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***Supplementary Information* for**

Absorbance enhancement of Aptamers/GNP enables sensitive protein detection in rat brain

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Experimental Procedures

Materials and Reagents. All the oligonucleotides were synthesized and purified by Sangon Biotechnology Co. Ltd (Shanghai, China) and their sequences are shown in Table S1. Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All the proteins were purchased from Beijing Biodee Biotechnology Co. Ltd. (Beijing, China). Trisodium citrate and all the other chemical reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. (Beijing, China). All the reagents were of at least analytical grade and were used without any other further purification. All the oligonucleotides solution in this work was obtained by diluting the stock solution with a 20 mM Tris-HCl buffer (pH 7.4) which contained 140 mM NaCl and 5 mM KCl. The protein solutions were prepared with 0.1 M phosphate buffer (pH 7.4) or artificial cerebrospinal fluid (aCSF, pH 7.4). Artificial cerebrospinal fluid (aCSF) was prepared by dissolving NaCl (126 mM), KCl (2.4 mM), KH_2PO_4 (0.5 mM), $MgCl_2$ (0.85 mM), $NaHCO_3$ (27.5 mM), Na_2SO_4 (0.5 mM), and $CaCl_2$ (1.1 mM) into millipore water.

Instrumentation. All the absorbance spectra were recorded using a Shimadzu 3600 UV–vis spectrophotometer (Shimadzu, Japan) at room temperature. The resonance light scattering (RLS) signals were measured using a Hitachi F-4600 fluorescence spectrophotometer (Hitachi Ltd., Japan) equipped with a Xenon lamp excitation source. The fluorescence spectrophotometer was set in the synchronous mode with the slit widths of 5 and 5 nm for excitation and emission, respectively. The PMT voltage was set at 400 V. Scanning electron microscopy (SEM) measurements were carried out on a HITACHI SU8010 Scanning Electron Microscope (Hitachi Ltd., Japan) at an accelerating voltage of 3 kV. Dynamic light scattering, and Zeta-potential analysis were conducted using a Zetasizer Nano ZS90 (Malvern Instruments Ltd., England). Water used throughout the experiments was purified by a Milli-Q system (Millipore, Bedford, MA, USA).

Preparation of Gold Nanoparticle (GNP). The water-soluble GNP were synthesized according to the classical sodium citrate reduction method.[1] The 100 mL of 1 mM $HAuCl_4 \cdot 4H_2O$ solution was prepared in a three-necked flask and was heated to reflux for 5 minutes under vigorous stirring, then 10 mL of 38.8 mM sodium citrate solution was immediately added into the boiling solution with a color change from light yellow to wine red. After the mixture was allowed reflux for another 15 minutes, the heating was stopped, but stirring was continued. Then the solution was cooled to the room temperature. And the solution was filtered through a 0.22 μm membrane filter and stored in a fridge of 4°C before being used. The concentration of GNP was calculated based on an extinction coefficient of $2.7\times10^8\ M^{-1}\cdot cm^{-1}$ at 520 nm according to the Beer−Lambert Law. The sizes of the GNP were verified by SEM and dynamic light scattering.

Fabrication of the probe (Apts/GNP). The principle of this experiment involves binding of Apt-1 functionalized GNP with Apt-2 to IFN γ. Taking the preparation of ssDNA-GNP nanoconjugates as an example. Typically, Apt-1 (10 μL of 1.0 μM) was added to GNP solution (molar ratio of Apt-1/GNP, 20:1) and the solution was allowed to incubate for 0.5 hour at room temperature. Then, Apt-2 (10 μL of 1.0 μM) was added to the mixture to form the Apts/GNP based probe. After the mixture incubation for another 0.5 hour, the resulting Apts/GNP based probe was used for sensing proteins.

