Supporting Information

A modification-free nanochannel platform for monitoring cerebral β-amyloid peptides based on the dispersion of stimuli-responsive polymer-modified gold nanoparticles

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Reagents and materials

Gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$) and 2,2′-azobis(isobutyronitrile) (AIBN) were purchased from Sinopharm Chemical Reagent Co., Ltd. *N,N*-diisopropylethylamine, sodium borohydride ($NaBH_4$) and all metallic salts were supplied by Aladdin. *N*-isopropylacrylamide (NIPAAm), S-benzyl dithiobenzoate (BDTB), proteins and amino acids were purchased from Sigma-Aldrich. A sialic acid derivative (SA) and acryloyl-3,5-bis(trifluoromethyl)-phenylthiourea (Ac-TP) were synthesized based on the method previously reported.[1] β-Amyloid peptides were purchased from ChinaPeptides Co., Ltd (Shanghai, China). Aβ monomer solution was prepared by mixing $A\beta_{40}$ and $A\beta_{42}$ in a ratio of 6:1. The oligomeric forms of Aβ were prepared by incubating Aβ monomer solution in PBS at 37 °C for 24 h. Artificial cerebrospinal fluid (aCSF) was prepared according to the reported method.[2] Borosilicate glass capillary (1 mm o.d., 0.78 mm i.d.) was purchased from Sutter Instrument Co..

Apparatus

Scanning electron microscope (Gemini SEM-300) was employed to obtain the morphology of glass nanopore. Transmission electron microscope (JEOL JEM-2100) was employed to obtain the morphology of gold nanoparticles. The UV-vis absorption spectra were acquired on a Shimadzu UV-1800 spectrometer. Zeta-potentials were performed on a Malvern Zetasizer Nano ZS90. The hydrogen nuclear magnetic resonance (^{1}H NMR) spectra was acquired from the Bruker AV-500 instrument. Isothermal titration calorimetric (ITC) experiments were performed on the Nano ITC at 25 ºC

Synthesis of copolymer PNI-TP-SA

The copolymer was synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization. A mixture of NIPAAm, Ac-TP, SA, BDTB, and AIBN (molar ratio 180:20:20:2:1) in 1 mL of 1,4-dioxane was degassed three times and stirred at 70°C for 24 h. Then, the reaction was quenched with chloroform, and the copolymer was precipitated by the addition of ethyl ether. The product was redissolved with chloroform and then precipitated with ethyl ether. This precipitation procedure was repeated three times. The resulting light pink product was dried under vacuum overnight.

Secondly, the above product (200 mg) was dissolved in 10 mL of methanol/water (1:1). The value of pH was adjusted to 9 using sodium methoxide (1M). After stirring at room temperature for 24 h, the value of pH was neutralized to 7 with cation exchange resin (2.5 g). The solution was dried over anhydrous Na_2SO_4 overnight. After evaporation of solvent, the product (PNI-TP-SA) was obtained. The number-average molecular weight M_n was estimated by gel permeation chromatography (GPC) as 10022 ($M_w/M_n = 1.05$).

Preparation of copolymer-modified gold nanoparticles

The preparation of gold nanoparticles has been reported in the literature.[3] 100 mL of $HAuCl_4$ solution (1 mmol) was heated to refluxed. Then, sodium citrate solution (38.8 mmol, 10 mL) was added quickly to the solution. The solution turned to red in several minutes. The solution was heated for an additional 10 min, and allowed to cool down to room temperature.

The copolymers were immobilized onto the surface of AuNPs via Au-S bonds. The copolymer (55 mg) was dissolved in methanol (10 mL) containing $NaBH_4$ (550 μL, 100 mM). The mixture was reacted overnight at 4 °C to obtain copolymer with sulfhydryl group. Then, the above solution was mixted with the AuNPs solution and kept overnight 4 °C. The copolymer-capped AuNPs were purified with dialysis bags (MWCO: 100 kDa), and stored at 4 °C for further use.

Preparation of glass nanopores

Firstly, the glass capillaries were washed with freshly prepared piranha solution (98% H_2SO_4: 30% H_2O_2 = 3:1) and then rinsed with a large amount of deionized water and dried at 80° C. The glass capillaries were pulled by a CO_2-laser-based pipette puller (P-2000, Sutter Instrument) to produce conical glass nanopores with the following parameters: Heat=320, Fil=4, Vel=40, Del=180, Pull =225.

