January 2024

Investigation of Alzheimer's Amyloid-β Protein Aggregation With a New Fluorescent Dye.

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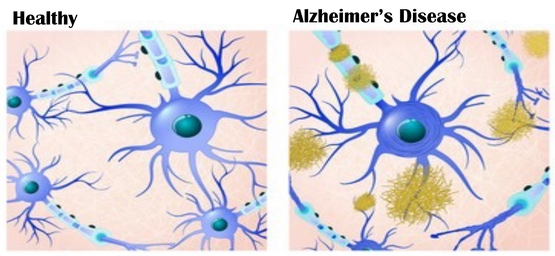
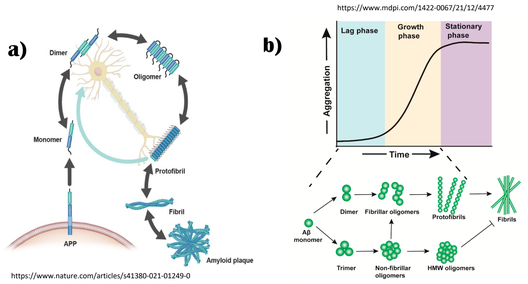
Introduction

- Alzheimer's Disease (AD) is the most common form of dementia characterized by the impairment of at least two brain functions such as memory loss and judgement. AD is a progressive illness that can last as many as 20 years. AD is largely considered to be caused by the formation of extracellular amyloid plaques and intracellular neurofibrillary tangles. A better understanding of the structure and function of these plaques may lead to clearer understanding of the disease. To analyze amyloid plaques, aggregation assays are often used. During these assays we begin with monomer and place the sample in biological conditions to see how long it takes for the monomer to aggregate. A key component of these assays is a tracer molecule such as Thioflavin T. The tracer molecule allows us to determine when the monomer has begun to aggregate. I have been analyzing a new fluorescent dye to determine if it may be a better fit for amyloid beta aggregation assays.



https://www.123rf.com/photo_74087894_neurons-in-the-healthy-brain-and-alzheimers-disease-with-amyloid-plaques-in-comparison-medical.html

Figure 1 Morphology of AD: Alzheimer's Disease is characterized by extracellular Amyloid plaques (shown in yellow) that form between neurons and disrupt their ability to communicate and send signals to each other.



https://www.mdpi.com/1422-0067/21/12/4477

https://www.nature.com/articles/s41380-021-01249-0

Figure 2 Pathogenic Pathway: a) Amyloid plaques are formed from the aggregation of Amyloid beta 42 monomer. b) Amyloid beta aggregation curve.

- Amyloid beta 42 is one product that can be cleaved from Amyloid Precursor Protein or APP which is a naturally occurring protein and its presence is not indicative of AD.
- In the pathogenic pathway APP is cleaved by beta secretase and gamma secretase to form Aβ- 42 which forms insoluble aggregates that eventually form plaques.

Objectives

- To determine if AT540 is a possible replacement for Tht in Amyloid beta aggregation assays.
- To compare results from the fluorometer and the plate reader instruments.

Methods

Amyloid Beta Aggregation Assay: Solutions were prepared in Amyloid beta specific Eppendorf tubes and placed on ice until the first measurement was completed. To measure each sample an aliquot was placed into a quartz cuvette and measured in the fluorometer or placed in a 96-well plate. After the sample was completed it was placed back into Eppendorf tube for incubation. The samples in the plates were left in the plate and transferred to incubator. Each samples were gently shaken at 37°C. A reading was taken in either the fluorometer or plate reader every 30 minutes for 3-4 hours.

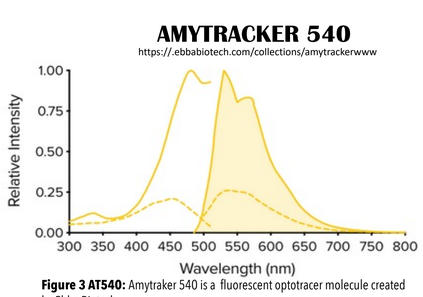


Figure 3 AT540: Amytraker 540 is a fluorescent optotracer molecule created by Ebba Biotech.

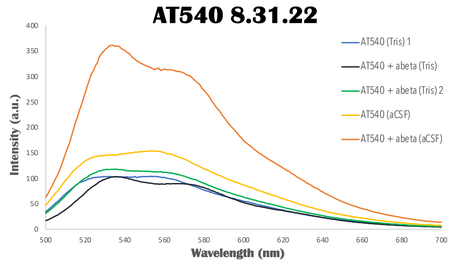


Figure 4: AT540 shows higher florescence in aCSF buffer pH 7.6 over Tris buffer pH 8.0. Amyloid beta prep #516 (aggregates). AT540 max excitation 480nm, max emission 540nm.

- AT540 is a yellow fluorescent optotracer molecule used for amyloid beta aggregation assays and is used to track when the Aβ monomer has started to aggregate.
- AT540 has an excitation peak at **480 nm** and an emission peak at **540 nm**.
- A large salt concentration may be required for optimal performance as shown by the higher intensity readings after switching to aCSF buffer in figure 4.

