Supporting Information

for

Paper-based electrochemiluminescence sensor for highly sensitive detection amyloid‑β oligomerization: Toward potential diagnosis of Alzheimer's disease

Hongxing Liu, Xiaoming Zhou*, Qi Shen, Da Xing*

MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science, College of Biophotonics, South China Normal University, Guangzhou 510631, China.

* The contact information of the corresponding authors is

Xiaoming Zhou, PhD, Professor, Da Xing, Ph.D., Professor

MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science, College of Biophotonics, South China Normal University

E-mail: zhouxm@scnu.edu.cn (X.Zhou) xingda@scnu.edu.cn (D.Xing)

1. Systhesis and characterization of [Ru(phen)$_2$dppz](PF6)$_2$

[Ru(phen)$_2$dppz](PF6)$_2$ was synthesized according to previously reported methods.[1] Briefly, [Ru(phen)$_2$dppz](PF6)$_2$ was obtained by putting dppz (0.100 g, 0.355mmol) and cis-Ru(phen)$_2$Cl$_2$ (0.188 g, 0.355mmol) into a component solvent of ethyl alcohol and water (3:1). The mixture was heated to reflux under a nitrogen atmosphere for 8h to give the product as anorange solid. The Cl- was then exchanged by PF6- and the raw product was purified with silica gel column (CH_3CN: toluene = 1:1). The volatiles were removed under reduced pressure and the remaining solid was recrystallized with CH_3CN and diethyl ether to give red crystals(0.224g, 0.216mmol; Yield: 59.9%).1H NMR (400 MHz, [D6]DMSO): δ 9.62 (d, J = 8.2 Hz, 2H; phen-H), 8.80 (t, J = 7.8 Hz, 4H; phen-H), 8.53 (dd, J = 6.4, 3.4 Hz, 2H; dppz-H), 8.41 (s, 4H; phen-H), 8.28 (d, J = 5.2 Hz, 2H; dppz-H), 8.24 – 8.16 (m, 4H; phen-H), 8.06 (d, J = 5.1 Hz, 2H; dppz-H), 7.91 (dd, J = 8.0, 5.6 Hz, 2H; dppz-H), 7.80 (ddd, J = 15.5, 8.0, 5.4 Hz, 4H; phen-H).; MS (ESI): m/z: 889.10. $[M-PF6]^+$, 372.07 $[M-2PF6]^{2+}$.

2. Dynamic light scatter assays

50 nM monomeric Aβ (1-42) incubated with pH 7.4 PBS buffer for 0, 0.5, 1, 2, 4, 8, 16, 24 , 48, 72 and 120 hours at 37 °C to apply for dynamic light scatter assay using Zetasizer Nano Series (Malvern).

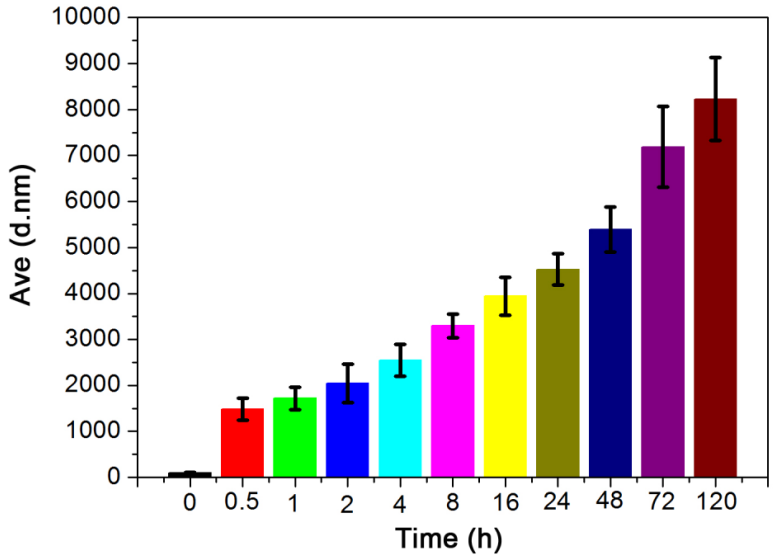

Figure. S1.dynamic light scatter assay for Aβ (1-42) incubated at 37 °C for different times.

