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Targeting β -amyloid plaques and oligomers: development of near-IR fluorescence imaging probes

Evidence indicated that shifting treatment to a presymptomatic stage may produce significant benefits to prevent/alleviate the progression of Alzheimer's disease (AD); in particular, early incorporation of noninvasive imaging and biomarker testing will be significantly beneficial for AD drug development. Based on amyloid cascade hypothesis and its revised version, both β -amyloid deposition and soluble oligomeric species could be good diagnostic biomarkers for AD. Near-IR fluorescence (NIRF) imaging, which so far is limited to animal studies, is a promising method for its incomparable advantages such as low cost, high-throughput and easy operation. This review focuses on recent reported NIRF probes that showed excellent binding to plaques and oligomers. We hope that this review will shed light on the future of NIRF probes' discovery.

First draft submitted: 19 September 2016; Accepted for publication: 30 November 2016; Published online: 27 January 2017

Keywords: Alzheimer's disease • amyloid cascade hypothesis • near-IR fluorescence imaging

As the most common cause of dementia, Alzheimer's disease (AD) is posing a serious threat to public health and healthcare systems in both developed and developing countries. AD is a progressive neurodegenerative disorder with cognitive impairment symptoms, including memory loss, language barrier, odd behavior, personality and mood changes. By the age of 65 years old, about 1% of the population has AD, and it will likely increase to 50% in people over the age of 85 years old [1]. Due to the globally aging trends, the number of people suffering from dementia is ever growing [2,3]. Moreover, the financial cost of AD is forecasted to grow rapidly with the aging trends [4]. In the USA, an estimated 17.9 billion hours of care for AD patients and other dementias were provided by 15 million family members and other unpaid caregivers in 2014, which is valued >US\$217 billion [5]. Clearly, AD has become one of the greatest healthcare challenges in the 21st century.

It has been >100 years since the first case report of AD was published by psychiatrist

Alois Alzheimer in 1906 [6,7], AD is still an insurmountable challenge for physicians and medical researchers. There is no efficient therapy for preventing AD. Six drugs (donepezil [Aricept[®], Eisai Co Ltd, Tokyo, Japan]; rivastigmine [Exelon[®], Novartis Pharma Ltd, Basel, Switzerland]; galantamine [Reminyl[®], Johnson & Johnson, NJ, USA]; tacrine [Cognex[®], Pfizer, NY, USA]; memantine [Namenda[®], Merz Pharm, Frankfurt, Germany]; and [Namzaric[®], Adamas Pharmaceuticals, CA, USA]) have been approved by the US FDA [6–8]. These approved drugs only provide symptomatic relief and short-term benefits, but they inadequately alleviate the progression of the disease. In fact, it is widely believed that severe pathological changes are already presenting in the brains of AD patients before the diagnosis becomes clinically apparent [9,10]. Previous research has shown that cerebrospinal fluid levels of $A\beta_{1-42}$ are fully changed 5–10 years before the onset of AD [11]. In humans, abnormal levels of β -amyloids ($A\beta$ s) in brain appear 30

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years before the symptom starts. However, the current positron emission tomography (PET) probes can only detect the abnormal A β deposits around 5 years before the clinical syndrome, which is obviously too late for early diagnosis.

There still is no general agreement about the pathogenesis of AD, but four main hypotheses have been intensively studied: amyloid cascade hypothesis, tau hypothesis, metal ion hypothesis and oxidative stress hypothesis (Figure 1). The amyloid cascade hypothesis states that A β species play key roles in the pathological progression of AD [12–14]; the tau hypothesis underlines that hyperphosphorylation and subsequent mislocalization of tau protein are identified as seminal steps for AD pathogenesis [15]; the metal ion hypothesis emphasizes that the impaired metal homeostasis likely contributes to the pathology, in particular of abnormal high concentrations of Zn, Cu and Fe are the underlying causes of AD. The impaired metal homeostasis also leads to the over-accumulation of A β s [16]; the oxidative stress hypothesis speculates involvement of the production of free radicals, which can influence metabolism and also promote A β aggregation [17]. Though various hypotheses focus on different features of the disease, as shown in Figure 1, these hypotheses all stress that A β species are involved in the physiological symptoms and play a vital role in the pathological process of AD [18].

The studies on the amyloid cascade hypothesis are an ongoing process. Initial hypothesis suggested that A β plaque in the brain was a central role in AD pathology, and this assumption had been the framework for research in the past 20 years. However, all of the A β -centric therapeutics that reached Phase III clinical trials failed (Bapineuzumab, Pfizer; Solanezumab, Eli Lilly and Company, etc.) [22–25], which led to question the roles of A β and amyloid deposition in AD pathology. Many researchers directed their attentions to tau protein, and considered that abnormal hyperphosphorylation of tau protein might be a key component in neurodegenerative processes [26–30]. Meanwhile, a redefinition of the amyloid cascade hypothesis was supported by increasing evidence from the literatures, in which smaller, soluble oligomeric species of A β were considered to contribute to either neuronal death and/or affect synaptic neurotransmission [19,31–34]. In addition, the results of Dominantly Inherited Alzheimer Network indicated AD mutation carriers exhibited high levels of CSF A β_{42} at least 30 years before the estimated symptom onset, but CSF tau levels were increased approximately 15 years before the onset [35], which implicated the aggregation of A β s might act as a critical early trigger in the chain of events [36,37]. Therefore, A β species, especially soluble A β , appear to be good diagnostic bio-

markers for AD, and also be good predictive biomarkers of progression of AD [38].

Research progress in noninvasive molecular imaging probes

Different molecular imaging methods, including MRI, PET, single-photon emission computed tomography (SPECT) and optical imaging, have been used in attempt to detect the progression of AD through visualization of A β s both *in vitro* and *in vivo* [39].

Current modalities of molecular imaging applied in clinical studies

MRI and PET are established imaging techniques for clinical investigations of AD with sensitivity and specificity reaching 85–90% [40]. However, on account of the low sensitivity, blurred signal contrast between A β plaques and surrounding tissues, and low blood–brain barrier (BBB) permeation of contrast agents, MRI is not ideal for molecular imaging of AD [41].

PET imaging is the most promising imaging modality for AD. Three ^{18}F -labeled ligands have been approved for clinical use by the European Medicines Agency and the FDA: Florbetapir (AmyvidTM, Eli Lilly), Florbetaben (NeuraceqTM, Piramal Imaging Limited), and Flutemetamol (GE Healthcare) (Table 1) [42,43]. Owing to the excellent sensitivity of 10^{-10} – 10^{-12} mol/L, limitless tissues' depth penetration and safety for biological material, PET is a high-performance tool used in clinical for AD [44]. Nonetheless, the expensive facilities and risk of radiation exposing, and short half-life of the tracers are inevitable limitations.

