**Part I: Preliminary Information**

**Title:** The Importance of Neurotransmitter-Stabilized Amyloid Beta Oligomers in the Cell Death Mechanisms of Alzheimer’s disease

**Abstract:**

 The mechanism by which neurons degenerate in Alzheimer’s disease remains unclear. There are significant data to suggest that oligomers of amyloid beta (Aβ) peptides are the driving force behind this mechanism,4,5,11,12 but it is unknown exactly which molecules in the brain react with these oligomers. Reactions between oligomers and tauproteins within the cell, however, have been suggested to trigger cell death mechanisms.8 This project will stabilize Aβ oligomers under physiological conditions, and then investigate the reaction between oligomers andtauproteins. Finally, the rate of mitochondria-mediated cell death that occurs as a result of this reaction will be measured and compared to rates caused by oligomers alone. The stabilization of oligomers under physiological conditions, by using neurotransmitters,6 may provide an accurate model of the mechanism in the human brain.

**Personal Statement:**

 During the process of selecting a research mentor, I found myself drawn to professors whose work focused largely on neurological issues. After looking at the research history of the available biochemistry mentors, I did some further investigation of possible neuroscience topics needing further study. I concluded that, as I have found myself so drawn to neuroscience projects, I would be interested in a neuroscience minor. During winter term this year, I took Behavioral Neuroscience and was surprised to find that the development of this research project was influenced by the things that I learned. The descriptions in the class textbook of Alzheimer’s disease informed my search for resources to use in writing this proposal. My passion for this field has inspired my still-forming career goals to pursue graduate school and eventually a career in medical research.

From an educational standpoint, I love the science. The intricate details of biochemistry are like the key to the biological code, finally revealing the reality of how life occurs. From a career standpoint, I would like to use this type of work to make a tangible difference in people’s lives. The study of diseases has the potential to improve the quality of life of millions of people, to make life safer and happier for people across the world. My work with Elon’s Periclean Scholars, where I have dedicated myself to learning how to improve the health and well-being of people living in a Habitat for Humanity community in rural Zambia, complements these ideas perfectly. Whereas in Periclean, we are searching for ways to bring health services and opportunities into underdeveloped communities, with this research I can contribute to a project that could help improve the lives of people around the globe. This research is a start to what I hope to be a long career in medical research, where I can discover the information that saves lives.

Receiving a Lumen Prize will provide the funds to purchase the materials that are necessary for me to fully realize my vision for this research. The funds from Lumen also provide amazing opportunities to travel to conferences around the country and see the work of my peers and leaders in the field. These conferences, particularly those that are for professors, graduates, and undergraduates, can provide opportunities to better understand the field in which I am studying and develop networks in the field. Conferences also provide the experience of presenting my research and having it viewed by experts, the benefits of which are vast for both my research and my professional development.

**Part II: Project Description**

**Focus:**

Many neurodegenerative diseases have root mechanisms involving protein aggregation, in which chains of amino acids known as peptides interact with each other to form larger aggregates known as oligomers and fibrils. Some examples of these diseases are Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and prion diseases.10 Large peptide aggregates are easily identifiable in the brains of patients, but there is considerable debate over what actually causes cell death and neurodegeneration in these diseases. In order to narrow down the possibilities, the toxic species needs to be determined. A theory that has been extensively studied presents the peptide aggregates, which typify patients’ brains, as the molecules causing cell death. In Alzheimer’s disease, many possible mechanisms have been suggested to explain the effects of the aggregating protein amyloid beta (Aβ) on the brain.

A study by Bemporad and Chiti in 20122 measured the relationship between toxicity and size of peptide aggregates. Toxicity was measured by the rate of cell death in the presence of the protein. Their findings suggest that, contrary to previous beliefs, larger peptide aggregates are, in fact, not the toxic species. Proteins aggregated in the small oligomer form caused the greatest percentage of cell death relative to other sizes of aggregates. These findings have been supported by the vast majority of recent studies.4,5,11,12 Research has begun to investigate the mechanisms by which Aβ oligomers might be inducing cell death.

