Summary:
The cause of both Alzheimer’s Disease (AD) and Parkinson’s Disease (PD) is an abnormal deposition of proteins in brain cells. In addition, there are 20 other neurological diseases caused similarly by protein deposition. Millions of people suffer from these diseases. The latest research shows that these diseases arise as a consequence of a specific series of molecular events. First, a protein assumes a non-native sticky “misfolded state.” Two or more such sticky proteins associate together to generate a multi-protein “oligomeric state.” These oligomers can associate with each other or can recruit newly formed sticky proteins, thereby growing into bigger thread-like structures called “amyloid fibrils.” These fibrils can deposit either inside or outside brain cells, disrupting normal biological functions and resulting in neuronal cell death. Depending on the region of the brain affected, this cell death leads to visible symptoms, such as memory loss, loss of cognitive ability, abnormal muscular movements, involuntary shaking and, in many cases, death.

Course Format:
The purpose of this course is to familiarize you with the primary scientific literature. Each week we will discuss two scientific papers. Our emphasis will be to understand the main objective of the article and learn the scientific approach of addressing it. We will learn how a combination of methods and techniques were used to achieve that objective. We will also learn how the data presented in the articles were interpreted. We will critically analyze the paper. You will be strongly encouraged to suggest your opinion. It is important that you read the papers carefully before you come to the class and prepare at least one question for discussion.

At the end of each class, you will be given a brief introduction to the papers for the next class discussion.

We will have a field trip to relevant facilities on campus or in a nearby company once during the course.

Attendance:
Since this course involves discussion physical presence (“mind and body”) is essential in all of the classes. You should not miss any class. If there is a special situation or in case you are aware of such a situation in your schedule before hand, please let me know as soon as possible and we will discuss the options.

Grading and Assignments:
You will be required to complete three assignments.

1. You will be given a scientific article without an abstract and asked to write an abstract for the paper based on its results and discussion.
2. You will be given a paragraph outlining the objectives of a scientific research project based on the articles discussed previously in the class. You will be asked to design experiments to achieve the goal of the proposal.

3. At the end of the course, you will be asked to give an oral presentation. You will choose any article of your liking related to the course and present it to the whole group in the form of slides or another audiovisual form. You will present a background introduction to the topic of your article, the goal researchers are trying to achieve in this article, the methods they have used to approach their goal and you will interpret the results from the experiments shown. You should comment about the utility or shortcomings of the techniques used in this article and answer critical questions from your classmates.

The grading is pass/fail. Attending all classes and completing all assignments satisfactorily will result in pass.

**Class 1-February 9: General introduction.**
We will introduce ourselves to the whole group, talk about our backgrounds and our reasons for taking the course. We will discuss the aim of the course and the syllabus. There will be an introduction to the various neurodegenerative disease and possibly videos of some disease symptoms and mechanism. You will be given two papers to be discussed at the next class. You will be introduced to the format of scientific papers and how to critically analyze papers.

**Class 2-Feb. 16: Involvement of Aβ42 in Alzheimer’s disease (AD) and detection of Aβ42 fibrils in the brain.**
It is of the utmost importance to know the mechanisms of biological processes that cause pathogenic disease. This will not only fulfill our scientific curiosity but in addition will help us to devise effective drug treatments. In the case of AD, research so far has shown the predominant factor in the genesis of AD is the deposition of Aβ peptides in extracellular space. Aβ peptides are 39-43 residue fragments produced by enzymatic cleavage of amyloid precursor protein (APP) by β and γ-secretase. Amyloid deposits in AD predominantly consist of a 42-residue peptide also known as Aβ42. Today we will discuss the identification and characterization of Aβ peptide from the cerebrovascular fluid and amyloid plaques of AD patients.


Class 3-Feb. 23: \textit{Aβ is produced from APP: mutations in Presenilin cause overproduction of Aβ}

\textit{Aβ} is a normal metabolic product of amyloid precursor protein (APP) present in the human body. The AD related abnormal deposition of \textit{Aβ} occurs only when it is overproduced. We will learn how \textit{Aβ} is produced and processed from APP in the biological systems. We will also gain insight into the effects of mutations in presenilins on the aggregation of \textit{Aβ42 in vivo}. Presenilins are a group of enzymes (including \(β\) and \(γ\)-secretase) that act sequentially on APP to produce \textit{Aβ}.


Class 4-March 2: \textit{Biophysical characterization of Aβ42/40 aggregation related to AD.}

Transformation of \textit{Aβ} into amyloid fibrils is not a single spontaneous process but rather involves various steps. The details of events taking place during the transformation of monomeric \textit{Aβ} to mature fibrils have been reported in the articles we will discuss today. In summary, \textit{Aβ} undergoes structural change before assembling into oligomers consisting of 20-90 monomers. These oligomers in turn assemble into small protofibrils that look like small flexible curved rods, which then form long thread-like mature amyloid fibrils.


