Supplementary information

Label-free Detection of Alzheimer’s Disease through ADP3
Peptoid Recognizing Serum Amyloid-beta42 Peptide

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Experimental section

Antibodies

Anti-human IgG (ab6759, Abcam), anti-Ab42 (ab10148, Abcam).

Subjects selection and sera sample collection

This study included 6 patients with Alzheimer’s disease (AD) and 6 age-matched, nondemented controls. Informed consent was obtained from all participants or their guardians. The diagnosis of AD was according to the criteria of NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association).1 The non-demented control subjects were healthy elderly volunteers without cognitive symptoms or other neurological disease.

The blood drawn by venous puncture was collected in blood collection tubes containing clot activator, and was allowed to clot for 15 min at room temperature. The serum was then separated by centrifugation at 3000 rpm for 10 min. The serum samples were stored at -80°C in aliquots.

Peptoid synthesis

Peptoids were synthesized using solid-phase submonomer method2 with modification. Briefly, the syntheses were conducted with Rink amide AM resin (substitution level 0.30 mmol/g), which was pooled into a 50 mL glass peptide synthesis reaction vessel. The amine on the resin was acylated by 2 M bromoacetic acid with 3.2 M diisopropylcarbodiimide (DIC) in dimethylformamide (DMF) for 30 min at 37°C. After the Sn2 reaction there was a nucleophilic displacement of bromide with 2 M primary amine for 90 min at 37°C. Reagents for each reaction were mixed by bubbling N₂ through the suspension. At the end of the
synthesis, the side-chain protecting groups were removed, and the peptoids were cleaved from
the resin by treating with 95% TFA, 2.5% water and 2.5% triisopropylsilane for 2 h. Then
after filtration, dilution, and lyophilization, peptoids were purified by HPLC and established
by mass spectrometry (MS).

**Aβ42 preparation**

Aβ42 preparation was performed according to the previously published protocol\(^3\) with
slight modification. Briefly, Aβ42 (GL Biochem, Shanghai, China) was dissolved in
1,1,1,3,3,3-hexafluoro-2-propanol (HFIP; Sigma, Shanghai, China) to the final concentration
of 0.8 mM, and aliquoted in microcentrifuge tubes. After removing HFIP under vacuum in a
SpeedVac, the HFIP-treated Aβ42 was stored at -20°C. Prior to AFM and SPRi experiments,
various concentrations of Aβ42 were prepared by dissolving the HFIP-treated Aβ42 with PBS
(or PBST for SPRi experiments) using DMSO as hydrotropic agent, and incubating at 4°C for
20h.

**Sensor chip fabrication and surface plasmon resonance imaging (SPRi)**

Sensor chip fabrication and SPRi measurements were performed according to the
previously published protocol\(^4\) with slight modification. Various concentrations of
ADP3 dissolved in water were manually printed onto the bare gold-coated (thickness
47 nm) PlexArray Nanocapture Sensor Chip (Plexera Bioscience, Seattle, WA, US) at
40% humidity. Each concentration was printed in replicate, and each spot contained
0.1 μL of ADP3 solution. The chip was incubated in 80% humidity at 4°C for
overnight, and rinsed with 10× PBST for 10 min, 1× PBST for 10 min, and
deionized water twice for 10 min. The chip was then blocked with 5% (w/v) non-fat
milk in water overnight, and washed with 10× PBST for 10 min, 1× PBST for 10
min, and deionized water twice for 10 min before being dried under a stream of
nitrogen prior to use.

