J. Chin. Inst. Chem. Engrs., Vol. 36, No. 6, 533-559, 2005

Review paper

An Overview of Alzheimer's Disease

Steven S.-S. Wang[1]
Department of Chemical Engineering, National Taiwan University
Taipei 106, Taiwan

Theresa A. Good[2]
Department of Chemical and Biochemical Engineering
University of Maryland, Baltimore, Maryland 21250, U.S.A.

Abstract—More than twenty different human proteins can fold abnormally resulting in the formation of pathological deposits and several feared degenerative diseases. These proteins lack primary sequence homology, yet they can self-assemble into fibrils with a characteristic cross β-sheet structure. Among them, β-amyloid of Alzheimer's disease is the best characterized. β-amyloid is the principal protein component of senile plaques seen in the brains of individuals diagnosed with Alzheimer's disease and is implicated in the neurotoxicity associated with the disease. Although Alzheimer's disease has been the center of intense research, much remains to be learned concerning the role of β-amyloid and the toxicity mechanisms that mediate its biological responses.

In the current review, the general background information on Alzheimer's disease and β-amyloid are first presented. Next, attention is focused on the relevant research works regarding structural aspect and molecular features of Aβ species as well as β-amyloid fribrillogenesis/aggregation process. Importantly, an emerging issue that an oligomeric intermediate is the primary toxic β-amyloid species is also discussed. Lastly, several β-amyloid-induced toxicity mechanisms are proposed and reviewed. It is our belief that the advances in basic understanding of the conformational changes as well as biological functions of β-amyloid and β-amyloid-elicited toxicity mechanisms will shed light on the development and/or design of potential interfering agents against amyloid formation associated with Alzheimer's disease.

Key Words : Amyloidosis, Alzheimer's disease, β-amyloid, Fibril, Aggregation

AMYLOIDOSIS AND AMYLOID

The amyloidoses are a group of protein conformational or misfolding diseases which arise when a constituent protein or peptide undergoes a fluctuation in shape or change in size, with resultant protein self-assembly and tissue deposition. These amyloidogenic diseases are indeed of enormous importance in the context of present-day welfare and human health. At least twenty different human proteins and peptides, including transthyretin, α-synuclein, calcitonin, β_2-macroglobulin, gelsolin, amylin, atrial natriuretic peptide, and β-amyloid, have been isolated as the fibrillar components of disease-associated amyloid deposits (Kelly, 1998b; Lansbury, 1999; Murphy, 2002; Dobson, 2004; Ross and Poirier, 2004; Uversky and Fink, 2004). These conformational diseases individually have their unique clinical, neuropathological, and biochemical characteristics and their corresponding amyloidogenic precursor proteins have unrelated functions and share no sequence homology (Table 1). However, the molecular events underlying these disease processes appear to be the same. The mechanism involves an aberrant structural transition in a normally innocuous and functional protein (Kelly, 1998a; Lansbury, 1999; Taylor *et al.*, 2002; Dobson, 2004; Ross and Poirier, 2004; Uversky and Fink, 2004).

The pathological conformers in these protein structural diseases are called amyloids. Amyloid is considered a general term delineating protein aggregates and structures of amyloids are recognized to possess several specific tinctorial and physicochemical features in common: exhibition of β-sheet rich secondary structure, a fibrillar morphology, birefringence upon staining with Congo red dye, insolubility in most solvents, and protease-resistance. Extensive protein fibrillogenesis or aggregation often occurs leading to an accumulation of the abnormally folded proteins that damages cells. The formation of the pathological conformers has been shown to be influenced by genetic mutations, protein concentration, chaperones, and environmental factors (*i.e.* the presence of salt, metal ion concentration, and pH of solu-

[1] 王勝仕
[2] To whom all correspondence should be addressed

Table I. A listing of some common amyloidogenic proteins and corresponding diseases.

Disease Type	Key Protein Involved
Alzheimer's disease	Amyloid-β protein
Primary systemic amyloidosis	Immunoglobulin light chain
Senile systemic amyloidosis	Transthyretin
Familial amyloid polyneuropathy I	Transthyretin
Familial mediterranean fever	Serum amyloid A
Secondary systemic amyloidosis	Serum amyloid A
Hemodialysis amyloidosis	β_2-Microglobulin
Finish hereditary systemic amyloidosis	Gelsolin
Type II diabetes	Islet amyloid polypeptide (IAPP)
Medullary carcinoma of the thyroid	Calcitonin
Hereditary renal amyloidosis	Fibrinogen
Injection-localized amyloidosis	Insulin
Atrial amyloidosis	Atrial natriuretic factor (ANF)
Hereditary non-neuropathic systemic amyloidosis	Lysozyme
Familial visceral amyloidosis	Lysozyme

tion) (Taubes, 1996; Carrell and Lomas, 1997; Carrell and Gooptu, 1998; Kelly, 1998a; Lansbury, 1999; Soto, 1999; Ji *et al.*, 2002). Generally, the content of β-sheet secondary structure increases with lower pH, increasing salt concentration, or rising protein/peptide concentration. However, Aβ solution exhibits α-helix rich conformation in membran-mimicking solvents (Fraser *et al.*, 1991, 1994; Barrow *et al.*, 1992; Talafous *et al.*, 1994; Kohno *et al.*, 1996; Ji *et al.*, 2002; Stine *et al.*, 2003; Petkova *et al.*, 2004; Beking *et al.*, 2005).

ALZHEIMER'S DISEASE

Alzheimer's disease (AD) was first described by German neurologist Alois Alzheimer nearly a century ago (Alzheimer, 1907). AD is the most common cause of dementia and also the fourth leading cause of death in the developed countries. It is estimated that approximately 18 million people worldwide suffer from AD and, tragically, nearly thirty-four million people worldwide will be afflicted by AD before 2025 (American Health Assistance Foundation, 2005).

AD is a disease characterized by progressive dementia, gradual memory loss, and decline in cognitive functions and functional capacity. It is considered one of the major diseases of dementia in the western world. The progression of AD is often variable but ultimately leading to death due to infections approximately 10 years after symptoms first appear. Histopathology of the disease consists of senile plaques of β-amyloid accumulation, neurofibrillary tangles, cerebrovascular amyloid deposits, neuronal damage, and loss of synapses (Terry and Katzman, 1983; Selkoe, 1989). AD is an age-dependent disorder that increases in prevalence with age. Currently no effective treatments are directed toward curing this debilitating disease.

Forms of AD

AD is divided into early-onset ('presenile') dementia affecting individuals below 65 years of age and accounting for 25% of cases and late-onset ('senile') dementia (Terry and Katzman, 1983). The disease is also divided into familial and sporadic forms of disease according to family history. Early-onset familial cases make up 10% of all AD patients while late-onset familial cases make up 30% of all AD patients (American Health Assistance Foundation, 2005). Early-onset Alzheimer's has been clearly shown to be genetic in origin. While a mutation on chromosome 19 (where the ApoE gene is located) has been correlated with late-onset AD (the most common form), not everyone having the mutation develops the disease. The relationship between genetics and late-onset Alzheimer's is not fully known (American Health Assistance Foundation, 2005). The mutation of APP on chromosome 21 is linked to early-onset AD (Lendon *et al.*, 1997; Saunders, 2001; Zekanowski *et al.*, 2004), while presenilin 1 (PS1) gene (chromosome 14) and PS 2 gene (chromosome 1) are responsible for 50% of early-onset familial AD (Rogaev *et al.*, 1995; Saunders, 2001; Zekanowski *et al.*, 2004).

Symptoms and diagnosis

Progressive memory loss, problem with adjustment, change in personality, difficulties in communication, and abnormal behaviors are key clinical symptoms of AD. The diagnostic procedure for AD consists of taking a thorough medical history, conducting the Mini Mental State Examination, and checking family members (Alzheimer's Association, 2005).

Until the accomplishment of effective cure for AD, available treatments currently are aimed mainly at retarding the disease progression not reversing the process or halting it completely. Probing the disease in early stages has been a major focus of a number of neuroimaging techniques. One of these techniques is magnetic resonance imaging (MRI), which can be used to detect the size change of some structures in the AD brain, the hippocampus shrinkage in particular. Researchers are exploring exactly how early this shrinkage in hippocampus can be probed. Another set of neuroimaging techniques, such as positron emission tomography (PET) scans and single photon emission computed tomography (SPECT) scans, provides a means to visualize the activity and interactions of some certain brain areas during neural cognitive operations. This window on the living brain can aid in probing early alterations in brain function or structure and further identifying those individuals who are at risk of AD even prior to the development of the symptoms of the disease (Burggren and Bookheimer, 2002; Petrella *et al.*, 2003; Masdeu *et al.*, 2005; Villemagne *et al.*, 2005). Although most of these neuroimaging techniques are currently utilized primarily as research tools, they certainly hold great promise, combined with other diagnostic measures, for the identification of persons at risk of developing AD at early stages.

Treatment

Several acetylcholine esterase inhibitors/drugs such as Cognex, Aricept, and tetrahydroaminoacridine, are designed to fortify the memory and slow the progression of cognitive degeneration in the early stages of AD. These drugs are used to prevent the level of acetylcholine in the brain from dropping acutely. However, the drugs become useless with the disease progression and wasting away of cholinergic neurons. Other potential treatments currently under investigation include: vitamin E (antioxidant), prednisone (targeting inflammatory damage on neurons), premarin (female hormone promoting neuronal survival), calcium channel blockers, and nerve growth factor (Alzheimer's Association, 2002). Although these treatment strategies seem to help a fraction of the patients to some degree, we are currently still far from a real cure (Marx, 1997).

There is a hope that vaccination would serve as a potential treatment for AD. Recent studies showed the deposition of amyloid plaques and associated memory impairment in transgenic mice of AD can be prevented or reversed by active immunization without dramatically changing the total level of Aβ (Janus *et al.*, 2000; Morgan *et al.*, 2000), while passive immunization with prolonged treatment with anti-Aβ antibodies in APP-overexpressing mice can prevent the development of amyloid fibrils (Bard *et al.*, 2000; DeMattos *et al.*, 2001). Since improvement of the disease with immunization/vaccination has been perceived in AD models, it is now considered a potential treatment strategy.