Characterization of the Apts/GNP. The SEM images of the synthetic GNP showed an average diameter of about 13 nm (50 nanoparticles, counted by Image J, Fig. S1a). After the GNP was functioned with two aptamers, the characteristic peak of aptamers at 260 nm was significantly

increased (Fig. S2a). It is interested that bare GNP also has a moderate absorption at 260 nm, which is related to GNP and their common impurities absorb light at the wavelength of aptamers characteristic peak. [2] What's more, the dynamic light scattering (DLS) experiments showed that the average hydrodynamic size increased about 8 nm (Fig. S2b) after two aptamers decorated to the GNP. In addition, Zeta-potential analysis indicated that the Apts/GNP had a more negative zeta potential (-8.76 mV) compared to the bare GNP (-6.47 mV) (Fig. S2c). Because GNP and aptamers were negatively charged, a higher aptamer concentration should result in a more negatively charged surface. The result further confirming that the GNP was successfully assembled with two aptamers. [3, 4]

Quantification of the oligonucleotides assembled on each GNP. To quantify the amount of the oligonucleotides on the surface of each GNP, the loading of DNA on GNP was determined according to the previous literatures.[5, 6] First, the sequence Apt-2 was modified with fluorescein FAM at the 3' end, the fluorescence intensity of series concentration of sequence Apt-2 was detected to establish a calibration curve (Fig. S3). As the concentration of Apt-2 increased, the fluorescence intensity was enhanced in a linear way. After the GNP was mixed to form FAM-DNA-GNP mixture solution, the system was centrifuged for 10 min at the speed of 12000 r min^{-1}, and then the supernatant containing free FAM-DNA was removed. Particles were washed two more times with deionized water. The number of FAM strands per GNP was estimated by subtracting the amount of FAM-DNA in the supernatant mixture from the total amount of FAM-DNA. And the mean fluorescence intensity of total amount of FAM-DNA and FAM-DNA in the supernatant mixture were measured. As a sequence, the concentration of DNA absorbed onto the surface of GNP was 30.5 nM and the number of duplexes loaded on each GNP (Finally concentration 2.16 nM) was calculated to be ~14 copies.

IFN γ detection using the Apts/GNP. In the IFN γ detection step, the sensing probes were incubated with IFN γ of a series of concentration in physiological buffer (phosphate buffer or aCSF). UV-vis absorbance measurements were thereafter performed after another 30 minutes incubation and every experiment was performed in triplicate. Only difference between the sensing assay is the concentration of proteins.

PDGF-BB detection using the Apts/GNP nanoconjugates. PDGF-BB were measured with the same procedure except Apt-1 and Apt-2 were changed to Apt-3 and Apt-4. Apt-3 functionalized GNP were incubated with Apt-4 and PDGF-BB to form a new locked-loop conformation. PDGF-BB with a series of concentration in phosphate buffer were tested with the same process mentioned above.

Sensing IFN γ in cerebrospinal fluid using the Apts/GNP. Adult male Sprague-Dawley rats (300-350 g) were purchased from Health Science Center, Peking University. The animals were housed on a 12:12 h light-dark schedule with food and water ad libitum. All animal procedures were approved by the Animal Care and Use Committee at National Center for Nanoscience and Technology of China and performed according to their guidelines. Cerebrospinal fluid (CSF) was obtained as followed. Briefly, the rat was anaesthetized with chloral hydrate and were placed in a stereotaxic frame. A guide cannula was implanted into the lateral ventricle (AP: −3.7 mm, L: 4.8 mm from the bregma, V: 6.4 mm from the surface of the skull). The cerebrospinal fluid was collected at a flow rate of 1 μL min^{-1} driven by a microinjection pump (CMA/100; CMA Microdialysis AB, Stockholm, Sweden). The sensing process here is same as mentioned above except the molar ratio of GNP and aptamers has been changed to 1:40 to protected GNP from aggregating. The

mean absorbance intensity was measured to be 0.601. According to the calibration curve established in the aCSF sensing system, the concentration of the protein in CSF was determined to be 2.09 ng mL^{-1}. Standard samples recovery experiment was also conducted to demonstrate the feasibility of our proposed probe for protein detection in CSF.

