Applying Gold Nanoparticles to Denature Proteins Characteristic of Alzheimer's Disease

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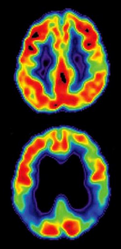
Alzheimer's in America

- **5.8 million Americans are afflicted with Alzheimer's**
- **1 in 10 adults over the age of 65 has Alzheimer's**
- **An American is diagnosed with Alzheimer's every 65 seconds**
- **By 2050, the number of Alzheimer's patients in America is expected to reach 13.8 million**
- **Alzheimer's care costs Americans $227 billion per year**
- **There is currently no cure or long-term treatment for Alzheimer's** [1]



[Graphic 1] Normal brain (above) and Alzheimer's brain (below) [2].

Introduction

With the number of Alzheimer's patients in America and around the globe climbing, putting an immense burden on the health care system, progress towards a cure is paramount. Drug developers have invested millions of dollars in the search for small-molecule therapeutics only to discover that many of those drug candidates which appear promising *in vitro* falter in clinical trials, demanding innovative alternatives.

Beta-secretase (BACE), an enzyme responsible for cleaving neuronal proteins to produce the hallmark amyloid plaques of Alzheimer's pathology, has been of considerable interest to the medical community as a target for drug development in recent years.

This experiment investigated whether the plasmonic properties of 800 nm near-infrared resonant gold nanorods, and their consequent ability to generate localized heat, could serve as a mechanism to denature BACE. The results suggest that by conjugating gold nanorods with BACE via anti-BACE antibodies and polyethylene glycol, the denaturation of BACE was induced upon irradiation with near-infrared light.

This novel tactic, which has never been translated from the cellular to the molecular level, indicates the potential of plasmonic nanogold in protein-specific therapeutics. These findings may serve as a platform for addressing the shortcomings of past drug development for Alzheimer's disease, and perhaps for a diverse array of applications.

Background

Alzheimer's Proteome:

One widely attributed cause of Alzheimer's is the buildup of amyloid-beta plaques in the brain. Amyloid-beta is a component of amyloid precursor protein, a surface protein on neurons. Gamma-secretase and BACE are enzymes that cleave amyloid precursor protein to produce the insoluble amyloid-beta [3].

Much of the current Alzheimer's research focuses on preventing BACE and gamma-secretase from splicing amyloid precursor protein. Generally, BACE is a favored target over gamma-secretase given BACE's high specificity and the negative repercussions of targeting gamma-secretase due to its involvement in the production of sAPPalpha, a soluble peptide cleaved from amyloid precursor protein. Research suggests that sAPPalpha is neuroprotective against oxygen and glucose deprivation and assists potassium currents that bolster membrane potential [4].

Gold Nanoparticles:

Gold nanoparticles are nanoscale aggregates of gold atoms that possess an integral property known as surface plasmon resonance, which denotes the nanoparticles' ability to absorb a specific wavelength of light, causing the particles to oscillate. Gold nanorods are a type of gold nanoparticle resonant at the 800 nm wavelength which is the ideal wavelength for safe tissue penetration. Because their oscillation is translated as localized heat, gold nanorods can be applied to tumor ablation (the destruction of tissue using thermal energy) [6].

To target specific sites within the body, researchers linked gold nanorods to an existing drug or antibody that binds to the target [7]. In this way, researchers at the University of Chicago successfully treated malignant glioma (a type of brain tumor) in mice. The researchers determined that gold nanoparticles crossed the blood-brain barrier of the mice, indicating the promise of nanogold therapeutics in treating neural illnesses [8].

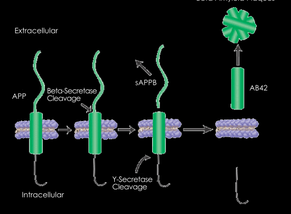
Gold nanoparticles' plasmon resonance and ability to cross the blood-brain barrier make them ideal therapeutic agents with a wide range of potential applications in the treatment of neurological illness such as Alzheimer's disease.

Polyethylene Glycol (PEG):

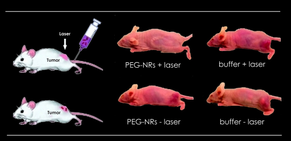
Polyethylene glycol (PEG) is an intermediary molecule used to connect two other molecules via functional groups on either end. The ease of adding desired functional groups to PEG makes it especially applicable in nanotechnology and protein engineering. In particular, thiols have a well-documented ability to interact with gold, enabling PEG to be attached to gold nanoparticles [10]. This ability has made possible a new spectrum of drug delivery utilizing bioreagent conjugated gold nanostructures [11].

Tryptophan Fluorescence:

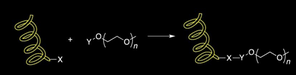
Tryptophan is an amino acid that is strongly hydrophobic and an intrinsic fluorophore. By exposing a tryptophan residue to 280 nm (ultraviolet) light, it will fluoresce at a wavelength that is representative of the tryptophan's current environment. Tryptophan tends to be found at the center of a protein where it is shielded from interaction with the protein's polar surroundings. A tryptophan will redshift (emit a longer wavelength of light) when the tryptophan has come into contact with the more polar environment of the surrounding solution, meaning that the tryptophan is no longer located centrally. This would indicate that the protein is denatured [12].



