Supporting Information

A sensitive colorimetric strategy for sensitively monitoring cerebral β-amyloid peptides in AD based on dual-functionalized gold nanoplasmic particles

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Experimental section:

Materials. Purified synthetic β-amyloid peptides ($A\beta_{-11}$, $A\beta_{-16}$, $A\beta_{-38}$, $A\beta_{-40}$ and $A\beta_{-42}$) were all obtained from ChinaPeptides Co,. Ltd (Shanghai, China) and stock solutions of these peptides (1 mg mL^{-1}) were prepared by independently dissolving desired amounts of them into 25 mM Tris-HCl buffer solution (pH 7.4) and diluted to approprite concentrations using 10 mM NaOH and freshly-prepared daily before experiments. The oligomeric forms of these peptides were made according to reference 1.[1] Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was purchased from Sinopharm Chemical Reagent Co., Ltd. Polyethylenimine (PEI) and copper (II) chloride were bought from Aladdin Reagent Co. Hemin (which is referred to as heme) and human serum albumin (HSA) were purchased from Sigma Co. Phosphate buffer saline (PBS, pH 7.4) containing 8.72 mM Na_2HPO_4, 1.41 mM KH_2PO_4, 136.7 mM NaCl and 2.7 mM KCl was employed as incubation buffer. The test kits of malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) were purchased from Beyotime Institute of Biotechnology. Human β-amyloid ELISA kits were obtained from Shanghai Saimo Biological Science and Technology Development Limited Company. Water (≥18 MΩ) used throughout the whole experiment was purified with Millipore system. All reagents were of the analytical grade commercially available and used without further purification.

Apparatus. UV-vis absorption characterizations were performed on a UV-vis 2450 spectrometer (Shimadzu, Japan). The size distribution and monodispersity of PEI/GNPs and the prepared nanoprobe in the absence and presence of Aβ were measured by using a FEI Tecnai G2 T12 transmission electron microscope (TEM, USA) operating at 120 kV. The TEM specimens were prepared by dropping the sample solutions onto 50 Å carbon coated copper grids with the excess solution being immediately wicked away. Zeta-potentials of PEI/GNPs and the probe were measured by a Zetasizer Nano ZS (Malvern, UK). Images of pathological sections of brain tissues from rats were observed and captured with a fluorescence microscope (IX51,

Olympus Corp., Tokyo, Japan).

Preparation of the PEI/GNPs-Cu-hemin probe. Firstly, polyethylenimine (PEI) functionalized GNPs with an average diameter of 11 nm were prepared as follows:[2] To a rapidly stirred $HAuCl_4$ solution (1 g $10mL^{-1}$, 100 mL), 25 mL ultrapure water and 1 mL PEI (1%) were successively added. The color of the solution turned from pale yellow to deep brown immediately. After that, the mixture was kept stirring for another 5 h at room temperature to obtain a wine red PEI/GNPs solution and stored in 4°C when not in use. In our typical experiment, after adjusted to pH 2.0, 200 μL of the as-prepared PEI/GNPs were centrifuged at 4°C, 14000 r/min for 15 min, followed by resuspending with 200 μL PBS (0.1M, pH 7.4). Then, 2 μL of 40 mM $CuCl_2$ (pH = 2.0) and 3 μL of 0.25 mM hemin were added in succession, vortex-mixted and incubated at 37°C, 140 r/min for 1 h. Finally, the mixture was centrifugated at 14000 r/min to remove any unreacted substances, resuspended with PBS, and the resultant PEI/GNPs-Cu-hemin probe was used for absorbance measurement at a wavelength of 525 nm (A_1). The colorimetric sensing of Aβ using the PEI/GNPs-Cu-hemin nanoprobe was performed by mixing different concentrations of Aβ standard solutions or real samples with the probe at a volume ratio of 1: 49. After incubated for another 1 h at 37°C, 140 r/min and then centrifugated, the UV-vis absorbance of the solution was measured again at 525 nm (A_2). The quantitative analysis of Aβ with varied concentrations was based on the calculated ΔA value at 525 nm ($\Delta A = A_1 - A_2$).

Animal experiments. Briefly, male Wistar rats, weighing 100-120g at the beginning of experiments, were obtained from Shanghai Yisen Biotechnology Co., Ltd. Rats were housed in plastic cages, with food and water available ad libitum, and kept under standard environmental conditions (12-h light/dark cycle, 22°C). All animal experiments were conducted with approval of the Animal Ethics Committee in Xuzhou Medical College, China. All efforts were made to minimize the number of animals used and their sufferings.

