Supplementary Information

Nanochannel array-based platform for aggregation monitoring and high-sensitive identification of neurotoxic amyloid-β oligomers

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1. Experimental Section

1.1 Peptide Preparation. Aβ42 powder was treated according to a previous work.[1] Lyophilized powdered Aβ peptide was dissolved in 1,1,1,3,3,3,3-hexafluoroisopropanol (HFIP) with a concentration of 1 mg/mL and shaken at ice-water bath for 2 h to further dissolve. It was separated into small aliquots and stored at -20 °C, and each aliquot was dried using by evaporation under a gentle stream of nitrogen. The dry peptide was then dissolved in 25 mM Tris-HCl buffer (containing 100 mM NaCl and 2% DMSO, pH 7.4) to 1 mg/mL. After sonicating for 15 min, the solution was diluted with 25 mM Tris-HCl, 100 mM NaCl solution (pH 7.4), and stored 4 °C until use. This freshly prepared sample was defined as AβM. To form aggregated peptide (AβO, AβF), 0.2 mg/mL Aβ42 monomer sample was incubated for different times (1 h, 3 h, 5 h, 7 h, 12 h and 15 h, respectively) at 37 °C in 25 mM HEPES, 100 mM NaCl solution (pH 7.4).

1.2 Thioflavin T (ThT) Fluorescence Measurements. The dye ThT was used to monitor the kinetics of Aβ aggregation, the fluorescence of which depended on the formation of amyloid fibrils. All fluorescent measurements were recorded in a FLSP920 spectrofluorometer (Edinburgh Instruments Ltd., Livingston, UK) at room temperature with excitation wavelength of 440 nm and emission wanelength of 480 nm. Fluorescence emission spectra were collected between 460 nm and 600 nm with a 5 nm slit. The peptide concentration was 2 μM, and the ThT concentration was 10 μM. At different incubation times, aliquots of the Aβ solution were taken for fluorescence measurements.

1.3 Atomic Force Microscopy (AFM) Assay. AFM measurements were performed using a Nano Scope V multi-mode atomic force microscope (Brucker Bioscope Resolve, Germany). The concentration of Aβ protein used for AFM imaging was 2 μM, and the sample of 20 μL was applied onto freshly cleaved mica sheets and allowed to dry. Tapping mode was used to acquire the images under ambient conditions. All the AFM images were further analyzed using Nanoscope Analysis 2.0.

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Table S1 The oligonucleotides and protein sequences used in this work.

Entry	Sequence (from 5′ to 3′)
AβO-Apt	NH_2-$(CH_2)_6$-CGG CCG CCT GTG GTG TTG GGG CGG GTG CGC G
AβO-Apt-T	NH_2-$(CH_2)_6$-CGG CCG CCT GTG GTG TTT GGG CGG GTG CGC G
AβO-Apt-TT	NH_2-$(CH_2)_6$-CGG CCG CCT GTG GTG TTT TGG CGG GTG CGC G
AβO-Apt-TTT	NH_2-$(CH_2)_6$-CGG CCG CCT GTG GTG TTT TTG CGG GTG CGC G
Aβ42	D-A-E-F-R-H-D-S-G-Y-E-V-H-H-Q-K-L-V-F-F-A-E-D-V-G-S-N-K-G-A-I-I-G-L-M-V-G-G-V-V-I-A

Table S2 Comparison of Aβ42 detection performance with other methods.