HYPOTHESES OF MAJOR CAUSE OF NEURODEGENERATION IN AD

Aβ accumulation

It is widely believed that the conversion of Aβ into amyloid deposits is a causative event in the molecular pathogenesis of AD. This so-called amyloid hypothesis has been buttressed by a number of evidence in animal model, pathological, genetic, epidemiological, and *in vitro* cell culture studies. Generation of amyloid deposits similar to AD pathology and neuronal death in the brains were observed in transgenic mice with overexpression of APP or fragments of APP carrying familial AD mutations (Games *et al.*, 1995; LaFerla *et al.*, 1995; Masliah *et al.*, 1996; Johnson-Wood *et al.*, 1997; Holcomb *et al.*, 1998; Tandon and Fraser, 2002). Additionally, evidence showed that the activation of microglia and significant loss of neurons were associated with the presence of fibrillar Aβ or Aβ deposits (Weldon *et al.*, 1998; Bornemann and Staufenbiel, 2000; Nagele *et al.*, 2004), suggesting the central role of Aβ accumulation in a cascade of events underlying AD pathogenesis.

Pathologically, a strong correlation has been observed between the dementia of a patient in life and the amyloid burden in the autopsy (Cummings *et al.*, 1996). Genetic mutations in APP, PS1, and PS2 genes, which cause the familial AD, result in a rise in Aβ production (Schellenberg, 1995; Selkoe, 1996; 1997; Holcomb *et al.*, 1998; Kowalska *et al.*, 2004). Evidence from numerous *in vitro* studies has demonstrated the toxicity of aggregated form of Aβ to cultured nerve cells (Pike *et al.*, 1993; Seilheimer *et al.*, 1997; Hartley *et al.*, 1999; Ward *et al.*, 2000; Small *et al.*, 2001; Wang *et al.*, 2001; Plant *et al.*, 2003; Ciccotosto *et al.*, 2004; Michaelis *et al.*, 2005). While there is some disagreement concerning the exact identity of the Aβ aggregated species (Lambert *et al.*, 1998; Hartley *et al.*, 1999; Ward *et al.*, 2000; De Felice *et al.*, 2004; Klein *et al.*, 2004), the toxicity is associated with the species that are part of the Aβ accumulation or aggregation process.

Tau hyperphosphorylation and paired helical filaments

Neurofibrillary tangles form intracellularly in neurons. They appear as dense and insoluble clots

found in or around damaged hippocampal cells (Hyman *et al.*, 1984). The chief component of neurofibrillary tangles is paired helical filaments consiting of the hyperphosphorylated isoform of the microtubule-associated protein tau, which are typical histological lesions found in AD brains (Hyman *et al.*, 1984; Iqbal *et al.*, 2003). The correlation between Aβ deposition and paired helical filament formation has been reported to be responsible for the onset and progression of AD (Gotz *et al.*, 2001; Lewis *et al.*, 2001). Such a correlation suggests a potential link between aggregation state, cytoskeletal abnormalities and cell death. However, the details of the mechanism remain to be further explored.

AD is an inflammatory disorder

Involvement of inflammatory processes in the etiology of AD has recently received considerable attention (Bamberger and Landreth, 2001; Meda *et al.*, 2001; Barger, 2004; Broytman and Malter, 2004; Zhang *et al.*, 2004). Senile plaques contain a variety of inflammatory molecules that are also elements of an inflammatory response, such as heparan sulfate proteoglycan, thrombin (Akiyama *et al.*, 1993), α1-antichymotrypsin (Aisen, 1996), and a few complement components (Rogers *et al.*, 1992). These inflammatory components are produced principally by activated microglia, the brain's version of macrophage. Microglia originate from myeloid lineage and serve as the key immune effector cells in the brain. They are observed to be clustered within and adjacent to senile plaques.

Many lines of evidence showed that microglia exhibit an activated, reactive phenotype and extend ramified processes into the senile plaques in AD brains as compared to the controls (Akiyama *et al.*, 1993; Le Prince, 1993) and that Aβ is responsible for the elevated level of activation. Alternatively, Aβ has been shown to be a chemoattractant for microglia (Davis *et al.*, 1992). In both cases, activated microglia could either phagocytose Aβ or stimulate an inflammatory response that over a period of time could lead to the destruction of surrounding tissue.

Numerous complement components, one type of proinflammatory molecules, have been detected with senile plaques in the AD brain. Typically, classical complement action is initiated via the binding to an activator, including amyloid fibrils. After binding of the first complement protein C1, a cascade of autocatalytic or proteolytic cleavage reactions take place, resulting in the formation of biological active complement proteins. Aβ was shown to bind C1 and activate complement in a number of tests (Rogers *et al.*, 1992; Emmerling *et al.*, 1997; Webster *et al.*, 1997) and this amyloid-dependent activation of the complement cascade eventually culminating in the formation of a membrane attack complex (MAC) (Emmerling *et al.*, 1997; Bradt *et al.*, 1998). In senile plaques, all complement components, including the MAC, can be detected, suggesting that the full classical complement cascade has been activated. Binding interaction between complement components and Aβ fibrils could either up-regulate phagocytosis of Aβ or lead to nonspecific cell lysis.

Clinically, non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, and naproxen commonly used in patients with rheumatoid arthritis, have been shown to significantly ameliorate symptoms of AD (Lim *et al.*, 2000; Yan *et al.*, 2003; Cole *et al.*, 2004; Imbimbo, 2004). The involvement of complement in AD progression is plausible due to increasing experimental support; however, it remains unclear whether this mechanism accounts for the specificity of the disease to given areas of the cortex. Presumably, an immune response to Aβ deposition would occur, but it is unlikely to serve as a primary event of neuronal loss in AD brains.

β-AMYLOID SPECIES

Generation of Aβ

β-amyloid (Aβ) is the principal proteinaceous constituent of senile plaques (amyloid plaques) and vascular amyloid of AD. An increase in production and abnormal accumulation of Aβ in the brain is implicated in the etiology of AD. Aβ is a peptide of 39 to 43 amino acid derived from a much larger integral protein, the amyloid precursor protein (APP) (Haass *et al.*, 1993), which appears to have a membrane spanning region and resembles a glycosylated cell-surface receptor (Kang *et al.*, 1987). Cloning studies (Kang *et al.*, 1987) and biochemical studies (Dyrks *et al.*, 1988; Selkoe *et al.*, 1988) showed that the APP gene encodes at least four alternatively spliced products ranging from 695 to 770 amino acids. These four types of human APPs have 695, 751, 770, and 714 residues, in which the first three are the major isoforms of APPs. Both APP751 and APP770 carry a domain that includes a 56 and 75-amino acid insert with high homology to the Kunitz-type protease inhibitor sequence (KPI) (Kitaguchi *et al.*, 1988) which is capable of inhibiting the proteolytic degreedation of Aβ (Naidu *et al.*, 1995). The Aβ domain is composed of 28 extracellular and 12 to14 transmembrane amino acids of APP (Martins *et al.*, 1991). The combined activities of β- and γ-secretases are required for generating the Aβ protein. Once formed, Aβ is immediately secreted into the culture medium or biological fluids including plasma and cerebrospinal fluid (Haass *et al.*, 1993). The normal processing

of the APP involves cleavage predominately by the secretase enzyme, γ-secretase, which cuts APP in the middle of the β-amyloid sequence and thus prevents the formation of Aβ (Ishiura, 1991). Ninety percent of Aβ in the brain consists of Aβ(1-40) while the remaining ten percent is the more fibrillogenic species Aβ(1-42).

Amyloid precursor protein (APP)

The function of APP or its role in the pathogenesis of AD is less than clear. APP is a highly conserved protein that is produced in most of the tissues throughout the body. It has been reported that protein kinase C can enhance APP secretion via the phosphorylation of an undetermined protein (Buxbaum *et al.*, 1992; Caporaso *et al.*, 1992) and decrease the production of Aβ (Buxbaum *et al.*, 1992; Gabuzda *et al.*, 1993). In addition, persistent depolarization of eurons results in APP cleavage and secretion, implying that APP processing is modulated by neuronal activity (Nitsch *et al.*, 1993). Large numbers of extracellular neuritic amyloid plaques or senile plaques, cerebrovascular amyloids, and to a lesser extent, intracellular neurofibrillary tangles, are the traditional molecular hallmarks found in the brains of AD patients (Selkoe, 1991). Transgenic mice expressing APP gene or fragments of APP gene carrying familial AD mutations exhibit many characteristics of the disease including amyloid plaques, cognitive deficits, and neuronal cell death (Games *et al.*, 1995; LaFerla *et al.*, 1995; Masliah *et al.*, 1996; Kowalska *et al.*, 2004). Genetic and epidemiological observations suggest that mutations in three genes (amyloid precursor protein, presenilin 1, presenilin 2) which cause the relatively rare familial forms of AD that affect the production and amyloidogenicity of Aβ (Selkoe, 1996; Kowalska *et al.*, 2004).

Pathologically, a strong correlation between the dementia experienced by patients in life and the amyloid burden detected upon autopsy was concluded in some careful studies (Cummings *et al.*, 1996). Although, up to date, the precise cause of AD and the mechanisms leading to the formation of these abnormal protein clusters remain unclear, they are believed to be involved in the pathogenesis of AD. Numerous lines of supporting evidence has pointed to the amyloid cascade hypothesis of Aβ formation, specifically highly ordered Aβ plaques (fibrils), as the central player in the molecular pathogenesis of AD.

