REVIEW

Near-infrared fluorescent probes for imaging of amyloid plaques in Alzheimer's disease

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Received 16 November 2014; received in revised form 8 December 2014; accepted 11 December 2014

**KEY WORDS**
Alzheimer's disease; Blood-brain barrier; Fluorescence probe; Near-infrared fluorescence; Optical imaging; Amyloid-β plaques

**Abstract**  One of the early pathological hallmarks of Alzheimer's disease (AD) is the deposition of amyloid-β (Aβ) plaques in the brain. There has been a tremendous interest in the development of Aβ plaques imaging probes for early diagnosis of AD in the past decades. Optical imaging, particularly near-infrared fluorescence (NIRF) imaging, has emerged as a safe, low cost, real-time, and widely available technique, providing an attractive approach for *in vivo* detection of Aβ plaques among many different imaging techniques. In this review, we provide a brief overview of the state-of-the-art development of NIRF Aβ probes and their *in vitro* and *in vivo* applications with special focus on design strategies and optical, binding, and brain-kinetic properties.

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**Abbreviations:** Aβ, amyloid-β; Ach, acetylcholine; AD, Alzheimer’s disease; APP, amyloid peptide precursor; BAP, BODIPY-based Ab imaging probe; BBB, blood-brain barrier; Cy, cyanine dyes; ICG, indocyanine green dyes; MRI, magnetic resonance imaging; NIR, near-infrared; NIRF, near-infrared fluorescence; PET, positron emission tomography; ROS, reactive oxygen species; SPECT, single photon emission computed tomography

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

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http://dx.doi.org/10.1016/j.apsb.2014.12.006
1. Introduction

Alzheimer’s disease (AD) is the most common type of dementia among older people, affecting approximately 35 million people worldwide, with 5 million new cases every year1. Clinical symptoms of AD include progressive cognitive decline, irreversible memory loss, disorientation, language impairment, and emotional instability2. The dilemma places significant mental, social and economic burdens on patients, families, and communities3. Unfortunately, there are no currently effective treatments available to reverse or stop the progress of this devastating disease, primarily due to difficulties in identification of disease etiology4-7.

Several pathological hallmarks of this disease have been identified, namely, the deposition of amyloid-β (Aβ) plaques and neurofibrillary tangles, elevated reactive oxygen species (ROS), imbalanced metal ion (e.g., Cu, Fe, and Zn) homeostasis, and decreased brain acetylcholine (Ach) levels. Three major theories have been proposed to explain these pathological hallmarks: amyloid cascade8-11, oxidative stress12,13, and the metal ion hypotheses14. The amyloid cascade hypothesis is currently the prevailing one. It is believed that the formation of Aβ plaques arises from aggregation of peptides Aβ1–42 and Aβ1–40, and is the initial event in the pathogenesis of the AD. Aβ1–40 and Aβ1–42 are degradation products of amyloid peptide precursor (APP), generated from cleavage by β- and γ-secretases. These cleaved peptides have a tendency to aggregate into different Aβ species such as dimers, oligomers, fibrils, and plaques, and may also interact with metal ions and produce ROS, with subsequent neuronal atrophy and death4. Regardless of the nature of the intertwined pathological pathways induced by Aβ aggregates, it is widely accepted that the formation of Aβ plaques precedes the clinical symptoms of AD. Therefore, they are excellent diagnostic and predictive biomarkers for the early detection of AD5,6,8. Moreover, the current clinical diagnosis of AD is primarily based upon family and patient’s medical history as well as neurological and neuropsychological observations. Thus, the diagnosis is often inaccurate. Confirmative AD diagnosis can only be made through postmortem histopathological examination of the brain Aβ plaques. There exists a great and urgent need to develop non-invasive and accurate probes for Aβ plaques to improve the current diagnosis of AD. Such probes will also be useful in monitoring disease progression and effectiveness of new AD treatments.

In the past decade, significant advances have been made in the design of molecular probes for specific labeling, detection, imaging of Aβ plaques both in vitro and in vivo. A number of different imaging modalities and approaches have been applied, including magnetic resonance imaging (MRI)10-11, positron emission tomography (PET)15-19, single photon emission computed tomography (SPECT)20-24, and optical imaging techniques25. MRI based approaches suffer from low resolution since the size of Aβ plaques typically range from 20 to 60 μm, while only large plaques (>50 μm) are detectable26. Compared with MRI, radio-labeled PET and SPECT probes are more sensitive methods. Many probes, such as [11C]PIB27-28, [11C]SB-130010, [11C]AZD218427, [18F]FPIC28, [11C]AZD469418, [18F]FDNDP19,20, [18F]AV-1, [201Tl]AV-1, and [123I]IMPY20, have been advanced in clinical trials. PET-based probes are more promising in terms of their translational applications. Three PET probes [18F]FPIC (Vizamyl™), [18F]AV-45 (Amyvid™), and [18F]AV-1 (Neuraceq™) were recently approved by the FDA. The clinical diagnostic utility of these PET imaging agents is limited: they cannot be used to confirmatively diagnose AD, only to support other diagnostic criteria27. Furthermore, the use of PET is limited by high cost and narrow availability, since generation of these probes needs specialized facilities that have a cyclotron for the generation of short-lived radionuclides (e.g., [11C], t1/2 = 20 min and [18F], t1/2 = 110 min) and an automated synthetic unit to produce radio-labelled probes. Compared with PET, SPECT has broader availability and lower cost as a routine diagnosis method due to the use of easily-generated radionuclides with longer half-lives (e.g., [123I], t1/2 = 60.1 d, [18F], t1/2 = 13.2 h, and [99mTc], t1/2 = 6.0 h). Current SPECT-based probes either have relatively high background for the radioiodinated probes due to high lipophilicity and nonspecific binding or have poor blood-brain barrier (BBB) penetration in the case of [99mTc]-labeled SPECT probes. Only one SPECT probe, [123I]IMPY, has advanced in clinical trials. In general, radionuclide-based imaging modalities PET and SPECT are limited by high cost, radiation exposure, and single signal readout.

In contrast to the radionuclide-based imaging techniques, optical imaging modalities are rather inexpensive; important merits include nonradioactive, real-time imaging with the option of multi-targets tracing in vitro and in vivo, wide availability, and high-resolution imaging depending on the specific technique used43-47. For in vivo applications, in order to avoid absorption and background autofluorescence and scattering of biological molecules, probe fluorescence emission wavelength in the near-infrared (NIR) region between 650 and 900 nm is advantageous so that one can achieve an optimal penetration depth and high sensitivity. Therefore, NIR fluorescence imaging has emerged as an attractive alternative to PET/SPECT and MRI techniques, and may provide a solution for the early diagnosis of AD. In the following sections, we discuss challenges and design strategies associated with the development of NIRF Aβ probes for in vivo applications, followed by a list of current reported probes and their optical, binding and brain-kinetic properties, as well as in vitro and/or in vivo studies (Table 1).