In this work, rats were randomly divided into four groups: normal group,

Alzheimer's disease (AD) group, CMC-Na-treated group (control) and ginkgo biloba extract (GBE)-treated group (n = 12 in each group). The AD model was conducted according to our previously reported procedures,[3] by daily administrating D-galactose (D-gal, Aladdin reagent Co., Ltd, 50 mg kg^{-1} in 0.5 mL saline, i.p.) for six weeks and bilateral infusions of 1 μL ibotenic acid (IBO, Enzo Life Science Inc., dissolved in ice PBS at 8 g L^{-1}) into nucleus basalis magnocellularis (NBM).

After confirming the successful modeling of AD rats, rats in AD group were received GBE administrations (i.g., 150 mg kg^{-1}) daily for successive two weeks to observe the rebound or recovery of Aβ monomer levels in rat brain. GBE was reported to be effective in protecing central nervous system, which has been extensively applied in clinical to treat cognitive function damaged diseases such as AD, Parkinson's disease.[4,5] Meanwhile, as a control, rats in CMC-Na group were treated with 1% CMC-Na with other procedures similar to those in GBE group. After the surgery, rats were allowed to recover for two weeks before experiments.

The successful modeling of rats is confirmed by comparing superoxide dismutase (SOD), catalase (CAT) activities and malondialdehyde (MDA) contents among these four groups (Table S1-S3) and hematoxylin-eosin and congo red immunostaining methods (Figure S1, S2).

Table S1-S3 summarize the variations in CAT, SOD activities and MDA contents among these groups. As can be observed, compared with normal group, SOD, CAT activities and MDA contents all decline in AD group ($P<0.01$). However, after GBE treatment, these indexes are found to increase ($P<0.001$, $P<0.01$ and $P<0.05$, respectively), which could be ascribed to the protective effect of GBE on neurons.

Table S1. Comparison results of CAT activities ($\times 10^3$ U/mg) in normal, AD, CMC-Na and GBE-treated groups (n = 12, mean ± S.D.)

Tissues	Normal	AD	CMC-Na	GBE
Hippocampus	2.2 ± 0.4	1.9 ± 0.2	2.0 ± 0.3	2.2 ± 0.3
Striatum	2.2 ± 0.1	2.0 ± 0.3	2.0 ± 0.1	2.3 ± 0.4
Prefrontal cortex	2.0 ± 0.1	1.8 ± 0.2	1.8 ± 0.02	2.0 ± 0.4

Table S2. Comparison results of SOD activities ($\times 10^{-2}$ U/mg) in normal, AD, CMC-Na and GBE-treated groups (n = 12, mean ± S.D.)

Tissues	Normal	AD	CMC-Na	GBE
Hippocampus	1.8 ± 1.2	0.9 ± 0.3	0.9 ± 0.3	2.2 ± 0.1
Striatum	1.6 ± 0.8	0.7 ± 0.4	1.1 ± 0.7	1.5 ± 1.2
Prefrontal cortex	1.0 ± 0.4	0.74± 0.5	0.6 ± 0.1	1.1 ± 0.3

Table S3. Comparison results of MDA contens (μmol/g) in normal, AD, CMC-Na and GBE-treated groups (n = 12, mean ± S.D.)

Tissues	Normal	AD	CMC-Na	GBE
Hippocampus	6.3 ± 2.0	9.8 ± 2.1	7.5 ± 2.6	5.6 ± 2.1
Striatum	4.6 ± 0.8	6.7 ± 0.9	6.6 ± 2.5	5.7 ± 0.9
Prefrontal cortex	6.5 ± 0.8	7.1 ± 1.1	7.2 ± 1.7	6.0 ± 1.9

Figure S1 displays the images of hematoxylin-eosin (HE) immunostained hippocampus tissues from normal, AD, CMC-Na and GBE groups. Hippocampus cones in AD (B) and CMC-Na treated group (C) are degenerated, which behave as

irregular arrangement, condensed nuclear as well as the vague boundaries between membrane and nuclear envelope. After the treatment with GBE, however, the cones become intact in shape again and the gaps between them reduce as well as a regular arrangement (D). Figure S2 shows the images of congo red immunostained prefrontal cortex tissues from normal, AD, CMC-Na and GBE groups. Compared with normal group (A), obvious senile plaques-like shapes appear in AD (B) and CMC-Na-treated (C) group, which could be ascribed to the Aβ deposition in the cortex. After the treatment with GBE, the density of plaques decreases obviously (D), suggesting that Aβ depositions in the cortex are weakened due to the protective effect of GBE on the damaged neurons. These results confirm the successful modeling of AD and GBE-treated rats.