Oligomers only exist in solution for a very brief amount of time before aggregating into the larger insoluble fibrils, which are readily visible in the brains of Alzheimer’s patients. This aggregation pattern is further described in Figure 1, which shows the progression of misfolded proteins from small monomers to larger, insoluble fibrils. Therefore, to further study this disease, it is important to find a way to stabilize peptides in oligomeric form. Previous studies have used aromatic phenolic molecules, such as 4-aminophenol or 3,4-dihydroxybenzoic acid 3 to stabilize Aβ oligomers. A study by Fischer and Matera, 2015,6 found that the presence of dopamine and norepinephrine (Figure 2), common neurotransmitters found in the brain, stabilized alpha-synuclein, the protein involved in Parkinson’s disease, in oligomeric form. These findings are particularly relevant because these neurotransmitters are present in large quantities in the brain, and may be contributing to Aβ oligomer formation in Alzheimer’s disease. Previous studies have largely been carried out under non-physiological conditions.3 By stabilizing Aβ oligomers using neurotransmitters under physiological pH, temperature, and other conditions, this study should provide a fully realized model for oligomer stabilization in the human brain.

**Figure 1**. **Aggregation of misfolded proteins7**

 As demonstrated by the equilibrium arrows in this diagram, these forms of a protein are transient unless acted upon by an outside force. Additionally, the reversibility of the aggregation decreases as a protein aggregates into the larger fibrils, explaining why fibrils are more stable molecules than oligomers. 

**Figure 2. The Structure of Dopamine and Norepinephrine.**

The aromatic phenol functional group in these neurotransmitters is comparable to the molecules used to stabilize oligomers in other studies 3,6.



The stabilization of Aβ oligomers under physiological conditions is the first stage of this project. Oligomers will be mixed in solution with several different neurotransmitters - dopamine, norepinephrine, and oxidized forms of these two neurotransmitters. Gel electrophoresis can be used to confirm that oligomers are present in solution and stabilization was successful, as the small oligomers will separate in the gel from larger amyloid fibrils.4,6 The purpose of this section of the project is to create a model for the stabilization of amyloid beta oligomers under physiological conditions, reacting with molecules that are present in high concentrations in the physiological environment.

The second stage of this project will be a structural analysis of the Aβ oligomers that are stabilized. This will be accomplished through a combination of hydrogen/deuterium exchange and Nuclear Magnetic Resonance (NMR), an instrumentation method used to examine the structural features of a molecule. Hydrogen/deuterium exchange, or H/D exchange, involves hydrogen atoms on functional groups of the peptides being replaced by deuterium, which is identical to hydrogen except for one additional neutron, making it unable to be detected by NMR.2 This exchange can only occur on the parts of the oligomer which are most exposed to solution. Coupling H/D exchange with solution NMR reveals which portions of the oligomer are exposed to solution and which parts are hidden in the hydrophobic interior; as those portions of oligomers exposed to the solution will more readily undergo H/D exchange. The groups that have undergone exchange will not show up on an NMR spectrum, and a comparison of this spectrum to one pre-exchange will indicate which hydrogens are most likely on the surface of the aggregates. This information will provide mechanistic detail of the formation of resulting toxic oligomers.

 While Aβ oligomers are known to be correlated with cell death,1,3,4,5,11,12,13 the mechanism by which this occurs is unknown. Aβ oligomers have been linked with the formation of reactive oxidized species, which result in oxidative stress, receptor-mediated calcium intake, disturbance of the mitochondrial membrane, and ultimately apoptosis.1,13 Aβ oligomers exist both inside and outside the cell,1,11 and model organisms show high levels of intracellular oligomers.5 While the presence of Aβ oligomers within the cell is correlated with cell death, no studies have been able to propose a thorough causation mechanism.

 One candidate for a molecule with which Aβ oligomers might directly interact is the tau protein, which is involved in holding microtubules, the structural determinants of the cell,8 in place. In Alzheimer’s disease, tau proteins are accumulated within the cell in the form of neurofibrillary tangles, along with Aβ fibrils.8,11 Interactions between Aβ and tau, such as those proposed in Figure 3, have been proposed as the first step in the mechanism that leads to cell death;8 however, the mode of this interaction, if any, is unclear. This uncertainty leads into the second stage of this project.

