Far Red Amyloid Binding Fluorophores for Alzheimer's Disease Drug Development

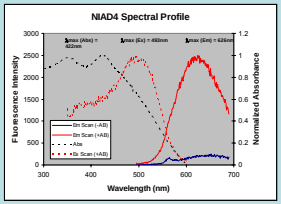
Dongyuan Piao[1], Brian J. Bacskai[2] , Randall H. Morse[1], Paul MacLean[1], Michelle Young[1], Lawrence F. Hancock[1]

1. Nomadics, Inc. 215 First Street, Cambridge, MA 02142 Email: dpiao@nomadics.com
2. Alzheimer's Disease Research Unit, Mass. General Hospital, Charlestown, MA 02129

Abstract

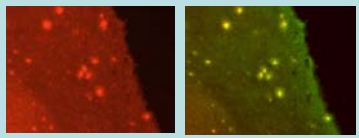
Alzheimer's disease (AD), the most common type of dementia, is a progressive degenerative disorder effecting tens of millions of people worldwide. Confirmation of AD still relies on post-mortem examination of amyloid senile plaque in brain tissue. Amyloid beta (A-beta) aggregates are an important indicator of AD. Hence, analytical methods to accurately assess aggregate formation are useful for monitoring the efficacy of AD therapeutic drugs. We present the development and evaluation of NIAD fluorophores, a unique class of proprietary small molecule fluorophores that can cross the blood-brain barrier and specifically bind to A-beta aggregates in the APP transgenic mouse model. These compounds are first in a series of small fluorophores that cross the blood-brain barrier and upon binding to amyloid-β plaques emits strong red or near-infrared fluorescence when excited. These non-antibody based molecular imaging agents promise to facilitate preclinical Alzheimer's disease drug development by enabling researchers to monitor Amyloid-beta aggregate formation *in vivo*.

NIAD4 Response to *in vitro* Aβ(1-40) fibrils



The absorption maximum red shift and the emission intensity increase upon binding **NIAD4** to Amyloid-beta aggregates. The Amyloid-beta fibrils was formed *in vitro* in PBS from synthetic A-beta (1-40) peptide. The Absorption and emission of NIAD4 was measured in the absence (blue lines) and presence (red lines) of A-beta fibrils in PBS.

NIAD-4 vs. Thioflavin-S Histochemical Staining

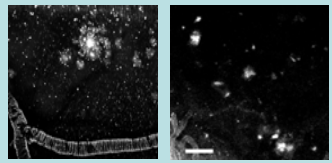


NIAD 4 staining of transgenic mouse brain tissue

Colormerge of NIAD 4 and Thioflavin-S stained brain tissue

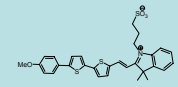
A tissue section from a 10 month-old transgenic mouse brain (APPswe:PS2dE9) was incubated in **NIAD4** at ~10 uM for 30 min. The section was then rinsed in PBS (3X), and imaged under epifluorescence with an Olympus microscope coupled to a color CCD camera. The section was then incubated in 0.001% thioflavin S for 20 min, rinsed 3X in PBS, and then re-imaged using the microscope. The senile plaques stained with NIAD4 were co-labeled with thioflavin S, demonstrating the specificity of the probe for amyloid pathology in the brain. The image is approximately 600 x 800 microns.

NIAD-4 In vivo Imaging of amyloid plaque in living mice

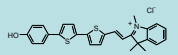


NIAD4 labels pathology *in vivo* after systemic administration in a Tg2576 mouse imaged with multiphoton microscopy. In vivo image was taken about 30 min after iv injection (3mg/kg) into an 8-mo-old APPswe:PS1dE9 transgenic mouse using multiphoton microscopy through a surgically implanted cranial window. 2-photon excitation at 800nm from a Spectra Physics MaiTai laser was used on a Biorad 1024MP microscope. Fluorescence emission was collected at 500-550nm with a photomultiplier tube.

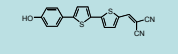
NIAD chemical structures and optical properties



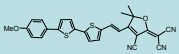
NIAD1



NIAD



NIAD4

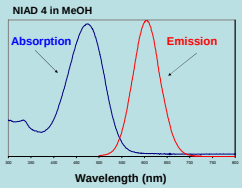


NIAD14A

	Absorption Max (nm)	Emission Max (nm)	Mol. Wt.
NIAD1A	560	734	563.75
NIAD3	551	750	478.07
NIAD4	475	625	334.41
NIAD14A	556	765	481.58

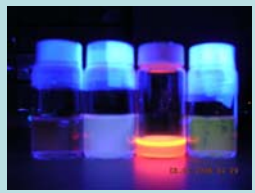
Advantages of NIAD Amyloid-binding fluorophores

- ✓ Large Stokes shift (>150 nm) that minimizes autofluorescence interference
- ✓ Reduced fluorescence background interference for higher signal to noise ratio
- ✓ Excite or emit at greater than 650 nm, avoiding the absorption and light scattering of hemoglobin
- ✓ Greater array of fluorophores provides greater staining flexibility and multiplexing capability
- ✓ NIAD fluorophores do not emit ionizing radiation—no radioisotope safety issues



NIAD fluorophores under sun light

NIAD1A NIAD 3 NIAD 4 NIAD 14A



NIAD fluorophores under ultraviolet light

Conclusion

NIAD4 is a proprietary small molecule fluorophore that readily crosses the blood-brain barrier in transgenic mouse and binds to amyloid-β plaques. Upon excitation, it emits strong far red fluorescence which can be detected through a cranial windows on the mouse skull. Paired with fluorescence in vivo imaging techniques, NIAD4 has utility for visualizing amyloid-beta aggregates in live animal. In addition to NIAD 4, several other amyloid-binding proprietary NIAD fluorophores with longer absorption and near-infrared (NIR) fluorescence have been synthesized, and are currently being evaluated. We will continue the synthesis and development of NIAD fluorophores as enabling molecular agents for optically monitoring amyloid-beta aggregates to facilitate the preclinical drug development for AD.

References

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