Mechanistic Insight into the Modulation of Amyloid-β Aggregation by Polymer-Functionalized Nanoparticles

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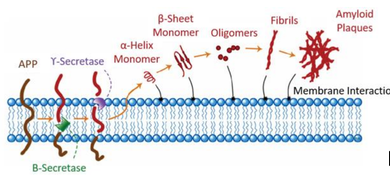
Introduction

Alzheimer's disease (AD), a progressive disorder involving the degeneration of neurons in the brain, affects 6.2 million people over the age of 65 in the United States[1]. Despite being the seventh leading cause of death in the world, there is no known cure for the deleterious disease. AD is characterized by the accumulation of amyloid-β (Aβ) plaques around neurons in the brain. Aβ stems from the transmembrane protein Amyloid Precursor Protein (APP), which is cleaved to form monomeric Aβ. Aβ's intrinsically disordered structure leads to the formation of amyloid fibrils which aggregate to form plaques. These plaques surrounding the brain cells lead to neuronal cell death, directly related to symptoms of the disease such as memory loss, aggression, and depression. As a result, inhibition of Aβ aggregation is one therapeutic approach.

Among cutting-edge research within the field, nanoparticles are under investigation to determine their efficacy in slowing the progression of Aβ aggregation. Gold nanoparticle's (AuNPs) biological inertness, versatile size and surface properties, and ability to modulate aggregation at substoichiometric ratios of AuNP to Aβ 1:10,000 make them a great therapeutic strategy for AD[2]. AuNPs have inhibited aggregation through evidence of lag phase extension and plateau reduction, though further studies are needed to determine the exact mechanism behind this inhibition. This experimental study stemming from Moss Lab graduate student work, aims to answer the question: does AuNP diameter and/or the degree of polymerization of the PAA-tethered molecule modulate aggregation? We hypothesize the AuNPs are modulating solvent conditions, as the low stoichiometric ratio of AuNPs to Aβ limits the feasibility of inhibition by binding mechanisms.

Amyloid-β Aggregation



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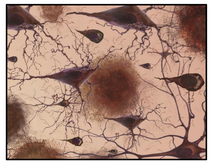


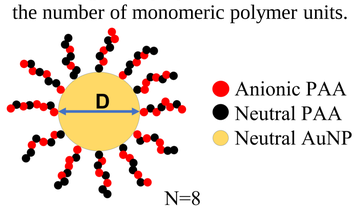
Aβ is an intrinsically disordered protein originating from APP. Fibrillization of Aβ is one characteristic of AD. The interstitial fluid of a healthy human brain contains Aβ monomer, but aggregation can occur due to the protein's disordered nature and internal body conditions. The Aβ monomer forms neurotoxic oligomers followed by fibrillar aggregates, which deposit as amyloid plaques accumulating around the neurons of the brain. $Aβ_{1-40}$ was selected for this study because of its increased β-sheet structure stability during fibrillization compared to $Aβ_{1-42}$, which forms more stable oligomers.

Polymer-Functionalized AuNPs

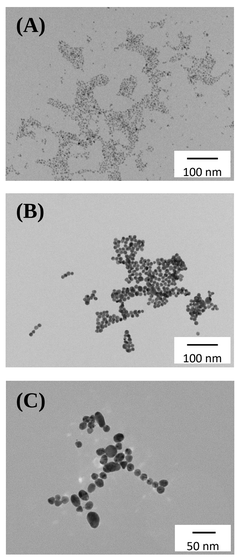
AuNP Configurations

	Degree of Polymerization (N)	AuNP Diameter (nm)
GMY #1	35.8	10
GMY #2	112	10
GMY #3	35.8	20
GMY #4	112	20
GMY #5	11	15
GMY #6	138	15
GMY #7	70	6.75
GMY #8	70	23
GMY #9	70	15

Table 1. Gold nanoparticles were synthesized with various core diameters (D) and degrees of polymerization (N) of poly (acrylic acid) (PAA) as modeled below. Degree of polymerization describes the number of monomeric polymer units.



AuNP Characterization



Spherical shape of AuNPs was visualized on JEOL JEM-1400 Plus TEM at 120 kV. (A) AuNP 7, 40k Mag (B) AuNP 5, 40k Mag (C) AuNP 8, 60k Mag.

Results

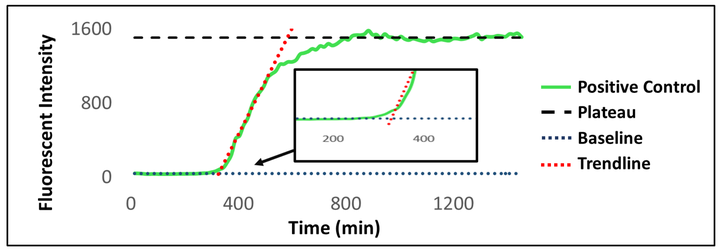


Figure 1. Lag time is defined by the intersection point of the tangent line of the steepest slope of the growth phase and the baseline of the first two hours of data. Plateau is defined by the average of the last seven hours of data.