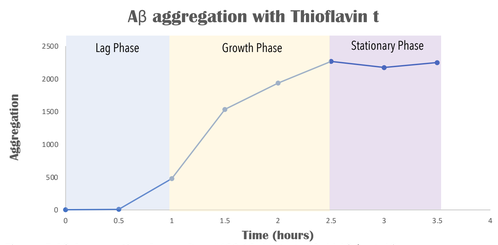


Figure 5 Aβ Aggregation Assay: Prep #628 19uM stock, 10uM Aβ final. Aβ monomer was incubated under gentle rotary shaking conditions at 37 °C in aCSF pH 7.6 with 100 mM NaCl and 20 uM Tht. Total volume 180 uL per tube. Integration taken every 0.5 hours for 4 hours. `

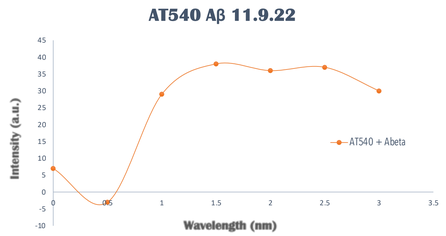


Figure 6 Aβ Aggregation Assay: Prep # 627, Final dilution AT5401500X. Aβ monomer was incubated under gentle rotary shaking conditions at 37°C in aCSF pH 7.6. Value taken at 535 nm for each time point.

- Tht is another tracer molecule that is widely considered a gold standard for amyloid aggregation with an excitation peak at 450 nm and an emission peak at 482 nm.
- Both Tht and AT540 will only show fluorescence if there are fibrils present in a sample allowing us to measure if a sample has aggregated into oligomers or fibrils. AT540 does show a growth curve, however it is not as clear as the one shown for Tht.

Conclusion

- AT540 does not show better results during amyloid beta aggregation assays than Tht. This molecule seems to be dependent on a large salt concentration which makes using the tracer for amyloid assays difficult since they need to stay at biological conditions.
- AT540 does not show high enough difference between the control and the sample to be preferred over Tht.

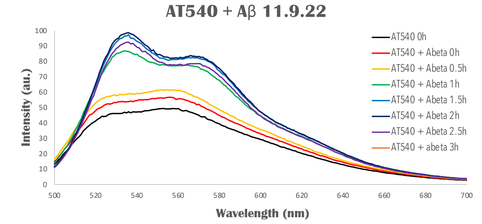


Figure 7 Aβ aggregation assay over time: Prep #627 Aβ, 1500x diln AT540. Aβ monomer was Incubated under gentle rotary shaking conditions at 37°C in aCSF pH 7.6. Values taken every 0.5 h for 3h.

- AT540 shows two peaks one at ~540nm and a smaller peak at ~560nm.
- AT540 does not show a large difference between the control sample (AT540 only) and the sample with Amyloid beta monomers.

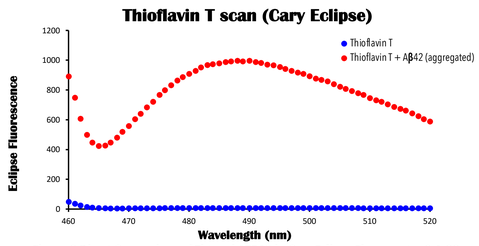


Figure 8 Direct Comparison of Tht and AT540 in Cary Eclipse Fluorometer 3.1.23.

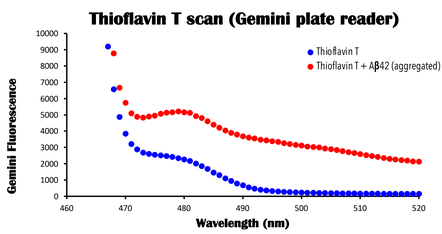


Figure 9 Direct Comparison of Tht and AT540 in Gemini Plate Reader 3.1.23.

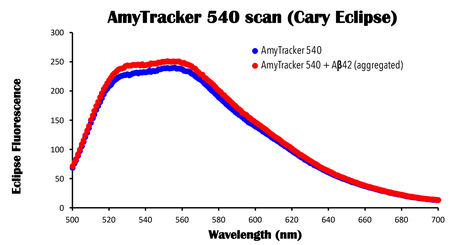


Figure 10 Direct Comparison of Tht and AT540 in Cary Eclipse Fluorometer 3.8.23

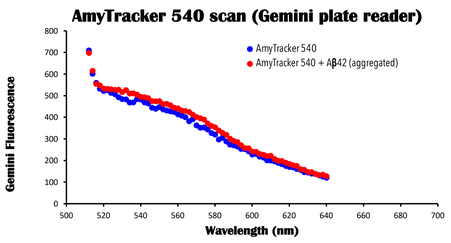


Figure 11 Direct Comparison of Tht and AT540 in Gemini Plate Reader 3.8.23.

- During a comparative analysis of AT540 in a fluorometer and plate reader not much difference was seen for either the Eclipse or the Gemini plate reader and comes no where close to the results shown for Tht.