BIOLOGICAL EFFECTS OF Aβ: Aβ-INDUCED NEUROTOXICITY

Accumulating evidence has demonstrated that Aβ is toxic to nerve cells both *in vivo* and *in vitro* (Delobette *et al.*, 1997; Nitta *et al.*, 1997; Shin *et al.*, 1997; Giovannelli *et al.*, 1998; Wang *et al.*, 2001, 2002; Plant *et al.*, 2003; Ciccotosto *et al.*, 2004; Michaelis *et al.*, 2005). The majority of toxicity studies have been performed on primary cultures of rat hippocampal neurons typically obtained from fetal tissue. However, studies have found that in mouse model of AD, AD-like symptoms do not develop in young animals (Games *et al.*, 1995). Developmental differences in tissues may bias the results from these toxicity studies. Rat PC12 and human SH-SY5Y cells, two neuron-like cell lines, have also been widely used in toxicity studies and were found to be susceptible to Aβ-induced neurotoxicity (Iversen *et al.*, 1995; El-Agnaf *et al.*, 2000; Blanchard *et al.*, 2002; Datki *et al.*, 2003; Sung *et al.*, 2003; Datki *et al.*, 2004; Wang *et al.*, 2005a). It has been suggested that microglial cells kill neurons upon activation by Aβ (Roher *et al.*, 1996; Wegiel *et al.*, 2000). In the past, with the exception of studies on transformed cell lines, microglia cells may or may not be present and have generally been ignored in *in vitro* cultures. Thus, the correlation between *in vitro* assays of toxicity and the *in vivo* disease process remains open to question.

Different Aβ peptide fragments have been used in these studies. In general, Aβ(1-16) was thought to be too short to aggregate. Aβ(1-28) is less toxic than other longer sequences. Aβ(25-35) was found to aggregate readily and elicit neurotoxic effects in many (Pike *et al.*, 1993; Weiss *et al.*, 1994; Xu *et al.*, 2001) but not all of the studies (Games *et al.*, 1992; Stein-Behrens *et al.*, 1992). The full-length sequence, Aβ(1-40), is capable of forming aggregates and the its aggregation is positively correlated with Aβ-induced toxicity (Pike *et al.*, 1993; Tomiyama *et al.*, 1994; Roher *et al.*, 1996; Cribbs *et al.*, 1997; Wang *et al.*, 2001, 2005; Xu *et al.*, 2001; Schuster *et al.*, 2005).

It should be pointed out that some recent studies demonstrated the toxic effect of smaller Aβ oligomeric species on neurons (Lambert *et al.*, 1998; Hartley *et al.*, 1999; Ward *et al.*, 2000; Klein *et al.*, 2001, 2004; De Felice *et al.*, 2004), suggesting an important and surprising concept that fibrillar species might not be the only neurotoxic form of Aβ and perhaps not even the most significant form for AD.

MOLECULAR AND STRUCTURAL ASPECTS OF Aβ SPECIES AND FIBRILLOGENESIS

Because aggregation or deposition of Aβ has been reported to be closely related to the biological effects or pathology of AD, understanding the aggregation of Aβ on a molecular basis might aid in the

development of new approaches to eliminate or reduce the destructive effects of Aβ (Jarrett *et al.*, 1993; Soreghan *et al.*, 1994; Shen and Murphy, 1995; Lomakin *et al.*, 1996).

Structure and conformation of Aβ species

The sequence of soluble (monomeric) Aβ peptides can be generally divided into four regions, two hydrophobic segments from 17-21 and 29-42, and two hydrophilic segments from 1-16 and 22-28. Two turns are predicted between residues 6 and 8, and residues 23 and 27 (Lynn and Meredith, 2000; Serpell, 2000).

Secondary structure or conformation of Aβ has been suggested to be the key determinant of structure changes in the peptide that would accompany the aggregation process (El-Agnaf *et al.*, 1998; Frears *et al.*, 1999; Demeester *et al.*, 2000, 2001; Festy *et al.*, 2001). As opposed to α-helix and random coil, the β-sheet secondary structure has been found to be associated with insolubility and protease resistance (Nordstedt *et al.*, 1994; Jacchieri 1998). Generally, soluble Aβ species has predominately a random coil or α-helical structure, aggregated Aβ species adopts a higher degree of β-sheet content, which has been suggested to correlate with neurotoxicity of Aβ (Simmons *et al.*, 1994). The secondary structure or conformation of Aβ peptide has proven to be exquisitely sensitive to its environment, including ionic strength, temperature, concentration, pH, and solvent components. In addition, different portions/primary sequences of Aβ peptide may exhibit different conformations even though those external environmental factors stay the same. Fraser *et al.* (1991) reported the pH-dependent behavior in Aβ conformational transition. Their results in Aβ(1-28) showed that largely random coil conformations were observed at either acidic or basic pH, while β-sheet assemblies dominated in the intermediate range. It has been shown that Aβ(1-42) were primarily β-sheet (~90%) in phosphate-buffered saline at pH 7.4. Under the same experimental condition, Aβ(1-39) adopted a mix of 50% random coil and 50% β-sheet conformation, while Aβ(1-28) peptide was virtually in 100% random coil conformation (Barrow and Zagorski, 1991). Similar dependency of pH on Aβ conformation was also observed in other studies (Ji *et al.*, 2002; Stine *et al.*, 2003).

Aβ conformation has been shown to be dependent of salt concentration. It was found that Aβ peptide fragments were soluble in water and adopted a dimeric form (Hilbich *et al.*, 1991). The secondary structure of the peptide was primarily β-sheet conformation. Upon addition of salt, large fraction of precipitates and small fraction of soluble peptide retained β-sheet and random coil conformations, respectively. Aβ secondary structure was also demonstrated to be concentration-dependent. Results from a thermodynamic study revealed that Aβ(25-35) underwent a reversible transition between monomeric random coil and oligomeric β-sheet in a concentration-dependent manner (Terzi *et al.*, 1994). It was observed in Aβ(1-28), Aβ(1-39), and Aβ(1-42) that a decreased percentage of intermolecular β-sheet content and an increased percentage of α-helical content as the concentrations of fluorinated alcohols such as trifluoroethanol or hexafluoro-2-isopropanol (Barrow and Zagorski, 1991; Barrow *et al.*, 1992; Sticht *et al.*, 1995) increased. In addition, previous studies of Aβ(1-40) showed that α-helix was the major structural motif in dimethyl sulfoxide (DMSO) (Snyder *et al.*, 1994) and sodium dodecyl sulfate (SDS). It was also reported that the percentage of β-sheet content of Aβ peptides varied in different solvents (Shen and Murphy, 1995; Crescenzi *et al.*, 2002).

Factors of aggregation process

Aβ is secreted as a monomer *in vivo*. Under either *in vivo* or *in vitro* environment, Aβ spontaneously self assembles into fibrils. It has been shown that Aβ aggregation process depends on pH of solution, temperature, Aβ peptide concentration, the presence of salt, and mixing protocol (Fezoui and Teplow, 2002; Kawooya *et al.*, 2003; Stine *et al.*, 2003). Stable monomers of Aβ can be formed, *in vitro*, by dissolving the peptide in 8M urea, 100% (v/v) DMSO, TFE, HFIP or in detergents such as SDS (Coles *et al.*, 1998; Szabo *et al.*, 1999; Gursky and Aleshkov, 2000). When dissolved in water, Aβ is observed to undergo a random coil to β-sheet conversion that can be controlled by temperature, pH, and peptide concentration (Szabo *et al.*, 1999; Gursky and Aleshkov, 2000). At low temperatures (0°C), the transition to β-sheet is not accompanied by dimerization or aggregation of the peptide (Gursky and Aleshkov, 2000). At low concentrations (10 μM) and in 10% DMSO, formation of stable Aβ dimers occurs (Garzon-Rodriguez *et al.*, 1997). Whether in water or physiological buffers, under higher concentrations and/or high temperature, reversible aggregation into higher molecular weight species may occur (Shen and Murphy, 1995; Gursky and Aleshkov, 2000). Moreover, the effect of dissolving solvents on the kinetic behavior of Aβ aggregation/fibril formation was quantitively examined (Wang *et al.*, 2005c).

Several studies have focused on identifying certain primary sequences or particular amino acids that are critical to Aβ aggregation. The C-terminus of Aβ has been shown to play a role in rate of aggregation. Studies using variants with different C-termini showed that shorter C-terminus slowed the aggregation process (Burdick *et al.*, 1992; Jarrett *et al.*, 1993;

Walsh *et al.*, 1997). Several lines of evidence indicate that the central hydrophobic cluster region Leu17-Ala21 is important for β-sheet formation as well as Aβ aggregation (Wood *et al.*, 1995; Tjernberg *et al.*, 1996; Mansfield *et al.*, 1998; Frears *et al.*, 1999; Pallitto *et al.*, 1999). Introduction of substitutions of two hydrophobic phenylalanine residues in positions 19 and 20 (Hilbich *et al.*, 1991; Esler *et al.*, 1996; Mansfield *et al.*, 1998) or Lys16 (Hilbich *et al.*, 1991) produced a peptide with high solubility in aqueous buffers and no propensity for aggregation. Substitution of V18 in Aβ(1-40) led to an enhanced propensity for α-helical conformation and a decreased ability to aggregate (Soto *et al.*, 1995). Abolished aggregation was observed in Aβ(15-23) and Aβ(12-26) as the replacement of a single amino acid with proline within the region 17-23 (Wood *et al.*, 1995). Furthermore, the segment ranging from 17 to 21 has been proposed to be a potential target for aggregation inhibitors (Pallitto *et al.*, 1999). Residues 14-23 are necessary for long fibril formation (Tjernberg *et al.*, 1999).

It has been suggested that not all N-terminal region of Aβ is necessary for aggregation (He and Barrow, 1999). Truncated peptide fragments from N-terminus of Aβ indicated that residues 1-9 were not necessary for aggregation (Hilbich *et al.*, 1991). However, residues between 11 and 16 are probably critical to the conformation stability responsible for aggregation (Mansfield *et al.*, 1998; He and Barrow, 1999).