SPECT, which is based on single-photon-emitting radioisotopes, is other form of nuclear imaging. The cost of SPECT is significantly less expensive than that of PET scans, partly because the nuclides commonly used in SPECT have a longer half-life (^{123}I , $t_{1/2} = 13.2$ h; $^{99\text{m}}\text{Tc}$, $t_{1/2} = 6.02$ h) and agents are relatively easily obtained than PET tracers. Currently, ^{123}I IMPY has been selected for human studies [45,46]. However, because SPECT collimator absorbs most of the photons, the sensitivity and spatial resolution of SPECT are lower than that of PET imaging [47].

In short, molecular imaging technologies that have been currently applied to image A β s can not completely fulfill the needs of both preclinical and clinical diagnosis.

An alternative plan: near-IR fluorescence imaging

In the last decades, fluorescent imaging has attracted wide attentions due to their broad applications coupled with high sensitivity and specific detection methods [48]. Compared with PET and SPECT imaging modalities,

Figure 1. An overview of four main hypotheses of Alzheimer's disease.

A β : β -amyloid; APP: Amyloid precursor protein; FAD: Familial Alzheimer disease; GSK3 β : Glycogen synthase kinase 3 β ; HIF: Hypoxia-inducible factor; PSEN₁: Presenilin 1; PSEN₂: Presenilin 2; ROS: Reactive oxygen species.

Data taken from [19–21].

near-IR fluorescence (NIRF) imaging has many advantages, such as high sensitive, safe detection without radiation, and moderate cost. In addition, the fluorochromes in the near IR (NIR) window have minimal autofluorescence from cellular or tissue components. Though NIRF imaging is so far limited to animal studies, it is an attractive tool for early AD detection in preclinical studies because of its excellent features.

Moreover, the proper NIRF probes can be easily modified to be PET or SPECT ligands with incorporating radionuclides. These dual-modal probes are favorable for multimodality imaging by merging nuclear imaging with optical imaging, which could be important complementarily for current imaging methods.

Herein, we provide a review of reported NIRF probes that bind various A β s, including insoluble A β s,

Table 1. A brief introduction of positron emission tomography tracers for clinical application.

Characteristic	Flutemetamol	Florbetapir	Florbetaben
Synonyms	GE-067,3'-fluoro-PIB	AV-45	BAY-94–9172, AV-1
Amyloid affinity (K_i , nM)	0.7	2.2	2.4
Plasma metabolites	Polar	Polar and nonpolar	Polar and nonpolar
Typical injected dose (MBq)	185	300	300
Typical imaging time (min)	80–100	50–70	90–130
Effective radiation dose (mSv; μ Sv/MBq)	6.3 (33.8)	5.8 (19.3)	4.4 (14.7)

Data taken from [42].

such as plaque, and soluble A β s, such as oligomers. Within this context, we summarized different types of compounds in a chronological order, including the strategies of structural modification and the optical properties of representative probes. We hope that our review can shed light on the future of NIRF probe discovery.

The progress in near-IR fluorescence imaging

The standards of small compounds as NIRF probes for A β s

A good NIRF probe for A β s in the brain should have the following prerequisites to be fulfilled: high selectivity and affinity for A β s (K_d < 20 nM); high brain penetration and fast washout kinetics from normal brain regions; high *in vivo* metabolic stability; low-affinity binding with bovine serum albumin; suitable emission wavelength between 650 and 900 nm, a large Stokes shift and high quantum yield (QY); fluorescence properties change upon binding to A β s (i.e., fluorescence intensity, fluorescence lifetime, emission wavelength

and QY); relatively easy of synthesis [49]. These requirements set the standards for us to evaluate the reported NIRF probes.

NIRF probes for imaging A β plaques

Based on amyloid cascade hypothesis, extracellular accumulation of A β peptides into plaques plays a vital role in AD onset [12]. Though the correlation between AD severity and the A β accumulation plaques is not linear, *in vivo* detection of A β plaques still is a way to predict the progression of AD and provides an accurate index for A β -related treatments. To date, substantial research has been dedicated to developing NIRF probes binding to A β plaques [50,51].

Fluorescent probes derivated from styryl dyes

As a class of fluorescent probes, styryl dyes are used to serve as mitochondrial labeling agents and membrane voltage-sensitive probes of cellular structure and function [52,53]. Chang and coworkers established a library of combinatorial wide-color range fluorescent styryl dyes of 320 compounds [54,55]. From screening of the

Figure 2. The structural optimization of styryl dyes and the chemical structures of 2C40, 2E10. Compared with 2C40 and 2E10, STB-8 is a new type of styryl dye that its free amino group is removed.

Table 2. Binding and optical properties of the near-IR fluorescence probes based on scaffolds 1–3 reported in the early exploration stage.

No.	Scaffold	Name	MW	$\lambda_{ex}/\lambda_{em}$	Affinity [†]	Φ (%)	Ref.
1	1	2C40	491.65	505/590 [‡]	–	–	[55]
2	1	2E10	381.50	464/503 [‡]	–	–	[55]
3	1	STB-8	383.15	373/407 [§]	3.2 μ M	–	[56]
4	2	AOI987	411.16	650/670 [¶]	0.2 μ M	61/41 [#]	[57]
5	2	ASG236	427.22	665/695 [¶]	–	28/13 [#]	[57]
6	2	ASG237	409.16	658/677 [¶]	–	61/ [#]	[57]
7	2	AMQ987	423.21	–	–	–	[57]
8	3	NIAD-4	334.41	475/612 ^{††}	10 nM	0.008/5 ^{††}	[58]
9	3	NIAD-11	400.47	545/690 ^{††}	–	11 ^{††}	[59]
10	3	NIAD-16	361.48	470/720 ^{††}	–	–	[59]