2. Challenges in developing NIRF Aβ probes

A number of NIR fluorophores such as cyanine dyes (Cy7), indocyanine green dyes (ICG), alexa fluor dyes (660–790 nm), and SRfluor dyes have been developed and employed for in vivo applications; many of them are commercially available47. Nonetheless, these known NIR fluorophores have large molecular weight and intrinsic charges. They are likely to be unsuitable for labeling Aβ plaques in the brain because of their limited BBB permeability. In order to use a fluorophore for in vivo brain Aβ imaging, several criteria are required48-50: (1) a suitable wavelength of excitation and emission within the NIR range (650–900 nm); (2) high BBB permeability (logP values between 2 and 3.5, or clogP<5.0 are considered optimal11,51); (3) high affinity for specific labeling of the Aβ plaques in the brain with low nonspecific binding to other proteins; (4) rapid clearance of the unbound dye from the brain; and (5) significant changes in the probe fluorescence properties upon binding to Aβ plaques. It is challenging to design probes meeting all the requirements. First, many NIR fluorophores are often highly-conjugated structures with molecular weight over 600 Da, while a small and compact scaffold with molecular weight less than 600 Da is required for NIRF Aβ probes. Secondly, the probes should have balanced lipophilicity to ensure good BBB penetration and avoid nonspecific binding. Moreover, high affinity and specific fluoroscence property changes require fluorophore scaffolds which are challenging to design. Ultimately, it is difficult to predict in vivo properties of a designed NIRF probe before synthesis and testing.
| Name      | M. W.  | $K_d$ (nmol/L) | $K_i$ (nmol/L) | $\log P^a$ | $\epsilon$  | $\lambda_{ex}$ (nm) | $\lambda_{em}$ (nm) (free) | $\lambda_{em}$ (nm) (binding) | $\Phi$ (%) | Intensity increment (fold) | References |
|-----------|--------|----------------|----------------|-----------|------------|---------------------|-----------------------------|-------------------------------|-----------|-----------------------------|------------|
| NIAD-4    | 334.41 | 10             | 4.52           | 35700     | 4.5        | 492                 | 603                         | 24                          | 0.008     | 400                         | 48         |
| NIAD-11   | 400.47 | -              | 4.77           | -         | -          | 545                 | 690                         | -                           | 11        | -                           | 53         |
| NIAD-16   | 361.48 | -              | 5.33           | 470       | -          | 720                 | 720                         | -                           | -         | -                           | 53         |
| A0I-987   | 324.35 | 220            | 1.66           | 61960     | 0.29 (1.8) | 627                 | 644                         | 650                         | 38.5      | 54                          | 49         |
| THK-265   | 350.37 | -              | 0.29 (1.8)     | 61960     | 0.29 (1.8) | 627                 | 644                         | 650                         | 38.5      | 54                          | 49         |
| CRANAD-2  | 410.26 | -              | 5.56 (3.0)     | 640       | 805        | 715                 | 700                         | 640                         | 0.6       | 70                          | 50         |
| CRANAD-3  | 420.55 | -              | 5.13           | -         | 700        | 640                 | 700                         | 640                         | -         | -                           | 55         |
| CRANAD-58 | 439.31 | -              | 5.67 (1.94)    | -         | -          | -630                | 750                         | -                           | -         | -                           | 56         |
| CRANAD-17 | 456.25 | -              | 5.11           | -         | -          | -600                | 700                         | -                           | -         | -                           | 56         |
| BODIPY7   | 530.18 | 108            | 9.08 (2.2)     | 606       | 613        | -                   | 36                          | -                           | -         | -                           | 57         |
| BAP-1     | 351.20 | -              | 5.47           | 610       | 613        | -                   | 36                          | -                           | -         | -                           | 57         |
| BAP-2     | 357.23 | -              | 5.24           | 651       | 708        | -                   | 11.4                        | -                           | -         | -                           | 59         |
| BAP-3     | 341.10 | -              | 4.65           | 665       | 705        | -                   | 4.5                         | -                           | -         | -                           | 59         |
| BAP-4     | 433.32 | -              | 26.7           | 623       | 704        | -                   | 9.3                         | -                           | -         | -                           | 59         |
| BAP-5     | 417.26 | -              | 6.75           | 639       | 723        | -                   | 4.3                         | -                           | -         | -                           | 59         |
| DANIR 2c  | 249.31 | 37             | 2.81           | 5119      | 597       | 665                 | 625                         | 4.09                        | -         | 12                          | 60         |
| MAAD-3    | 327.37 | 354            | 4.28           | 674       | 674        | -                   | 4.7                         | -                           | -         | 15                          | 61         |
| DMDAD-3   | 323.43 | 645            | 4.36           | 694       | 694        | -                   | 2.68                        | -                           | -         | 7                          | 61         |
| MCAAD-3   | 282.34 | 106            | -              | 885       | 654        | -                   | 1.23                        | -                           | -         | 26                          | 61         |
| DMMAD-3   | 315.36 | 652            | -              | 687       | 642        | -                   | 0.10                        | -                           | -         | 8                          | 61         |

\[a\]Calculated using ChemBioDraw 12.0 software.
\[b\]Experimental value.
\[c\]Quantum yield after binding to $A\beta$ fibrils/aggregates.
\[d\]Quantum yield before binding.
\[e\]For $A\beta_{40}$ fibrils/aggregates.
\[f\]For $A\beta_{42}$ fibrils/aggregates.
\[g\]For $A\beta_{40}$ fibrils/aggregates.
3. NIRF Aβ probes

Compared with a vast number of PET/SPECT probes for Aβ plaques in the literature, there have been relatively few reports on the development of NIRF probes. This is no doubt due to the many challenges discussed in the previous section. In this section, we cover six different kinds of NIRF Aβ imaging probes in chronological order according to their publication dates. Their structures are shown in Fig. 1. Most of them are highly conjugated molecules containing the donor-acceptor or donor-acceptor-donor architecture. Their structural features are characterized by an electron-donating group linked to an electron-withdrawing group by an highly polarized conjugated π-electron chain, leading to nonlinear optical properties, such as fluorescence intensity change in response to environmental change. Such architecture is particularly useful in the design of NIRF Aβ probes, since the recognition process often involves surrounding environmental changes of the probe. Moreover, physical, optical, and binding properties can be rationally tailored by varying the conjugated π-chain, the donor, and the acceptor groups.