3. TEM to observe and monitor the formation of fibrils

The oligomer and aggregated Aβ(1–42) was negatively stained with 1% uranium acetate and applied to TEM for further verification. According to the TEM images, the oligomer Aβ(1–42) show a 150 nm spherical shape, while aggregated Aβ(1–42) show a fibrous shape. The experimental results also directly verified the formation of amyloid-beta-oligomer/fibrils.

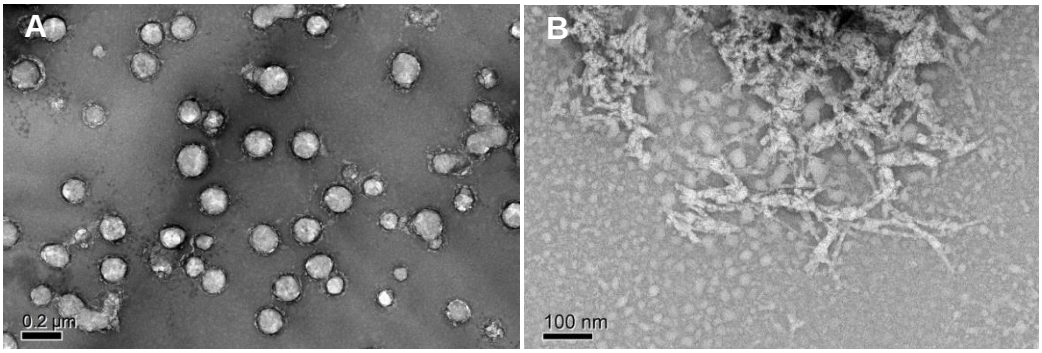

Figure. S2. TEM to observe and monitor the formation of fibrils. **(A)** oligomer and **(B)** fibrils.

4. Cultivation and identification of transgenic mice

The APPswe/PSEN1dE9 (APP/PS1) transgenic mouse model used in this study was produced by coinjection with the APPswe and PS1dE9 vectors[2]. The APPswe/PSEN1dE9 (APP/PS1) transgenic mouse model harbours two human transgenes: the amyloid beta precursor protein gene (APP) containing the Swedish mutation; and the presenilin-1 gene (PS1) contains a deletion of exon 9. Two transgenes inserted at a single locus in Chromosome 9 between Arpp21 and Pdcd6ip. (Figure 4C) The DeltaE9 mutation of the human presenilin 1 gene is a deletion of exon 9 and corresponds to a form of early-onset Alzheimer's disease.

All the experimental mice were of a C57BL/6 background, and Wild-Type (WT) and transgenic mice were paired from the litters and housed under the same living conditions, at 6 months of age. For mouse genotyping, PCR primers for APP (377 bp) and PS1 (608 bp) was prepared from Sangon，Shanghai. APP (374 bp) and PS1 (608 bp) were identified by PCR with primers for APP (forward, 5’-AGGACTGACCACTCGACCAG-3’, reverse,5’-CGGGGGTCTAGTTCTGCAT-3’) and PS1 (forward, 5’-AATAGAGAACGGCAGGAGCA-3’, reverse, 5’ -GCCATGAGGGCACTAATCAT-3’). PCR conditions for APP and PS1 consisted of 35 cycles of 94 °C for 30 s, 58 °C for 40 s, and 72 °C for 45s. All PCRs started with 94 °C for 5 min and ended with 72 °C for 5 min. The PCR products were confirmed by electrophoresis on 1% (w/v) agarose gels.