[†]Binding constant to β -amyloid aggregates.[‡] λ_{ex} and λ_{em} measured in aqueous HCl (pH 1.4) recorded with a Hitachi F-2500 FL spectrophotometer.[§] λ_{max} (nm) upon binding to β -amyloid fibrils determined by use of a Hitachi F-2500 FL spectrophotometer in 100 mg/ml fibril in PBS solution (1 mM EDTA, pH 7.4).[¶] λ_{ex} and λ_{em} measured in mouse serum.[#]QYs of compounds in methanol and mouse serum.^{††} λ_{ex} and λ_{em} measured in combination of 10 μ M aggregated A β_{40} in PBS and fluorophores at 5 μ M concentration.^{††}QYs of compounds were obtained with or without aggregated A β_{40} ; the standard of QY calculated for NIAD-4 was aqueous rhodamine 6G (Φ = 0.76) and standard for NIAD-11/16 was methanol solution of methylene blue (Φ = 0.03). λ_{em} : Maximum emission wavelength; λ_{ex} : Maximum excitation wavelength; MW: Molecular weight; QY: Quantum yield.

library, 2C40 and 2E10 were found to be the best candidates for imaging of the amyloid plaques in brain slices (**1–2**, **Figure 2**). However, the wavelength of excitation/emission of 2C40 and 2E10 are far from the NIR region as shown in **Table 2**. Meanwhile, the presence of the charged moieties limits their penetrability of BBB. Thus, these two candidates are only suitable for staining plaques *in vitro*.

In order to increase the hydrophobicity of the compounds for better permeability of BBB, a series of styryl-based neutral derivatives of 2C40 and 2E10 were reported by same workgroup in 2007 via removing both the pyridinium positive charge and the free amino group [56]. On the basis of structure of 2C40, the charged moiety is replaced by styrylpyridine and styrylbenzene respectively; for 2E10, the unique quin-alidine-styryl structure is retained. They found that STB-8 (**3**, **Figure 2**) could successfully penetrate BBB and specifically binds to A β plaques in *ex vivo* studies. However, these styryl-based neutral derivatives can not be used for *in vivo* imaging because their excitation and emission are still outside of NIR region. In addition, they have low affinities for A β plaques.

NIRF probes derivatived from oxazine dyes

Based on the scaffold of oxazine dye (**Scaffold 2**), Hintersteiner *et al.* reported a series of NIRF probes for senile plaque imaging (**4–7**, **Figure 3**) [57,60]. The compound AOI987 (**4**, **figure 3**) has excellent optical properties, such as maximum absorption/emission in the

NIR domain (λ_{ex} = 650 nm, λ_{em} = 670 nm) and high QY (Φ = 41%), that are favorable for *in vivo* imaging. The long wavelengths of absorption/emission owe to the structure of benzo[1,4]oxazin and the high value of QY benefits from the rigid and planar conformation. Both these two characters are necessary for *in vivo* imaging. It is noteworthy that AIO987 is a charged molecule, which likely has low BBB permeability. Nevertheless, as reported, the maximal plasma concentration of AOI987 was achieved within 15 min in brain after administration, which indicates that significant amount penetration of AOI987 passed through the intact BBB [57]. The fluorescent properties of AOI987 are very attractive and meet most of the requirements for NIRF probe for the detection of A β plaques both *in vitro* and *in vivo*. However, its moderate affinity (K_d = 220 nM) to A β aggregates indicates that further structure optimization is needed, and its small Stokes shift (25 nm) may lower the detection sensitivity [61].

NIRF probes derivatived from 2, 2'-bithiophene

Compared with the complex structures of compounds that were reported in early exploration stage, a series of 2, 2'-bithiophene compounds NIADs (**8–10**, **Figure 4**) possess relatively simple structures and excellent features.

The design philosophy of NIADs roots in Congo Red (CR) and Thioflavin T (Th-T) that have planarized or easily planarizable π system [62–65]. Small planar structures that match with the features in amyloid fibrils sur-

Figure 3. The synthesis scheme of novel oxazine dyes. Compound AOI987 has optimal optical properties for *in vivo* imaging (maximum excitation wavelength = 650 nm, minimum excitation wavelength = 670 nm; quantum yield = 41%).

face are in part responsible for the high-binding selectivity to aggregated amyloids. With a classical push–pull architecture, terminal donor and acceptor moieties are interconnected by a highly polarizable bridge (Figure 4). Various donor and acceptor groups can be used to manipulate the relative energy gaps of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO). Therefore the appropriate groups can lead to a smaller HOMO–LUMO gap, which can lead to the desired long excitation/emission wavelengths [66]. In addition, with the difference from derivatives of styryl and oxazine, NIAD probes bear no charged groups, and have high BBB permeability. To avoid high lipophilicity that will lead to its aggregation and accumulation in blood proteins and red blood cell membranes, NIADs possess some hydrophilic groups, such as thiophene and hydroxyl, to balance BBB permeability and affinity to A β fibrils. More interestingly, the significant increase of fluorescence QY of the bound probe indicates that NIADs are ‘smart’ fluorescence probes.

Swager and coworkers reported the first compound NIAD-4 (**8**, Figure 4) in 2005 [58]. Notably, in the amyloid-binding pocket, the rings of NIAD-4 are rigidly held in a close planar conformation that has better electron conjugation, thereby, fluorescence QY is improved [58,66]. However, the wavelengths of excitation/emission peaks are out of NIR region (λ_{ex} = 475 nm, λ_{em} = 612 nm, Table 2). In order to further red-shift the spectral wavelengths and increase fluorescence QYs, in 2008, the same group introduced compounds NIAD-11 and NIAD-16 (**9–10**, Figure 4) [59]. Compared with the structure of NIAD-4, the ring of NIAD-11 is more rigid and has an extended electron conjugation. The results show that NIAD-11 exhibits 11% fluorescence QY with A β aggregates, which is ten-times higher compared with NIAD-11 in saline alone. It also has an emission peak above 700 nm (Table 2) [59].

The design strategy of NIADs is a promising approach for designing novel scaffolds with consideration of molecular characteristics of both probe and binding pocket. A

Figure 4. Structural model of nonsteroidal anti-inflammatory drug and three typical compounds with sufficient research.

thin hydrophobic groove along the long axis of the filament of fibrils has been identified as the binding site of fluorochromes [67]. It is an essential strategy to utilize the constraints of the fibril-binding pocket to enhance fluorescence smart probes for A β in future research.

NIRF probes derivatived from natural product curcumin

Fluorescent compounds named CRANADs are the first designed NIRF probes derivated from natural product curcumin, which has high binding for plaques. Ran *et al.* reported a series of NIRF probes by modifying the structure of curcumin. Studies demonstrated that curcumin displayed high-affinity binding for A β aggregates ($K_d = 0.20$ nM). However, curcumin is not suitable for *in vivo* imaging because of its short emission wavelength, limited BBB penetration and rapid metabolism [68,69]. On the basis of the structure of curcumin, Ran and coworkers employed 2,2-difluoro-1,3,2-dioxaborines as an acceptor to bridge two N,N'-dimethyl groups on aromatic rings that serve as donor groups, which are beneficial for electron delocalization in the whole structure (Figure 5). In addition, replacing the phenolic hydroxyl groups with some donor groups, such as N,N'-dimethyl, not only enables red-shifted absorption but also improves the lipophilic of curcumin for its reasonable BBB penetration. In addition, the replacement can avoid rapid metabolism, thus increase the *in vivo* stability of curcumin.