**Figure 3. Interactions between Aβ and Tau8**

 Presented are three different potential mechanisms of interaction between Aβ and tau that may be related to toxicity, as proposed by Ittner and Gotz, 2011. 

 The second major stage in this project is to combine the neurotransmitter-stabilized oligomers with tau proteins. Tau proteins will be combined with the neurotransmitters alone, with Aβ fibrils, and with the different stabilized oligomers that were formed in the first stage of this project. In order to quantify the interaction between tau and neurotransmitter-stabilized Aβ oligomers, western blot analysis 13 can be used to identify whether or not the two molecules form any kind of complex between them. Additional methods, such as UV/vis spectroscopy or immunoprecipitation, may be used as supplementary sources of information.

 Whether or not tau and Aβ oligomers directly interact, the final stage of the project will examine the impact of the presence of both molecules on mitochondrial-mediated cell death. The neurotransmitter-stabilized Aβ oligomers will be placed into a cell culture, and their impact will be compared to that of Aβ oligomers in combination with tau proteins. The rates of mitochondrial-mediated cell death will be measured using cytochrome c, a molecule which is released during the chain reaction which results in apoptosis.13 The amount of cytochrome c that is present in the cell cultures will be analyzed by spectroscopy using a cytochrome c releasing apoptosis assay kit. A comparison of the levels of cell death between stabilized AB oligomers alone and stabilized Aβ oligomers in solution with tau proteins may indicate whether or not these two molecules interact during the mechanism in which cell death occurs in Alzheimer’s disease.8

**Outline of Project Procedure:**

* Stabilization of Aβ oligomers under physiological conditions.
	+ Types of Neurotransmitters
		- Dopamine
		- Norepinephrine
		- Oxidized Dopamine
		- Oxidized Norepinephrine
	+ Physiological conditions: pH 7.4, 37°C
	+ Gel electrophoresis: to identify that stabilized oligomers are present
* Structural Determination
	+ Nuclear Magnetic Resonance (NMR)
		- Oligomers + Tau: Combine stabilized oligomers with tau proteins
			* Neurotransmitter alone + Tau
			* Aβ fibrils + Tau
			* Neurotransmitter-stabilized oligomers + Tau
	+ Western Blot Analysis: To determine whether stabilized oligomers interact with tau
		- Supplemental methods: UV-vis spectroscopy, immunoprecipitation
* Mitochondrial Model
	+ Analyze the effects of stabilized oligomers on mitochondrial apoptotic mechanisms
		- Stabilized oligomers
		- Aβ fibrils
		- Tau
		- Tau + stabilized oligomers

**Proposed Experiences:**

The summer of 2016 will mark the beginning of my research. Through the Summer Undergraduate Research Program (SURE) here at Elon, I will spend the summer stabilizing Aβ oligomers using dopamine and norepinephrine neurotransmitters. Depending on how effective these neurotransmitters are, much of this time may be spent focusing on this topic and ensuring that we have developed an effective procedure for stabilizing Aβ oligomers under physiological conditions. The final portion of the summer will be learning to perform H/D exchange and running and interpreting NMR experiments. During Fall 2016, Spring 2017, Fall 2017, and Spring 2018, I plan to be enrolled in 2 credit hours of research each semester, during which I will conduct the subsequent steps of the research project. Fall 2016 and Spring 2017 will involve finishing any remaining structural determination and creating the mitochondrial model. By Fall 2017, the mitochondrial model will be wrapped up and I will start analyzing the reaction between tau proteins and amyloid beta. Spring 2018 will consist largely of addressing any final questions and writing an Honors Thesis, and potentially an article to be submitted for publication. During these years, I also plan to attend several conferences. In Fall 2016, I plan to attend the Southeastern Regional Meeting of the American Chemical Society, as well as submit an application for the National Goldwater Scholarship. In Spring 2017, I plan to attend the National American Chemical Society Meeting, as well as present my work at SURF Day at Elon. Spring 2018 will involve the presentation and defense of my Honors Thesis, and I plan to attend SYNAPSE: Symposium for Young Neuroscientists and Professors of the Southeast during this semester as well.