Mechanisms of aggregation process/ fibrillogenesis

Nucleation-dependent growth

In vitro work has led to the proposal of nucleation-dependent pathway in amyloid fibrillization (Jarrett and Lansbury, 1992; Jarrett *et al.*, 1993; Lomakin *et al.*, 1996). This mechanism primarily involves three main steps: (1) a slow nucleation phase, in which Aβ undergoes a series of thermodynamically unfavorable association steps to generate an ordered oligomeric nucleus, (2) a growth phase, involving the nucleus which readily grows to form larger polymeric species, and (3) a steady state phase, where the ordered aggregated species and the monomeric species are at equilibrium. Results showed that no detectable turbidity was perceived after a few days (with a longer lag time) with Aβ(1-39) or Aβ(1-40) at 20 μM (below the critical concentration), whereas Aβ(1-42) solution with identical concentration formed aggregates or fibrils readily. Other sedimentation results also supported this point (Burdick *et al.*, 1992). Apart from the elimination of lag time, the elongation rate of Aβ(1-39) or Aβ(1-40) was drastically enhanced and the aggregation kinetics of these fragments were observed to follow the first order kinetics as seeded with a trace of Aβ(1-42). A similar conclusion was also established by others (Esler *et al.*, 1996; Naiki and Nakakuki, 1996).

Other researchers (Snyder *et al.*, 1994) have also found that the aggregation kinetics of Aβ(1-40) and Aβ(1-42), alone and in combination, were tremendously different. The aggregation rate of Aβ(1-42) was retarded by adding monomeric Aβ(1-40) as compared with Aβ(1-42) alone. However, the mixture of Aβ(1-40) and Aβ(1-42) was still aggregated faster than Aβ(1-40), suggesting that aggregation kinetics was determined by Aβ(1-40).

Amyloid fibril fromation model

The model is proposed in the work of Shen and Murphy (1995). Fibrils are initiated from nuclei formed by monomers of Aβ and could either elongate by adding of partial fragments (*i.e.* entire nuclei, small fibrils), or dimers to lengthen the fibril ends. The same research group (Tomski and Murphy, 1992; Shen and Murphy, 1995) has quantified changes of fibril dimensions with time, and also developed mathematical models to verify this mechanism of amyloid fibrillization. Other investigators have proposed conceptually somewhat similar models, but did not account for the fibril-fibril interactions, or reactions among the variety of known oligomeric species in existence. Only one other investigator has constructed a mathematical model of fibril assembly (Lomakin *et al.*, 1996), in which monomers of Aβ form a micelle, from which nuclei are spontaneously released. Monomers of Aβ then add to the nuclei, resulting in fibril elongation. The model, although capable of describing the sedimentation data on which it was based, still does not explain the fibril elongation observed during light scattering experiments (Shen and Murphy, 1995).

Micelle formation

Other investigators proposed a micelle formation hypothesis to describe the mechanism of amyloid aggregation (Soreghan *et al.*, 1994). They found that Aβ displayed properties commonly associated with surfactants or detergents that form micelle in solution and also lowered the surface tension of water. Different degrees of surface tension reduction were observed in various Aβ analogs, depending on whether these analogs contained the hydrophobic residue 29-43. In addition, based on the SDS-PAGE and gel filtration analyses, the results showed that, with the exception of Aβ(1-40), Aβ(1-41), Aβ(1-42), Aβ(1-43) formed SDS-resistant aggregates with mass of about 16 kDa. On the other hand, Aβ(1-40) exhibited higher molecular weight aggregates in gel filtration results. Furthermore, two populations, a

dimer species and a larger aggregate species, were observed in both Aβ(1-40) and Aβ(1-42) solutions at concentrations above the critical concentration of 25 μM.

This model postulates that Aβ peptide was organized into a "tubular micelle", known as a hexagonal I type phase. The polar domain (approximately the first 28 residues) formes the outer wall of the amyloid fibril, while the hydrophobic domain forms the inner wall surrounding the center of the fibril. This tubular micelle model, although consistent with the β-crystallite model deduced from X-ray fiber diffraction (Inouye *et al.*, 1993), does not explain the cross β-sheet conformation.

Advanced glycation modification

Vitek *et al.* (1994) postulated that the deposition and the accumulation of Aβ in AD might be triggered by the formation of advanced glycation end products (AGEs), which have been implicated in amyloidogenesis of β2-microglobulin associated with hemodialysis-assocaied amyloidosis (Miyata *et al.*, 1994). The thioflavin T fluorescence results indicated that aggregation rate induced by AGE-Aβ(1-40) was about ten times faster than that induced by preformed aggregated Aβ(1-40). Based on this AGE modification model, Aβ aggregation can be induced by chemical modification of the peptide.

While numerous models have been proposed, the great detail of the aggregation process remains to be unequivocally discovered. Exploring, at the amino acid residue level, which parts of monomeric, oligomeric, and fibrillar Aβ species are accessible to the environment media/solvent will aid in recognizing a critical contact sites that are pivotal to aggregation. Additionally, elucidating, at the residue level, how Aβ-Aβ interaction or Aβ self-associate in the aggregation event will certainly provide new targets for the development of aggregation modifiers that could potentially inhibit the neurotoxicity elicited by Aβ. Hydrogen/deuterium exchange (HX) technique provides significant insight into structure information by determining the portions of the peptide backbone that are protected from H/D exchange. Hydrogen/deuterium exchange along with mass spectrometry (MS) or nuclear magnetic resonance spectroscopy (NMR) has proven to be a relatively powerful tool for mapping residue-level aggregation of Aβ (Kheterpal *et al.*, 2000, 2001, 2003a, 2003b; Wang *et al.*, 2003b; Whittemore *et al.*, 2005; Williams *et al.*, 2005). Wetzel and coworkers reported that HX-MS was able to demonstrate the structural difference(s) among monomeric, protofibrillar, and fibrillar Aβ species by their different protection patterns. Their results further showed that, relative to Aβ monomer, approximately 40% and 60% of the backbone amide hydrogens resistant to H/D exchange in protofibrillar and fibrillar species, respectively (Kheterpal *et al.*, 2000, 2003a). In order to reveal the degrees of protection from exchange in different regions of the full-length Aβ, the proteolytic digestion was employed after HX. Wang *et al.* (2003b) found that the N-terminus (residues 1-4) was completely unprotected from exchange and the fragment containing residues 5-19 was over 50% protected from exchange while the C-terminal segment was approximately 35% protected in the fibrillar Aβ. However, recent HX-NMR data presented a good solvent accessibility of the C-terminus of Aβ (Whittemore *et al.*, 2005). Further investigations are needed to strengthen our knowledge concerning the amyloid fibril structure and the fibril formation process.

Role of size/aggregation state of Aβ species in the Aβ-induced neurotoxicity

The relationship between structure and toxicity has been well documented for β-amyloid of AD. In aggregated solutions (containing fibrils, protofibrils, and low molecular weight species) Aβ has been consistently shown to be toxic to different cultured nerve cells such as PC12 cells, primary mixed brain cells and B12 cells (Yankner *et al.*, 1990; Busciglio *et al.*, 1993; Howlett *et al.*, 1995; Seilheimer *et al.*, 1997; Ward *et al.*, 2000). While there has been some disagreement as to the exact structure of the aggregated species associated with toxicity, whether it be a protofibril (Hartley *et al.*, 1999; Ward *et al.*, 2000), a diffusible, non-fibrillar ligand (Lambert *et al.*, 1998; Klein *et al.*, 2001), some other low molecular weight intermediate (Hartley *et al.*, 1999), or spherical aggregates (Hoshi *et al.*, 2003), it is certain that toxicity is associated with peptide structures that are part of an aggregation pathway associated with amyloid fibril formation.

Several investigators have attempted to characterize the size of the toxic Aβ species (Lambert *et al.*, 1998; Hartley *et al.*, 1999; Walsh *et al.*, 1999; Ward *et al.*, 2000; Klein *et al.*, 2001, 2004; Hoshi *et al.*, 2003). Toxicity was attributed to a non-fibrillar species with molecular weight ranging from 17 to 42 kD (Lambert *et al.*, 1998; Klein *et al.*, 2001, 2004) or with hydrodynamic radii between 3 and 8 nm (Chromy *et al.*, 2003). These oligomers, referred to as the slowly sedimenting Aβ-derived diffusible ligands (ADDLs), were formed via incubation of Aβ(1-42) with clusterin (Apo J) or via incubation at low temperatures, and were not associated with Aβ fibril formation or an aggregation pathway. ADDLs were showed to be toxic at nanomolar doses, when bound to cell surface toxin receptors and Fyn, a non-receptor protein tyrosine kinase overly expressed in AD, leading to a disruption in long-term potentiation

(LTP) and synaptic plasticity.

Other researchers further confirmed the nature of toxic Aβ oligomers in the brain (Walsh *et al.*, 2002). Their results showed that these Aβ oligomeric species were produced soon after generation of the peptide within certain intracellular vesicles and are subsequently secreted from the cell. Microinjection of cell medium containing both Aβ oligomers and monomers markedly blocked hippocampal LTP in rats *in vivo*. Immunodepletion from the culture medium of all Aβ species entirely prevented the inhibition of LTP. Cell medium treated with insulin-degrading enzyme, which selectively degraded monomeric Aβ, did not prevent the block of LTP, suggesting the toxic effect of these low molecular weight Aβ oligomers (Walsh *et al.*, 2002). Besides, Ward and coworkers have performed fractionation of aggregated (fibrillar) Aβ and then measured its biological activity (Ward *et al.*, 2000). They used density-gradient centrifugation to isolate different fibrillar forms of Aβ including protofibrils, fibrils, and low molecular weight oligomers. The hydrodynamic radii of the protofibrillar species were characterized by photon correlation spectroscopy to be on the order of 22-35 nm and 97-367 nm, whose corresponding molecular weights would be significantly greater than 42 kD. The species were found to be toxic as determined via a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. It was concluded that the detectable effects on cell viability were found to correspond to the presence of protofibrillar and fibrillar structures, but not to the monomeric species.