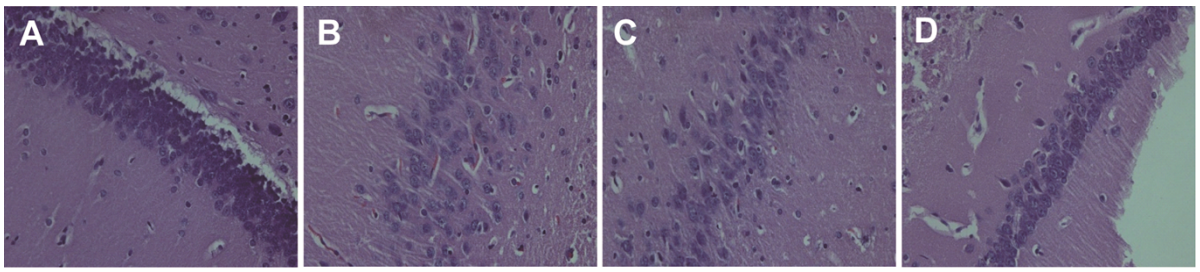

Figure S1. Images of hematoxylin-eosin (HE) immunostained hippocampus tissues from (A) normal, (B) AD, (C) CMC-Na and (D) GBE groups.

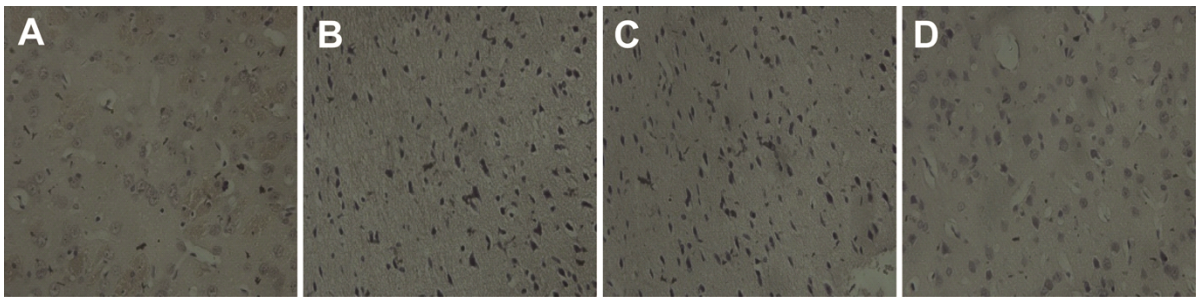

Figure S2. Images of congo red immunostained prefrontal cortex tissues from (A) normal, (B) AD, (C) CMC-Na and (D) GBE groups.

Characterizations of the probe and the optimizations of experimental conditions. The modifications of PEI/GNPs with Cu^{2+} and hemin are confirmed by zeta-potential and UV-vis measurements. Firstly, the zeta-potentials of the prepared probe at each step show obvious differences (Figure S3A). The PEI/GNPs are positively charged due to the abundant amino groups on PEI, with the potential value of + 52.5 mV. After chelation of PEI with Cu ions (II), the zeta-potential of the obtained PEI/GNPs-Cu nanocomposite increases to a value of + 56.7 mV, which confirms the successful assembly of Cu^{2+} onto PEI/GNPs. Then, this value decreases to + 43.0 mV after the following hemin modification, which is smaller compared with that of PEI/GNPs-Cu due to the negatively charged hemin dissolved in NaOH solution. Figure S3B displays the typical UV-vis absorption spectrum of free hemin, free Cu^{2+}, PEI/GNPs and PEI/GNPs-Cu-hemin in aqueous solutions. The initial PEI/GNPs shows a characteristic absorption peak at around 525 nm (green curve), belonging to the surface plasmon resonance of GNPs, and no obvious change is observed in the spectra when PEI is functionalized with GNPs. Free Cu^{2+} doesn't display any distinctive absorption peak in the inspected 400-700 nm regison (red curve) and free hemin shows three characteristic absorption peaks at around 350, 390 and 500 nm (black curve), corresponding to the Fe (II) moiety in hemin. After the formation of PEI/GNPs-Cu-hemin complex, however, only the peak at 525 nm is detectable with others belonging to hemin completely disappeared or weakened dramatically (blue curve), suggesting that hemin has been involved in the binding to PEI/GNPs. These results verify the successful assembly of Cu^{2+} and hemin onto PEI/GNPs.