CRANAD-2, the first reported curcumin-derived probe, possesses considerable good optical properties (11, Figure 5 & Table 3) [49]. Upon interacting with A β aggregates, CRANAD-2 underwent a range

of changes, such as a 70-fold fluorescence intensity increase, a 90 nm blue shift (from 805 to 715 nm) and a large increase in QY. Meanwhile, this probe also shows a high affinity for A β aggregates ($K_d = 38.0$ nM), a reasonable log p-value (LogP = 3) and high *in vivo* metabolic stability (Table 3) [49]. In brief, CRANAD-2 nearly meets all requirements of an NIRF probe and can be potentially applied for drug screening. In the subsequent research, based on CRANAD-2, Ran *et al.* reported more curcumin analogs with better optical properties. Results suggested that these derivatives were capable of detecting soluble and insoluble A β s both *in vitro* and *in vivo*. We provided more details in the following section (see the 'NIRF probes for binding to oligomers non-specifically' section) of NIRF probes for imaging amyloid- β oligomers, such as CRANAD-3, CRANAD-28 and CRANAD-58.

Modification of natural products could be a promising method to search novel NIRF probes. Especially, researchers should focus on the natural products that bear therapeutic effects on AD and possess large conjugation system. These lead compounds can be easily modified to be dual functional probes that are not only useful for diagnosis but also have the capacity to treat the disease, which could be an active area of research over the next few years.

NIRF probes derivatived from boron dipyrromethene

Since first reported in 1968, boron dipyrromethene (BODIPY) and its derivatives, especially in the last decade, have been proposed for applications in many diverse areas such as molecular sensors/logic gates, pho-

todynamic therapy, dye-sensitized solar cells and light harvesting/energy transfer cassettes [78–80]. In 2009, Suzuki and coworkers reported a new series of long-wavelength fluorescent dyes based on BODIPY with sophisticated optical properties [81], named Keio Fluors. More importantly, Keio Fluor dyes allow the easy and fine tuning of absorption/emission peaks by changing the substituents attached to the BODIPY cores, which is helpful to establish a compound library of BODIPY fluorescent dyes.

Ono *et al.* developed dual-modal (SPECT and NIRF) probes [^{125}I] BODIPY7 (Figure 6) based on the structure of BODIPY in 2011 [82]. Though the dual-modal probes have been applied successfully in the field of cancer imaging [83], such design strategy for imaging A β plaques has not been reported before. In theory, dual functional nuclear/fluorescent imaging probes can provide complementary information to improve diagnosis and management of AD patients, and facilitate the validation of optical imaging by standard nuclear imaging techniques. However, shorter wavelengths of absorption/emission and poor brain uptake indicate that further optimization of the structure is necessary.

To overcome the above limitations of BODIPY7, in 2012, Ono *et al.* introduced dimethylamino styryl group to improve not only wavelengths but also affinity for A β plaques (12, Figure 6) [70]. However, obviously, the excitation and emission wavelengths of BODIPY-based probe-1 (BAP-1) were still shorter than the ideal wavelengths for optical imaging *in vivo* (Table 3). Meanwhile, results also showed that BAP-1 had nonspecific accumulation both in Tg2576 mice and age-matched controls. Based on these findings, Ono *et al.* designed and synthesized novel BAPs for NIRF imaging *in vivo*: BAP2–5 (13–16, scaffold 5–6, Figure 6) [71]. According to the

previous report of Suzuki *et al.*, they replaced a phenyl group with a thiophenyl or furanyl group to extend the wavelengths of excitation and emission. Results demonstrated that BAPs had better optical properties than BAP-1: BAPs exhibited excitation and emission wavelengths of 636–663 nm and 704–723 nm, respectively. In addition, *ex vivo* fluorescent staining of brain sections of Tg2576 mice after the injection of BAP-2 showed selective binding of A β plaques with low nonspecific binding.

Aminophenylethenyl unit is a ‘star’ group, which appears in many probes for A β plaques, such as Th-T, CRANAD-2 and some PET probes. Ono *et al.* investigated the function of aminophenylethenyl in different position of BODIPY core [72]. The first set (17–19, scaffold 7) of the dimethylaminostyryl groups is placed at the meso position of the BODIPY core, and the second (20–21, scaffold 8) is at the 3- and 5-positions of BODIPY core. Among these probes, EUA-5 have the longest excitation/emission wavelength (721 nm/763 nm); EUA-1, EUA-2 and EUA-4 show considerably good staining patterns in the brain sections of Tg2576 mice; especially EUA-4 possesses relatively a good affinity ($K_d = 48.6 \pm 10$ nM).

Rich chemistry and easy derivatization at every position on the core of BODIPY dyes are attractive properties for being new NIRF probes scaffolds. Future derivatization strategy should focus on balancing the polarity of compounds and obtaining desirable emission properties.

NIRF probes derivatived from the classic donor–acceptor architecture

The donor–acceptor architecture bridged by a conjugated π -electron chain is an important class of organic

Figure 5. Design philosophy of CRANAD-2. Introduction of a difluoroboronate group into curcumin to form a red-shifted dye, and employing 2,2-difluoro-1,3,2-dioxaborines as an acceptor and two N,N'-dimethyl groups on aromatic rings as donor groups.