SPRi measurements were performed with PlexArray HT (Plexera Bioscience,
Seattle, WA, US). Collimated light (660 nm) passes through the coupling prism,
reflects off the SPR-active gold surface, and is received by the CCD camera. Buffers
and samples were injected by a non-pulsatile piston pump into the 30 μL flow cell
that was mounted on the coupling prim. Sample preparation was performed prior to
SPRi measurement. Each serum sample was diluted in PBST proportionately (1:5000).
Each antibody and the proteinase K was diluted in PBST proportionately (1:1000). Each
measurement cycle contained four steps: washing with PBST running buffer at a
constant rate of 2 μL/s to obtain a stable baseline, sample injection at 5 μL/s for
binding, surface washing with PBST at 2 μL/s for 300 s, and regeneration with 0.5% (v/v) H$_3$PO$_4$ at 2 μL/s for 300 s. For serum samples, regeneration was performed with 0.5% (v/v) H$_3$PO$_4$ at 2 μL/s for 300 s followed by proteinase K solution at 2 μL/s for 300 s. All the measurements were performed at 4°C. The signal changes after binding and washing (in AU) are recorded as the assay value.

Selected peptoid-grafted regions in the SPR images were analyzed, and the average reflectivity variations of the chosen areas were plotted as a function of time. Real-time binding signals were recorded and analyzed by Data Analysis Module (DAM, Plexera Bioscience, Seattle, WA, US). Kinetic analysis was performed using BIAevaluation 4.1 software (Biacore, Inc).

**Atomic force microscopy (AFM)**

Various concentrations of ADP3 was deposited onto freshly cleaved mica, incubated at 25°C for 5 min and dried in air. For Aβ42 binding measurements, after incubation 5.94 mM of ADP3 on freshly cleaved mica, 2.22 μM of Aβ42 was deposited onto the sample, incubated at 25°C for 5 min, and carefully rinsed with water. Samples were air dried before measurements. AFM measurements were performed with Dimension 3100 AFM (Bruker, MA, US) in tapping mode in air. Images were flattened and plan-fitted as required.
S1. Mass spectrum of the purified ADP3.
S2. Representing SPRi sensorgram showing the binding of AD serum to different concentrations of ADP3. The black, red, blue, cyan, and purple curves correspond to the ADP3 concentrations of point I, II, III, IV, and V in Fig1(a) respectively: I, 48 μM; II, 0.23 mM; III, 1.2 mM; IV, 5.9 mM; V, 30 mM.

S3. Representing SPRi sensorgram showing the binding of ADP3 and scrambled ADP3 to Aβ42. The black and red curves correspond to ADP3 and scrambled ADP3, respectively. Compared to ADP3, scrambled ADP3, which had the same residues with ADP3 but the residues are arranged in a random order, had negligible binding signal to Aβ42, indicating no binding between scrambled ADP3 and Aβ42.
S4. AFM image of ADP3 adsorbed on gold-coated glass chip. After increasing the scanning force, materials were scratched from the surface by the cantilever tip, confirming ADP3 nano-cluster formed on the surface. The areas before and after scratching are marked with green cross and pink triangle respectively.

S5. Schematic illustration of ADP3 immobilization on the surfaces. According to the height (< 1.2 nm) and length (~4 nm) of ADP3 peptoid, if the peptoid layed flat on the surface, the height of the nano-clusters should be less than 1.2 nm. However, the nano-clusters with height of ~4 nm on the gold surface, and ~1.7 nm on mica, indicate that the peptoids stay upright on both surfaces as a brush, but with steeper slope on mica.
S6. AFM images of Aβ42 adsorbed on mica. Aβ42 was deposited onto freshly cleaved mica at the concentration of 2.2 μM. Compared to Aβ42 adsorbed on ADP3 nano-cluster (Fig 3c), not so many materials adsorbed on the surface, confirming the capability of ADP3 nano-cluster to capture Aβ42.

S7. AFM images of Aβ42 adsorbed on incomplete ADP3 nano-clusters. ADP3 was adsorbed on freshly cleaved mica at the concentration of 5.9 mM. Aβ42 was deposited onto ADP3 nano-clusters at the concentration of 2.2 μM. Aβ42 binds to pieces of ADP3 nano-clusters (marked with blue arrows), but not to bare mica. Fig 3e is the zoom image taken from (a), (c-e) are zoom in images from (b), Fig 3f is the zoom image from (f).
References