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In vivo near-infrared fluorescence imaging of amyloid-β plaques with a dicyanoisophorone-based probe

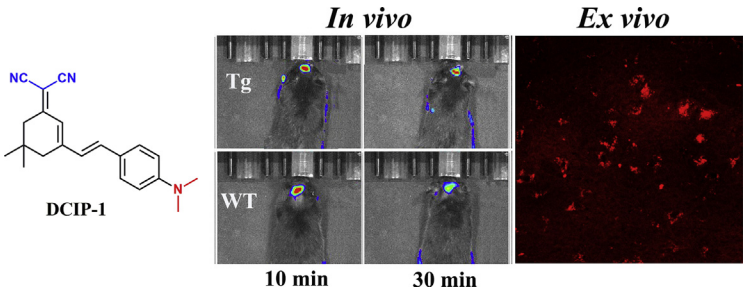
Jia-ying Zhu [a, 1], Lin-fu Zhou [a, 1], Yu-kun Li [a], Shuo-bin Chen [b], Jin-wu Yan [a, *], Lei Zhang [a, **]

[a] School of Bioscience and Bioengineering, South China University of Technology, Guangzhou, 510006, PR China
[b] School of Pharmaceutical Science, Sun Yat-sen University, Guangzhou, 510006, PR China

HIGHLIGHTS

- A two-photon probe (DCIP-1) with NIR emission based on dicyanoisophorone group, for the *in vivo* fluorescence imaging of amyloid-β plaques, was reported.
- The probe showed turn-on fluorescence (13-fold) with a large Stokes shift upon inserting into the hydrophobic pockets of Aβ aggregates.
- The *in vivo* imaging studies indicated that the probe can penetrate the blood–brain barrier efficiently and discriminate APP/PS1 transgenic mice from WT controls.

GRAPHICAL ABSTRACT



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ABSTRACT

A dicyanoisophorone-based probe with two-photon absorption and NIR emission was developed for the *in vivo* fluorescence imaging of amyloid-β plaques, which exhibited high selectivity toward Aβ aggregates over other intracellular proteins. The detection limit was calculated to be as low as 109 nM. *In vivo* imaging studies indicated that the probe could penetrate the blood–brain barrier and label Aβ plaques in the living transgenic mice, and its specific binding to cerebral Aβ plaques was further confirmed by one- and two-photon *ex vivo* fluorescence imaging. All these results featured its promising application prospects for amyloid-β sensing in basic research and biomedical research.

1. Introduction

Alzheimer's disease, (AD), a progressive neurodegenerative brain disorder, is the most common type of dementia [1]. With the acceleration of the global population aging process, AD has become a serious disease that affects the health of the elder people in the world. To date, the etiology is not completely clear, and diverse pathologic factors responsible for the AD have been proposed. Major neuropathological observations from postmortem AD brains include the plaques (β-amyloid, Aβ), and neurofibrillary tangles (hyper-phosphorylated Tau) [2,3]. It is believed that the formation

* Corresponding author.
** Corresponding author.
E-mail addresses: yjw@scut.edu.cn (J.-w. Yan), lzhangce@scut.edu.cn (L. Zhang).
[1] These two authors contributed equally to the work.

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Aβ plaques is the initial event in the pathogenesis of the AD [3]. Currently, there are no clinically effective treatments useful to stop or reverse the progression of AD, which may attribute to unspecified etiology of AD and late-stage administration [4].

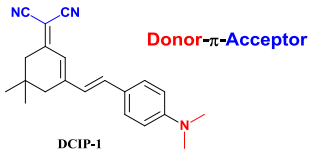
As we stated above, on one hand, there are still many problems of Aβ protein deposition cascade hypothesis to be further studied, and there are also some arguments that the role of the deposition of Aβ in the pathogenesis of the course of AD, and whether the Aβ related target is feasible or not. On the other hand, the failure of many drug candidates has been attributed to their administration at a late stage, when the pathology is too advanced [4]. Early diagnosis of AD, before elder people appear mild cognitive impairment, even preceding clinical symptoms, would greatly benefit the prevention and treatment of AD [5]. As an early pathological feature of AD, Aβ plaques are now accepted as biomarkers for AD diagnosis.

Along with the development of molecular imaging techniques, in the past decades, the development of Aβ plaques imaging probes for early diagnosis of AD has been of a great interest, including positron emission tomography (PET), single photon emission computed tomography (SPECT) [6], and optical imaging [7]. Compared with radioactive imaging, optical imaging has the limitation of depth penetration and clinical translation, but it is a rapid, inexpensive, and usable for frontline screening and basic research [8–10]. More recently, numerous efforts have been devoted to develop fluorescent probes for detecting Aβ plaques deposits [11,12].

Fluorescent probes with both two-photon absorption [13,14] and near-infrared emission (NIR) [15–19] have many advantages including the real-time and 3D visualization, deeper penetration depths, lower tissue auto-fluorescence and self-absorption, reduced photo-bleaching and photo-damage, and high spatial resolution [14,16]. However, up to now, very few two-photon fluorescent probes for early diagnosis of AD have been developed [20–24], and most of them with emission in the visible region, which greatly restricts their applications in biological imaging. To this end, development of probes with two-photon absorption and NIR emission (≥650 nm) for the fluorescence imaging of amyloid-β plaques is highly desired and urgent. Keeping the above in mind, we presented a two-photon probe (DCIP-1) with NIR emission (≥650 nm) based on dicyanoisophorone electron-withdrawing group, for the *in vivo* fluorescence imaging of amyloid-β plaques (Scheme 1).

Dicyanoisophorone-based fluorophores have currently attracted considerable attention because of their excellent properties in dye-sensitized solar cells and organic nonlinear optical crystals due to their typical donor-π-acceptor (D-π-A) structure, two-photon absorption, long emission wavelength in the NIR region, and large Stokes shift [25–28]. However, the usage of dicyanoisophorone-based platform as fluorescence probes have rarely been reported [29–31].

In order to develop a fluorescent probe with two-photon absorption and NIR emission and also good binding affinity to β-amyloid fibrils, *p*-dimethylamino phenyl moiety, an important electron-donating group was introduced to construct our push-pull chromophore (**DCIP-1**) as a candidate fluorescence probe for amyloid-β plaques, which is firstly reported by Lemke in 1974 [32]. To the best of our knowledge, this is the first example of dicyanoisophorone-based probes studied for the detection of β-amyloid fibrils in the brain.



Scheme 1. Chemical structure of **DCIP-1**.

2. Experimental

2.1. Materials and general information

All solvents and reagents (analytical grade) were obtained commercially and used as received unless otherwise mentioned. Column-layer chromatographic silica gel was purchased from Branch of Qingdao Haiyang Chemical Co., Ltd. ^{1}H and ^{13}C NMR spectra were recorded using TMS as the internal standard in $CDCl_3$ with a Bruker BioSpin GmbH spectrometer at 400 MHz and 100 MHz, respectively. High resolution mass spectra (HRMS) were taken on a Thermo-Fisher LTQ Orbitrap XL instrument. Flash column chromatography was performed with silica gel (200–300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd. All chemicals were purchased from commercial sources unless otherwise specified. All the solvents were of analytical reagent grade and were used without further purification.

The synthetic trifluoroacetic acid salt forms of $A\beta_{(1-42)}$ peptides, human serum albumin (HSA) and bovine serum albumin (BSA) were purchased from Qiang Yao Biological Technology Co. (China). Acetylcholinesterase (AChE, E.C. 3.1.1.7, from electric eel), butyrylcholinesterase (BuChE, E.C. 3.1.1.8, from equine serum) were purchased from Sigma–Aldrich. $A\beta_{(1-42)}$ powder was dissolved in 0.1% ammonium solution and stored at −80 °C until usage. The aggregated $A\beta_{(1-42)}$ was prepared by diluting the stock solution of $A\beta_{(1-42)}$ to 200 μM with PBS solution (pH 7.4) and incubating at 37 °C for 7 days.

The UV–vis absorption spectra were recorded on a UV-2450 spectrophotometer (Shimadzu, Japan). Fluorescence measurements were performed on an FL-4500 fluorescence spectrophotometer (Hitachi, Japan) and Fluoro Max-4 (HORIBA, Japan) equipped with quartz cell of 10.0 mm path length. Fluorescence QYs were measured using an ethanol solution of Rhodamine B as a standard.

The two-photon excited fluorescence was measured by using a Ti: sapphire femtosecond oscillator (SpectraPhysics Mai Tai) as the excitation source. The output laser pulses have a tunable central wavelength from 690 nm to 1000 nm with pulse duration of less than 100 fs and a repetition rate of 100 MHz. The laser beam was focused onto the samples using a lens with a focus length of 3.0 cm. The emission was collected at an angle of 90° to the direction of the excitation beam to minimize the scattering. The emission signal was directed into a CCD (Princeton Instruments, Pixis 400B) coupled monochromator (IsoPlane160) with an optical fiber. An 800 nm short pass filter was placed before the spectrometer to minimize the scattering from the excitation light. A PBS solution of **DCIP-1** (5 μM) in the presence of Aβ aggregates (25 μM) was used as the sample for cross-section determination. Rhodamine B was selected as reference (2 μM in ethanol) [33]. Since the input beam parameters are the same for all experiments, we can calculate the two-photon absorption cross-section (δ_s) of **DCIP-1** in the presence of Aβ aggregates using the known two-photon absorption cross-section of rhodamine B (the reference) according to the following equation:

$$\delta_s = \frac{S_s \Phi_r \phi_r C_r}{S_r \Phi_s \phi_s C_s} \delta_r$$

where S_s and S_r are the integrated fluorescence intensities measured at the same power of the excitation beam; Φ are the