Table 3. Binding and optical properties of the near-IR fluorescence probes from scaffolds 4–13.									
No.	Scaffold	Name	MW	$\lambda_{\text{ex}}/\lambda_{\text{em}}$	Fold increase [†]	Affinity (nM)	LogP	Φ (%)	Ref.
11	4	CRANAD-2	410.26	640/802 [‡]	70	38.0	3	0.6/40 [§]	[49]
12	5	BAP-1	351.17	614/648 [¶]	–	44.1	–	46.8 [¶]	[70]
13	5	BAP-2	357.23	650/708 [¶]	–	55	–	11.4 [¶]	[71]
14	5	BAP-3	341.16	663/705 [¶]	–	149	–	4.5 [¶]	[71]
15	6	BAP-4	433.32	636/704 [¶]	–	27	–	9.3 [¶]	[71]
16	6	BAP-5	417.26	649/723 [¶]	–	18	–	4.3 [¶]	[71]
17	7	EUA-1	365.23	518/654 [¶]	–	320	–	12 [#]	[72]
18	7	EUA-2	483.36	597/667 [¶]	–	230	–	7 ^{††}	[72]
19	7	EUA-3	601.49	677/736 [¶]	–	320	–	1 ^{††}	[72]
20	8	EUA-4	511.41	624/673 [¶]	–	48.6	–	13 ^{††}	[72]
21	8	EUA-5	642.59	721/763 [¶]	–	97	–	3 ^{††}	[72]
22	9	DANIR-2a	197.24	452/487 [†]	1.5	1590	2.19	0 ^{§§}	[73]
23	9	DANIR-2b	223.27	539/577 [†]	2.9	35.8	2.79	0.66 ^{§§}	[73]
24	9	DANIR-2c	249.31	597/665 [†]	12.0	26.9	3.37	4.09 ^{§§}	[73]
25	10	DANIR-3a	247.29	614/538 ^{¶¶}	331	44.8	3.46	4.8/0.1 [§]	[74]
26	10	DANIR-3b	273.33	682/615 ^{¶¶}	716	8.8	4.05	29.9/0.3 [§]	[74]
27	10	DANIR-3c	299.37	783/678 ^{¶¶}	280	1.9	4.56	9.0/0.009 [§]	[74]
28	10	DANIR-3d	325.41	828/716 ^{¶¶}	5	–	5.10	3.7/0 [§]	[74]
29	10	DANIR-3e	351.44	830/- ^{¶¶}	–	–	5.62	0.1/0 [§]	[74]
30	11	DANIR-8a	291.35	616/550 ^{¶¶}	57	–	–	10 ^{††}	[75]
31	11	DANIR-8b	317.38	683/619 ^{¶¶}	321	38.5	3.69	22.3 ^{††}	[75]
32	11	DANIR-8c	343.42	798/678 ^{¶¶}	629	14.5	4.30	7.1 ^{††}	[75]
33	11	DANIR-9a	321.37	613/573 ^{¶¶}	3	–	–	7.5 ^{††}	[75]
34	11	DANIR-9b	347.41	685/642 ^{¶¶}	17	197	3.04	15.7 ^{††}	[75]
35	11	DANIR-9c	373.45	787/673 ^{¶¶}	194	19.9	3.73	9.3 ^{††}	[75]
36	12	Probe 5	227.26	400/532 ^{##}	49.9	1590	2.55	–	[76]
37	12	Probe 6	255.31	440/554 ^{##}	58.9	2300	3.24	–	[76]
38	13	ANCA-6	452.54	410/545 ^{##}	7.7	1400	3.81	–	[77]
39	13	ANCA-14	467.56	385/530 ^{##}	5.0	4600	2.79	–	[77]
40	13	ANCA-15	454.52	380/525 ^{##}	5.1	13800	2.74	–	[77]
41	13	ANCA-16	497.58	430/540 ^{##}	2.9	6700	2.53	–	[77]
42	13	ANCA-17	496.60	410/550 ^{##}	8.4	1600	3.60	–	[77]
43	13	ANCA-18	434.53	–	–	–	–	–	[77]
44	13	ANCA-19	394.46	410/535 ^{##}	6.6	1600	3.14	–	[77]

[†]Fold increase in fluorescence intensity upon binding with A β aggregates.

[‡] λ_{ex} and λ_{em} were measured in PBS.

[§]QYs were measured in PBS/A β 40 aggregates respectively.

[¶]Fluorescence excitation and emission and QY of compounds were determined with 10 μ M of the compounds in CHCl₃.

[#]In reference to rhodamine 6G in H₂O (λ_{exc} = 488 nm, Φ_{f} = 95%).

^{††}In reference to sulforhodamine in ethanol (λ_{ex} = 550 nm, Φ_{f} = 90%).

^{¶¶}In reference to crystal violet in methanol (λ_{ex} = 610 nm, Φ_{f} = 66%).

^{§§}QYs were determined using a calibrated integrating sphere. QYs were determined in dichloromethane.

^{¶¶¶}Determined in PBS (λ_{em1}) and upon binding with A β aggregates (λ_{em2}).

^{##} λ_{ex} and λ_{em} were measured upon binding with A β aggregates.

λ_{em} : Maximum emission wavelength; λ_{ex} : Maximum excitation wavelength; A β : β -amyloid; MW: Molecular weight; QY: Quantum yield.

Figure 6. Near-IR fluorescence probes derivated from boron dipyrromethene. (A) The structure and properties of BODIPY7; **(B)** design strategies of new series of BODIPY derivatives.
BODIPY: Boron dipyrromethene.

nonlinear optical chromophores, and this architecture has been selected as the backbone structure in the NIRF probes of donor-acceptor NIR probes (DANIRs),

which were designed by Cui *et al.* (Figure 7). DANIR probes possess three kinds of scaffold that are likely to be the simplest A β -binding structures. Compared with

those complicated compounds, DANIRs provided some useful information on the interaction between small molecules and A β fibers, and new insight of design strategy of AD probes.

Possessing only one benzene ring is the most obvious characteristic of the scaffold 9 (**22–24**, Figure 7), which significantly reduces the lipophilicity and MW compared with CR and Th-T [73]. While the high affinity to A β aggregates of DANIR 2b–c (**22–24**) indicated that these small and planar structures were likely bound to the clefts on the surface of the A β fibrils. In addition, DANIRs showed high brain penetration *in vivo* and fast washout kinetics from normal brain regions. A direct comparison of the brain kinetics between 2c and the BAP-1 showed that probe 2c ($\text{brain}_{2\text{min}}/\text{brain}_{30\text{min}}$: 5.66) cleared much faster than BAP-1 ($\text{brain}_{2\text{min}}/\text{brain}_{30\text{min}}$: 1.82). Compared with

CRANAD-2 ($K_d = 38.7\text{ nM}$), the probes of scaffold 9 exhibited outstanding affinities for A β aggregates (2b, $K_d = 35.8\text{ nM}$; 2c, $K_d = 26.9\text{ nM}$, Table 3). However, the short π -conjugation system led to short excitation and emission wavelengths, and thus the compounds of scaffold 9 were not suitable for *in vivo* imaging.