Ionic current measurement

During the ionic current test, the Ag/AgCl electrode in the nanochannel was used as the working electrode, while the reference/counter electrode (Ag/AgCl) was placed outside the glass capillary. The electrolyte solution used for measuring the *I-V* curve is 10 mM KCl solution. Each test was repeated five times to obtain the average current at different voltages.

In vivo **microdialysis**

All experiments involving animals were performed following the approval of the Animal Ethics Committee of Nantong University and performed in accordance with the ARRIVE guidelines. Male C57BL/6J mice and APP/PS1 double transgenic mice at 8 months of age were housed on a 12:12 h light-dark schedule with food and water ad libitum. The animals were anaesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.) and positioned onto a stereotaxic frame. The microdialysis probe was implanted in the hippocampus (bregma -2.3 mm, 2.5 mm lateral to midline, and 1.8 mm below dura). Next, the aCSF solution was infused at the flow rate of 2.0 μL/min. After equilibration for 90 min, the microdialysates were collected. The dialysate was diluted 10-fold with artificial cerebrospinal fluid (aCSF). To determine the Aβ monomers in the microdialysate, glass nanopores were first immersed in the microdialysates with P-AuNPs solution, and then *I-V* curves were recorded in KCl solution.

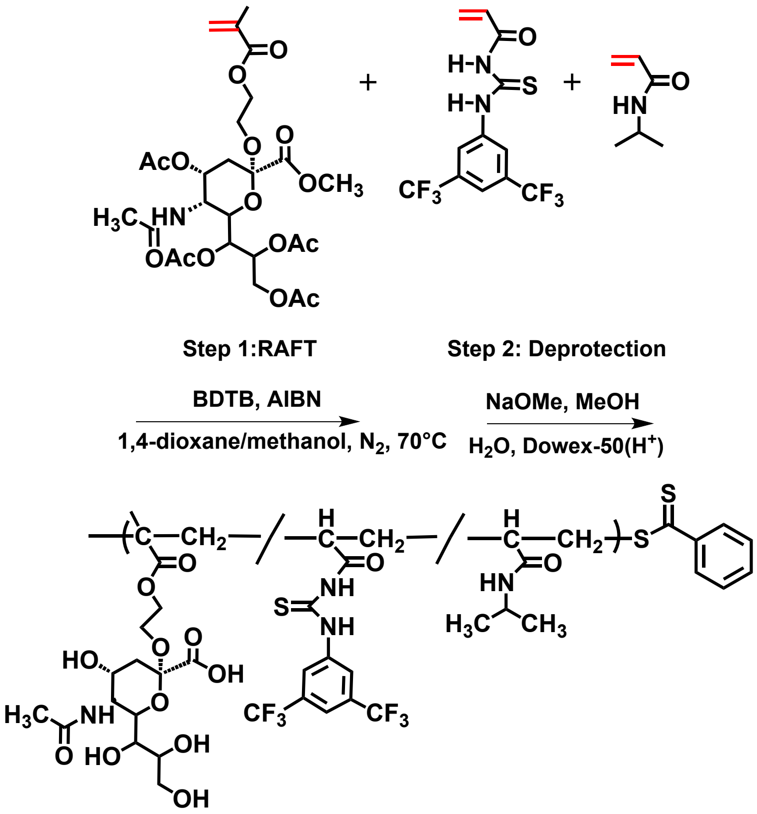


Figure S1. Synthetic route to PNI-TP-SA

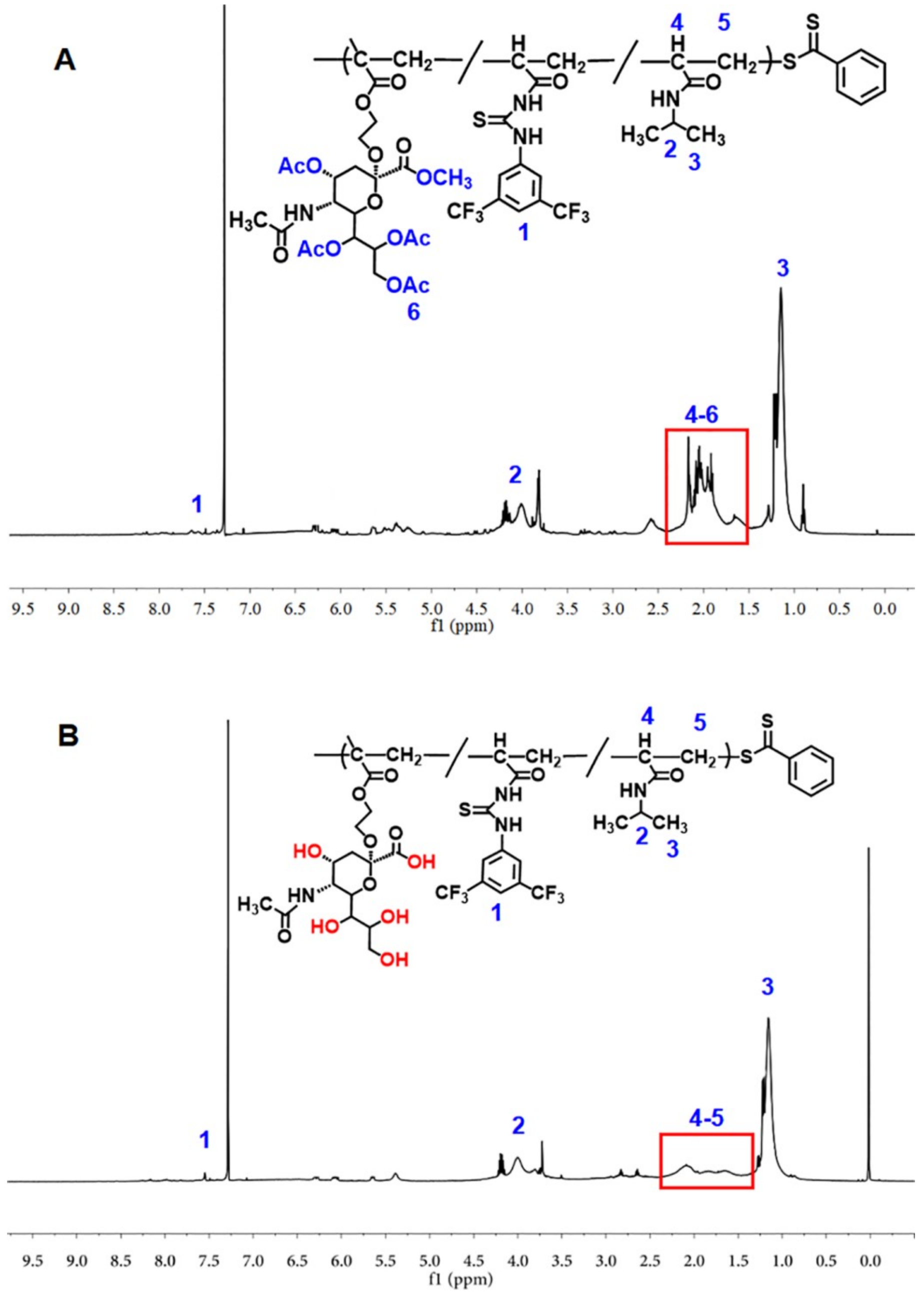


Figure S2. ^{1}H NMR spectrum of PNI-TP-SA copolymer in $CDCl_3$. (A) The signal at 7.0-8.8 ppm corresponded to the protons of phenyl group in TP unit. The peak observed at 3.9 ppm and 1.1 ppm was ascribed to the protons of $CH(CH_3)_2$ in NIPAAm unit. In addition, the peaks located at 2.0-1.5 ppm were attributed to the protons of acetyl group (-Ac) in SA unit and methine protons (-CH) and methylene protons ($-CH_2$) in the backbone. (B) After deprotection, the peak at 2.0-1.5 ppm decreased, indicating the remove of acetyl group (-Ac).

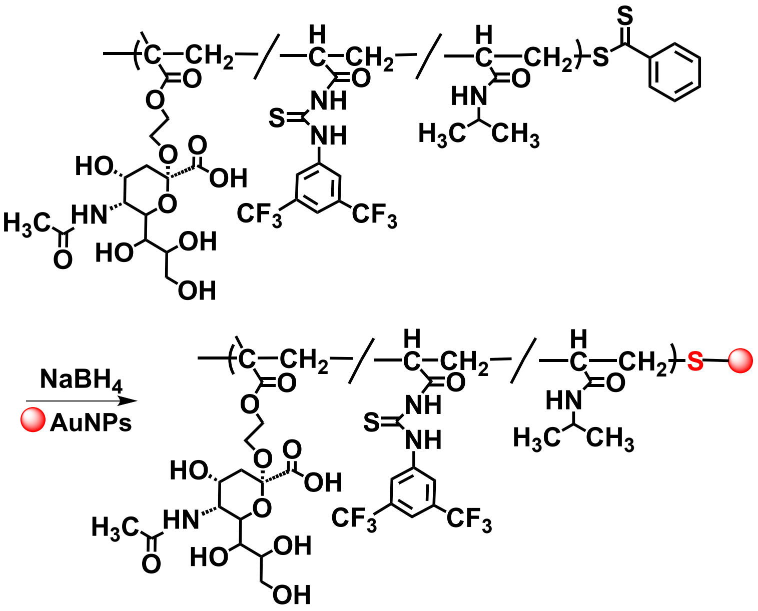


Figure S3. The modification of PNI-TP-SA polymers on the surface of gold nanoparticles.

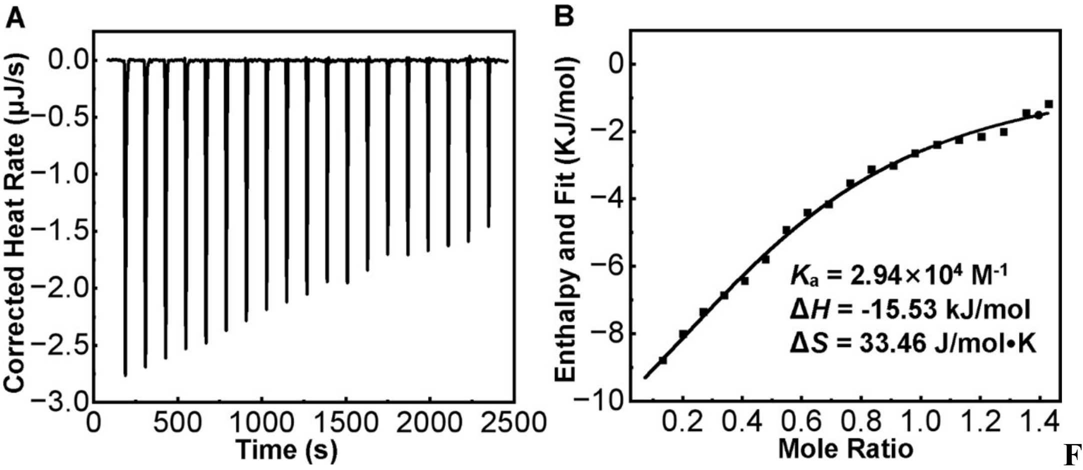


Figure S4. Isothermal titration calorimetric data for the titration of PNI-TP-SA solution (0.01 mmol/L) with Aβ monomer (0.1 mmol/L) in DMSO. (A) The calorimetric titrations for 2 μL injections with 120 s between injections. The stirring speed was 350 r/min. (B) The integrated heat values as a function of the PNI-TP-SA / Aβ monomer molar ratio in the cell. The solid line represents the fitting curve using one set of independent binding sites model. Inset in B: the corresponding thermodynamic parameters.

The exothermic data demonstrated significant heat release, providing direct evidence of bond formation between the PNI-TP-SA polymer and Aβ monomers.[4,5] In addition, the thermodynamic parameters suggested that the binding of PNI-TP-SA with Aβ monomer was enthalpy-driven, implying that the hydrogen bonding underlied the interaction.[6] Meantime, the increase in entropy indicated that binding induced the expansion of PNI-TP-SA polymer chain.[7-9]

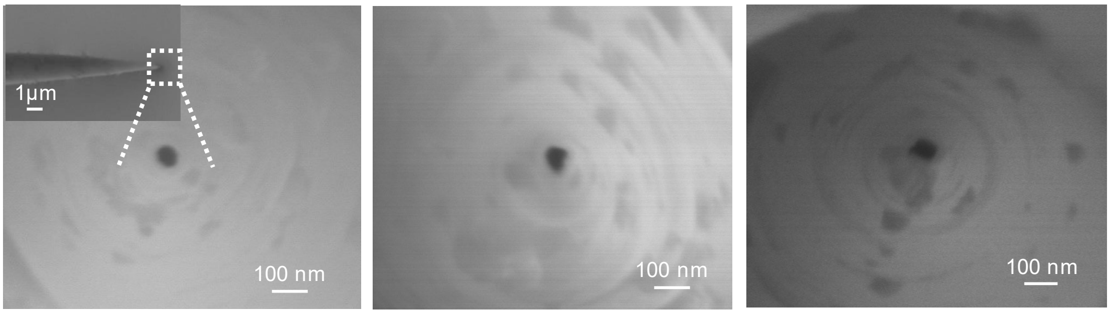


Figure S5. Representative SEM images of the unmodified glass nanopores.

Table S1. The tip diameters of ten independently fabricated glass nanopores

	1	2	3	4	5	6	7	8	9	10	Mean ± SD
Diameter (nm)	62.5	71.42	57.14	66.67	83.33	83.21	65	75	62.5	65	69.2 ± 8.9

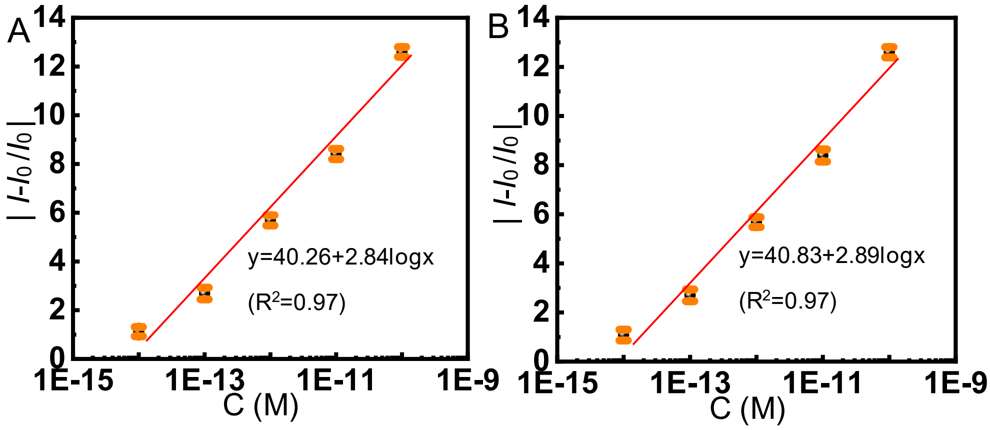


Figure S6. Calibration curves illustrating the change in rectification ratio against the Aβ monomers concentration (10^{-10} M, 10^{-11} M, 10^{-12} M, 10^{-13} M, 10^{-14} M). Each data point represented the mean ± SD of three replicate measurements.

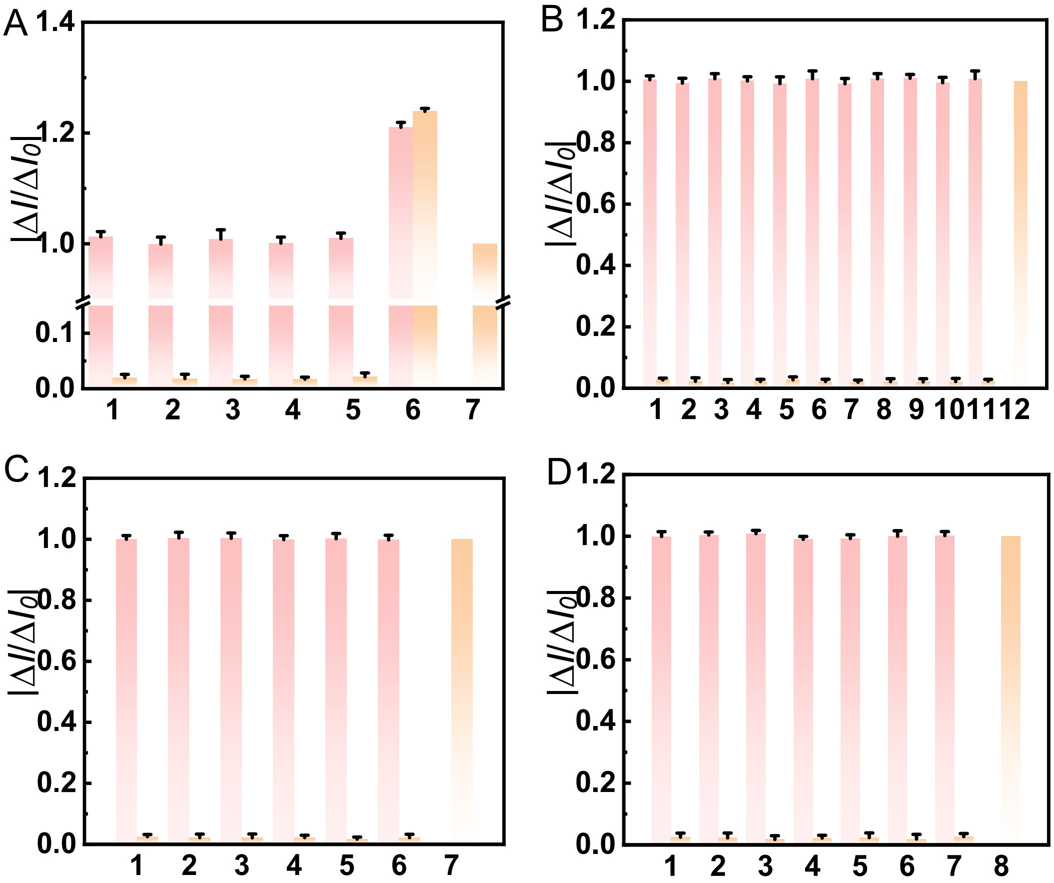


Figure S7. Selectivity and competition experiments of (A) proteins against Aβ monomers (1-7: Tau, actin, α-syn, presenilin, VDAC1, Aβ oligomers, Aβ monomers, 0.1 nM for Aβ monomers and oligomers, 0.2 mg/mL for other proteins) ; (B) amino acids against Aβ monomers (1-12: Glu, Gly, Phe, Ser, Val, Cys, His, Iso, Arg, Lys, Thr, Aβ monomers, 10 μM for all the tested amino acids, 0.1 nM for Aβ monomers) ; (C) metal ions against Aβ monomers (1-7: K^+, Na^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cu^{2+}, Aβ monomers, 100 mM for K^+, Na^+; 10 mM for Ca^{2+}; 1 mM for Mg^{2+}; 10 μM for Fe^{3+} and Cu^{2+}, 0.1 nM for Aβ monomers) and (D) other biological molecules against Aβ monomers (1-8: 500 μM AA, 20 μM UA, 20 μM DA, 50 μM DOPAC, 20 μM 5-HT, 5 mM glucose, 1 mM lactate, 0.1 nM Aβ monomers). The orange bars mean the addition of potential interferences. The red bars mean the subsequent addition of Aβ monomers. ΔI_0 represents the current change induced by Aβ monomers, ΔI represents the current change induced by potential interferences or the mixture of potential interferences and Aβ monomers.