Detection mechanism	sample	Linear range	Limit of detection	References
Liposome-based aptasensor	AβO	5 nM ~1 μM	2.27 nM	2
Optical and electrochemical sensor	AβO	10^{-5} to 10 μM	10 nM	3
Electrochemical immunosensor	AβO	1pM ~1μM	0.4pM	4
Colorimetric aptasensor	AβO	10~100 nM	3.03 nM	5
CeONP-Res-PCM@ZIF-8/PDA/Apt nanocomposite	AβO	10nM~10 μM	3.2 nM	6
Surface plasmon resonance sensor	AβO/AβF	0 ~10 pM/0.05 ~10 pM	0.2 pM /0.05 pM	7
Ratiometric fluorescent probe	Aβ	0~6 μM	89.9 nM	8
Near-infrared fluorescent probe	AβO	2~10 μM	9.3 nM	9
NC-Apt-based biosensor	AβO	0.01 ~ 5000 μg/mL (2.3 nM~113μM)	0.384 ng/mL(85.1 pM)	This work

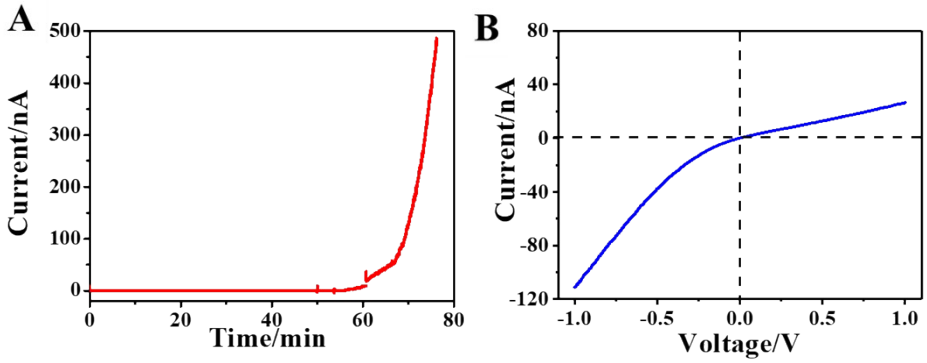

Fig. S1 (A) The ion-current curve on PET-etching process *vs* time. (B) I-V curve of successful etching PET conical nanochannels, which obtained in 25 mM Tris-HCl buffer containing 100 mM NaCl, pH7.4.

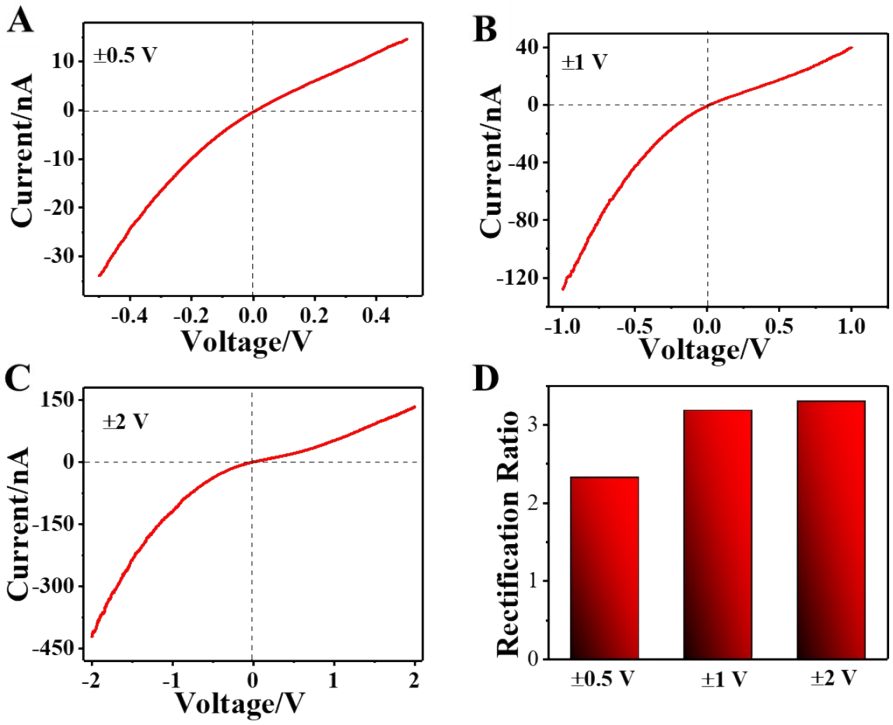

Fig. S2 I-V curves of bare nanochannel (B-NC) obtained in 25 mM Tris-HCl containing 100 mM NaCl (pH7.4) with scanning range of (A) ±0.5 V, (B) ±1V and (C) ±2 V, respectively, and (D) the corresponding rectification ratios ($|I_{-0.5\ V}/I_{0.5\ V}|$) statistics.