In order to shift the emission wavelength to the NIR range and improve the values of QY, Cui and coworkers modified the structure of scaffold 9 by replacing the benzene ring with a naphthalene ring [74,75]. Results showed that the emission wavelengths of probe 3b–e (**25–29**, scaffold 10, Figure 7) red-shifted to the NIR range ($>680\text{ nm}$ in PBS) with large Stokes shifts ($>120\text{ nm}$). In addition, as reported, for aromatic hydrocarbons such as benzene, naphthalene, anthracene and pyrene, a remarkable increase in QY was observed when the size increased from 1 to 4 rings [84]. It was

Figure 7. Near-IR fluorescence probes derived from the classic donor–acceptor architecture. (A) Structural modes of DANIR probes and three reported scaffolds by Cui and coworkers. (B) Representative images of transgenosis (*APPswe/PSEN1*) mouse (top row) and wild-type control mice (bottom row) at different time points before and after intravenous injection of DANIR-3C/8C, which indicates 8c possesses comparatively good brain kinetics [74,75].

reasonable to speculate that the value of QY could be improved by replacing the benzene ring with a naphthalene ring. Meanwhile, multiple torsions along the single bonds of the polyenic chains can remarkably affect the global geometry of the molecules in a solution [85]. These effects also have a noticeable impact on the QYs of the probes and the interaction between probes and A β aggregates. With increasing polyenic chain length, the values of QY had a remarkable decrease from 3b to 3e (Table 3).

By incorporating hydroxyethyl groups into the electron donor moiety of scaffold 10, the compounds of scaffold 11 (30–35, Figure 7A) had an improvement of hydrophilicity [75]. Compared with 3c (brain_{2min}/brain_{60min} = 3.3), 8b and 8c possess comparatively good brain kinetics (8b, brain_{2min}/brain_{60min} = 10.8; 8c brain_{2min}/brain_{60min} = 5.6). However, this kind of improvement did not fit for all cases. Meanwhile, compared with DANIR3b-c, the affinity for A β aggregates dropped to a certain extent (such as 8c, K_d = 14.5 nM; 3c, K_d = 1.9 nM, Table 3). In order to explain this phenomenon, the authors speculated that the larger substitutions at the N position forced the molecules to prefer geometries with greater degrees of nonplanarity that were inappropriate to fit the binding pocket. In addition, *in vivo* NIR fluorescent imaging revealed that 8c could efficiently distinguish between AD transgenic model mice and normal controls (b, Figure 7).

Due to better fitting to the binding channel with lower binding energies, small molecules that possess larger conjugated systems have more potential to bind to A β aggregates and plaques. This strategy has been successfully applied for the design of NIADs. However, the overlong conjugated double-bond bridge might play bad roles in binding of targets and QYs of the probes. Therefore, more attentions should be paid to the balance between length of π -conjugation system and properties of probes to make the molecules fill in the grooves on the fibril surface [74].

NIRF probes based on the other scaffolds

Besides the scaffolds described above, other novel molecular scaffolds can serve as smart A β probes as well. Though poor optical properties of these fluorescent dyes may prevent their applications from *in vivo* imaging, the design strategy could shed light on the future research.

NIRF probes derivatived from chalcone

Studies show that flavones dose-dependently inhibit the formation of A β aggregates, as well as destabilize preformed A β aggregates, indicating that these molecules can directly interact with A β aggregates [86]. In 2007, Ono and coworkers reported a series of chalcone

derivatives as PET/SPECT probes for *in vivo* imaging A β plaques. These chalcone-mimic compounds showed diversified binding affinities for A β aggregate, which were varied from 3 to 105 nM [87]. Inspired by the work of Ono, Jung *et al.* designed a series of chalcone derivatives served as NIRF probes by modifying chalcone with a focus on the aromatic furan ring to improve characteristics, such as A β plaque affinity and fluorescent properties (36–37, scaffold 12, Figure 8). Though these probes were able to specifically stain the A β plaques in a brain section from a transgenic AD model mouse, especially probe 5 exhibited an approximately 50-fold increase in emission intensity after mixing with A β 42 aggregates, low micromolar affinity (K_d = 1.59 μ M) and short excitation/emission wavelength (400 nm/532 nm) prevent their application from *in vivo* imaging (Table 3) [76].

NIRF probes derivatived from amino naphthalenyl-2-cyano-acrylate

In 2011, Chang *et al.* designed a new family of fluorescent probes based on the amino naphthalenyl-2-cyano-acrylate motif (38–44, scaffold 13, Figure 8). Through modifications of the hydrophilic group and nitrogen donor group, they validated donor part of the molecule, which was likely to bind the pocket of the aggregated protein [77,88]. However, in view of the low affinity and selectivity for A β plaque, the properties of this series of fluorescent probes were not so well fit for *in vivo* imaging.

Compounds for imaging A β oligomers

With evidence that the extent of deposited amyloid poorly correlated with cognitive impairment, researchers have shifted their focus to soluble forms of A β . Data indicate that small soluble oligomers of A β inhibit hippocampal long-term potentiation *in vitro* and *in vivo*, and induce synapse degeneration in the brain of AD patients [31,89]. These results support the hypothesis that diffusible oligomers of A β initiate a synaptic dysfunction, which is likely an early event in presymptomatic of AD. Compared with A β plaques, oligomers may be an earlier and more precise biomarker for early AD diagnosis. However, due to heterogeneous and transient nature of A β oligomers, it is remarkably difficult to detect these species specifically. Thus, reports related to development of NIRF probes for binding to A β oligomers are very rare, nonetheless a summary of these rare cases are highly necessary to provide useful information for further probe development.

NIRF probes for binding to oligomers nonspecifically

With the modification of curcumin, Ran and coworkers first designed and synthesized a family of NIRF probes for binding to various A β species [90]. Compared with

the previously reported NIRF probes, these compounds were capable of binding soluble A β species, which were the likely biomarker in the early stage of AD [89,91,92].

A β_{13-20} fragment (HHQKLVFF, as shown in Figure 9) possesses hydrophilic/hydrophobic regions and appropriate structural stereo-hindrance compatibility. As mentioned, CRANAD-2 is the first reported curcumin-based NIRF imaging probe, but it lacks the capability of detecting soluble A β species. Considering the hydrophobic property of CRANAD-2, its symmetric structure does not match with the hydrophobic and hydrophilic properties of A β_{13-20} fragment [90]. In order to enhance interaction with the hydrophobic LVFF segment (A β_{17-20} , Leu17, Val18, Phe19, Phe20), Ran *et al.* attempted to cut the CRANAD-2 in half and obtained the compound CRANAD-54. As expected, CRANAD-54 showed significant fluorescence changes with KLVFF and much stronger fluorescence intensity increase with monomeric A β_{40} than that of CRANAD-2. However, CRANAD-54 is not suitable for *in vivo* imaging, due to its short excitation and emission wavelengths.

Based on the structure of CRANAD-54, CRANAD-58 (47, Figure 10) was designed to have a longer π -conjugation system, in which its pyridyl moiety was conjugated to match the hydrophilic HHQK segment [90]. CRANAD-58 displays excellent fluorescence properties ($\lambda_{\text{ex}} = 630 \text{ nm}$, $\lambda_{\text{em}} = 750 \text{ nm}$) and 91.9- and 113.6-fold fluorescence intensity increases at 672 nm for A β_{40} and A β_{42} monomers, respectively. However, its binding affinity declines to a certain degree compared with CRANAD-2 (A β_{40} : $K_d = 105.8 \text{ nM}$, A β_{42} : $K_d = 45.8 \text{ nM}$). In an attempt to enhance the interaction with A β s, Ran and coworkers designed CRANAD-3 by replacing the phenyl rings of CRANAD-2 with pyridyl to introduce potential hydrogen bonds [93]. As the experimental results suggested, CRANAD-3 (46, Figure 10) exhibited strong binding with A $\beta_{40/42}$ monomers, dimers and oligomers ($K_d = 24 \pm 5.7 \text{ nM}$, $23 \pm 1.6 \text{ nM}$, $16 \pm 6.7 \text{ nM}$ and $27 \pm 15.8 \text{ nM}$, respectively, Table 4) [93]. Both CRANAD-3 and CRANAD-58 can differentiate transgenic and wild-type mice as young as 4-months old, the age that assembles soluble A β in brain. Meanwhile, its fluorescence properties nearly

Figure 8. Near-IR fluorescence probes derived from other scaffolds. (A) Chemical structures of chalcone derivative; **(B)** structures of amino naphthalenyl-2-cyano-acrylate-based β -amyloid-binding probes.

Figure 9. The schematic of binding site: $A\beta_{13-20}$ fragment (the model of $A\beta_{1-42}$ hexamer from x-ray [4NTR]). $A\beta$: β -amyloid.

meet all the requirements for an NIRF probe for the detection of $A\beta$ s both *in vitro* and *in vivo*.

However, these curcumin analogs for detecting soluble and insoluble $A\beta$ s both *in vitro* and *in vivo* always have low QY. To overcome the low QY limitation of these probes, Ran *et al.* replaced the phenyl rings with pyrazoles to increase the brightness [94]. As expected, CRANAD-28 (**45**, Figure 10) displayed high QY in PBS and ethanol. Although CRANAD-28 is an excellent

two-photon imaging probe for $A\beta$ plaques and cerebral amyloid angiopathies (CAAs), its short emission wavelengths (578 nm) prevent it from *in vivo* NIRF imaging.

Ran and coworkers have successfully designed a series of curcumin analogs for detecting soluble and insoluble $A\beta$ s both *in vitro* and *in vivo*. Though these compounds exhibited low selectivity between $A\beta$ subspecies, some have showed the potential for monitoring $A\beta$ species at a presymptomatic stage of AD. In addition, the struc-

Figure 10. A new series of curcumin analogs for binding to β -amyloid oligomers nonspecifically.

Table 4. Binding and optical properties of the near-IR fluorescence probes for binding β -amyloid oligomers from scaffold 12–16.

No.	Scaffold	Name	M.W.	$\lambda_{\text{ex}}/\lambda_{\text{em}}^{\dagger}$	Fold increase [‡]	Affinity [§] (nM)	Affinity [¶] (nM)	Log P	Φ (%)	Ref.
45	14	CRANAD-28	484.30	498/578	–	52.4	68.8 159.7 162.9 85.7	–	32 [#]	[94]
46	15	CRANAD-3	468.35	605/730	12.3 39.5 16.3 16.1	–	24 23 16 27	2.50	–	[93]
47	15	CRANAD-58	439.31	630/750	91.9 113.6	–	105.8 45.8	1.94	–	[90]
48	16	BODIPY-5	481.35	520/550 ^{††}	25 ^{††}	–	–	–	3 ^{††}	[95]
49	16	BODIPY-6	537.45	529/540 ^{††}	35 ^{††}	–	–	–	1 ^{††}	[95]
50	16	Aza-BODIPY	497.34	–/670	16 ^{††}	–	–	–	–	[96]
51	–	BD-Oligo	571.64	580/640 ^{§§}	~15 ^{††}	–	480	–	8.7 ^{§§}	[97]

[†] λ_{ex} and λ_{em} were measured in PBS.[‡]Fold increase in fluorescence intensity upon binding to soluble A β s (A β_{40} monomer/A β_{42} monomer/A β_{42} dimers/A β_{42} oligomers) for CRANAD-3, and fold increase in fluorescence intensity upon binding to A β_{40} monomer and A β_{42} monomer for CRANAD-58.[§]Binding constant to A β aggregates.[¶]Binding constant to soluble A β s (A β_{40} monomer, A β_{42} monomer, dimers and oligomers in sequence for) CRANAD-3/28/58.[#]QYs were measured in PBS.^{††} λ_{ex} and λ_{em} , and QY were measured in 10 mM TRIS-NH₄OH, pH 8.7.^{†††}Fluorescence enhancement in the presence of ordered A β 1–42 oligomers.^{§§} λ_{ex} and λ_{em} , and QY were measured in DMSO. λ_{em} : Maximum emission wavelength; λ_{ex} : Maximum excitation wavelength; A β : β -amyloid; BODIPY: Boron dipyrromethene; QY: Quantum yield.

ture–activity relationship studies of these curcumin analogs are very important to guide further work about designing more selectivity NIRF probes.

Based on previous research work, our group has designed a series of NIRF probes with the curcumin scaffold to selectively detecting soluble A β s. This development is currently underway.