Effect of IFN γ concentration higher than critical red-shift concentration on the Apts/GNP. We investigated the effect of IFN γ concentration higher than critical red-shift concentration (CRSC) on the effect of the Apts/GNP. We assumed that when the IFN γ binding onto the surface of particle, IFN γ was firstly specifically interacted with Apt-1 and Apt-2 to form locked-loop conformation. After every two oligonucleotides (Apt-1 and Apt-2) matched with one protein, the excrescent IFN γ will directly absorbed onto the surface of gold nanoparticle where there were no DNA located recognition sites, inducing the aggregation of gold nanoparticle via electrostatic interaction between GNP (negatively charged) and the target protein (positively charged). Fig. S7c shows the SEM pictures of the probe in the presence of 2.0 μg mL^{-1} protein, the nanoparticle aggregated compared with the Apts/GNP in the presence of 40 ng mL^{-1} protein (Fig. S7b). It was insufficient to induce the obvious color change of GNP to make it observed by the naked eye because the maximum concentration of protein used here is only 4.0 μg mL^{-1}. The result suggested that for the protein concentration higher than CRSC, the absorption peak's red-shift was related to the excrescent protein induced aggregation of GNP. We further investigated the influence of the molar ratio between GNP and aptamers on the critical red-shift concentration. As shown in Fig. S8, CRSC gently increased with the increase of the aptamers concentration. CRSC is about 8 ng ml^{-1}, 40 ng ml^{-1} and 80 ng ml^{-1} when the molar ratio is 1:10 ,1:20, 1:40 (GNP: aptamer), respectively. These results suggested that the highly density of negative charge on the surface of GNP protected the GNP from aggregation with the increase of two aptamers (Apt-1 and Apt-2) concentration, together with the CRSC increasing**.**

Absorption and scattering effect on the Apts/GNP. The sensing system is a heterogeneous system, except for the absorption of the nanoparticle, light-scattering would also affect the sensing assay, therefore, Beer−Lambert Law should be changed as the following formula,

$$I = I_0 e^{-cd(E+A)} \qquad \textit{(eq 1)}$$

E is the absorption coefficient, *A* is the scattering coefficient which is related to the size of the nanoparticle, *(E+A)* is the extinction coefficient of the nanoparticle. In our present manuscript, considering that no red-shift of the UV-vis spectrum indicating no refractive index change of the particle, and the large absorption coefficient produced by GNP couldn't be easily altered by single strand DNA or protein because of their low absorption coefficient. Therefore, absorption coefficient (*E*) didn't change when IFN γ concentration was lower than CRSC. The limited size change of the nanoparticle indeed affected the scattering coefficient (*A*) as shown in Fig. S5. As a result, the enhanced absorbance would mostly relate to the light-scattering change of Apts/GNP after interacting with IFN γ.

In summary, for the protein concentration lower than CRSC, the absorbance increment was mostly related to light-scattering effect; for the protein concentration higher than CRSC, the absorption peak's red-shift and the absorbance increment were related to SPR phenomenon and light-scattering effect.

Table S1. List of DNA sequences used in the study

Name	Sequence (5'-to-3')	Notes
Apt-1 (Aptamer 1 of IFN γ)	GGGGTTGGTTGTGTTGGGTGTTGTGT GTCGTGGGTCTAAAAAAAAAA	Apt-1 and Apt-3 contain three functional regions: aptamer of IFN γ or PDGF (red), a short stem sequence (black) and poly 10A (blue). Apt-2 and Apt-4 contain two functional regions: aptamer of IFN γ or PDGF(red) and a short stem sequence (black).
Apt-2 (Aptamer 2 of IFN γ)	AGACCCACGACCCGCCCAAATCCCTAA GAGAAGACTGTAATGACATCAAACCA GACACACTACACACGCA	
Apt-3 (Aptamer 1 of PDGF)	CAGGCTACGGCACGTAGAGCATCACC ATGATCCTGGTCGTGGGTCTAAAAAAA AAA	
Apt-4 (Aptamer 2 of PDGF)	AGACCCACGACACTCAGGGCACTGCA AGCAATTGTGGTCCCAATGGGCTGAG TA	