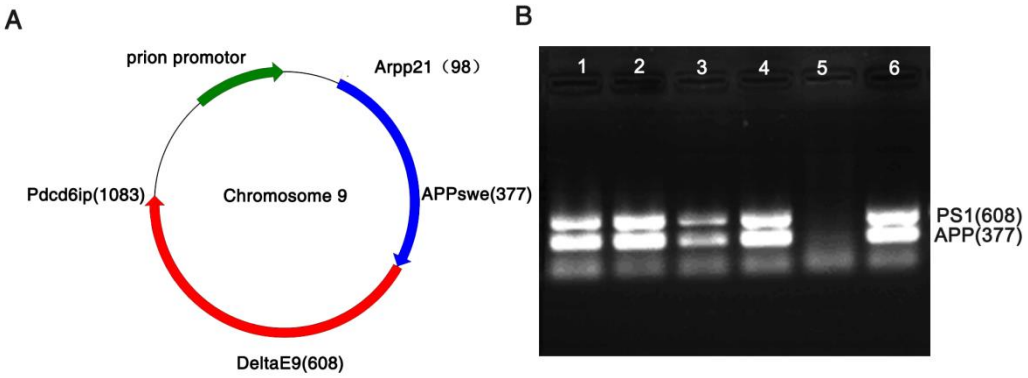

Figure. S3 (A) The APPswe/PSEN1dE9 (APP/PS1) transgenic mouse model harbours two human transgenes: the amyloid beta precursor protein gene containing the Swedish mutation (APPswe); and the presenilin-1 gene contains a deletion of exon 9 (PSEN1dE9). Two transgenes inserted at a single locus in Chromosome 9 between Arpp21 and Pdcd6ip. **(B)** Conventional 1% gel electrophoresis identified that the PCR production in the gene identification. Lanes 1~4 and lanes 6 represent the PCR production of APP/PS1 mouse gene, while lanes 5 represent the PCR production of C57BL6 wild type mouse gene. The gel electrophoresis was performed for 35 min at 100 V constant voltages.

5. Sequential coronal brain sections Thioflavin T staining analysis for APP/PS1 AD models mouse and C57BL6 wild type mouse.

In the experiment, the saline-perfused brains were excised; and the hemisphere was fixed in 4% paraformaldehyde for 48 hours and stored in 15% and 30% sucrose at 4℃. Sequential coronal brain sections (10 μm thick) were obtained and mounted on SuperFrost-Plus glass slides (Thermo Scientific, Rockford, IL, USA). Sections were stained with Thioflavin T, a highly sensitive marker of Aβ deposits. Staining was performed with 0.05% Thioflavin T (Sigma-Aldrich) solution in phosphatebuffered saline for 8 minutes at room temperature. Excitation wavelength was 440nm. The results shown that APP/PS1 AD models mouse group produced significant fluorescence spots which was significantly higher than that in C57/BL6 wild-type (WT) mice.

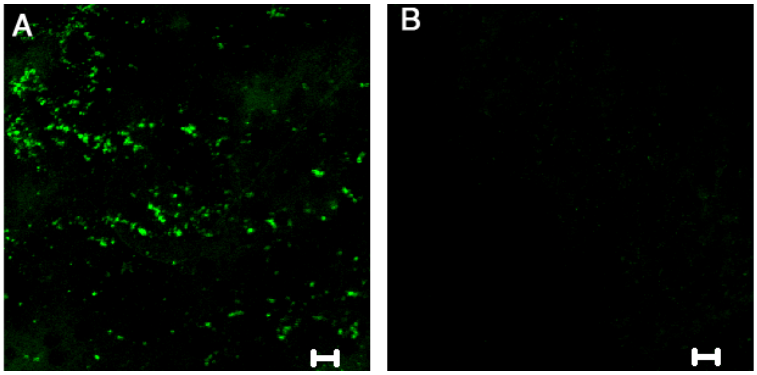

Figure S4. Sequential coronal brain sections Thioflavin T staining analysis for **(A)** APP/PS1 AD models mouse **(B)** C57BL6 wild type mouse Scale bar=20 μm.