Class 5-March 9: \textit{Is there a way we can develop an early stage diagnosis kit for AD?}

Perhaps if we were able to detect AD patients in an early disease stage we would be able to identify changes related to AD that take place in the patient and have better opportunity to develop therapeutic treatments. One extraordinary step in this direction was the generation of an antibody that can specifically recognize the “oligomeric state” of aggregation by the group of Prof. Charles Glabe. This makes it possible to check the levels of \textit{Aβ} oligomers in the cerebrospinal fluid (CSF) of patients and thus possibly indicate the severity of the disease state. Another method proposed in this direction is to monitor the levels of metals, especially Cu\(^{2+}\), in the CSF. Abnormalities in homeostasis of Cu\(^{2+}\) have been reported to be intricately involved in AD. Our two articles today are related to these two topics.


**First Assignment due today - March 9, 2006:**

**Class 6-March 16:** *Oligomers are more toxic than fibrils. Understanding the cell biology related to the AD - cytotoxicity of Ab40/42 aggregates.*

Recent studies have shown that more labile Aβ oligomers are cytotoxic or neurotoxic. These two papers discuss the preparation of oligomers from synthetic peptides *in vitro*. The oligomers have been found to cause necrosis and cell death more than the mature fibrils as monitored by two assays - lactate dehydrogenase (LDH) release assay and a tetrazolium salt (called MTT) reduction assay, respectively.


**Class 7-March 23:** *Rational drug design for AD.*

AD is associated with Ca²⁺ dyshomeostasis. A heavy Ca²⁺ influx into cells through various receptors and channels is thought to take place. Memantine, an antagonist of one such channel - the NMDA receptor can prevent Ca²⁺ influx into the cell and has been proposed as a drug for AD. Similarly, high cholesterol levels have been found to correlate with the severity of AD. Statins are believed to reduce the levels of cholesterol concomitantly with Aβ40 and Aβ42 levels in animal models. However, they have been found to be less effective in studies involving humans.


**Class 8-April 1:** *Introduction to Parkinson’s disease (PD) and involvement of synuclein*

Parkinson’s disease is thought to be caused by deposition of the protein synuclein in the substantia nigra region of the brain. These papers report the localization of synuclein in PD patients. We will also learn how synuclein fibrils produced *in vitro* and bearing the characteristics of fibrils in Parkinson’s patients are cytotoxic as monitored by the MTT assay.


Class 9-April 7: Field trip (To Amgen or on campus - Bioinstrumentation facility (18/511), EM facility (68/304a), Fluorescence spectrophotometer (68/322))

Class 10-April 14: Biophysical characterization of α-synuclein, its other forms and its mutants related to familial PD

Rochet et al., have followed the events in aggregation of α-synuclein bearing an A30P mutation. The mutant shows a higher propensity to aggregate compared to the wild-type protein. Besides mutant forms, synuclein exists in three different forms differing in length - α, β and γ synuclein. Anthony Fink’s group has studied the aggregation kinetics of these various forms of synuclein. β-synuclein does not undergo fibrillation under ordinary conditions, unlike the other two forms, and we will learn about this anomaly today.


Second assignment due today - April 14, 2006:

Class11-April 21: Some of the trial and error drugs proposed for PD.

Anthony Fink’s group has proposed an RNA polymerase inhibitor and an antibiotic, rifampicin, as a drug candidate for PD. Their claim is based on the ability of rifampicin to dissolve the fibrils into lower aggregates and convert of fibrils into the native-like soluble protein. The drug is in animal trials. Peter Lansbury’s group showed the effects of various dopamine derivatives on inhibiting synuclein aggregation.


Class12- April 28: Prions
Prof. Stanley Prusiner received the Nobel Prize for reporting the protein nature of the source of infection in scrapie, mad cow disease or kuru. We will learn experimental findings that led him to propose his theory. This paper redefined the nature of infectious agents and their propagation. The second paper describes methods of detecting prions in patients.


Class 13- May 5: Prions continued
Prion proteins assume distinct conformational states that bear the ability to self-propagate. Here we will discuss two yeast prion proteins, Sup35 and Ure2p, that have been used as models to understand the mechanism underlying the prion-related disorders. Krishnan and Lindquist give details of arrangement of protein in amyloid fibrils, whereas Catharino et al., provide further insight into the characterization of Ure2p intermediate states on the pathway to its toxic fibril formation.


Class 14- May 12 Assignment 3 - Oral presentations

Optional further reviews: