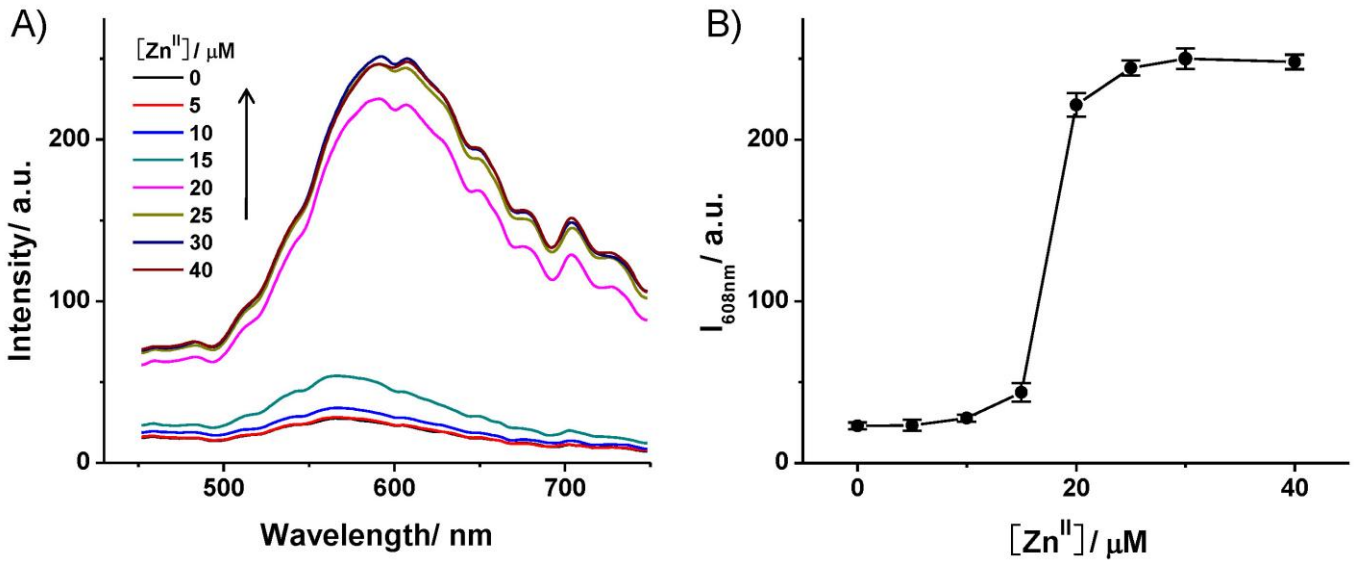

Fig. S7 The RLS spectra of SA-GNPs after incubated with Zn^{II} induced $A\beta_{1\text{-}16}$(biotin) aggregates in 1 % HP (A); and the RLS intensity at 608 nm as a function of the concentration of Zn^{II} (B), respectively. The concentration of $A\beta_{1\text{-}16}$(biotin) is 2.0 μg mL^{-1}, and the concentration of SA-GNPs is 1.2 nM, respectively.

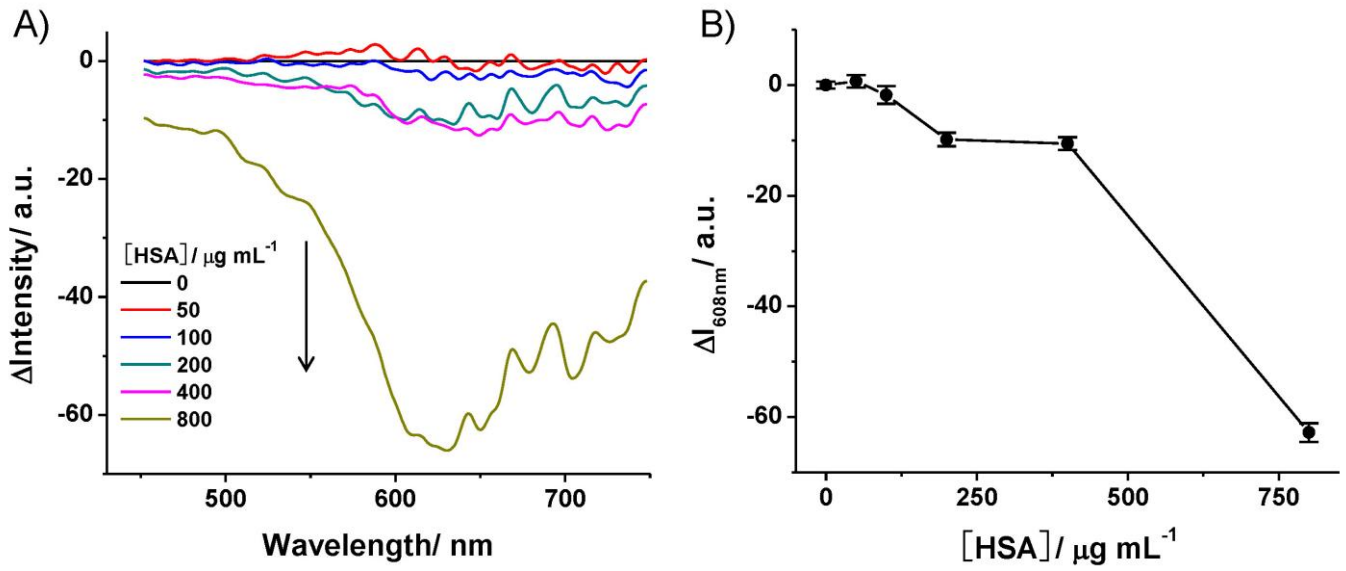

Fig. S8 The RLS spectra changes of SA-GNPs with Zn^{II} induced $A\beta_{1-16}$(biotin) aggregates by adding different amounts of HSA in 1 % HP (A); and the RLS intensity changes at 608 nm as a function of the concentration of HSA (B), respectively. The concentration of $A\beta_{1-16}$(biotin) is 2.0 μg mL^{-1}, the concentration of Zn^{II} is 25.0 μM, and the concentration of SA-GNPs is 1.2 nM, respectively. The RLS spectrum of SA-GNPs with Zn^{II} induced $A\beta_{1-16}$(biotin) aggregates in the absence of HSA was used as blank.