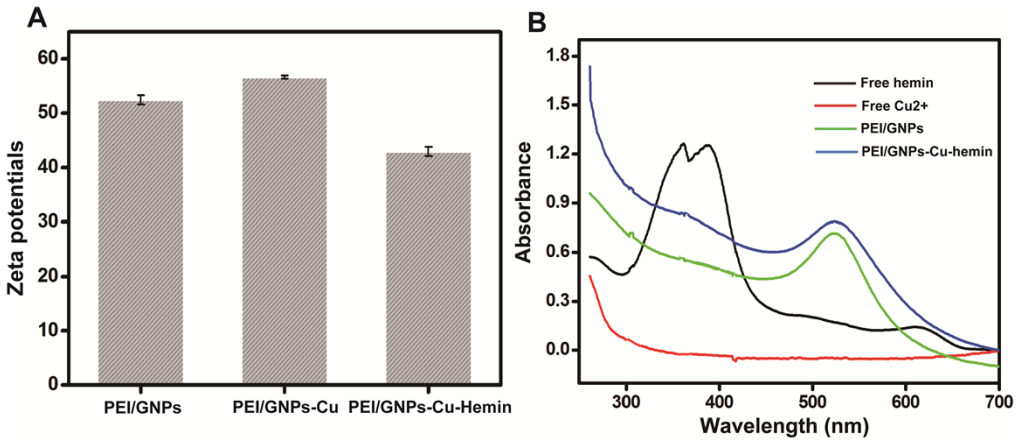

Figure S3. (A) Zeta-potentials of PEI/GNPs, PEI/GNPs-Cu and PEI/GNPs-Cu-hemin. (B) UV-vis absorption spectrum of free hemin (black), free Cu^{2+} (red), PEI/GNPs (green) and PEI/GNPs-Cu-hemin (blue) in aqueous solutions.

Optimizations of incubation conditions. In this work, the GNPs are pre-functionalized with PEI, which is positively charged due to the abundance in $–NH_2$ group, in favor of the assembling of negative hemin moeity and coordination with Cu^{2+}, respectively. Figure S4A displays the influence of $HAuCl_4$ amount in the preparation of PEI/GNPs on the absorption intensity of the resultant probe. Obviously, when 0.005 g $HAuCl_4$ was added and reacted with PEI, the obtained PEI/GNPs-Cu-hemin probe shows a rather weak absorption at 525 nm, which is not beneficial for improving the sensitivity. However, when this amount is increased by three times to 0.015 g, the maximum absorption intensity reaches nearly 1.6, as a result of which, the solution of PEI/GNPs would need to be diluted before the next step to strictly control the absorption within the optimal detection range of the UV-vis instrument (0.2 ~ 0.9). Therefore, 0.010g is considered as the ideal amount of $HAuCl_4$ to prepare the probe. In our experiment, pH of the PEI/GNPs solution is found to be another factor required to be optimized. Due to the characteristic of easy-to-aggregation of the positive-charged PEI/GNPs under alkaline medium, the pH test is performed in the range of 1.0 ~ 7.0 by adding 1 M HCl to the PEI/GNPs solution (Figure S4B). As can be seen, along with the increase of pH value from acidic to neutral, the absorption

intensity of the obtained PEI/GNPs-Cu-hemin probe at 525 nm becomes weaker and weaker under an identical condition. However, for the same reason, considering that when the pH of the PEI/GNPs solution is lower than 2.0, the absorption is too high, pH 2.0 is therefore adopted as the optimal acidic condition for the following Cu^{2+} and hemin modifications. By varying the molar ratio between hemin and copper ion from 1: 320 to 1: 40, we determine the optimal incubation condition for the following recognition of Aβ molecule. The strongest absorption at 525 nm is obtained at 1:107 (Figure S4C). When this value is higher than 1:107, *i.e.*, excessive hemin is employed, the absorption gradually decreases, indicating an equilibrium between the two recognition elements coexisting in the probe to reach a high determination sensitivity. Meanwhile, we observe the distinct ratio-resolved color change of the probe in the presence of $Aβ_{-40}$ (inset in Figure S4C) from red to purple and followed by blue grey. Figure S4D depictes the incubation time-dependent spectral responses. Within 10 min after adding $Aβ_{-40}$ into the probe, an obvious red-shift of the maximum absorption wavelength and decrease in the intensity are captured, which is attributed to the fast clustering of GNPs by the dual-recognition capability of the designed probe. For a longer incubation time, this change is continuous but with a more slight extent. However, in general, the influence of incubation time on the aggregation of the probe is comparatively weak among the four inspected parameters. On the basis of the above optimization results, we select 0.010g $HAuCl_4$ addition, pH 2.0 of the PEI/GNPs solution, molar ratio = 1:107 (hemin: copper ion), 60 min incubation time as the optimal incubation conditions for a proof-of-concept demonstration of the proposed method and apply to the subsequent Aβ determination.