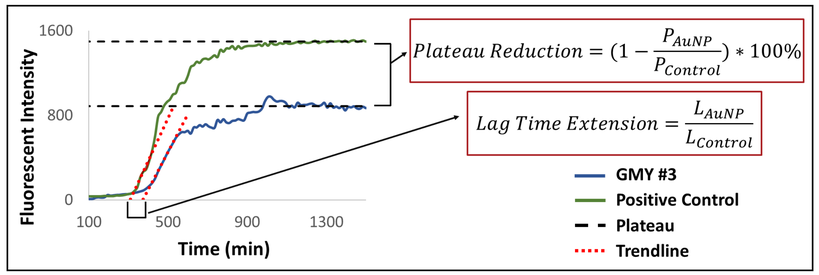


Figure 2. Lag time extension and plateau reduction provide quantitative values to describe the inhibition of Aβ aggregation.

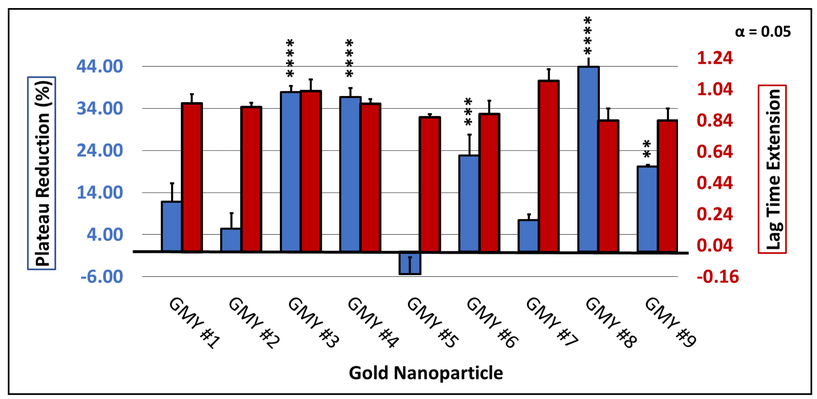


Figure 3. Lag time extension is represented by values larger than 1.00. Positive plateau reduction value represents plateau reduction. GMY #5 has negative plateau reduction, thus the AuNP increased the plateau. Error bars represent SEM, N=3. ** p=0.0015; *** p=0.0004; **** p<0.0001.

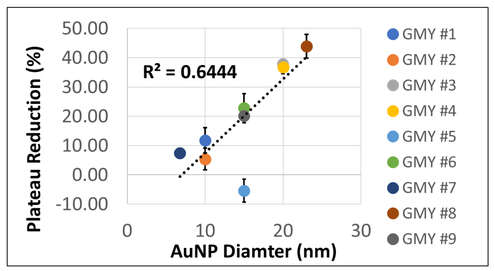
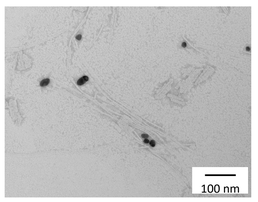


Figure 4. AuNP core diameter correlates with plateau reduction. Error bars represent SEM, N=3. GMY #5 has the shortest polymer chain length describing its outlier position. All additional analysis between independent and dependent variables demonstrated no correlation.

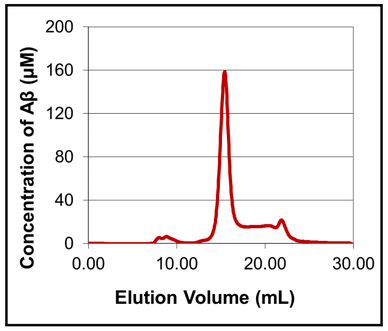


GMY #8 and Aβ fibrils visualized on JEOL JEM-1400 Plus TEM at 120 kV and 40k magnification.

Methods and Materials

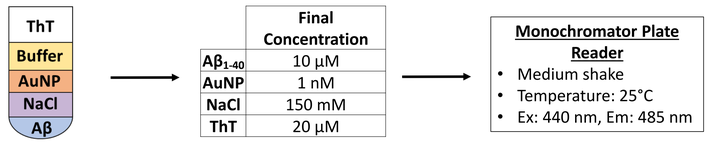
Aβ Monomer Purification

- Pure $Aβ_{1-40}$ monomer (95%) was isolated via Fast Protein Liquid Chromatography in 40 mM Tris-HCl (pH 8.0).
- Aβ monomer elutes at 14-17 mL. These fractions are collected for experimentation.
- Ultraviolet-visible spectrometer measured sample absorbance at 278 nm for $Aβ_{1-40}$ concentration determination.



Aβ Monomer Aggregation Assay with Thioflavin T

- Samples containing purified $Aβ_{1-40}$, NaCl, AuNP, Thioflavin T (ThT), and Tris-HCl (pH 8.0) were prepared and loaded into a 96 well plate.
  - ThT fluorophore binds to β-sheets of aggregated Aβ, providing a direct correlation to amyloid aggregate concentration.
  - Reaction accelerated by NaCl and agitation.
- Fluorescent intensity values determined by monochromator-based plate reader.



Conclusion

Increasingly larger diameters of PAA-functionalized AuNPs correlate to significant Aβ plateau reduction. AuNP core diameter has no significant correlation with lag time extension.

PAA-length has no significant correlation to Aβ plateau reduction or lag time extension.

Low stoichiometry and large core diameter are consistent with the modulation of solvent conditions as a mechanism of inhibition.

Future Work

Further investigate the efficacy of AuNPs with core diameters between 15-23 nm at various PAA-lengths and negative charges.

Investigate the efficacy of AuNPs functionalized with different negatively charged polymers.

References

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[2] N. Van der Munnik, "Understanding Early Amyloid-β Aggregation to Engineer Polyacid-Functionalized Nanoparticles as an Inhibitor Platform', Ph.D. dissertation, University of South Carolina, Columbia, SC, 2018.

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