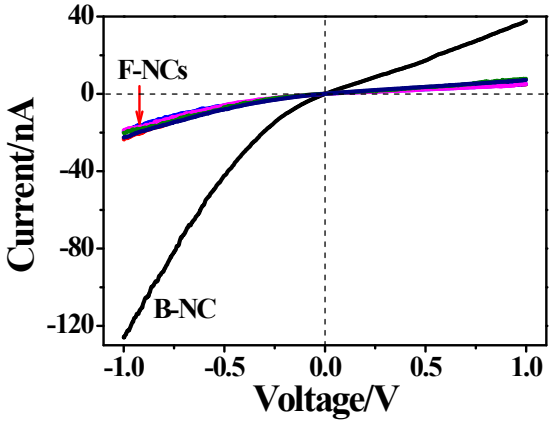

Fig. S3 I-V curves of 5 different functionalized nanochannels (F-NCs) obtained in 25 mM Tris-HCl containing 100 mM NaCl, pH7.4.

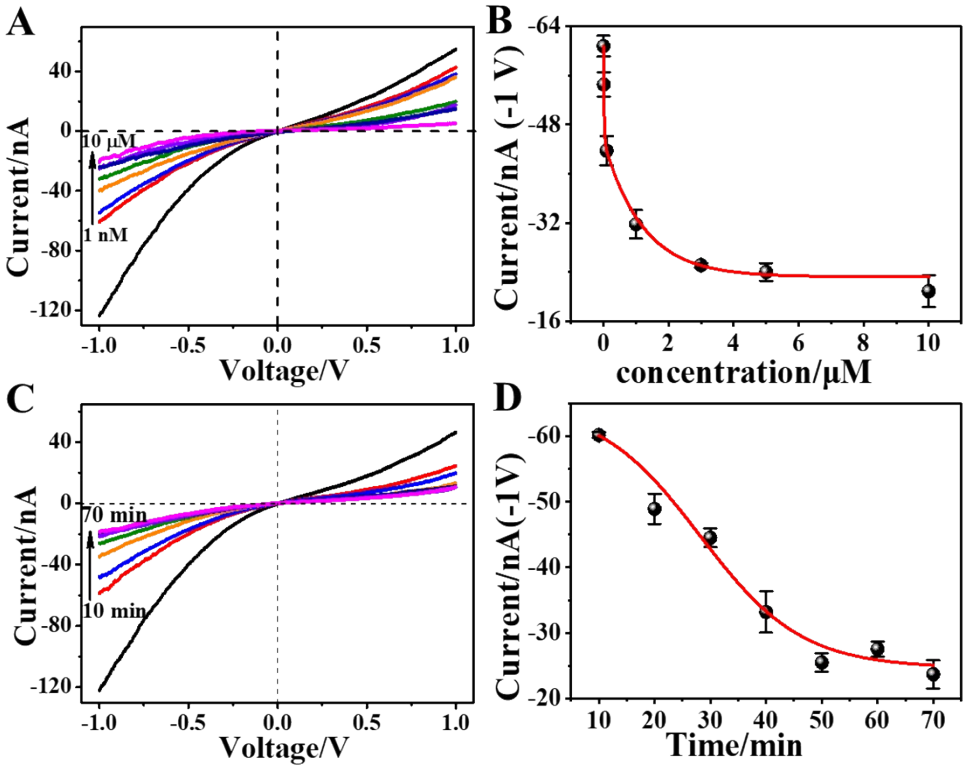

Fig. S4 (A) I-V curves of the nanochannels modified with AβO-Apt with different concentrations (0.001 μM, 0.01 μM, 0.1 μM, 1 μM, 3 μM, 5 μM, 10 μM, respectively), and (B) their corresponding rectified ion-current change curve (at -1V) with increasing concentration. (C) I-V curves of nanochannels treating with 5 μ M AβO-Apt at different times (10 min-70 min with an interval time of 10 min), and (D) their corresponding rectified ion-current change curve (at -1V) with extending interacting time.

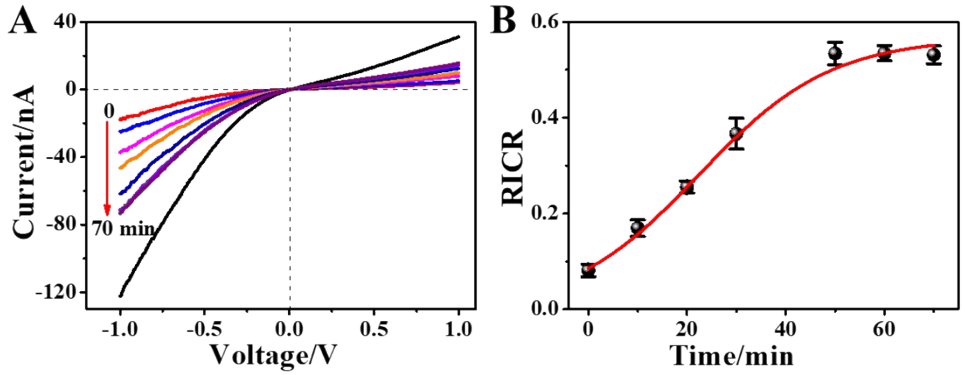

Fig. S5 (A) I-V curves of the F-NC after treating with 0.2 mg/mL AβO with capture time ranging from 10 min to 70 min at 4 °C (with an interval time of 10 min), and (B) its corresponding RICR changes curve (at -1 V) with extending AβO capture time.

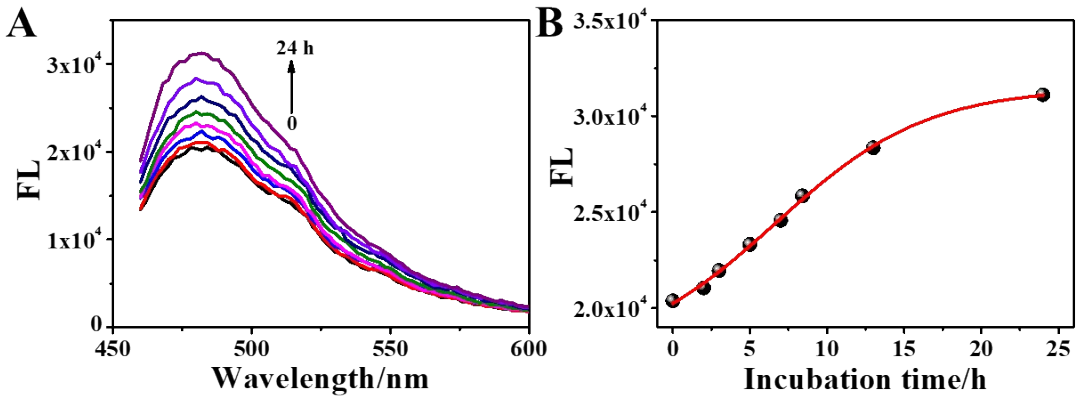

Fig. S6 (A) The fluorescence emission spectra of ThT (10 μM) in the presence of Aβ42 (2 μM) with different incubation time (0, 2 h, 3 h, 5 h, 7 h, 9 h, 13 h, 24 h, respectively) at 37 °C. (B) The plot of the ThT fluorescence intensity at 480 nm against incubation time.