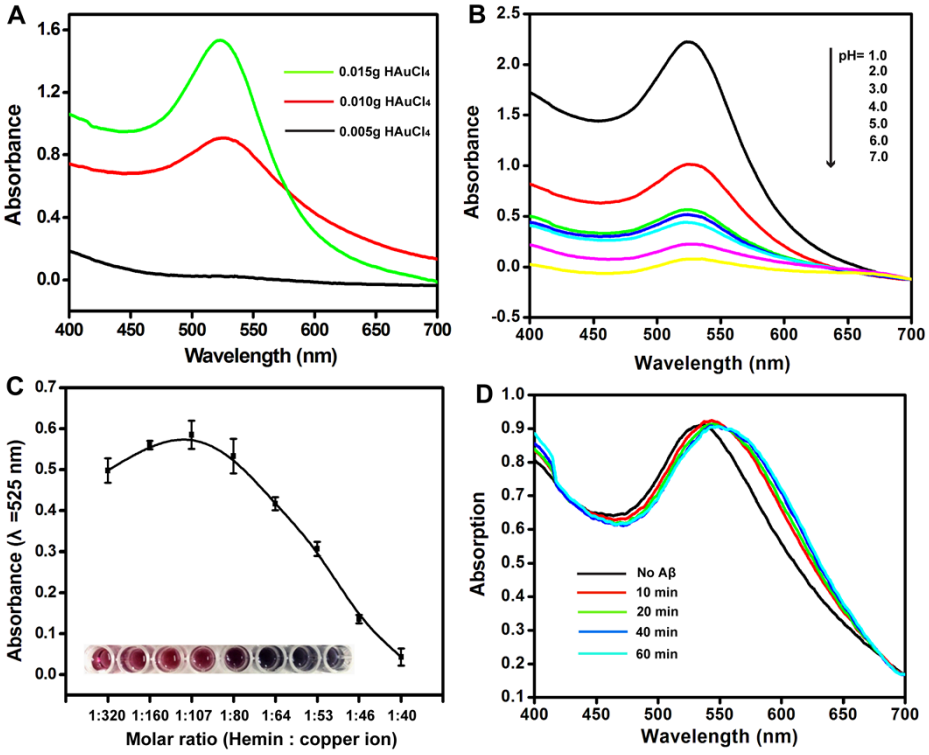

Figure S4. Optimization results of (A) $HAuCl_4$ additions, (B) pH of the PEI/GNPs solution, (C) molar ratio between hemin and Cu^{2+} and (D) incubation time. Inset in (C): direct observation of color changes upon the variation of molar ratios between hemin and copper ion.