Table S2. Comparison of different methods for IFN γ detection

Detection technique	**Detection of limit**	**Linear range**	**Reference**
Electrochemistry	1.92 ng mL^{-1} (60 pM)	1.92 ng mL^{-1} – 377.6 ng mL^{-1} (60 pM - 11.8 nM)	7
Electrochemistry	0.37 ng mL^{-1} (11.56 pM)	0.71 ng mL^{-1} – 3.52 ng mL^{-1} (22.22 pM - 0.11nM)	8
Surface Plasmon Resonance	0.32 ng mL^{-1} (10 pM)	0.32 ng mL^{-1} – 3200 ng mL^{-1} (10 pM-100 nM)	9
Chemiluminiscence	12.8 ng mL^{-1} (400 pM)	16 ng mL^{-1} – 3200 ng mL^{-1} (500 pM-100 nM)	10
Fluorescence	160 ng mL^{-1} (5 nM)	160 ng mL^{-1} – 3200 ng mL^{-1} (5 nM - 100 nM)	11
UV-vis	0.8 ng mL^{-1} (25 pM)	0.8 ng mL^{-1} – 4000 ng mL^{-1} (25pM - 125 nM)	**Present work**

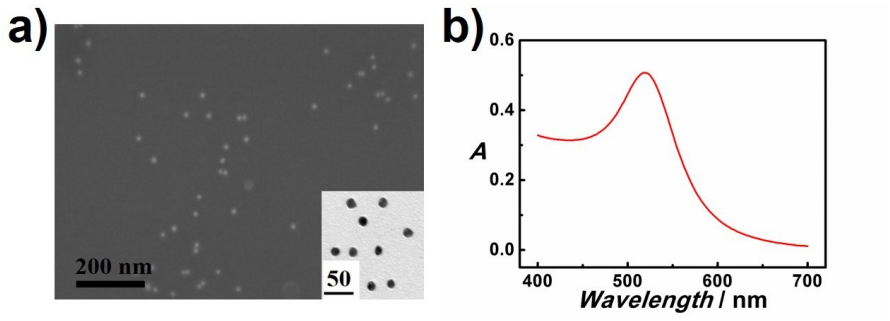

Fig. S1 (a) Scanning electron microscope (SEM) image of bare GNP. (b) Typical absorbance spectra of GNP in H_20.

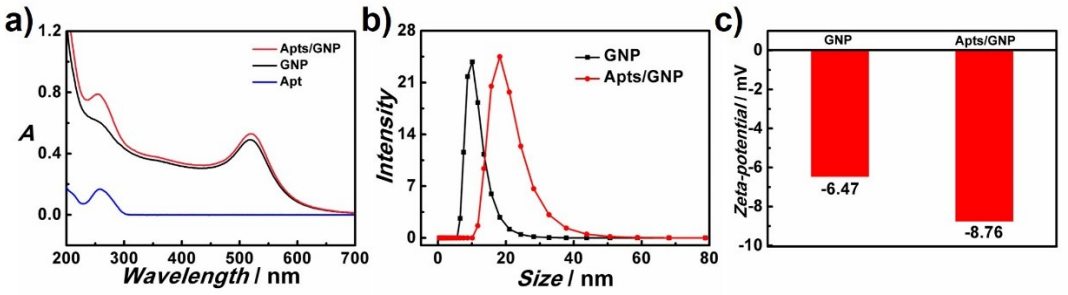

Fig. S2 (a) UV−vis spectra of bare GNP, aptamer and the developed Apts/GNP probe in H_20. (b) Hydrodynamic diameter distribution determined by dynamic light scattering in H_2O. (c) Zeta-potentials of GNP and the Apts/GNP in H_2O.

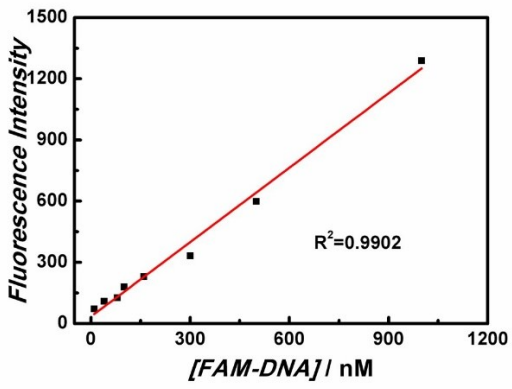

Fig. S3 The linear relationship between fluorescence intensity and concentration of FAM-DNA.

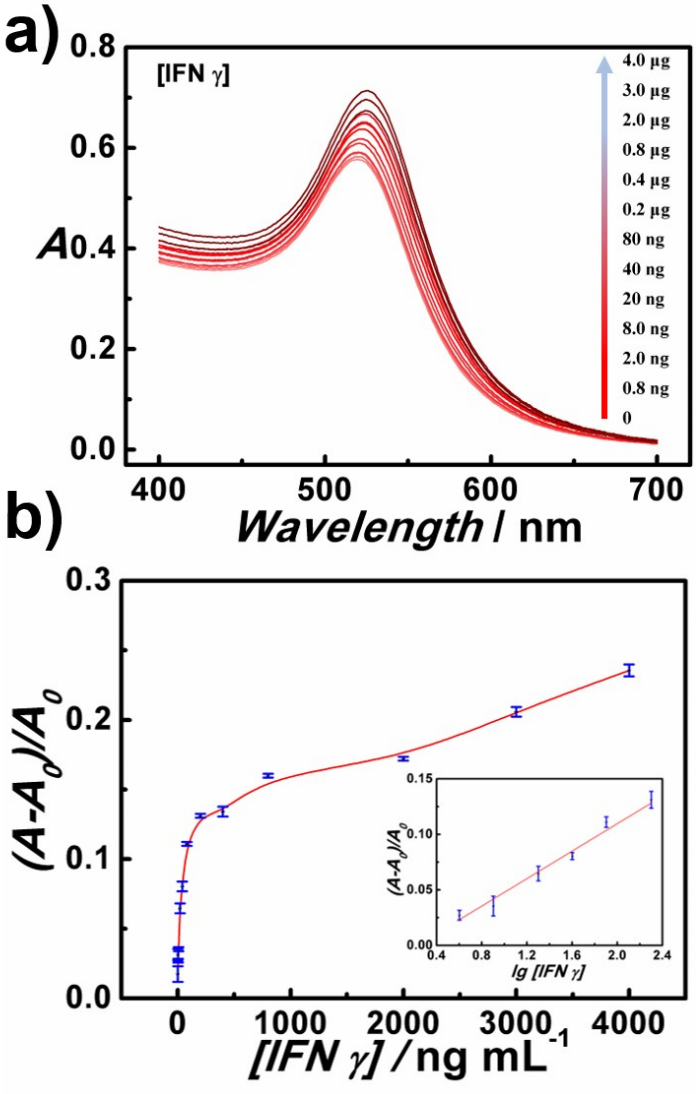

Fig. S4 Detection of IFN γ in phosphate buffer using the Apts/GNP. (a) The absorbance spectra of the Apts/GNP in phosphate buffer in the presence of different concentration of IFN γ (0–4.0 μg mL^{-1}). (b) Relationship between the corresponding values of absorbance ratio $(A-A_0)/A_0$ and the protein concentration, inset shows the linear plot ranging from 4.0 ng mL^{-1} to 200 ng mL^{-1}.