Walsh *et al.* (1999) used size exclusion chromatography (SEC) to fractionate fibrillar Aβ into low molecular weight species (LMW), protofibrils, and fibrils, and found that protofibrillar intermediates, not the LMW Aβ, significantly affected the physiology, as evaluated via the alteration in neuronal MTT metabolism, of the primary rat cortical neurons in a concentration-dependent manner. However, Hartley *et al.* (1999), comparing the biological effects of different Aβ assemblies on primary mixed brain cultures, suggested that low molecular weight Aβ (with hydrodynamic radius of 1.5 to 2 nm) and prefibrillar or protofibrillar Aβ (4 to 100 nm diameter, length under 200 nm) were toxic as assessed via lactate dehydrogenase release after 3 days of exposure, while, only protofibrils induced acute rise in neuron electrical activity, for instance, action potentials and excitatory postsynaptic currents. Authors pointed out that these findings were consistent with the results that some transgenic mice over-expressing human APP have exhibited altered electrophysical responses before or during the initiation of Aβ fibril formation (Holcomb *et al.*, 1998; Chapman *et al.*, 1999; Hsia *et al.*, 1999).

Other than protofibrils, fibrils, and ADDLs, recent findings showed that spherical aggregates ASPDs with radus of 10-15 nm acquired the highest neurotoxicity (Hoshi *et al.*, 2003). This toxic ASPD species was highly correlated to SDS-resistant oligomeric bands in Western bloting. An elevation in TPKI/GSK-3β activity was observed on neuronal culture treated with ASPDs. This ASPD-induced TPKI/GSK-3β activity and resultant cell death were inhibited by lithium in a dose-dependent manner. Additionally, Aβ-elicited toxicity was still detected even with the addition of the β-sheet breakers, suggesting that the formation route of ASPDs might be different from that of Aβ fibrils (Hoshi *et al.*, 2003). Recently, using oligomer-specific recognition antibody, an important report demonstrated that the soluble oligomeric species of various amyloid diseases exhibited a unique conformation-dependent structure that was different from those in low molecular weight Aβ and fibrillar Aβ species (Kayed *et al.*, 2003). Also, judging from toxicity experiments, the authors commented that the soluble oligomeric species perhaps served as the actual culprit responsible for toxicity mechanism of Aβ (Kayed *et al.*, 2003).

It should be noted, however, that during the time course of a toxicity experiment, protofibrillar species were observed to convert both into monomeric or dimeric Aβ species and into fibrillar species (Walsh *et al.*, 1999). Due to the dynamic structure of Aβ, it has been fairly difficult to determine definitely and precisely which species are toxic. By taking advantages of the plaque-forming assay in virology and the diffusion concept, a new diffusion-based method was developed to simultaneously separate and detect the toxic Aβ oligomeric species formed during the Aβ aggregation process (Wang *et al.*, 2002). This method provides a new estimate of the smallest species associated with Aβ-induced neurotoxicity, a species with hydrodynamic radius of ~10 nm, which are considerably smaller than typical fibrils and protofibrils (Wang *et al.*, 2002).

Most importantly, identifying the size and/or structure of the Aβ species responsible for toxicity will aid in the development of agents that can intervene the aggregation pathway of Aβ and prevent the formation of toxic species either from preformed plaques or from newly synthesized and proteolytically cleaved monomeric/dimeric/oligomeric Aβ species.

MECHANISMS OF Aβ-INDUCED NEUROTOXICITY

The detailed mechanisms involved in the Aβ-elicited neurotoxicity remain elusive, but a variety of

hypotheses regarding the mechanisms of the toxicity induced by Aβ have been proposed in the literature. These mechanisms include membrane depolarization (Whitson *et al.*, 1994), membrane destabilization (Kilsdonk *et al.*, 1995; Neufeld *et al.*, 1996), pore formation (Arispe *et al.*, 1993a, 1993b, 1994a, 1994b), free radical generation (Butterfield *et al.*, 1999; Varadarajan *et al.*, 2000), Aβ-induced alterations in intracellular signaling (Ferrari-DiLeo *et al.*, 1995; Soomets *et al.*, 1999), ion channel function (Weiss *et al.*, 1994; Good, 1996; Good *et al.*, 1996), and calcium homeostasis (Mattson *et al.*, 1993; Weiss *et al.*, 1994; Good, 1996; Good *et al.*, 1996).

Free radical generation

The over-production of reactive oxygen species (ROS) and free radicals has been widely accepted as one of the key theories underlying the Aβ-induced toxicity mechanism (Butterfield, 1997; Carr *et al.*, 1997; Markesbery, 1997; Holscher, 1998). A correlation between the Aβ peptides and the production of ROS and free radicals has been demonstrated in various *in vitro* experiments (Davis, 1996; Pereira *et al.*, 1999), in transgenic mice models of AD (Pappolla *et al.*, 1998), and in AD brain (Yatin *et al.*, 1999a, 1999b).

Butterfield et al., using electron paramagnetic resonance (EPR) and spin tapping techniques, showed that Aβ generated free radicals in solution. Aβ(25-35) rapidly quenched spin labels within prepared synaptosomal membranes, but did not quench labels on the surface of the membrane (Butterfield *et al.*, 1994). Subsequent study showed that Aβ(25-35) produced a detectable free radical in solution within minutes, while full-length peptide Aβ(1-40) generated free radicals only after 3 to 24 hours, depending on the solvent type. Aβ(35-25), the non-neurotoxic counterpart of Aβ(25-35) also produced a comparable EPR signal by an oxygen dependent reaction (Hensley *et al.*, 1994). Mass spectroscopic measurement suggests that the peptide fragmentation occurs along the same time scale as free radical production. The identity of the peptide fragments is still undetermined. ROS generated by Aβ peptide had a short life-time (Mattson and Goodman, 1995). Preincubation of the peptide prior to measurement yielded no ROS production. In addition, different lots of Aβ(1-40) showed different patterns of free radical formation that correlated with toxicity (Aksenov *et al.*, 1995). Besides Aβ peptides, other amyloid-forming peptides including amylin and β2-microglobulin, also caused ROS production and increased the level of intracellular calcium ion $[Ca^{2+}]_i$ (Mattson and Goodman, 1995).

Generation of ROS could be associated with increased intracellular concentration of Ca^{2+} (Mark *et al.*, 1995). It was reported that exposure of rat hippocampal neurons or synaptosomes to Aβ(25-35) or Aβ(1-40) led to a reduction in Na/K-ATPase activity and Ca^{2+}-ATPase activity, respectively. The decline in ATPase activity in cultured neurons closely paralleled the rise in $[Ca^{2+}]_i$. Impairment of the Na/K exchanger or Ca^{2+} pumps from cells would lead to a loss of intracellular ion gradient, depolarization of the membrane, and increase in concentration of intracellular Ca^{2+}. Antioxidants, such as vitamin E and propylgallate, function as free radical scavengers that attenuate the impairment of Na/K-ATPase activity, impede the rise in $[Ca^{2+}]_i$ and prevent neurotoxicity induced by Aβ peptide (Mark *et al.*, 1995), all of which are suggestive of a role for ROS. The conclusion that Aβ-induced neurotoxicity could be inhibited by free radical scavengers was also suggested in other studies (Goodman and Mattson, 1994a, 1994b; Behl *et al.*, 1995).

Free radical production and ROS may indeed be involved in the pathogenesis of AD (Lockhart *et al.*, 1994). Until further evidence can be found to support these results, there is still too much uncertainty regarding the appropriateness of the findings and their consistency with the pool of literature to consider the free radical hypothesis of Aβ-mediated toxicity an acceptable proposal.

Membrane perturbation

The importance of the interaction(s) of most amyloid proteins including Aβ with membranes has been drawing a lot of attentions in the mechanism of Aβ-induced neurotoxicity. A variety of evidence demonstrates that plasma membrane may play a potential role, either by interaction with or perturbation by Aβ, in the Aβ-mediated neurotoxicity associated with AD (Ginsberg *et al.*, 1993; Mason *et al.*, 1993; Roth *et al.*, 1995; Terzi *et al.*, 1997; Wood *et al.*, 2003).

Aβ has been shown to decrease the fluidity of membranes derived from human lymphocytes, cerebral cortex, hippocampus, striatum, cerebellum and rat cerebellum embedded with DPH in a concentration-dependent fashion (Hartmann *et al.*, 1994; Muller *et al.*, 1995). In addition, immunostaining of cultured neurons incubated with Aβ by antibodies to β(1-40) and β(1-38) fragments clearly depicted aggregated species at the plasma membrane (Muller *et al.*, 1995). Subsequent study suggested that the longer fragments had greater impact on membrane fluidity than the shorter fragments under the same condition. Furthermore, the effect of Aβ peptides on the fluidity of phospholipid bilayer was observed to be greater in the hydrocarbon core than the hydrophilic heads (Muller *et al.*, 1998). Kremer *et al.* (2000) examined the size of Aβ species and its asso-

ciation with aggregation state, hydrophobicity, and changes in the membrane fluidity. Via light scattering and bis-ANS fluorescence, the authors noted that considerably greater aggregation rate and surface hydrophobicity were observed to occur at pH 6.0. Moreover, these two properties were strongly correlated with the extent of reduction in membrane fluidity measured in model lipid vesicles embedded with DPH. Recently, it has been observed that Aβ perturbed membranes of rat, mouse, and human brain resulted in enhanced membrane-bound DPH anisotropy (inversely correlated with membrane fluidity). They also showed that membrane-disordering or perturbing effect of Aβ occurred not only on synaptic plasma membranes (SPM) but also on mitochondrial membranes (Muller *et al.*, 2001).

In contrast, other researchers reported a rise in membrane fluidities (annular or bulk) induced by Aβ(1-40) or Aβ(25-35) in biological or model membrane embedded with the fluorescent dye pyrene via the fluorescence-based method (Avdulov *et al.*, 1997; Mason *et al.*, 1999; Chochina *et al.*, 2001). The lipid peroxidation was observed to be correlated with the fluidizing action of Aβ peptides (Avdulov *et al.*, 1997). Mason *et al.* (1999) explored the distribution and fluidizing action of soluble and aggregated Aβ species in rat SPMs by using small angle X-ray diffracttion and fluorescence spectroscopy for quantifying bulk and annular fluidities. The fluorescence anisotropy data presented an increase in both bulk and annular fluidities of SPM induced by both soluble and aggregated Aβ(1-40). Also, according to the electron density profile, the authors postulated that the soluble species were intercalated into a membrane hydrocarbon core, while aggregated species interacted with membrane bilayer at the headgroup/water interface and thus led to dramatic reorganization of the lipid bilayer.