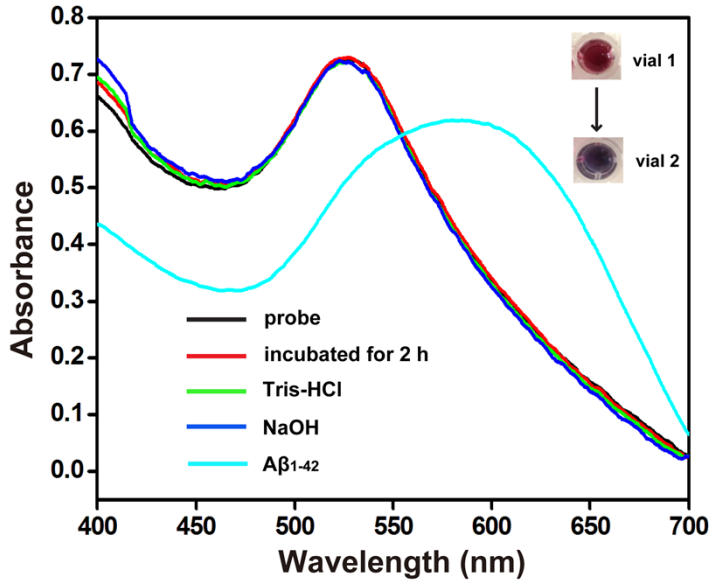

Figure S5. UV-vis absorption spectra of the designed probe without Aβ addition (black), incubated for 2 h without Aβ addition (red), addition of 0.005 mg mL^{-1} $Aβ_{-42}$ into the probe and incubated for 1 h (cyan), addition of Tris-HCl (pH 7.4) (green) and NaOH (blue) into the probe and incubated for 1 h. Inset: direct observations of the corresponding color change of the probe following $Aβ_{-42}$ addition.

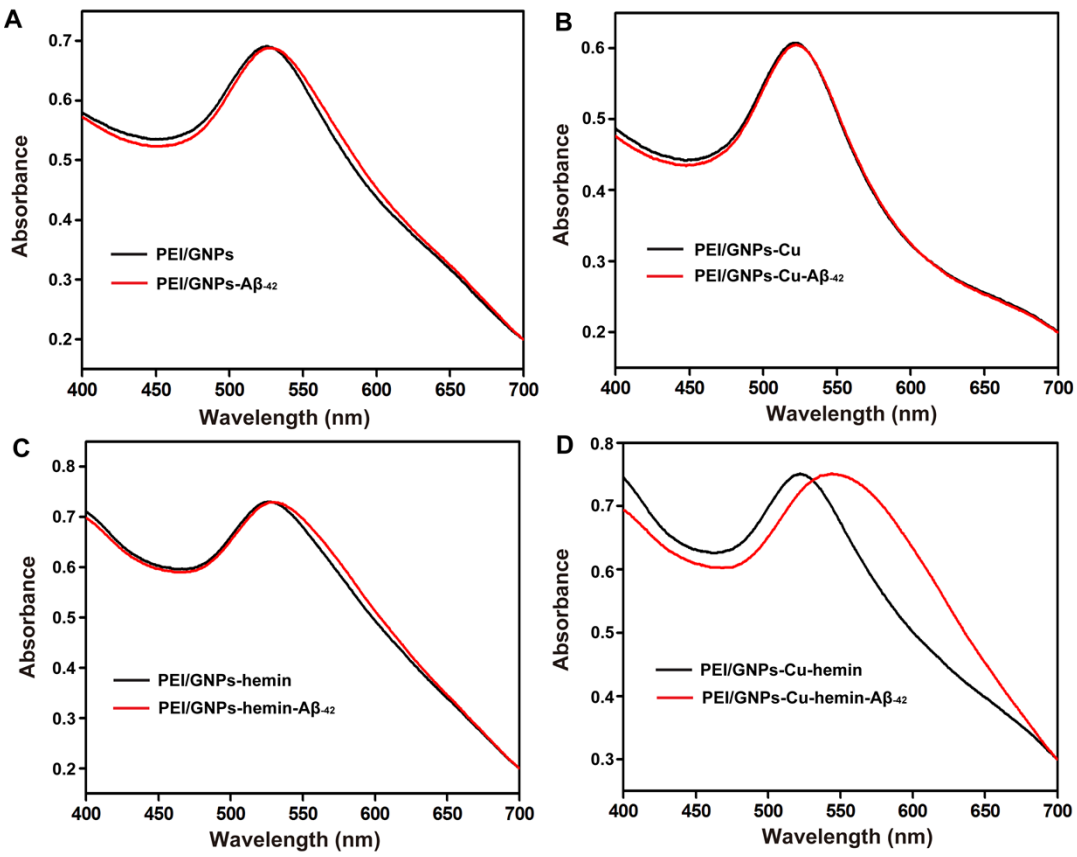

Figure S6. UV-vis absorption spectra of (A) PEI/GNPs, (B) PEI/GNPs-Cu, (C) PEI/GNPs-hemin and (D) the designed probe in the absence (black line) and presence (red line) of $A\beta_{-42}$.