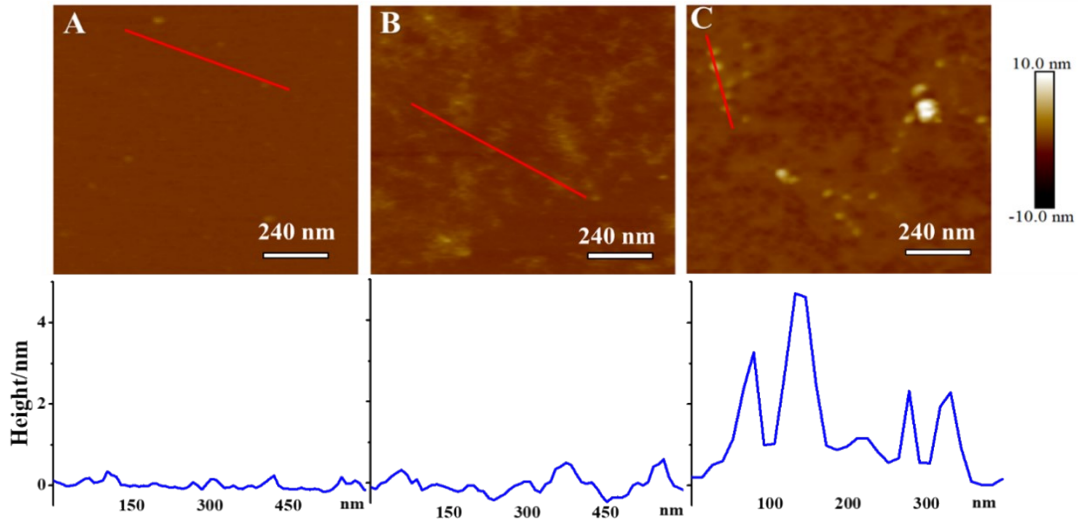

Fig. S7 AFM images and line-scanning height analysis of Aβ42 (with a monomr concentration of 2 μM) incubated for (A) 0 h, (B) 9 h and (C) 12 h, respectively.

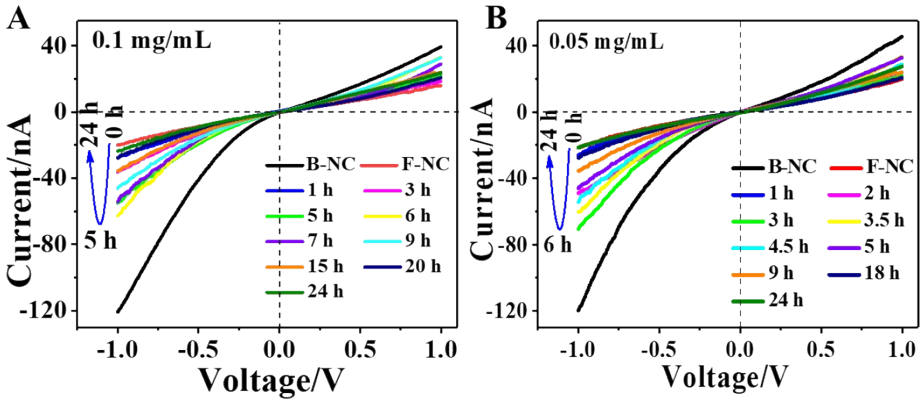

Fig. S8 I-V curves of F-NCs after interacting with (A) 0.1 mg/mL and (B) 0.05 mg/mL Aβ42 with various incubation.