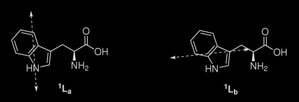
[Graphic 2] This graphic provided by Sigma Aldrich [5] indicates the process of amyloid precursor protein cleavage that produces amyloid-beta plaques.



[Graphic 3] This graphic by Dykman and Khlebtsov [7] depicts mice that were injected with human cancer cells to form a tumor. Heating the tumor with a near-infrared laser did not kill the cancer cells in the absence of gold nanorods, but completely killed the tumor in the presence of gold nanorods. The gold nanorods themselves did not kill the cancer cells in the absence of light from the laser, indicating that photothermal therapy (the resonance of the gold nanorods) was responsible for killing the tumor.



[Graphic 4] This graphic by Dozier and Distefano [9] depicts the reaction between a functionalized PEG molecule (shown in an abbreviated form) and a side chain of an amino acid.



[Graphic 5] This graphic by Ghisaidoobe and Chung [12] depicts the change in fluorescence characteristics that occurs following denaturation of a protein containing tryptophan.

Engineering Goal

Because gold nanorods absorbing near 800 nm light have successfully heated and lysed cancerous cells through their property of surface plasmon resonance, and because proteins can be denatured through heat or vibration, causing the breaking of their secondary and tertiary structure, the heating of the protein beta-secretase with 800 nm resonant gold nanorods denatures beta-secretase, which can be tested through an assay measuring the fluorescence of its tryptophan residues.

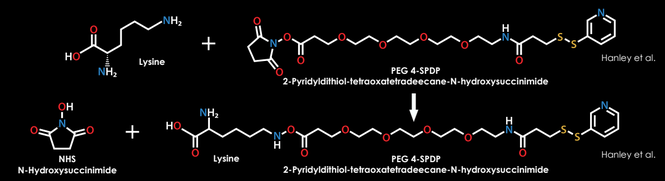
Materials

- Gold Nanorods
- Beta-Secretase (BACE)
- Anti-BACE Antibody
- PEG4-SPDP
- Monobasic Sodium Phosphate Monohydride
- 10 M Sodium Hydroxide
- 8 M Urea
- 10 W, 800 nm LED Lamp
- Arduino Uno
- Tecan Microplate Reader
- Spectrophotometer

Methods

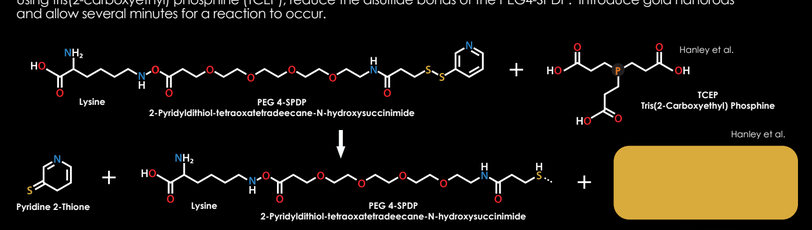
Bind PEG to Antibody:

At pH 7, perform a nucleophilic reaction of the NHS-ester functional group of PEG4-SPDP with the lysine residues of the antibody. Allow at least four hours to ensure that the reaction goes to completion.



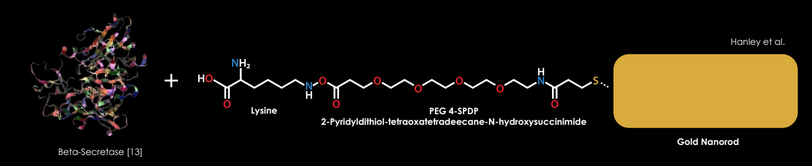
Bind PEG-Antibody Conjugate to Gold Nanorods:

Using tris(2-carboxyethyl) phosphine (TCEP), reduce the disulfide bonds of the PEG4-SPDP. Introduce gold nanorods and allow several minutes for a reaction to occur.



Bind Gold Nanorod-PEG-Antibody Conjugate to BACE:

Introduce BACE to the gold nanorod-PEG-antibody conjugate and allow several minutes for antibody-BACE bonds to occur.



Measure Tryptophan Fluorescence in a Plate Reader:

Perform a fluorescence intensity scan with an excitation wavelength of 280 nm and an emission wavelength range of 310 nm to 450 nm of 1 µL of the gold nanorod-PEG-antibody-BACE conjugate in 100 µL monobasic sodium phosphate buffer.

Irradiate Gold Nanorod-PEG-Antibody-BACE Conjugate:

Expose the gold nanorod-PEG-antibody-BACE conjugate in buffer to 800 nm light for a duration of 40 minutes.