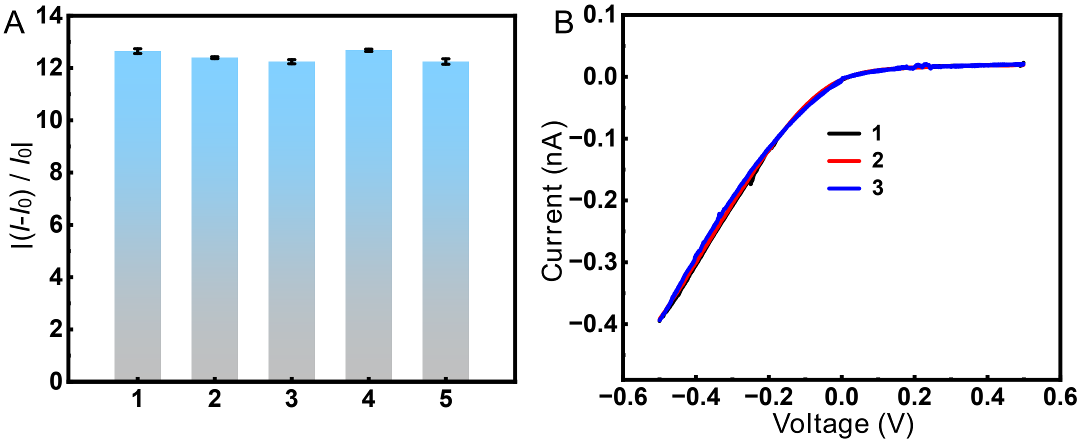


Figure S8. (A) The change of rectification ratio against the Aβ monomers (10^{-10} M) for five glass nanochannels. (B) The *I-V* curves were recorded from a single unmodified glass nanochannel in response to Aβ monomers (10^{-10} M) across three consecutive measurements.

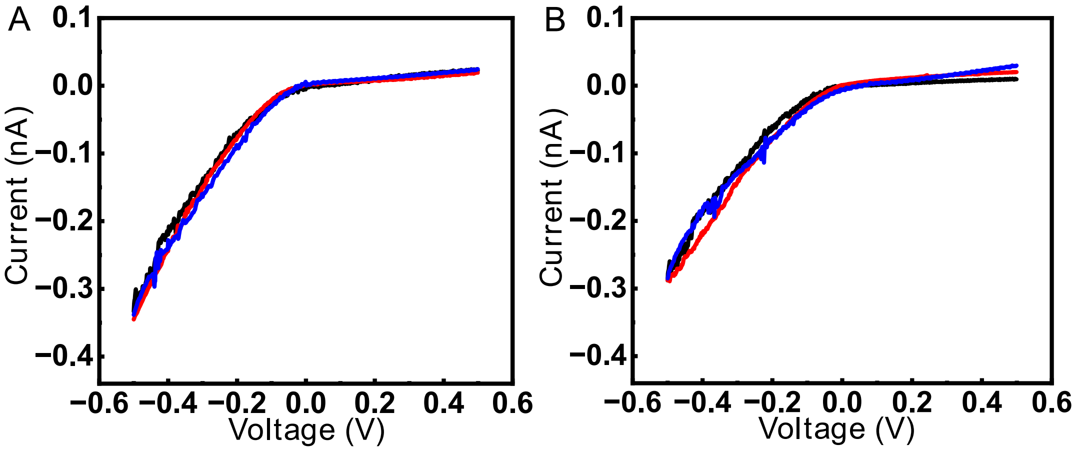
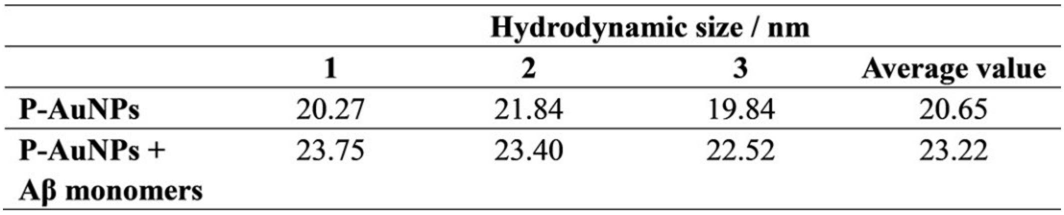


Figure S9. *I-V* curves recorded at the modification-free glass nanochannels after pretreatment with microdialysates from (A) three normal mice and (B) three AD mice.

Table S2. Hydrodynamic sizes of polymer-capped AuNPs obtained by DLS experiment.



	Hydrodynamic size / nm			
	1	**2**	**3**	**Average value**
P-AuNPs	20.27	21.84	19.84	20.65
P-AuNPs + Aβ monomers	23.75	23.40	22.52	23.22

The influence of Aβ monomers on the polymer shell of gold nanoparticles was characterized by dynamic light scattering measurements at 25 °C. The average hydrodynamic size of the polymer-capped AuNPs was about 20.65 nm. In the presence of 10^{-5} M Aβ monomers, the average hydrodynamic size increased to 23.22 nm.

References

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