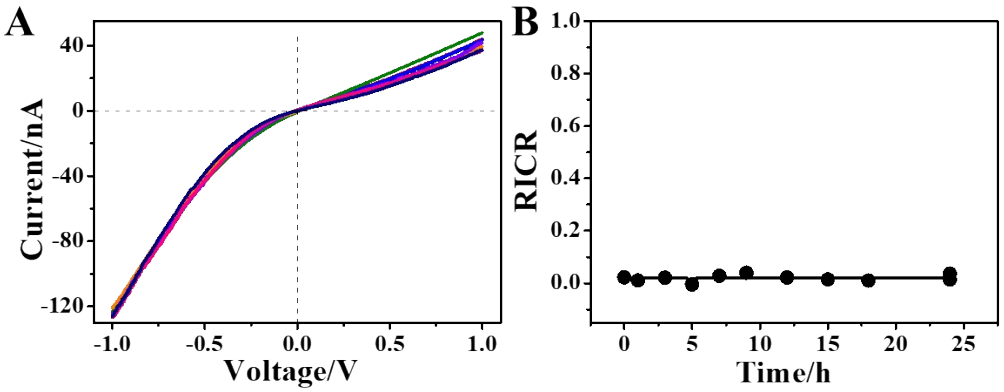

Fig. S9 (A) I-V curves of B-NCs interacting with 0.2 mg/mL Aβ with various incubation time from 0 to 24 h (0 h, 1 h, 3 h, 5 h, 7 h, 9 h, 12 h, 15 h, 18 h, 24 h, respectively), and (B) corresponding time course of their RICR.

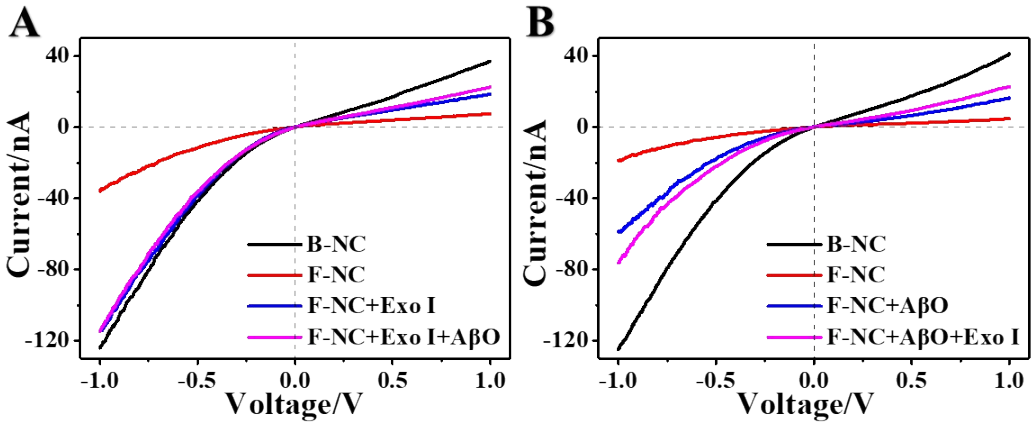

Fig. S10 I-V curves of (A) EXO I-treated F-NC after interaction with AβO，(B) AβO-binding F-NC after treatment with EXO I. All I-V curves were obtained in 25 mM Tris-HCl buffer containing 100 mM NaCl, pH7.4, EXO I and AβO were 20 U/L and 0.2 mg/mL, respectively.

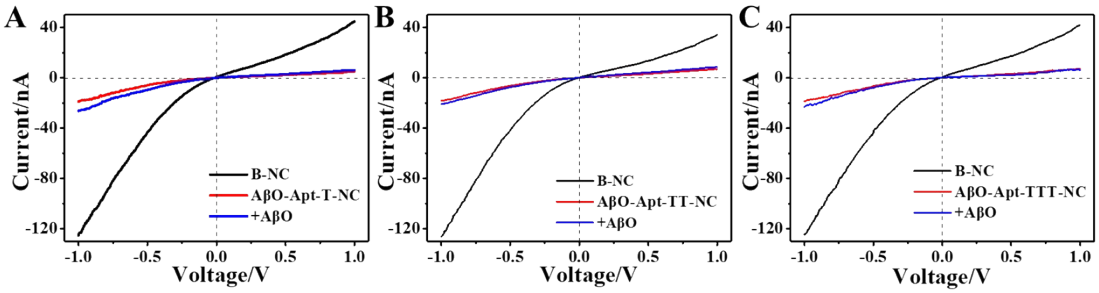

Fig. S11 I-V curves of (A) AβO-Apt-T, (B) AβO-Apt-TT, and (C) AβO-Apt-TTT functionalized nanochannels before and after treating with AβO with a monomer concentration of 0.2 mg/mL.