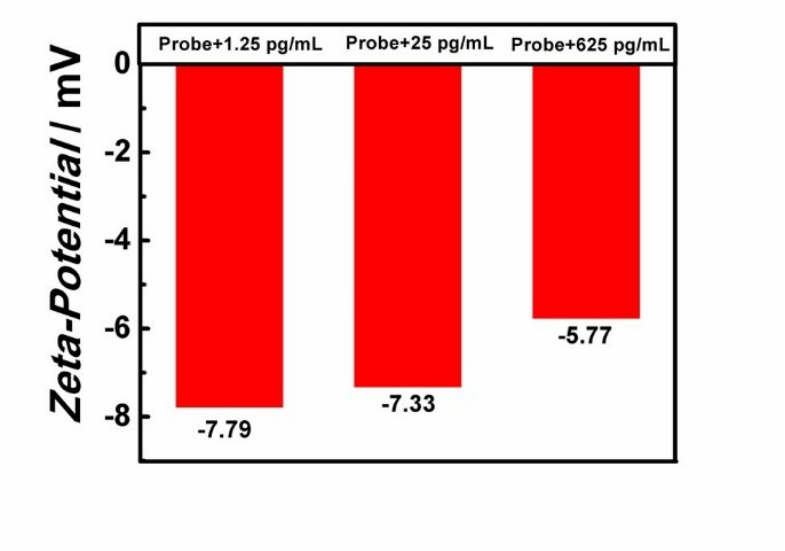

Fig. S5 Zeta potentials of Apts/GNP in H_2O after recognizing various concentration of IFN γ.

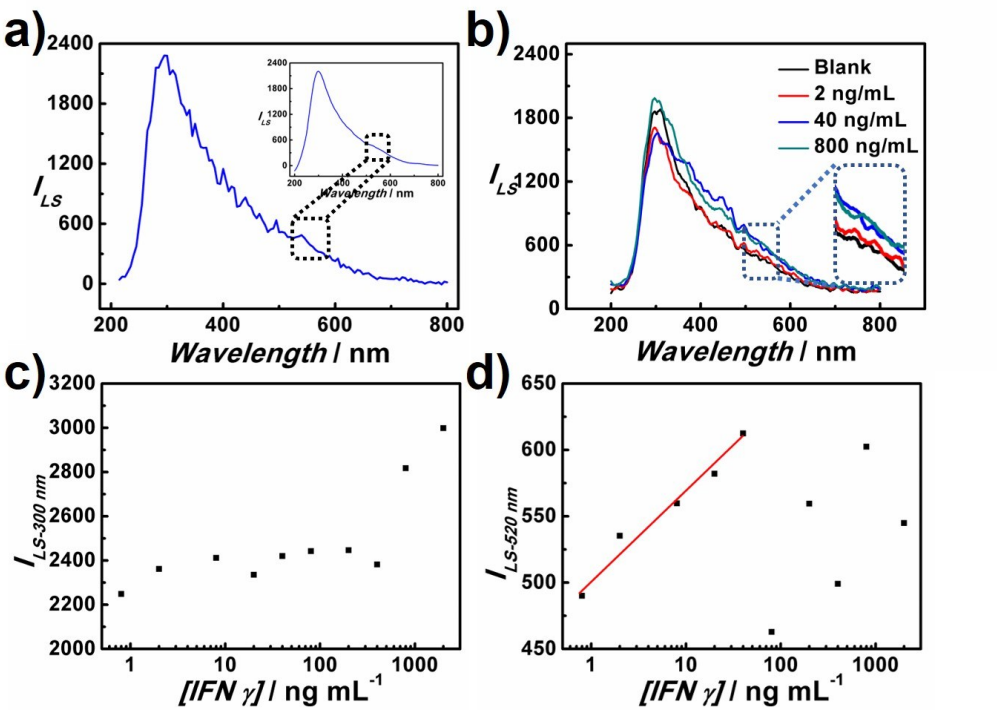

Fig. S6 (a) The light-scattering spectra of bare GNP, inset is the light-scattering spectrum after smooth. (b) The light-scattering spectra of the Apts/GNP in the presence of target protein at different concentrations. (c) The plot of scattering intensity of the assay vs. protein concentration at the λ of 300 nm. (d) The plot of scattering intensity of the assay vs. protein concentration at the λ of 520 nm. (The linear ranges from 0.8 ng mL^{-1} to 40 ng mL^{-1}).

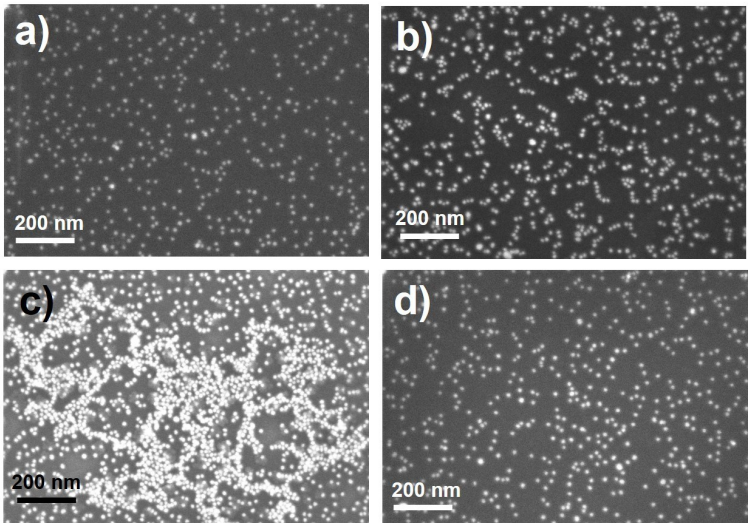

Fig. S7 Scanning electron microscope (SEM) images of Apts/GNP in 0.1 M phosphate buffer containing (a) 0 ng mL^{-1}, (b) 40.0 ng mL^{-1}, (c) 2.0 µg mL^{-1} IFN γ and d) CSF sample. (Molar ratio of GNP: aptamers = 1:40)

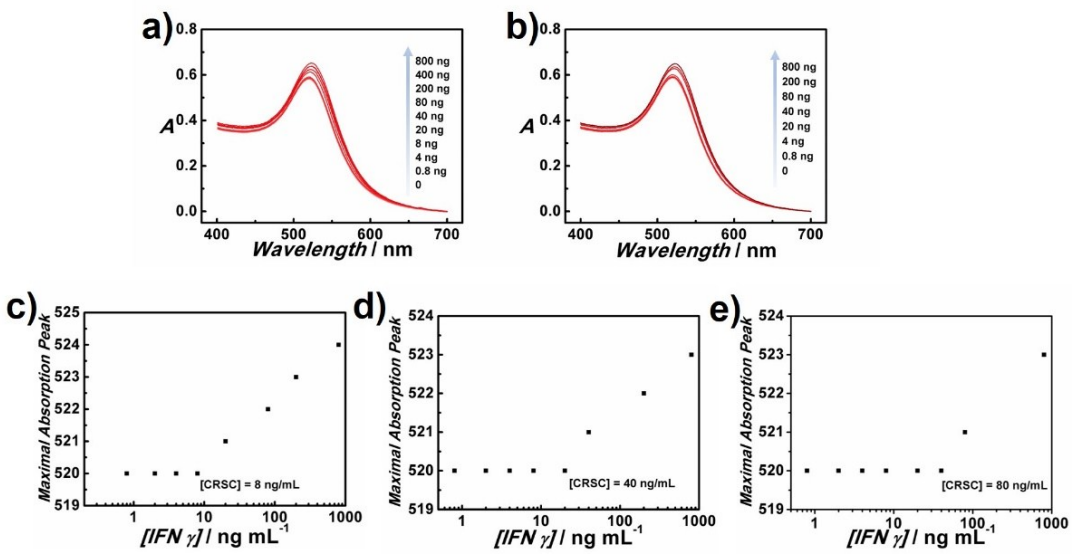

Fig. S8 The absorbance spectra of the Apts/GNP for detection of IFN γ (0–0.8 μg mL^{-1}) in phosphate buffer, the molar ratio of GNP and aptamers are 1: 10 (a) and 1: 40 (b), respectively. (c-e) critical red-shift concentration when the molar ratio of GNP and aptamers are 1: 10, 1:20 and 1: 40, respectively.