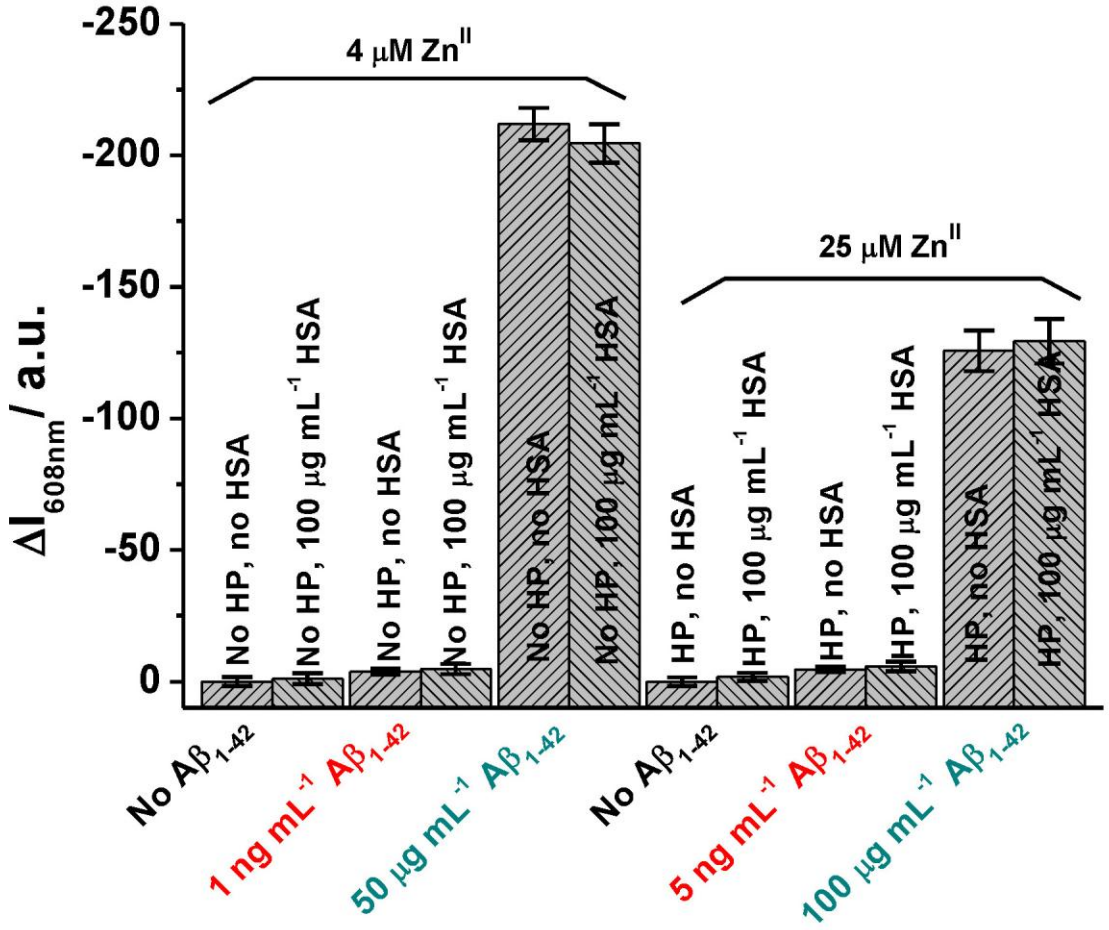

Fig. S9 The interference of HSA on the detection of $A\beta_{1-42}$ in aqueous solutions and in diluted HP, respectively. The concentration of $A\beta_{1-16}$(biotin) is 2.0 μg mL^{-1}, and the concentration of SA-GNPs is 1.2 nM, respectively. The solutions in the absence of $A\beta_{1-42}$ and HSA in aqueous solutions or in diluted HP were used as blanks, respectively.

Table. S1 Comparison of our work with previous methods to detect Aβ related molecules in aqueous solutions and cell derived samples.

Samples	Detected species	Methods	Linear range/ Limit of detection	References
Aqueous solution	Tau protein	Two-photo rayleigh scattering (using antibody)	5-350 ng mL^{-1}	*ACS Nano* **2009**, *3*, 2834-2840
Aqueous solution	Tau protein	Fluorescence analysis (designed fluorescent molecule)	0-1250 μg 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^{-1}	*Talanta* **2008**, *74*, 1038-1042
Aqueous solution	$Aβ_{1-40}$/ $Aβ_{1-42}$	Electrochemistry (using antibody)	2-40 μg mL^{-1}	*Bioelectrochemistry* **2008**, *74*, 118-123
Aqueous solution	$Aβ_{1-28}$/ $Aβ_{1-40}$	Fluorescence analysis	0-5 μg mL^{-1}	*Protein Sci*. **1993**, *2*, 404-410
Aqueous solution	$Aβ_{1-40}$/ $Aβ_{1-42}$	Electrochemistry	Limit of detection:0.7 μg mL^{-1}	*J. Am. Chem. Soc.* **2005**, *127*, 11892-11893
Cell derived samples	$Aβ_{1-42}$	Flow cytometry-fluorescence resonance energy transfer (using antibody)	0.04-10 ng mL^{-1}	*J. Alzheimer's Dis.* **2007**, *11*, 117-125.
Cell derived samples	$Aβ_{1-40}$/ $Aβ_{1-42}$	Immunoprecipitation-HPLC-MS	0.0004-0.012 ng mL^{-1}	*FEBS letters* **1998**, *430*, 419-423
Cell derived samples	$Aβ_{1-40}$	Liquid chromatography - capillary electrophoresis -electrospray mass spectrometry	Limit of detection:25 ng mL^{-1}	*J. Chromatogr. A*. **2002**, *974*, 135-142
Aqueous solution	**$Aβ_{1-42}$**	**RLS**	**1 ng mL^{-1}-2.5 μg mL^{-1} and 2.5-50 μg mL^{-1}**	**Our present work**
1 % human plasma	**$Aβ_{1-42}$**	**RLS**	**5 ng mL^{-1}-20 μg mL^{-1} and 20-100 μg mL^{-1}**	

References

S1 (*a*) J. Turkevich, P. Stevenson and J. Hillier, *Discuss. Faraday Soc.*, 1951, **11**, 55-75. (*b*) G. Frens, *Nature Phys. Sci.*, 1973, **241**, 20–22.

S2 (*a*) C. Wang, J. Wang, D. Liu and Z. Wang, *Talanta*, 2010, **80**, 1626-1631. (*b*) C. Wang, J. Wang, D. Liu and Z. Wang, *Anal. Methods*, 2010, **2**, 1467-1471.