3.1. NIAD-4 and its analogs (NIAD-4, NIAD-11, and NIAD-16)

In 2005, Swager et al. rationally designed the fluorescent probe NIAD-4 based upon the donor-π-bridge-acceptor architecture, which utilizes a highly polarizable bishistephen to link the donor group (p-hydroxyphenyl) and the acceptor group (dicyanomethlyene). NIAD-4 showed excellent binding affinity ($K_i = 10$ nmol/L) for Aβ aggregates and a dramatic enhancement of the fluorescence intensity (about 400-fold) when mixing with Aβ aggregates. The increased fluorescence intensity was caused by reduced free rotation of aromatic rings in the excited state. In vivo two-photon imaging experiments in transgenic mice demonstrated NIAD-4 readily crossed the BBB after intravenous injection and labeled Aβ plaques in brain and cerebrovascular amyloid angiopathy on blood vessels (Fig. 2). In a separate study, NIAD-4 showed a broader pH tolerance than Thioflavin T in monitoring amyloid formation process, especially under acidic condition. NIAD-4 presented the first example of the rational design of Aβ specific probes to achieve emission wavelength over 600 nm upon binding with Aβ aggregates. However, the maximum emission wavelength of the probe NIAD-4 is only 603 nm, not in the optimal range of...
650–900 nm. Studies with this probe required the use of the invasive cranial window technique to perform in vivo fluorescence imaging, which is impractical for clinical diagnosis\textsuperscript{48}. To achieve longer emissions, the same group subsequently developed a series of NIAD-4 analogs, including NIAD-11 and NIAD-16\textsuperscript{53,65}. NIAD-16 could distinguish vascular and nonvascular A\textsubscript{β} plaques from background signal through fluorescence lifetime imaging\textsuperscript{53}.

### 3.2. AOI-987

In the same year, Gremlich et al.\textsuperscript{49} at Novartis designed and synthesized longer wavelength benzophenoxazine dyes, as NIRF probes for A\textsubscript{β} plaques. They could monitor the progression of A\textsubscript{β} deposition in APP23 transgenic mice, an animal model of AD. Among them, AOI-987 offered the best in vivo results. Although AOI-987 is a charged molecule and has only moderate affinity for A\textsubscript{β} aggregates ($K_d=220$ nmol/L), the probe was able to penetrate the BBB and specifically label A\textsubscript{β} plaques from background signal through fluorescence lifetime imaging\textsuperscript{53}.

#### 3.3. Curcumin derivatives (CRANAD-2, CRANAD-3, CRANAD-58, CRANAD-17, etc.)

To improve detection signals with noticeable fluorescence intensity alternation and a large Stokes shift, Moore and colleagues\textsuperscript{50} designed and synthesized a novel class of NIRF probes derived from curcumin structure. In the structure, a difluorobororate moiety and two p-dimethylamino phenyl groups were integrated into the curcumin scaffold to form a donor-acceptor-donor architecture, significantly increasing the emission wavelength to 805 nm. In this series, CRANAD-2 showed high affinity.
(\(K_d=38.7\) nM) and drastic fluorescence changes (70-fold fluorescence intensity increase, 90 nm hypochromic shift) upon binding to \(A\beta\) aggregates. Since bulky analogs CRANAD-6 and CRANAD-23 (structures not shown) did not show significant fluorescence change, it was assumed that the binding site of CRANAD-2 is stereo-hindered, likely to be the hydrophobic site containing the core fragment (KLVFF)\(^{30}\). \(In vitro\) staining experiments revealed that CRANAD-2 was capable of selective detection of \(A\beta\) plaques in a brain section from a 12-month old APP-PS1 transgenic mouse (Fig. 4a). Notably, CRANAD-2 could differentiate Tg2576 mice from wild type at an early time point (30 min) after injection by comparison of fluorescence intensities in \(in vivo\) studies (Fig. 4b). CRANAD-2 meets most of the requirements as a NIRF \(A\beta\) probe \(in vitro\) and \(in vivo\). Compared with PIB, a well-studied PET probe for \(A\beta\) plaques, CRANAD-2 has lower brain entrance/clearance rates.\(^{50}\) In another study reported by the same group, CRANAD-2 was used in combination with CRANAD-5 as a non-conjugated FRET pair for differentiating \(A\beta\) monomers from higher aggregated \(A\beta\) species including dimers.\(^{36}\)