Inhibition of the probe aggregation by Aβ. According to the above illustrations, the aggregations of the PEI/GNPs-Cu-hemin probe would take place when the probe encounted Aβ in the same system. We further investigate the inhibition of the probe aggregation by adding Aβ inhibitors into the probe. This concept is firstly tested with human serum albumin (HSA), which is reported to be one of well-known kinds of Aβ inhibitors to lower Aβ levels in blood and CSF by dynamic equilibrium across the blood brain barrier.[6,7] Following the respective pre-mixing of 10 μM HSA with $Aβ_{-16,}$ $Aβ_{-40}$ and $Aβ_{-42}$ for 1 h and then added into the probe solution, no obvious aggregation of the probe is detected, as the obtained absorption spectrum show much similarity to that of the original probe in the absence of Aβ (Fig. S7, B, D, E). This can be attributed to the decrease of the amount of the accessible Aβ monomers for the aggregations due to the high affinity of monomeric Aβ for HSA. However, for $Aβ_{-11}$ and $Aβ_{-38}$, there is not any difference in the UV-vis spectra whether the two peptides are treated with HSA or not (A and C), affirming the above-mentioned conclusion again that the devised probe is only suitable for $Aβ_{-16,}$ $Aβ_{-40}$ and $Aβ_{-42}$ determination, but not $Aβ_{-11}$ and $Aβ_{-38.}$ Considering that Aβ monomers are apt to form oligomeric or larger fibrillar forms under repeated freezing and thawing conditions, an alternative experiment to verify the above conclusion is carried out. Equally to be seen in Fig. S7F, for $Aβ_{-16,}$ $Aβ_{-40}$ and $Aβ_{-42}$, at the first freeze-thawing cycle of Aβ solution, the absorbance at 525 nm all decrease relative to that of freshly prepared Aβ solution. After that, the absorbance begins to rise and more freeze-thawing cycles are adopted, the stronger of the intensity is observed, indicating that along with repeated freeze-thawings, Aβ tends to aggregate in itself and thus the Aβ-induced aggregations is successfully inhibited with this treatment. The result also reveals that the suggested detection method is more applicable to Aβ monomers than any oligomeric or fibrillar forms.

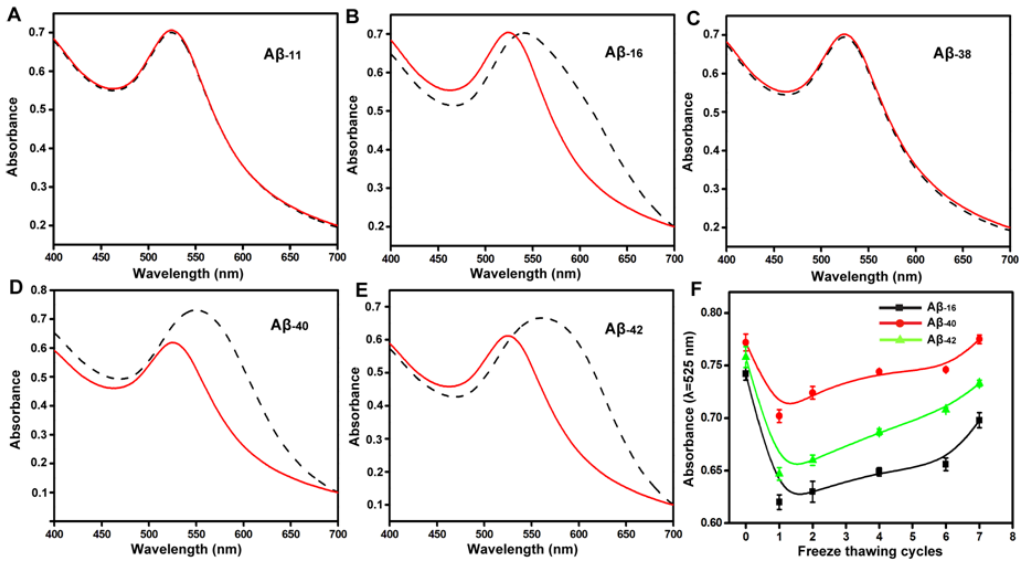

Figure S7. Demonstration of the inhibition of the PEI/GNPs-Cu-hemin probe aggregation by treatment with HSA (A-E) and repeated freeze-thawings (F). The HSA experiments were performed by incubating the probe with $A\beta_{-11}$, $A\beta_{-16}$, $A\beta_{-38}$, $A\beta_{-40}$ and $A\beta_{-42}$ (dashed lines) and the mixture of 10 μM HSA and the five Aβ peptides (solid lines), respectively.