Consistent with the observation that Aβ interacts with the hydrophilic headgroups of phospholipid membranes, the interaction between phospholipid membranes and both Aβ(25-35) as well as Aβ(1-40) through the electrostatic-mediated force was observed (Terzi *et al.*, 1994, 1995, 1997; Seelig *et al.*, 1995). Using circular dichrosim spectroscopy, titration calorimetry, and ultracentrifugation, investigators proposed the role of plasma membrane in the process of aggregation (Terzi *et al.*, 1994, 1995; Seelig *et al.*, 1995). It was hypothesized that both Aβ(25-35) and Aβ(1-40) exhibited cooperative and reversible random coil to β-sheet transition. However, the concentration-dependent conformational conversion between random coil and β-sheet structure shifts almost completely toward the β-sheet structure upon the addition/binding of negatively charged, small unilamellar lipid vesicles. The binding isotherms were exothermic and the apparent binding constants for both peptide fragments were also determined. The authors concluded that the presence of the lipid membrane served as a catalyst to Aβ aggregation which occurred at the lipid/water interface. The local elevation of peptide concentration was the result of cationic peptide attraction to negatively charged membrane surface (Terzi *et al.*, 1994, 1995; Seelig *et al.*, 1995). Researchers also tested the effects of several membrane process-related compounds on the Aβ-induced neurotoxicity and demonstrated that polyhydroxylated aromatic dipolar compounds such as phloretin, exifone, and phenolphthalein prevent association of Aβ to negatively charged lipid vesicles and Aβ-mediated neurotoxicity (Hertel *et al.*, 1997). All the aforementioned findings implied that there is a nonspecific dipole-dipole interaction between Aβ and cell membranes (Hertel *et al.*, 1997). Moreover, due to identical maximum chemical shielding anisotropy and similar ordering and average orientation of the labeled headgroup observed with and without Aβ from phosphorus-31 and deuterium NMR studies, the same authors concluded that Aβ binds electrostatically to the outer envelope of the polar headgroup region but does not penetrate through the polar groups (Terzi *et al.*, 1997).

However, there have been experiments suggesting that Aβ might be incorporated into the membrane rather than interacting with the membrane surface or the polar lipid head groups. This idea was supported by the work of Butterfield *et al.* (1994) in which Aβ(25-35) was found capable of reacting with a spin label, quenching it, when the label was within the membrane, but not when the label was on the surface.

Additonally, there have been other studies suggesting that Aβ(1-40) formed ion channels in cellular membranes (Arispe *et al.*, 1993a). These experiments proposed that Aβ inserted into lipid vesicles upon sonication of the vesicles. The liposomes containing Aβ peptide were then fused with a planar bilayer, and the conductance of the membrane was measured under various conditions. Discrete change in ion channel activity (conductance) was measured a few minutes after the addition of Aβ-liposome to the planar bilayer. The ion channels formed were permeable to cations including Ca^{2+} (Arispe *et al.*, 1993a; Lin *et al.*, 1999). Researchers also reported giant conductances in cation channels formed by Aβ and concluded that Aβ peptide was able to form similar channels irrespective of the mechanism involved in the insertation of Aβ into cell membrane, providing supportive evidence that cell death might be due to amyloid ion channel activity (Arispe *et al.*, 1993a, 1993b; 1994b, 1996; Pollard *et al.*, 1995). Besides, a study was proposed to describe numerous channel subtypes with properties likely to cause toxicity (Kourie *et al.*, 2001).

Fragments with neurotoxicity such as Aβ(1-42) and Aβ(25-35) were also shown to form Ca^{2+}-permeable pores on lipid bilayers (Mirzabekov *et al.*, 1994; Rhee *et al.*, 1998; Hirakura *et al.*, 1999). Both Aβ(1-42) and Aβ(25-35) have been demonstrated to readily insert into planar bilayer membranes to establish slightly cation selective, voltage dependent, ion channels. In addition, it has been found that C-terminal fragment of APP was able to change the cellular ionic activity either via interaction with existing channels or through *de novo* ion channel formation (Fraser *et al.*, 1997). It was also found that Aβ activated a similar conductance, but only upon transition from random coil to β-sheet (Li *et al.*, 1995). If the peptide were over-aged (already β-sheet structure), it could not have caused the conductance, nor would it have caused the conductance if the peptide were freshly prepared and hence random coil in configuration. Only if the peptide underwent the conformational transition from random coil to β-sheet would the conductance occur. Species of Aβ that were over-aged, and hence not capable of causing the conductance, were still toxic to neurons, which makes the relevance of this Aβ induced-conductance somewhat ambiguous (Li *et al.*, 1995).

Taken together, based upon the findings in aforementioned studies, there seems to be a link between ion channel formation and AD and Aβ does interact with cell membranes. However, the detail of this hypothesis of ion channel formation needs to be thoroughly explored.

Role of Cell Membrane Composition in Aβ-induced Neurotoxicity

Mounting evidence indicates that the neuronal cell membrane constituents, either in the cell membrane or in culture medium, are pivotal to the mechanism of Aβ toxicity. Studies have indicated that membrane components such as cholesterol and gangliosides altered the affinity of Aβ or other amyloid proteins for phospholipid membranes (Choo-Smith *et al.*, 1997; Ariga and Yu, 1999; Avdulov *et al.*, 1999; Matsuzaki and Horikiri, 1999; Wang *et al.*, 2005b). Once associated with membranes, negatively charged phospholipids, cholesterol, and gangliosides have been shown to increase the β-sheet content and/or rate of aggregation of Aβ or bovine calcitonin, another amyloid protein (Choo-Smith *et al.*, 1997; McLaurin *et al.*, 1998; Del Mar Martinez-Senac *et al.*, 1999; Matsuzaki and Horikiri, 1999; Mizuno *et al.*, 1999; Wang *et al.*, 2005b). Both *in vivo* and *in vitro* studies elucidated that the inhibition of cholesterol synthesis led to decreased Aβ formation (Frears *et al.*, 1999; Simons *et al.*, 1998).

Cholesterol

Generally, cholesterol has a broad variety of effects on the physical properties of cell membranes such as increasing ordering and rigidity, reducing permeability, and decreasing lateral diffusion (Yeagle, 1991). Cholesterol exerts a homogenizing effect in natural membranes and lipid bilayers. It has been shown to be correlated with some membrane proteins and is responsible for specific regulation of membrane-associated protein activity (Sinha *et al.*, 1977; Klein *et al.*, 1978; Mitchell *et al.*, 1990; Bretscher and Munro, 1993; Klein *et al.*, 1995). Cholesterol may serve as a regulator of protease access to its substrate or a structural membrane component of the lipid bilayer that alters the activity of embedded proteins, either by changing membrane fluidity or by a mechanism independent of its membrane-ordering effect (Schroeder *et al.*, 1991).

Epidemiological and genetic studies have supported the fact that cholesterol serves as a *bona fide* risk factor in the pathogenesis of AD. An increased risk of AD has been linked with a natural genetic variant of apolipoprotein E (apoE), a molecule associated with cholesterol metabolism. The apoE-4 allele, the allele involved in increased risk for sporadic and late onset AD (Corder *et al.*, 1993; Saunders *et al.*, 1993; Schmechel *et al.*, 1993; Graham *et al.*, 1999; Sadowski *et al.*, 2004), has been associated with elevated total serum cholesterol levels (Jarvik *et al.*, 1995; Notkola *et al.*, 1998). Demented patients homozygotic for apoE-4 had the highest total plasma cholesterol levels among a referral population of 40 patients with clinically diagnosed AD in comparison to a sample of non-demented elderly controls (Czech *et al.*, 1994). In addition, in a population-based study, the risk of AD has been positively correlated with elevated serum cholesterol levels (Evans *et al.*, 2000).

Alterations in soluble cholesterol and/or cholesterol biosynthesis have also been shown to affect the normal processing of APP in several *in vitro* cell-based studies (Howland *et al.*, 1998; Simons *et al.*, 1998; Frears *et al.*, 1999). Bodovitz and Klein, (1996), using cyclodextrin-solubilized cholesterol as a delivery system, examined the link between cholesterol and the stability of APP in human embryonic kidney 293 cells transfected with APP human plasmid. The results showed that cholesterol solubilized by methyl-β-cyclodextrin or ethanol downregulated the levels of the soluble N-terminal derivative following α-secretase cleavage (sAPP). However, APP holoproteins remained unchanged or increased. In COS-1 fibroblast cells, increasing concentrations of cellular non-esterified cholesterol carried by rabbit β-very low-density lipoprotein or human low-density lipoprotein were found to cause an inhibitory effect on sAPP release in a dose-dependent fashion. This inhibition in sAPP release was observed to be inde-

pendent of receptor-mediated lipoprotein metabolism (Racchi *et al.*, 1997). Simons *et al.* (1998) noted that the production of Aβ but not the generation of sAPP in transfected hippocampal cells was reduced by cholesterol-depleting drugs such as lovastatin and methyl-β-cyclodextrin, suggesting that cholesterol is essential for Aβ formation. Via immunoprecipitation, immunofluorescent labeling, and surface-enhanced laser desorption/ionization mass spectrometry, Frears *et al.* (1999) confirmed that cholesterol shifted the APP metabolism from α-secretase pathway to β-secretase pathway. Their results showed that the presence of the β-hydroxy-β-methylglutaryl-CoA (HmG-CoA) reductase inhibitor lovastatin, on human HEK cells transfected with APP, attenuated intracellular cholesterol/protein ratios by one half, and dramatically inhibited β-secretase cleavage of newly synthesized APP. Exogenous water-solubilized cholesterol at 200 μg/mL concentration increased newly synthesized β-amyloidogenic products four-fold. By examining three different primary cultures including rat neurons, astrocytes, and microglial cells, Galbete *et al.* (2000) demonstrated a reduced secretion of sAPP in glial and neuronal cells induced by cholesterol. In addition, a slight decrease in APP mRNA expression and a substantial effect on protein maturation were observed in neurons. Via cell-surface biotinylation study, the authors concluded that cholesterol interfered with maturation of APP and inhibition of glycoslation of the protein.

Several lines of evidence have also demonstrated a link between Aβ production and cellular cholesterol levels in regards to *in vivo* animal studies. Durham *et al.* (1998) reported that Aβ level in the brain was elevated in apo E deficient mice fed cholesterol diet. It has been indicated that Aβ production and enzymatic processing of APP could be modulated by cellular cholesterol levels. Most importantly, APP gene-targeted mice that lack apoE, and had high serum cholesterol levels, did not show changes in APP processing. Such finding was suggestive of the importance of apoE in this enzymatic processing (Howland *et al.*, 1998). In contrast, one group observed an rise in brain Aβ level of mice with APP695 Swedish mutation fed with a high cholesterol diet but not wild type mice fed on cholesterol (Shie *et al.*, 1999). In the mouse model that exhibited over-expression of the C-terminal fragment of APP after β-cleavage, a cholesterol diet led to significant accumulation of AD-like pathology (Li *et al.*, 1999). Fishman *et al.* (1999) used mice with APP v717f mutation fed on high fat and cholesterol diet as an animal model and found that these mice exhibited both increased serum lipids and brain Aβ levels. Several observations were made in transgenic mouse model with diet-induced hypercholesterolemiadramatic: dramatic rise of Aβ in the CNS, a strong correlation between the levels of plasma and CNS total cholesterol, a significant decrease in sAPP, and an increase in C-terminal fragment of APP (Refolo *et al.*, 2000). Finally, recent reports have shown that cholesterol-lowering drugs were capable of decreasing accumulated Aβ levels in animal models such as guinea pigs fed with high doses of the hydroxymethyl co-enzyme A (HMG-CoA)-reductase inhibitor, simvastatin (Fassbender *et al.*, 2001). Similar results were found in APP/PS1 co-transgenic mice fed with (4-(2-[1-(4-chlorocinnamyl) piperazine-4-yl]ethyl]benzoic acid) (Refolo *et al.*, 2001) or 7-dehydrocholesterol-reductase inhibitor (Golde and Eckman, 2001), suggesting the critical role of cholesterol in Aβ-induce neurotoxicity responsible for AD.

Gangliosides

While present only in relatively small quantities in most tissues, gangliosides are abundant components of nervous tissues. Gangliosides compose of approximately one-tenth of total neuronal membrane lipids (Wiegandt *et al.*, 1982; Tettamanti and Riboni, 1993), and, specifically, ganglioside GM1 tends to be highly concentrated in certain regions of neurons, especially in pre- and postsynaptic membranes (Hansson *et al.*, 1977). In the respect of functionality, gangliosides have been associated with a number of important neurological events such as neuritogenesis, neurodifferentiation, synaptogenesis, and synaptic transmission.

Several lines of evidence implied the importance of gangliosides in pathology of AD. Working with isolated gangliosdie-bound Aβ(1-42), Yanagisawa and coworkers (Yanagisawa *et al.*, 1995, 1996) reported that brain tissue which contained increasing levels of diffuse plaques also included increased amounts of ganglioside-bound Aβ(1-42). They also suggested that some of the Aβ peptides in diffuse plaques were bound to GM1 or its related members. The same research group, via different anti-Aβ monoclonal antibodies such as BC05 (specific for Aβ(1-42)), BAN50 (raised against Aβ(1-16)), BAN052 (raised against Aβ(1-16) and specific for N-terminus), and 4G8 (specific for Aβ(17-24)) along with immunoprecipitation, noted that Aβ bound to a GM1 ganglioside in a certain way that the bound Aβ could only be characterized by BAN052 monoclonal antibody. They proposed a possible mechanism whereby the membrane-bound Aβ species might be shed into the extracellular space and served as a "seed" for fibril formation (Yanagisawa and Ihara, 1998). Large body of data suggested that gangliosides might play a role in Aβ fibrillogenesis after binding to membranes or in acceleration of Aβ fibril formation *in vitro* (Choo-Smith *et al.*, 1997; McLaurin *et al.*, 1998; Matsuzaki and Horikiri, 1999; Ariga

et al., 2001; Kakio *et al.*, 2001, 2002; Tashima *et al.*, 2004; Yamamoto *et al.*, 2004; Wakabayashi *et al.*, 2005).

Cholesterol and/or gangliosides have been linked to certain biological activities of Aβ (Wang *et al.*, 2001). Investigations showed that cholesterol reduction and depletion of membrane associated sialic acid residue both not only significantly reduced the Aβ-induced GTPase activity but also protected cells from Aβ-induced toxicity, indicating the importance of Aβ-membrane interactions in the mechanism of Aβ toxicity. Similar results have also been observed with other amyloid-forming peptides such as bovine calcitonin (Wang *et al.*, 2005a).

G-protein activation (GTP hydrolysis)

G-protein-linked receptors influence the concentration of second messengers and thereby affect the behavior of other target proteins in cells. In most cases, the interactions between the receptors and second messengers in the signal transduction cascade are mediated via enzymes or ion channels activated by GTP-binding or G-proteins. G-proteins are the membrane bound proteins and provide the site for the exchange between GDP and GTP. Signal transduction is typically initiated by the binding of a ligand, hormone, or neurotransmitter to a G-protein-linked receptor located in the membrane. This binding stabilizes the conformation of the receptor, transmits information across the membrane, and stimulates G-proteins (Gilman, 1987; Bourne *et al.*, 1990a, 1990b; Simon *et al.*, 1991; Linder and Gilman, 1992). On the other hand, signal transduction can also be started by directly activating G-protein through external stimuli. Upon activation, the α-chain of G-protein exchanges GDP for GTP, and α-GTP complex dissociates from the rest of the G-protein due to the lower affinity for the Gβγ subunit. It diffuses along the inner surface of plasma membrane to bind and activate an effector. Different types of effectors such as adenylyl cyclase, phospholipase C, and ion channels have been discovered. The signal terminates when the α-bound GTP hydrolyze to GDP and the α subunit reassociates with the rest of the G-protein (Gβγ subunit).

Increasing evidence suggests that G protein activation and other signal transduction events such as phospholipase D and adenylate cyclase activation are associated with the biological activity of Aβ (Schnecko *et al.*, 1994; Singh *et al.*, 1998; Soomets *et al.*, 1999; Jonsson *et al.*, 2000; Zambrzycka *et al.*, 2000; Vaudry *et al.*, 2004). A report showed that G-protein activation and Aβ aggregates were capable of upregulating tyrosine phosphorylation of focal adhesion kinase (FAK Tyr(P)) through a mechanism associated with protein kinase C and F-actin (Zhang *et al.*, 1996). After one day of incubation with aggregated Aβ(1-42), cellular FAK Tyr(P) level was elevated on immunoblots; however, this stimulation was blocked by cytochalasin D, an F-actin disrupting drug. Furthermore, the toxicity of Aβ(1-42) was inhibited by the addition of bisindolylmaleimide, suggesting the role of protein kinase C in the stimulation of FAK Tyr(P) by Aβ fibril. Soomets et al., found that GTPase activity was upregulated by Aβ(25-35) and Aβ(1-42) in ventral hippocampal and cortical membranes. The effect of on GTPase activity followed a dose dependent manner and exhibited a bell-shaped curve for Aβ(1-42) case. The bell-shaped concentration-dependent effect on adenylate cyclase activity was observed for both Aβ(1-42) and Aβ(25-35). At high concentrations of Aβ(25-35) the GTPase activity was pertussis toxin sensitive, suggesting the involvement of a $G_{i/o}$ G protein (Soomets *et al.*, 1999). Their study implied that the G protein and adenylate cyclase response to Aβ was associated with Aβ neurotoxicity (Soomets *et al.*, 1999).

It was observed that, in reconstituted phospholipid vesicles containing G_0 and APP, the turnover rate of G_0 GTPase activity was elevated when a monoclonal antibody (22C11) against N-terminal domain was added (Okamoto *et al.*, 1995). Authors concluded that the mode of G_0 activation by APP was highly similar to that of receptor-stimulated G protein activation suggested by others (Nishimoto *et al.*, 1993). However, a proposal has been made stating that the intracellular interaction between APP and $G_0\alpha$ can be regulated by extracellular signals and the high-affinity G_0 GTPase activity reduced upon incubating neuronal membranes with N-terminal APP recognition antibody (Brouillet *et al.*, 1999). It was found that $G_0\alpha$ interacted with APP, especially the C-terminal part of APP, in axonal microdomain. Moreover, in COS cell NK1 clone the activation of G_0 in reconstituted vesicles by the familial AD-associated APP mutant, V642I APP in particular, led to pertussis toxin-sensitive apoptosis and the impairment of cAMP response element via Gβγ-protein complex and $G_0\alpha$-related mechanisms respectively (Giambarella *et al.*, 1997).

It has been previously shown that aggregated Aβ (25-35 and 1-40 fragments, but not 1-16 fragment) was able to increase GTP hydrolysis in membranes derived from PC12 cells while inhibition of GTPase activity attenuated the toxicity of the Aβ peptides (Rymer and Good, 2001). Similar results were shown for bovine calcitonin; only when in an amyloidogenic form, bovine calcitonin increased GTP hydrolysis, and inhibition of the GTPase activity attenuated the toxic effects of calcitonin (Rymer and Good, 2001). An Aβ-induced increase in GTPase hydrolysis was observed with purified G_i and G_0 alpha subunits reconstituted in liposomes, and with cell membranes in which the receptors had been proteolytically removed. These results sug-

gested that the G protein activation observed upon incubation with Aβ was receptor-independent and was probably membrane-mediated. Recent evidence pointed that the mixtures of Aβ(25-35) and Aβ(1-42) or Aβ(1-40) induced an increase in GTP-binding several-fold higher than Aβ alone (Molnar *et al.*, 2004). This GTP-associated potentiation was found to be accompanied with the secondary structure of Aβ peptide mixtures, revealing that structural alterations are behind the enhanced biological activity (Molnar *et al.*, 2004).