By replacing of benzene with pyridine and dimethylamino with diethylamino groups in CRANAD-2, the same group reported another probe, CRANAD-3, in 2012. CRANAD-3 displayed significant fluorescence property changes upon binding to \(A\beta\) aggregates. What was different from CRANAD-2 was that it also interacted with soluble \(A\beta\) monomers and dimers, and displayed fluorescence signal change. \(In vivo\) imaging studies using transgenic APP/PS1 mice exhibited that CRANAD-3 could differentiate 2 month-old APP/PS1 mice from wild type mice. Furthermore, notably, CRANAD-3 could separate specific and nonspecific binding fluorescence signal of the probe in spectral unmixing imaging studies.\(^{35}\)

More recently, new CRANAD-2 analogs were designed and synthesized aiming for NIRF imaging of soluble and insoluble \(A\beta\) species and inhibition of copper-ion induced \(A\beta\) aggregation.\(^{38}\)

Among them, CRANAD-58 showed different fluorescence response towards soluble and insoluble \(A\beta\) species. Significant fluorescence intensity increase (91.9-fold for \(A\beta_{40}\), 113.6-fold for \(A\beta_{42}\) and high affinity (\(K_d=105.8\) nM for \(A\beta_{40}\), 45.8 nM/L for \(A\beta_{42}\) for \(A\beta\) monomers was observed. Similar fluorescence intensity changes were also seen with \(A\beta\) dimers, but to a lesser extent. \(In vivo\) experiments revealed that CRANAD-58 was able to detect soluble \(A\beta\) species in transgenic APP/PS1 mice at the age of 4 months. Another analog, CRANAD-17, containing two copper coordinating imidazoles, could compete and interfere with copper induced crosslinking of \(A\beta\). CRANAD-17 induced 68% more of \(A\beta\) monomers as compared with non-treated samples in \(in vitro\) anti-crosslinking studies, indicating potential usage as theranostic agent.\(^{56}\)

### 3.4. BODIPY based probes (BODIPY7, BAP-1 to BAP-5)

The high quantum yield, biocompatibility, and high lipophilicity of the BODIPY fluorophore render it attractive for the design of NIRF probes. In this regard, inspired by NIAD-4, Ono and his team reported their first BODIPY-derived fluorophore/SPECT dual probe BODIPY7. It contains a conjugated thiophene-phenyl chain similar to NIAD-4. BODIPY7 has modest affinity (\(K_d=108\) nM) for \(A\beta\) aggregates and is able to detect \(A\beta\) plaques in \(in vitro\) staining of brain slides from an animal model of AD. The low BBB permeability, the short absorption/emission wavelength (606/613 nm), and the narrow Stokes shift restricts \(in vivo\) imaging applications.\(^{57}\) Two years later, the same group developed a new BODIPY-based \(A\beta\) imaging probe (BAP-1) with a “privileged” \(p\)-dimethylamino phenyl group to improve \(in vivo\) properties. BAP-1 showed high affinity (\(K_d=44.1\) nM) and a significant fluorescence intensity increase upon binding to \(A\beta\) aggregates. It also has exceptional brain kinetic profiles and demonstrated specific labeling of \(A\beta\) plaques based on \(in vitro\) and \(ex vivo\) staining studies (Fig. 5). Nonetheless, it failed in \(in vivo\) imaging...
Inspired by the reported studies, Okamura and colleagues screened a collection of simple conjugated compounds, which led to the new NIRF Aβ probe, THK-265. This probe possess an emission wavelength around 650 nm combined with favorable physical properties such as high quantum yield, high molar absorption coefficients, and moderate logP value. A high binding affinity (Kₐ = 97 nmol/L) and 6-fold fluorescence intensity increase upon mixing with Aβ₁₋₄₂ fibrils were observed, albeit no significant change in emission wavelength. THK-265 was further evaluated for its in vivo imaging performance using AβPP transgenic mice. Such studies demonstrated that this probe crossed the BBB and selectively labeled Aβ plaques in the brain following intravenous administration. Compared with AOI-987 under the same experimental conditions, THK-265 showed an earlier differentiation time and better imaging contrast between transgenic mice and wild type and higher sensitivity for plaque detection in vivo. Most importantly, the fluorescence intensity of THK-265 correlated well with Aβ plaque burden, indicating its potential in monitoring progression of the Aβ aggregation in AD. Subsequently, Schmidt and Pahnke demonstrated that indeed THK-265 could be used for direct monitoring and evaluating different cerebral Aβ aggregation levels in different stage of AD progression in an animal AD model.