NIRF probes for binding to A β oligomers specificity

Due to A β oligomers' heterogeneous and transient nature, it is considerably difficult to selectively detect these species. As stated before, BODIPY dyes could be used as NIRF probes for amyloid aggregates when they were attached to a pharmacophore [70–72,79–82,95]. And in 2009, Dzyuba and coworkers first described novel triazole-containing BODIPY dyes (**48–50**, scaffold 16, **Figure 11**) to distinguish the unordered and ordered conformations of soluble A β_{1-42} oligomers [95]. The investigation of triazole-containing BODIPY dyes showed that the dyes provided up to an 8-fold and 35-fold fluorescence increase in the presence of the unordered and ordered, β -sheet-rich conformations of soluble A β_{1-42} oligomers, respectively. However, the emission spectra of these compounds are too short to meet the requirements of classic NIRF probes (**Table 4**). In 2013, the same group reported the compound Aza-BODIPY (**50**, **Figure 11**),

which has the wavelengths of excitation/emission maxima above 600 nm, owing to larger conjugated systems [96]. Results showed that the fluorescence intensity of the dye increased 6-fold in the presence of unordered soluble oligomers and 16-fold when the ordered form was added. However, these BODIPY analogs lack capability of discriminating oligomers from fibrils.

In 2015, BoDipy-Oligomer (BD-Oligo) (**51**, **Figure 12A**) was reported by Chang and coworkers as the first oligomer-specific sensor [97]. BD-Oligo is picked out from high-throughput screening of 3500 fluorescent candidates. With BD-Oligo, the highest fluorescence enhancement is observed upon incubation with A β oligomers. During the path of fibril formation, the fluorescent signal of BD-Oligo increased as monomers aggregate into oligomers but decreases as more A β assemble into fibrils, which indicated that this dye had oligomer-sensing ability (**Figure 12B**). Though BD-Oligo successfully penetrates BBB and shows A β oligomers detection capabilities in the brains of the AD transgenic mice model without toxicity, the low binding affinity ($K_d = 0.48 \mu\text{M}$, **Table 4**) and short wavelength of excitation ($\lambda_{\text{ex}} = 530 \text{ nm}$, **Table 4**) definitely hinder the application of BD-Oligo *in vivo* NIRF imaging.

The first attempt of designing of oligomer-specific NIRF probe seems unsuccessful, but this tedious

Figure 11. Chemical structures of triazole-containing boron dipyrromethene dyes and Aza-boron dipyrromethene that were reported by Dzyuba and coworkers.

approach provides a model for the interactions of BODIPY with A β oligomers and a good starting point for further NIRF probe development applicable.

Conclusion

Noninvasive molecular imaging of A β plaque plays a key role in the clinical assessment of patients with suspected AD. Compared with established imaging modalities,

such as positron emission tomography (PET) and MRI, near-IR fluorescence imaging has many advantages, such as low cost, high throughput, and easy operation. In this review, we focused on recently reported NIRF probes that showed excellent binding to plaques and oligomers, and discussed the advantages and disadvantages of these probes as NIRF tracers. Substantial research work has been dedicated to developing NIRF probes binding to A β plaques, but research on probes that can bind to A β oligomers, the most toxic A β s, are very limited. We hope that this review will promote more research to develop imaging probes that can bind to soluble A β s. With such probes, early diagnosis of AD could be feasible.

Future perspective

The exploitation of more NIRF probes that are selectively targeting A β oligomers may be a priority for future research. Studies have suggested that soluble oligomers play key roles in the early phase of AD before the formation of the plaques. Thus, oligomers are likely the earlier and more precise biomarkers for AD. Meanwhile these oligomers-specific probes that are capable of monitoring A β species at the presymptomatic stage of AD are beneficial to the development of anti-A β drugs and pathological studies. To date, BD-Oligo is an 'orphan' probe for binding oligomers, which is obviously inadequate. Except with the help of high-throughput screening from fluorescent libraries, the new structures can be derived from existing scaffolds, such as Th-T and CR, which are capable of binding to oligomers with high affinities but no specificity over insoluble A β s [98]. In addition, the

Figure 12. The properties of compound BD-Oligo. Chemical structure of BD-Oligo, and absorbance maximum, emission maximum and quantum yield of BD-Oligo, measured in DMSO; emission spectra indicate that BD-Oligo shows the different fluorescence enhancement when incubated with monomers, oligomers and fibrils of A β , and which proves that BD-Oligo is oligomer-specific sensor.

A β : β -amyloid.

Data taken from [97].

modification of established NIRF probes is faster and more efficient way to obtain new targeted probes.

Bifunctional NIRF probes may be another active area of research over the next few years. Natural products with potential therapy for AD have provided several new scaffolds for probes in the last decades. Data indicate that mangosteen, curcumin and flavonoids are capable of inhibiting the formation of A β aggregates and destabilizing preformed A β aggregates, which suggest that the NIRF probes derived from these natural products may possess therapeutic potential. As mentioned above, curcumin analog CRANAD-28 is not only useful for two-photon imaging but also has the capacity to attenuate crosslinking of A β 42.

Converting the NIRF probes to PET tracers may be a promising strategy to promote the application of NIRF probes in a clinical setting. Incorporation of isotopes into NIRF molecules will facilitate the use of the probe for PET imaging. In theory, dual functional nuclear/fluorescent imaging probes can provide complementary information, and facilitate the valida-

tion of optical imaging by standard nuclear imaging techniques.

Last, rapid advances in fluorescent imaging technology are likely to provide a huge leap for NIRF probe development. Although NIRF imaging has been only used in brain imaging on small animals, and it is still difficult for imaging of A β s inside the human brain. Fluorescence molecular tomography, which is potentially capable of detecting signals at depths of 7–14 cm, has the potential capacity to translate NIRF imaging into clinical applications in future [99,100].

Financial & competing interests disclosure

The authors of this review were supported by Outstanding Scientific and Technological Innovation Team of Jiangsu Province of China in 2015. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in this manuscript.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Background

- Currently, no treatment is available to stop/slow the disease's progression, and most promising strategy for treating Alzheimer's disease (AD) might be to the early intervention is based on early diagnosis at the early stage.
- Based on amyloid cascade hypothesis and its modification version, both β -amyloid (A β) deposition and soluble oligomeric species may be good diagnostic biomarkers for AD.

The development of molecular imaging technologies

- Noninvasive molecular imaging of A β plaque plays a key role in the clinical assessment of patients with suspected AD, and MRI and positron emission tomography are established imaging techniques for diagnosis of AD. However, the molecular imaging technologies that have been currently applied to imaging A β s can not completely fulfill the needs of clinical diagnosis.
- Compared with positron emission tomography and single-photon emission computed tomography imaging modalities, near-IR fluorescence imaging has many advantages, and it may have the potential for clinical use.
- Substantial research work has been dedicated to developing near-IR fluorescence probes binding to A β plaques, and CRANAD-Xs and boron dipyrromethenes are the most representative probes among the reported structures.
- NIRF probes for selectively binding to A β oligomers are very limited, and more investigations for developing oligomer-specific probes are highly desirable.

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