6. Collection of cerebrospinal fluid from the cisterna magna in mice

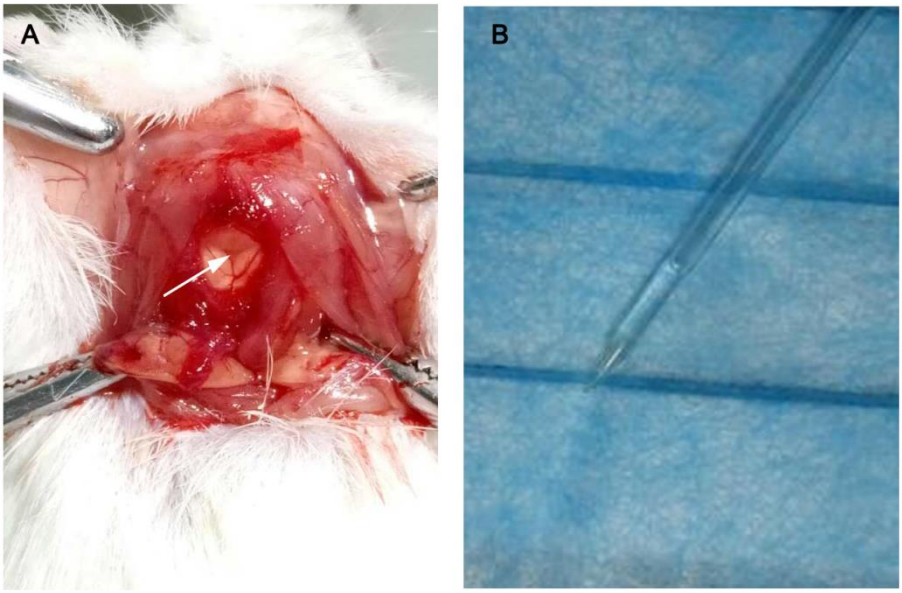

Figure S5. **(A)**The cisterna magna of The APPswe/PSEN1dE9 (APP/PS1) transgenic mice **(B)** Collected cerebrospinal fluid from mice

7. The fabricate process of the molds used for screen printing and wax-screen printing

The pBPE–ECL system contains a DC power supply, a cassette which a PMT and the pBPE assembled into it. The mold used for screen printing and wax-screen printing were produced as the following process: Firstly, a 300 nylon mesh was paved to a hollow frame with a stretcher machine to use as underlayer of the photosensitive material to coat onto. A photo mask is produced by printed the screen printing and wax-screen printing shapes designed by Adobe Illustrator on a silver halide photosensitive film. Subsequently, the as-prepared nylon mesh was exposed to ultraviolet (UV) light. Finally, a desired mold was obtained by wash out the unexposed photosensitive material, dried and properly mended. The wax in the wax-screen printing process can melt into the paper through screen mesh interspace and form the hydrophobic barriers. While areas with a cross-linked photosensitive material blocked the melted wax and yielded the hydrophilic channel in the screen printing process.