**Proposed Products:**

Over the course of this research, I will produce an Honors Thesis, to be defended in Spring 2018. I also plan to develop a scientific research paper that can be submitted for publication to a journal, such as Bioorganic and Medicinal Chemistry and Neurochemical Research. I will also produce a PowerPoint and poster format of my findings that can be presented at any conferences attended and displayed at Elon to share the research results with the broader community.

**Part III: Feasibility**

**Feasibility Statement:**

With the funds provided by Lumen, I will be able to purchase all of the chemicals, products, proteins, kits, and consumables necessary to carry out this project. In order to be capable of performing the tests proposed here, I will spend a significant amount of time learning lab techniques. First, I will learn techniques during the summer of 2016 through the SURE program, in which I will work with Dr. Matera in the lab to acquire the skills necessary to begin my project. After Summer 2016, I will learn and practice techniques as necessary through HNR 498 research hours over four semesters beginning Fall 2016.

Over the course of this project, there are several problems that could arise. In the first step, if the oligomers are not stabilized by dopamine and norepinephrine, as expected, I will proceed by oxidizing these neurotransmitters and conducting a follow-up trial. Subsequently, I can test oligomer stabilization with other types of neurotransmitters, in accordance with the findings of previous studies. Other issues at this stage may be that the oligomers do not originally stabilize under physiological conditions. Due to the timeline of a SURE program, I will be able to address this concern by conducting several different trials over the course of the program, guided by existing research. At the second stage of the project, if the tau and Aβ oligomers do not associate, this does not prevent us from continuing to the third stage. It may be that, despite not physically associating with one another, the presence of both tau and Aβ changes the levels of mitochondrial-mediated cell death. Throughout this project, I am prepared to address any problems that arise by consulting the existing research and reevaluating my project design accordingly.

**Budget:**

|  |  |
| --- | --- |
| **Amount** | **Purpose** |
| $10,000 | Tuition |
| $500 | Tau proteins: https://www.rpeptide.com/products/proteins/tau/t-1001-1 |
| $300 | Tau protein antibodies<https://www.thermofisher.com/order/genome-database/antibody/Tau-Antibody-clone-HT7-Monoclonal/MN1000>  |
| $400 | Anti-oligomer antibodies  |
| $1,000 (several mgs)  | Aβ <https://www.rpeptide.com/products/beta-amyloid-peptides/beta-amyloid-peptides-human-native-and-mutant-recombinant/>  |
| $1,000 | Travel to conferences  |
| $600 | Cytochrome c Releasing Apoptosis Assay Kit <http://www.abcam.com/cytochrome-c-releasing-apoptosis-assay-kit-ab65311.html>  |
| $1,200 | Consumables (pipet tips, gloves, solvents, electrophoresis gels) |

**Timeline:**

|  |  |  |
| --- | --- | --- |
|  | **Proposed Experiences** | **Proposed Product(s)** |
| **Summer 2016** |  SURE  | SURE Research PresentationStructural Determination of NT-stabilized Aβ oligomers |
| **Fall 2016** | Tau proteins and Aβ oligomer reaction | Southeastern Regional Meeting of the American Chemical SocietyDetermination of the mechanism of reaction between oligomers and tau proteins (if any) |
| **Winter 2017** |  Abroad |  None |
| **Spring 2017** | Tau proteins and Aβ oligomer reaction Begin Mitochondrial System | SURF PresentationNational American Chemical Society MeetingFinalize mechanism of reaction between tau and oligomers.Mitochondrial model designed and tested.  |
| **Summer 2017** |  Non- Elon REU |  None |
| **Fall 2017** | Mitochondrial model | Relative toxicity of tau + oligomer complex, as compared to oligomers alone. |
| **Winter 2018** |  Abroad |  None |
| **Spring 2018** |   Thesis writing/ Paper writing | SYNAPSE: Symposium for Young Neuroscientists and Professors of the SoutheastHonors Thesis/ Scientific Paper to be published |

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