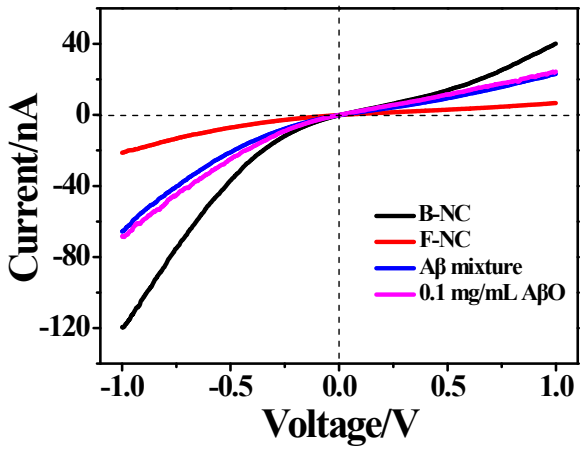

Fig. S12 F-NCs after interacting with 0.1 mg/mL AβO and Aβmixture of AβO, AβM and AβF with a monomeric concentration of 0.3 mg/mL.

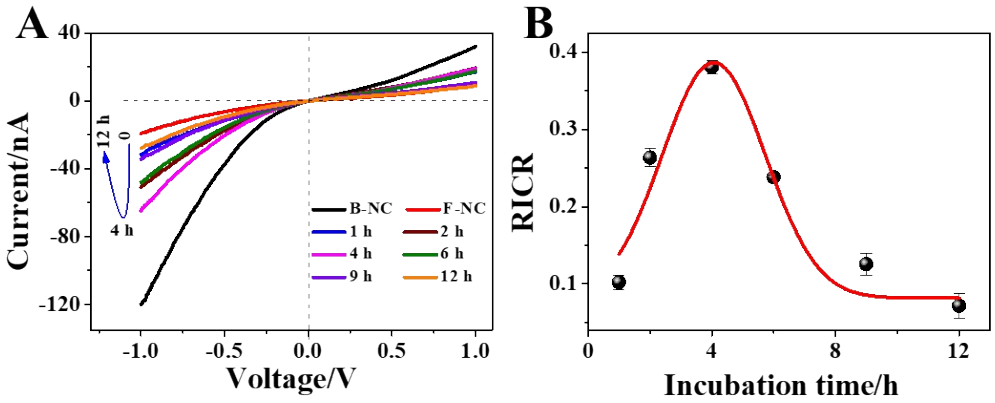

Fig. S13 I-V curves of F-NCs after interacting with 0.1 mg/mL Aβ in artificial cerebrospinal fluid (aCSF) with various incubation time (1 h, 2 h, 4 h, 6 h, 9 h, 12 h, respectively), and (B) the corresponding time courses of RICR values of F-NCs.