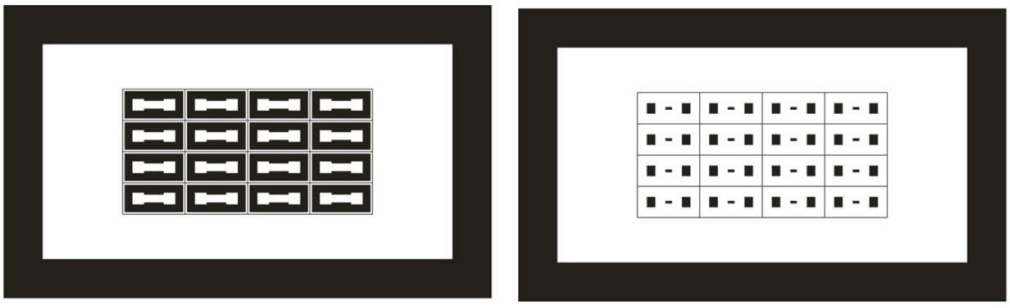

Figure S6. The as-prepared mold used for screen printing and wax-screen printing

8. Thioflavin T assay for the presence of amyloid-beta-oligomer/fibrils when Aβ (1–42) aggregation inhibitor screening.

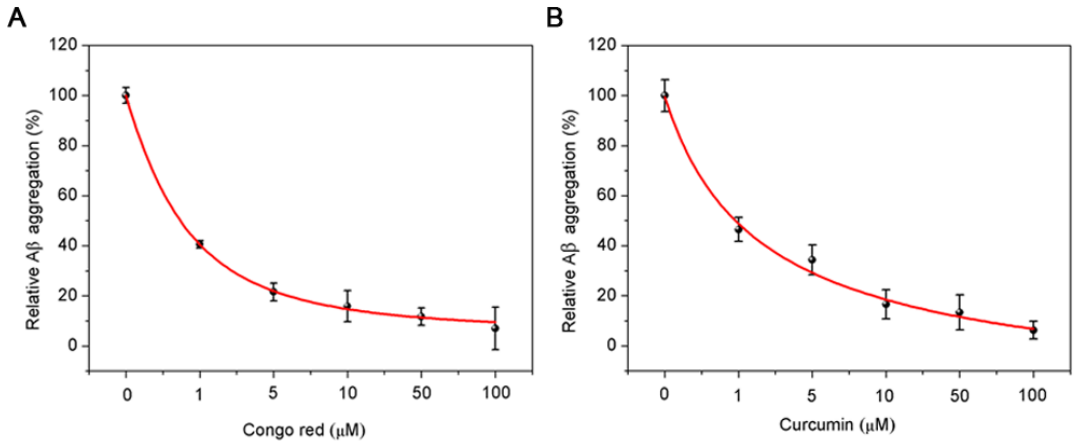

Figure S7.50 nM monomeric Aβ(1-42) incubated with different concentrations of (A)Congo Red, (B)Curcumin from 0 to 100 μM in pH 7.4 PBS buffer for 24 hours at 37 °C.

Table S1 Comparison of the proposed ECL methods for Aβ(1-42) aggregation with other existing method

Analytical method	Instrument	Operation	label free	Cost	references
Magnetic resonance imaging (MRI)	complex	complex	Yes	expensive	[3]
Computerized X-ray tomography (CT)	complex	complex	Yes	expensive	[4]
Fluorescence correlation spectroscopy (FCS)	complex	complex	Yes	expensive	[5]
Fluorescence assay	complex	simple	No	expensive	[6]
Electrochemical assay	complex	complex	No	expensive	[7]
pBPE–ECL	simple	simple	Yes	Low cost	Current work

Reference:

1. Hartshorn RM, Barton JK. Novel dipyridophenazine complexes of ruthenium (II): exploring luminescent reporters of DNA. J Am Chem Soc. 1992; 114: 5919-25.
2. Feng J, Meng C, Xing D. Aβ induces PUMA activation: a new mechanism for

Aβ-mediated neuronal apoptosis. Neurobiol Aging. 2015; 36: 789-800.
3. Erkinjuntti T, Ketonen L, Sulkava R, Sipponen J, Vuorialho M, Iivanainen M. Do white matter changes on MRI and CT differentiate vascular dementia from Alzheimer's disease? J Neurol Neurosurg Psychiatry. 1988; 51: 318.
4. Liu Y, Yang Y, Sun M, Cui M, Fu Y, Lin Y, et al. Highly specific noninvasive photoacoustic and positron emission tomography of brain plaque with functionalized croconium dye labeled by a radiotracer. Chem Sci. 2017; 8: 2710-6.
5. Pitschke M, Prior R, Haupt M, Riesner D. Detection of single amyloid [beta]-protein aggregates in the cerebrospinal fluid of Alzheimer's patients by fluorescence correlation spectroscopy. Nat Med. 1998; 4: 832-4.
6. H Levine. Thioflavine T interaction with synthetic Alzheimer's disease beta-amyloid peptides: detection of amyloid aggregation in solution. Protein Sci. 1993; 2: 404.
7. Zhou Y, Zhang H, Liu L, Li C, Chang Z, Zhu X, et al. Fabrication of an antibody-aptamer sandwich assay for electrochemical evaluation of levels of β-amyloid oligomers. Sci Rep. 2016; 6: 35186.