

# Proteomics of Alzheimer's Disease

Steven Bhutra  
Biochem 118  
Dr. Brutlag

Alzheimer's disease (AD) is the most common form of dementia in the elderly. This incurable neurological degenerative disease is typically diagnosed in people over 65. Alzheimer's can be incredibly difficult to diagnose because many of the symptoms can be mistakenly attributed to age-related concerns: the most common symptom is memory loss. As the disease manifests, symptoms include confusion, irritability, mood swings, and language breakdown. Eventually, control of the body is lost, leading to death. Since the disease can be undiagnosed for years, latency has not been determined. When AD is suspected, the diagnosis can be confirmed with brain scans or behavioral evaluations. Since this disease is degenerative, management is essential but it comes at a huge cost, both to the checkbook and to the family. This epidemic of the elderly still has no treatment to delay the progression of the disease. With an increasingly aging population, this disease will only become even more prevalent. Doctors and researchers, hope to end this disease using a new development called proteomics. (4)

Proteomics seeks to identify protein structure, modulations and protein-protein interactions in addition to protein expression levels. This procedure attempts to study biological processes comprehensively through the systematic analysis of the proteins expressed in a cell and is often thought of as the next step after genomics. Genomics is much simpler, as it focuses on the genome, which is consistent throughout its cells. However, proteomes change from cell to cell depending on their function. Previous assays of proteins, based on sequencing of the genome, were largely inaccurate because these studies were based on mRNA hypothetical translation. Many enzymes and miRNA inhibit translation altering the expected protein from the original strand. Moreover, the single mRNA strand may not translate continuously giving rise to multiple types of proteins through the process of alternate splicing. Any protein can undergo a wide range of

posttranslational modifications, including attaching to another protein or RNA molecule. The old way of detecting proteins wasn't easy as a hodgepodge of proteins within the same cells can make any study quite complicated, but with this complication comes a plethora of opportunity to investigate the protein spectrum of a cell and its biological functions; consequently to detect novel drug targets and diagnostic markers. (5, 7)

Previous protein analysis techniques examined proteins one by one; in proteomics this is done on a large scale. Despite a variety of new approaches, proteomics (in the study of Alzheimer's) largely relies on the classic two-dimensional (2-D) electrophoreses and mass spectrometry (MS) to identify proteins. 2-D electrophoreses is used to separate proteins based on two criteria: difference in net charge via pH gradient and molecular masses or molality. MS is an identification technique that identifies the chemical composition of a protein based on the mass to charge ratio. First a gel separates the proteins by specific proteases in the 2-D electrophoreses and then the mass spectrum is obtained via peptide mass fingerprint (PMF). In this process, the protein activation site is catalyzed with a specific enzyme, the resulting peptides are weighed and cross-referenced with computer generated theoretical PMFs, identifying the protein. In this way both 2-D gel and mass spectrum can be compared against databases to identify proteins. (1) Recently, however, a process called protein microarray has revolutionized proteomics. In this process a piece of glass is fixed with different proteins at different locations forming an array. These proteins are bound to similar proteins revealing fluorescent protein-protein interactions. (7)

Many recent studies involving proteomics attribute oxidative stress as the major cause of the disease. Oxidative stress can cause irreversible modulations to susceptible proteins leading to structural and functional modifications. Protein modulations such as carbonylation, nitration and protein-protein cross linking are generally associated with loss of function and may lead to

degradation of the damaged protein, or accumulation which can have even more detrimental effects. Identifying proteins has given rise to understanding the mechanism of the disease. The oxidatively modified proteins closely catalogue known developments in the disease: accumulation of damaged proteins, shortened dendrite lengths, neurotoxicity, excess ubiquination, and dysfunction of energy metabolism. (2, 3)

Alzheimer's disease is defined as a neurodegenerative disorder characterized by neurofibrillary tangles composed of hyperphosphorylated tau proteins connected with paired helical filaments and senile amyloid plaques that accumulate in inferior parietal lobule and hippocampus. Tau is a microtubule protein that is involved in assembly and stabilization. Neurofibrillary degeneration involves the hyperphosphorylation of tau, which critically impairs its binding capacity to a microtubule disrupting axonal cytoskeleton. Peptide-prolyl cis-trans isomerase or Pin1 has been confirmed to regulate phosphorylation of tau by proteomic methods. In Alzheimer's disease however, Pin1 is oxidized which impairs its regulatory function. (See diagram below) In effect, the oxidation of Pin1 plays a role in the accumulation of phosphorylated tau linking oxidative damage to tangle formation causing the disease. Through the use of proteomics, oxidation of certain proteins such as Pin1 have been linked in to the development of the disease. Pin1 could be used as a target for drug designers. (2, 3)

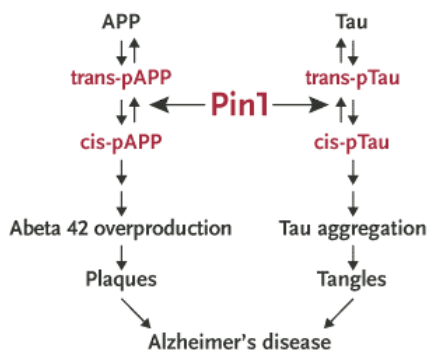
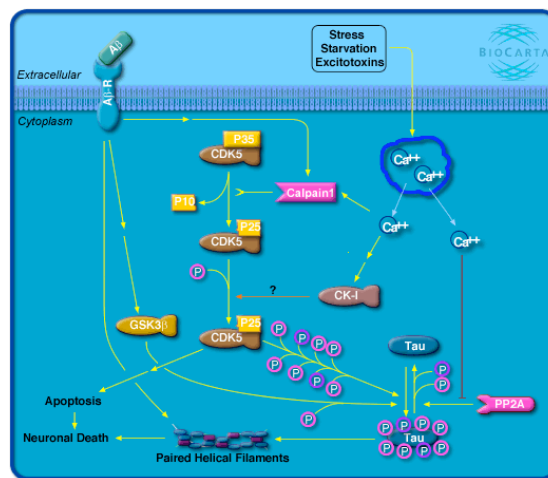


Diagram adapted by Rachel Eastwood from original courtesy of Kun Ping Lu

The disease can be further characterized by neuronal death and loss of synaptic connections within the already stated brain regions. Dihydropyrimidinase protein (DRP) is also an identified protein in the development of AD. DRP is involved in axonal expansion and guiding through the transmission and adjustment of extracellular signals. Already mentioned, one of the traditional symptoms of AD is memory loss, which is related to a decrease in neuronal connections and a shortened length of the dendrite. In fact, through the use of protein identification, DRP has been found in the neurofibrillary tangles of Alzheimer's disease patients, suggesting that the protein may be attempting to repair damaged neurons in the brain. Proteomic data suggests that in this case neuronal connections have failed due to an increase in carbonyl levels of DRP.  $\beta$ -actin is a specifically oxidatively modified protein in AD brain involved in cytoskeleton network integrity. In adult brain, actin is concentrated in dendrite spines where it can produce rapid changes in shape that are involved in recollection of memory. Therefore, decrease function of both DRP and actin, as a consequence of oxidative modifications, are consistent with the memory impairment and synapse loss observed in Alzheimer's disease. The addition of non-oxidized actin could possibly stabilize memory loss. (2, 3)

Most research points to the amyloid precursor protein as the principal cause of the disease and increase of accumulation of fibrillary amyloid- $\beta$  in the brain. In Alzheimer's, amyloid- $\beta$  peptide produces a specific protein that oxidizes glutamate transporter. Unwarranted concentrations of extracellular glutamate over-activates ionotropic glutamate receptors, resulting in a calcium overload and a surge of other events leading to neural cell death. Deficiency in the glutamate transporters can cause a backup of glutamate in the synaptic cleft leading to neurological problems. Hence, decreased glutamate transporter, caused by a build up of amyloid- $\beta$ , links glutamate neurotoxicity with neurodegeneration, supporting the possibility that abnormal

functioning of this system might be involved in the synaptic damage found in Alzheimer's disease. The amyloid- $\beta$  deposits that are typically found in Alzheimer's disease brains juxtapose themselves with a set of oxidative stress markers suggesting that there exists a close correlation between the two. (See diagram below) Given this link between the deposition and oxidative stress, agents that alter free radicals, known to combat neurotoxicity, may be useful in the therapy of Alzheimer's disease. For instance, application of the free radical antioxidant vitamin E has been used to prevent neurotoxicity from amyloid- $\beta$ . (2, 3, 4)



Recent protein analysis has found that the ubiquitin carboxyl-terminal hydrolase system becomes abnormal in Alzheimer's patients. The main function of the ubiquitin proteome is the degradation of misfolded proteins. Ubiquitin-proteasome is a protein quality control that protects proteins from unwanted protein-protein interactions and aggregation. In Alzheimer's patients mutated and oxidized proteins overload the ubiquitin system leading to the accumulation of abnormal proteins and to the selective degeneration of neurons. Recent studies have found that the ubiquitin proteome is one of the specific targets of protein oxidation in Alzheimer's patients, establishing a relationship between the effect of oxidative stress on protein and the proteasomal

dysfunction in Alzheimer's. With the addition of untainted ubiquitin to the brain, abnormal proteins could be broken down. (2, 3)

Moreover, most AD patients have a reduction of the metabolic rate of glucose. In Alzheimer's a group of enzymes involved in energy metabolism and ATP production become oxidatively modified. The modifications of these proteins alter the production capacity of ATP which is imperative to nerve terminals for communication among axons. Low levels of ATP may lead to a loss of synapses and cause cognitive problems. Any damage in neuronal glucose metabolism and its control may cause disturbances in memory formation retrieval. To balance these levels, the enzymes involved in the energy pathway could be targeted within the brain. (2)

One of the most powerful pieces of evidence in the proteomic analysis of AD was the detection of large amounts of Glial fibrillary acidic protein (GFAP). GFAP is expressed in the CNS in astrocyte cells. It is involved in many cellular functioning processes, such as cell structure, mitosis, cell communication, and the functioning of the blood brain barrier. GFAP is believed to be key in the long-term upkeep of normal CNS myelination, and in certain AD brains, GFAP is up 10 times its average level. This means that the pathway is overcompensating for its lack of control. In addition, GFAP has always been linked to Alexander's disease and in 2005; the enzyme plectin was identified to regulate GFAP in this disease. With the aid of this information, drug designers should try plectin to fight Alzheimer's. (7)

However, most designers are focusing their efforts on  $\beta$  secretase, which creates amyloid- $\beta$ -protein, causing plaque to build up in the patient's brain. Formation of amyloid fibrils is incredibly difficult to control and almost impossible to degrade. The events leading to its formation involve proteolytic cleavage by two enzymes,  $\beta$ -secretase, which fold the amino and carboxyl terminal. The excessive accumulation of this 42 amino acid long protein could be explained by a

deficiency in degrading amyloid- $\beta$ . One therapeutic strategy, an enzyme, called metalloendopeptidase increases the stability of the amyloid protein by curbing aggregation simply by inactivating the  $\beta$ -secretase. (4)

Nevertheless, there still remains a lot that eludes us. For example, many brain samples are tainted or mishandled, tissues only give a small amount of proteins, hydrophobic proteins and acidic proteins, likely drug targets, have been misidentified by the 2-D gel because it can not identify accurately anything under 3.5 on the pH scale, and due to posttranslational changes, many proteins can not be tracked back to their original strand. Because of the relatively small sample size and of gel problems, many protein changes in neuronal diseases may exist but have been missed because they did not reach a statistical significance. Despite current limitations of this technique, proteomics still represents a promising tool to gain insight into the molecular basis of disease. The challenge for the future will be ways of combating these limitations by developing techniques to replicate samples of proteins with specific post-translational modifications. This challenge is already addressed with the advent of protein microarray process. (6)

The application of proteomics technologies in the investigation of neurological diseases started just a few years ago. As an emerging technique, proteomics has identified oxidatively modified proteins, accounts for biochemical and morphological alterations in AD. In this time many advancements have already taken place. Many changes at the levels and the modifications of mainly abundant brain proteins have been detected by proteomics technologies; some of these are potential drug targets. The information presented in this paper reveals that there is not just one cause to the disease and proves that it is essential for the further study of the brain and of neurodegenerative diseases. However, it has probably not yet resulted in the detection of the majority of the important brain proteins. Proteomics offers unique possibilities and approaches to



study the brain, and its contribution to the investigation of central nervous system disorders is expected to increase in the future. With some degree of advancement of proteomics, researchers are now focusing on the application of their newfound knowledge. The goal of proteomics is to find a target protein in the drug-discovery process; the search for drug targets can be made easier with the search for proteins that are associated with certain disorders like those already mentioned. For example, if a certain protein is found to be important in a disease, its identification and 3-D structure provides the information to design drugs to interfere with the action of the protein. A molecule that fits the active site of an enzyme, but cannot be released by the enzyme, will inactivate the enzyme. (7)

#### Works Cited

1. Butterfield, D. A., Marzia Perluigi, and Rukhsas Sultana. "Oxidative stress in Alzheimer's

disease brain: New insights." (2005).

2. Butterfield, D. A "Proteomics: a new approach to investigate oxidative stress in Alzheimer's disease brain." Center of Membrane Sciences and Sanders-Brown Center on Aging (2004).

3. Chong, Zhao Z., Faqi Li, and Kenneth Maiseseab. "Stress in the brain: novel cellular mechanisms of injury linked to Alzheimer's disease." (2004).

4. Dobson, C.M. "Protein folding and human disease". Conformational Diseases- A Compendium. The Center for the Study of Emerging Diseases.

5. Fountoulakis, Michael. "Application of proteomics technologies in the investigation of the brain." Center for Medical Genomics (2003).

6. Lubec, Gert, Kurt Krapfenbauer, and Michael Fountoulakis. "Proteomics in brain research: potentials and limitations."

7. Travis, Dunckley, Keith D. Coon, and Dietrich A. Stephan. "Discovery and development of biomarkers of neurological disease." A Neurogenomics Division, The Translational Genomics Research Institute (2005).