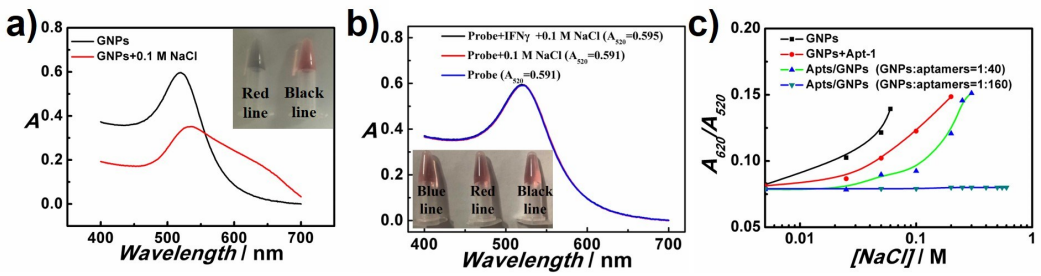

Fig. S9 High salt concentration stability assays. (a) Absorbance spectra of bare GNP (black line) and GNP in the presence of 0.1 M NaCl (red line), inset was the photographs of bare GNP and GNP with 0.1 M NaCl. (b) Absorbance spectra of the Apts/GNP (blue line), the Apts/GNP in the presence of 0.1 M NaCl (red line), and the Apts/GNP in the presence of IFN γ (2.0 ng mL^{-1}) and 0.1 M NaCl (black line), inset was the corresponding photographs. (c) Aggregation induced changes in corresponded nanoparticle resulting from varying salt concentration.

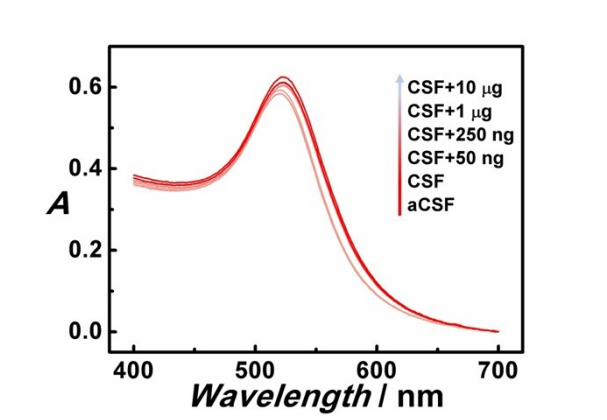

Fig. S10 Detection of IFN γ added in cerebrospinal fluid (CSF) of rat brain, molar ratio of GNP and aptamers here is 1:40. The absorbance spectra of the Apts/GNP for detection of IFN γ added in CSF (0–10 μg mL^{-1}).

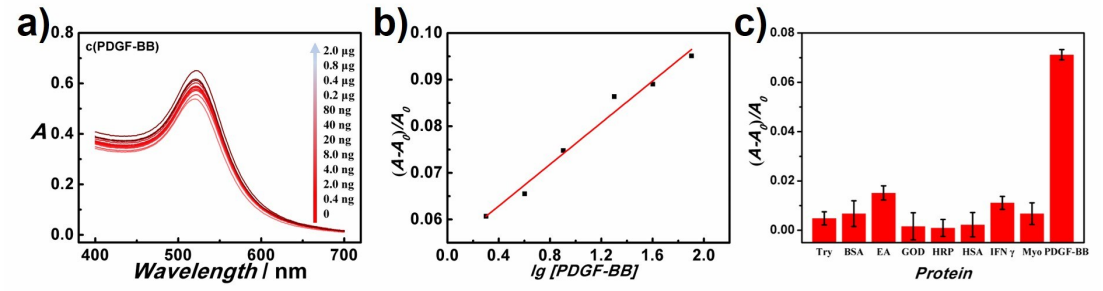

Fig. S11 Detection of PDGF-BB in the phosphate buffer. (a) The absorbance spectra of the Apts/GNP (Apt 3 and Apt 4 decorated GNP) for detection of PDGF-BB in phosphate buffer (0–2.0 μg mL^{-1}). (b) Linear relationship between the corresponding values of absorbance ratio $(A-A_0)/A_0$ and the logarithm of the protein concentration spiked in phosphate buffer, the linear ranges from 2.0 ng mL^{-1} to 80 ng mL^{-1}. (c) The specificity test of the assay method. Comparing the absorbance ratio $(A-A_0)/A_0$ from the target PDGF-BB and other non-target proteins (Try, BSA, EA, GOD, HRP, HSA, IFN γ, and Myo). The concentration of PDGF-BB is 40 ng mL^{-1} and all other proteins are 80 ng mL^{-1}.

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