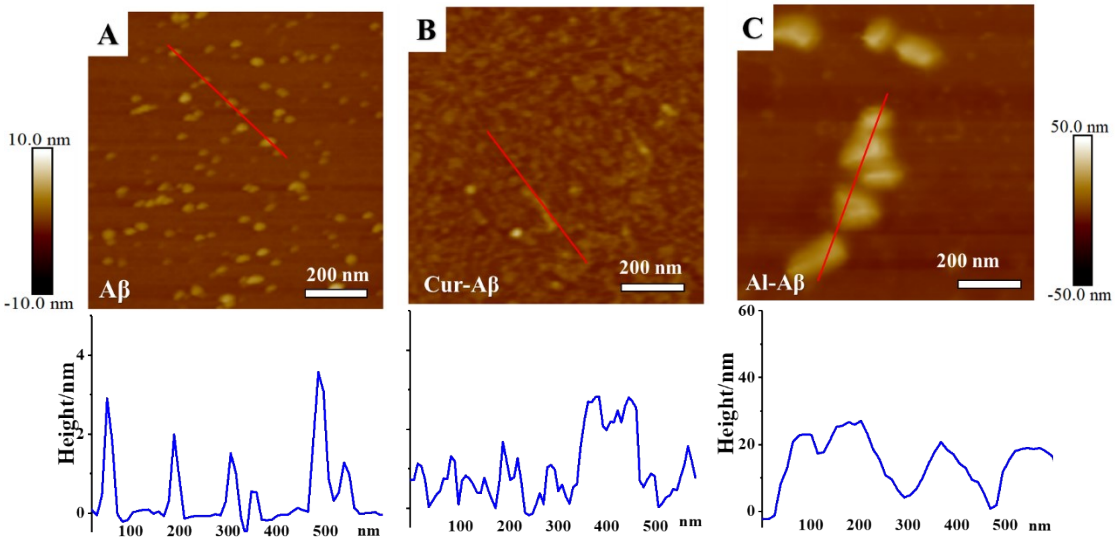

Fig. S14 AFM images and line-scanning height analysis of (A) pure Aβ42 and co-incubated Aβ42 with (B) 10 μM curcumin (Cur-Aβ) and (C) 10 mM $Al(NO_3)_3$ (Al-Aβ) for 3h at 37 °C, respectively.

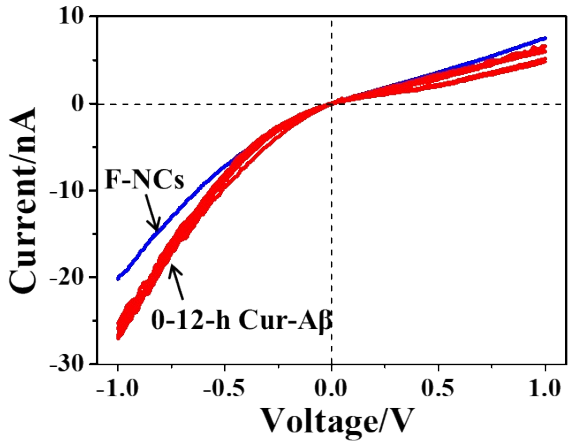

Fig. S15 I-V curves of F-NCs after interaction with Cur-Aβ at different co-incubation time ranging from 0 to 12 h (0, 50 min, 2h, 3 h, 6 h, 9 h and 12 h).

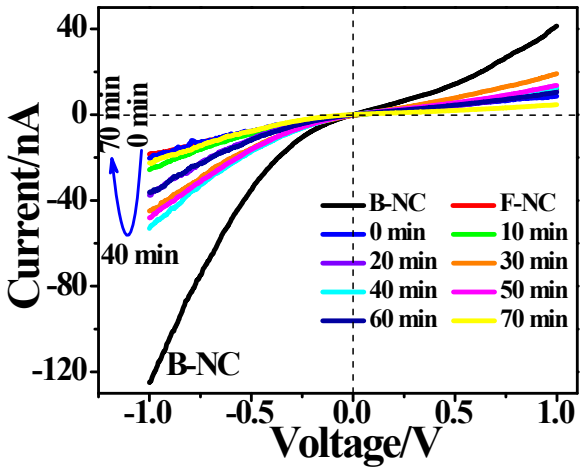

Fig. S16 I-V curves of F-NCs after interaction with Al-Aβ at different co-incubation time ranging from 10 min to 70 min (with an interval time of 10 min).

3. References

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