3.6. DANIR 2c and its analogs (MAAD-3, DMDAD-3, MCAAD-3, and DMMAD-3)

Earlier experiences from previous studies of the BODIPY series of NIRF Aβ probes led to the design of improved ones. By replacement of the undesired BODIPY with simple conjugated systems, Ono and Cui designed and synthesized a new series of structurally simplified Aβ fluorophores DANIRs. The p-dimethylamino phenyl moiety was used as the donor group on one side of polymethine, with dicyanomethylene as the acceptor on the other end. This design significantly reduced molecular weights of the probes and improved brain kinetics. The best probe in the series, DANIR 2c, was able to efficiently cross the BBB and label Aβ plaques with a fast washout rate of the unbound probe. This probe differentiated between Tg mice and wild type as early as 30 min after in vivo administration of the probe (Fig. 6b), a significantly shorter time as compared with that from AOI-987. DANIR 2c also has favorable optical properties (emission wavelength at 665 nm), a 12-fold increase in intensity upon mixing with Aβ aggregates, and excellent affinity for Aβ aggregates (Kₐ = 37 nmol/L). DANIR 2c meets most of the requirements as an optimal probe for in vivo imaging of Aβ plaques. One shortcoming is the blue-shift of this probe’s emission wavelength to only 625 nm (shorter than 650 nm) following binding to Aβ plaques.

Encouraged by the excellent performance of DANIR 2c, Cui et al. then turned to its analogs for better NIRF probes with longer emission wavelength. Four analogs MAAD-3, DMDAD-3,
MCAAD-3, and DMMAD-3 were synthesized, by differing in the donor group. These analogs showed extended emission wavelength and significant reduced binding affinity to Aβ aggregates compared with DANIR 2c. Docking simulations suggested that these probes likely bind to the same binding site as IMPY, which was a thin hydrophobic groove parallel to the fibrillar axis formed by VAL 18 and PHE 20. Increased bulkiness of the acceptor group within these analogs caused a reduced binding efficiency. One analog, MCAAD-3, which had the highest affinity of 106 nM/L among the series, was selected for in vivo imaging studies. Similar to DANIR 2c, MCAAD-3 exhibited good brain kinetics, including rapid initial uptake and fast egress. Furthermore, the latter could differentiate Tg from wild type mice at the earliest point of 30 min after dosing as well. Overall, MCAAD-3 may be a better NIRF probe for in vivo imaging than DANIR 2c, as the emission wavelength was at 654 nm when bound to Aβ aggregates.

4. Conclusions

This review highlights the development of NIRF imaging probes for in vivo detection of Aβ plaques in the past ten years. Six structurally distinct NIRF fluorophore scaffolds of Aβ probes have been developed. Most of these probes present high affinity for Aβ in vitro. As for in vivo imaging applications, pharmacokinetics-related properties are as vital as optical properties. Such pharmacokinetics considerations include in vivo stability, low-affinity for serum albumin, and reasonable lipophilicity, all of which are required for fast initial uptake into brain and fast washout to reduce nonspecific binding. In addition, significant fluorescence signal changes upon binding to Aβ are required. Other considerations regarding optical properties include absorption/excitation/emission in NIR region, high molar absorption coefficient, high quantum yield, and longer Stokes shifts. The currently reported probes fell short on one or several aspects of these required properties. NIAD-4 and DANIR 2c display a short emission wavelength, but charged AOI-987 is difficult to penetrate the BBB. CRANAD-2 has a slow egress, and BODIPYs suffer from a short Stokes shift and nonspecific binding in the scalp. Marginal fluorescent signal changes are observed with THK-265 upon binding to Aβ aggregates. MCAAD-3 has a relatively lower binding affinity than that of NIAD-4 or DANIR 2c.

Despite these concerns, development of these probes demonstrate the feasibility of NIRF imaging using Aβ specific fluorescence probes as a low-cost, convenient, readily available, and real-time approach for early diagnosis of AD in mice AD models. We believe that, in the future, the NIRF Aβ probes with enhanced pharmacokinetics and optical properties will be great benefits to human health through improved early AD diagnosis, evaluation of disease progression and clinical therapeutic outcomes.

Acknowledgments

The work was supported by the Fundamental Research Funds for the Central Universities and East China University of Science and Technology (start-up funds, Wei Wang), and the China 111 Project (Grant B07023, Wei Wang) is gratefully acknowledged.

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