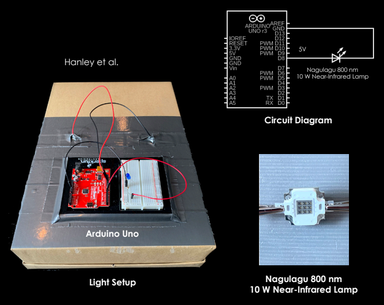
Measure Tryptophan Fluorescence:

Perform a second fluorescence intensity scan of the same excitation and emission wavelengths of the gold nanorod-PEG-antibody-BACE conjugate in buffer following irradiation.

Controls:

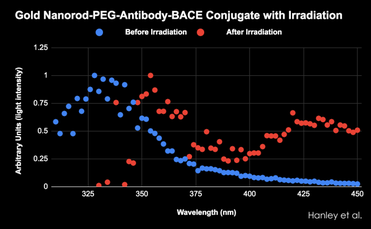
Perform control tests, repeating tryptophan fluorescence and irradiation on solutions with:

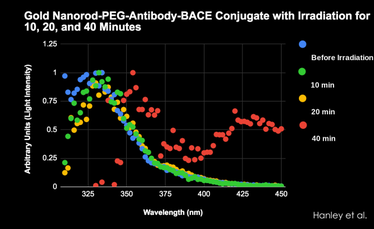
- Gold nanorod-PEG-antibody conjugate only
- Gold nanorods and BACE (not conjugated)
- BACE only

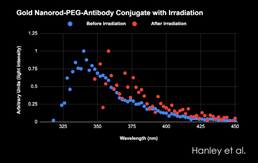


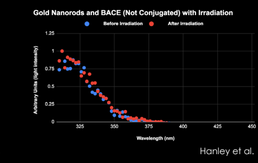
[Graphic 6] An Arduino was used to power the light source irradiating the samples with near-infrared light. An Arduino is a circuit board that can be programmed from a computer through a USB port. The Arduino provides 5 Volts of power, the necessary voltage for the light source. The LED is a 10 Watt, 5 Volt, 800 nm light with nine diodes. By downloading a pre-made Arduino program known as "Blink," the LED can be programmed to turn on for a desired length of time while it is plugged into the Arduino.

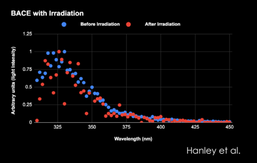
Results











Conclusions

Gold Nanorod-PEG-Antibody-BACE Conjugate:

Based on the tryptophan fluorescence graphs, there was a significant difference between the tryptophan fluorescence peaks before and after irradiation for the gold nanorod-PEG-antibody-BACE conjugate. This suggests that the irradiation of the conjugate with 800 nm near-infrared light successfully denatured BACE.

Gold Nanorod-PEG-Antibody Control:

Based on the tryptophan fluorescence graphs, there was a significant difference between the tryptophan fluorescence peaks before and after irradiation for the gold nanorod-PEG-antibody conjugate. This suggests that the irradiation of the conjugate with 800 nm near-infrared light denatured the antibody.

Gold Nanorod and BACE (Not Conjugated) Control:

Based on the tryptophan fluorescence graphs, there was no significant difference between the tryptophan fluorescence peaks before and after irradiation for the gold nanorod and BACE control. This suggests that the irradiation of gold nanorods in colloid with BACE using 800 nm near-infrared light did not denature the BACE.

BACE Control:

Based on the tryptophan fluorescence graphs, there was no significant difference between the tryptophan fluorescence peaks before and after irradiation for the BACE control. This suggests that the irradiation of BACE alone with 800 nm near-infrared light did not denature the BACE.

The significant redshift observed in the tryptophan fluorescence analysis of the gold nanorod-PEG-antibody-BACE conjugate suggests that gold nanorods, through their property of surface plasmon resonance, successfully heated and denatured the enzyme beta-secretase. Furthermore, the results of the control tests suggest that only by targeting BACE using an antibody-gold nanorod complex with near-infrared light was BACE denatured.

Next Steps

Next Steps in Alzheimer's Research:

The conclusions drawn from this experiment offer promising applications for the treatment of Alzheimer's. With further funding, more data points, and more characterization, this therapeutic could move into clinical trials. However, in order to serve as a viable treatment, further research must confirm that the gold nanorod complex is capable of crossing the blood-brain barrier and that the structural integrity of neurons is not compromised by the localized heating of the nanorods.

Targeting Enteroviruses:

SETD3 is a protein recently discovered to be involved in the replication of enteroviruses, a strain of viruses common in mammals that includes the common cold and polio. According to previous research by Diep et al. of Stanford University, in which the gene coding for SETD3 was knocked out in mice, enteroviruses were unable to reproduce in the absence of the protein. Currently, SETD3 can only be knocked out embryonically through gene editing, eliminating this protein for life [14]. Gold nanorods may have the potential to target and denature SETD3 *in vivo*, which could generate immunity against a broad spectrum of enteroviruses.

[Graphic 7] SETD3 (above) [15].

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