Selectivity. The data presented above demonstrated that our method is a simple and efficient way to monitor Aβ. The complexity of the brain environment put forward a great challenge for *in vivo* detection of Aβ. Therefore, the selectivity of our designed probe toward Aβ is inspected by comparing the absorbances after additions of target molecules with that of potential interference biomolecules. Firstly, some representative co-existing proteins in the rat brain such as human tau peptide, actin, human α-synuclein peptide, voltage-dependent anion channel 1 (VDAC1) and prealbumin peptide are selected for the interference test. Remarkably, no obvious change is observed following the additions of these interferences into the probe in comparison with that of $A\beta_{-42}$ (Figure S8A). Besides proteins, metal ions, the concentrations of which are more than doubled in the cerebral amyloid deposits of AD brains compared with the neuropil of normal age-matched brains,[8] are also

investigated. Likewise, the responses from these cation ions (Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}, Fe^{3+}, Cu^{2+} and Zn^{2+}) are rather low and ccan be neglected relative to that of $A\beta_{-42}$ (Figure S8B). Taking into account that amino acids in the biological system are capable of interacting with a lot of metal ions, interferences from a series of amino acids such as glutamate, arginine, lysine, isoleucine, serine, glycine, etc are also evaluated in the selectivity. Similarly, the additions of these amino acids into the probe in their physiological concentrations don't induce obvious changes in the UV-vis spectra, compared with that of $A\beta_{-42}$ (Figure S8C). All these evidences indicate the high selectivity of the devised PEI/GNPs-Cu-hemin probe for Aβ biosensing against proteins, metal ions and amino acids coexisting in the cerebral system, which could be ascribed to the dual-recognition ability of Cu^{2+} and hemin functionalized GNPs to specifically bind Aβ monomers.

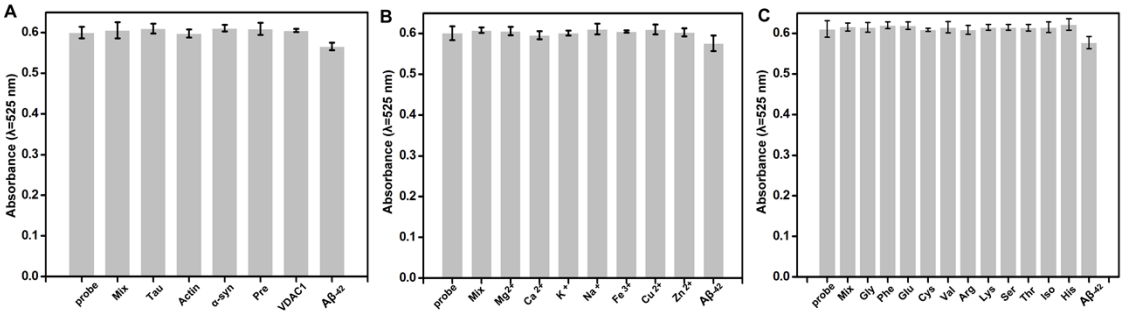

Figure S8. Selectivity investigations of the designed PEI/GNPs-Cu-hemin probe toward (A) proteins, (B) metal ions and (C) amino acids under identical conditions. (A) 0.2 mg mL^{-1} for all the tested proteins and 10^{-6} mg mL^{-1} for $A\beta_{-42}$; (B) 1 mM for Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}, 10 μM for Fe^{3+}, Cu^{2+}, Zn^{2+} and 10^{-6} mg mL^{-1} for $A\beta_{-42}$; (C) 10 μM for all the tested amino acids and 10^{-6} mg mL^{-1} for $A\beta_{-42}$.

Table S4. ELISA evaluation of the variations in total Aβ monomer levels in normal, AD, CMC-Na and GBE-treated rat brains (n = 12, mean ± S.D.)

Aβ content (ng mL^{-1})	CSF	Hippocampus	Prefrontal cortex	Striatum
Normal [a]	31.0 ± 4.6	25.3 ± 6.2	22.8 ± 7.7	18.2 ± 5.1
AD	26.7 ± 6.6	13.6 ± 5.0	12.0 ± 4.4	8.3 ± 3.8
CMC-Na [b]	27.5 ± 9.5	14.1± 4.9	12.2 ± 2.1	7.3 ± 1.9
GBE	30.5 ± 10.3	23.9 ± 7.0	24.2 ± 2.1	15.3 ± 2.9

[a] Untreated healthy rats.

[b] AD rats administrated (i.g.) with CMC-Na daily for two weeks as a control for GBE-treated group.

Supporting references

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