While the link between G protein activation and Aβ actions was observed, the issue regarding how the activation of G protein occurs remains largely unclear. The kinetic analysis on Aβ-induced GTP hydrolysis was performed to achieve the above goal (Wang *et al.*, 2003a). Further mechanistic understanding of reaction pathways/cascades of the interaction between Aβ and G protein remains to be examined.

In summary, it is unequivocal that Aβ triggers adverse impacts on cellular function via interactions with cell membranes. Given the ample of aforementioned evidence describing the possible actions of Aβ, there might be more than one mechanism involved in the interaction between Aβ and neurons as well as the Aβ-induced neurotoxicity. Therefore, it is most likely that AD is a heterogeneous disease. However, the underlying mechanism(s) of Aβ-membrane interaction remain to be investigated. Is the mechanism governed by the electrostatic interaction, dipole-dipole interaction, or membrane fluidity alteration? Closer examinations on the mechanism(s) of this interaction will certainly provide an exploratory guide for rational design of potential inhibitor molecules.

CONCLUSION

In the present review, the general background information on Alzheimer's disease and β-amyloid are first presented. Next, attention is focused on the relevant research updates regarding structural aspect and molecular features of Aβ species as well as β-amyloid fribrillogenesis/aggregation process. Importantly, an emerging issue that an oligomeric intermediate is the primary toxic β-amyloid species is also discussed. Finally, a number of β-amyloid-induced toxicity mechanisms are proposed and discussed. It is our belief that further knowledge on novel approaches for the understanding of the conformational changes as well as biological functions of β-amyloid and β-amyloid-elicited toxicity mechanisms will shed light on the development and/or design of potential therapeutic strategies for Alzheimer's disease.

REFERENCES

Aisen, P. S., "Inflammation and Alzheimer Disease," *Mol. Chem. Neuropathol.*, **28**(1-3), 83 (1996).

Akiyama, H., K. Ikeda, H. Kondo, M. Kato, and P. L. McGeer, "Microglia Express the Type 2 Plasminogen Activator Inhibitor in the Brain of Control Subjects and Patients with Alzheimer's Disease," *Neurosci. Lett.*, **164**(1-2), 233 (1993).

Aksenov, M., M. V. Aksenova, M. E. Harris, K. Hensley, D. A. Butterfield, and J. M. Carney, "Enhancement of Beta-Amyloid Peptide A Beta(1-40)-Mediated Neurotoxicity by Glutamine Synthetase," *J. Neurochem.*, **65**(4), 1899 (1995).

Alzheimer, A., "Uber Eine Eigenartige Erkrangkung der Hirnrinde," *All. Z. Psychiatr.*, **64**, 146 (1907).

Ariga, T. and R. K. Yu, "GM1 Inhibits Amyloid Beta-Protein-Induced Cytokine Release," *Neurochem. Res.*, **24**(2), 219 (1999).

Ariga, T., K. Kobayashi, A. Hasegawa, M. Kiso, H. Ishida, and T. Miyatake, "Characterization of High-Affinity Binding between Gangliosides and Amyloid Beta-Protein," *Arch. Biochem. Biophys.*, **388**(2), 225 (2001).

Arispe, N., H. B. Pollard, and E. Rojas, "Giant Multilevel Cation Channels Formed by Alzheimer Disease Amyloid Beta-Protein [A beta P-(1-40)] in Bilayer Membranes," *Proc. Natl. Acad. Sci. U.S.A.*, **90**(22), 10573 (1993a).

Arispe, N., E. Rojas, and H. B. Pollard, "Alzheimer Disease Amyloid Beta Protein Forms Calcium Channels in Bilayer Membranes: Blockade by Tromethamine and Aluminum," *Proc. Natl. Acad. Sci. U.S.A.*, **90**(2), 567 (1993b).

Arispe, N., H. B. Pollard, and E. Rojas, "The Ability of Amyloid Beta-Protein [A beta P (1-40)] to Form Ca^{2+} Channels Provides a Mechanism for Neuronal Death in Alzheimer's Disease," *Ann. N. Y. Acad. Sci.*, **747**, 256 (1994a).

Arispe, N., H. B. Pollard, and E. Rojas, "Beta-Amyloid Ca(2+)-Channel Hypothesis for Neuronal Death in Alzheimer Disease," *Mol. Cell. Biochem.*, **140**(2), 119 (1994b).

Arispe, N., H. B. Pollard, and E. Rojas, "Zn^{2+} Interaction with Alzheimer Amyloid Beta Protein Calcium Channels," *Proc. Natl. Acad. Sci. U.S.A.*, **93**(4), 1710 (1996).

Avdulov, N. A., S. V. Chochina, U. Igbavboa, E. O. O'Hare, F. Schroeder, J. P. Cleary, and W. G. Wood, "Amyloid Beta-Peptides Increase Annular and Bulk Fluidity and Induce Lipid Peroxidation in Brain Synaptic Plasma Mmbranes," *J. Neurochem.*, **68**(5), 2086 (1997).

Avdulov, N. A., S. V. Chochina, U. Igbavboa, C. S. Warden, F. Schroeder, and W. G. Wood, "Lipid Binding to Sterol Carrier Protein-2 is Inhibited by Ethanol," *Biochim. Biophys. Acta.*, **1437**(1), 37 (1999).

Bamberger, M. E. and G. E. Landreth, "Microglial Interaction with Beta-Amyloid: Implications for the Pathogenesis of

Alzheimer's Disease," *Microsc. Res. Tech.*, **54**(2), 59 (2001).

Bard, F., C. Cannon, R. Barbour, R. L. Burke, D. Games, H. Grajeda, T. Guido, K. Hu, J. Huang, K. Johnson-Wood, K. Khan, D. Kholodenko, M. Lee, I. Lieberburg, R. Motter, M. Nguyen, F. Soriano, N. Vasquez, K. Weiss, B. Welch, P. Seubert, D. Schenk, and T. Yednock, "Peripherally Administered Antibodies Against Amyloid Beta-Peptide Enter the Central Nervous System and Reduce Pathology in a Mouse Model of Alzheimer Disease," *Nat. Med.*, **6**(8), 916 (2000).

Barger, S. W., "An Unconventional Hypothesis of Oxidation in Alzheimer's Disease: Intersections with Excitotoxicity," *Front Biosci*, **9**, 3286 (2004).

Barrow, C. J. and M. G. Zagorski, "Solution Structures of Beta Peptide and Its Constituent Fragments: Relation to Amyloid Deposition," *Science*, **253**(5016), 179 (1991).

Barrow, C. J., A. Yasuda, P. T. Kenny, and M. G. Zagorski, "Solution Conformations and Aggregational Properties of Synthetic Amyloid Beta-Peptides of Alzheimer's Disease. Analysis of Circular Dichroism Spectra," *J. Mol. Biol.*, **225**(4), 1075 (1992).

Behl, C., M. Widmann, T. Trapp, and F. Holsboer, "17-Beta Estradiol Protects Neurons from Oxidative Stress-Induced Cell Death In Vitro," *Biochem. Biophys. Res. Commun.*, **216**(2), 473 (1995).

Beking, K., X. Hao, S. Basak, and A. Basak, "Modulatory Effects of pH, Cu^{+2} and Sheet Breakers on Aggregation of Amyloid Peptides," *Protein Pept. Lett.*, **12**(2), 197 (2005).

Blanchard, B. J., V. L. Thomas, and V. M. Ingram, "Mechanism of Membrane Depolarization Caused by the Alzheimer Abeta1-42 Peptide," *Biochem. Biophys. Res. Commun.*, **293**(4), 1197 (2002).

Bodovitz, S. and W. L. Klein, "Cholesterol Modulates Alpha-Secretase Cleavage of Amyloid Precursor Protein," *J. Biol. Chem.*, **271**(8), 4436 (1996).

Bornemann, K. D. and M. Staufenbiel, "Transgenic Mouse Models of Alzheimer's Disease," *Ann. N. Y. Acad. Sci.*, **908**, 260 (2000).

Bourne, H. R., D. A. Sanders, and F. McCormick, "The GTPase Superfamily: A Conserved Switch for Diverse Cell Functions," *Nature*, **348**(6297), 125 (1990a).

Bourne, H. R., L. Wrischnik, and C. Kenyon, "Ras Proteins. Some Signal Developments," *Nature*, **348**(6303), 678 (1990b).

Bradt, B. M., W. P. Kolb, and N. R. Cooper, "Complement-Dependent Proinflammatory Properties of the Alzheimer's Disease Beta-Peptide," *J. Exp. Med.*, **188**(3), 431 (1998).

Bretscher, M. S. and S. Munro, "Cholesterol and the Golgi Apparatus," *Science*, **261**(5126), 1280 (1993).

Broytman, O. and J. S. Malter, "Anti-Abeta: The Good, the Bad, and the Unforeseen," *J. Neurosci. Res.*, **75**(3), 301 (2004).

Burdick, D., B. Soreghan, M. Kwon, J. Kosmoski, M. Knauer, A. Henschen, J. Yates, C. Cotman, and C. Glabe, "Assembly and Aggregation Properties of Synthetic Alzheimer's A4/beta Amyloid Peptide Analogs," *J. Biol. Chem.*, **267**(1), 546 (1992).

Burggren, A. C. and S. Y. Bookheimer, "Structural and Functional Neuroimaging in Alzheimer's Disease: An Update," *Curr. Top. Med. Chem.*, **2**(4), 385 (2002).

Busciglio, J., J. Yeh, and B. A. Yankner, "Beta-Amyloid Neurotoxicity in Human Cortical Culture is not Mediated by Excitotoxins," *J. Neurochem.*, **61**(4), 1565 (1993).

Butterfield, D. A., K. Hensley, M. Harris, M. Mattson, and J. Carney, "Beta-Amyloid Peptide Free Radical Fragments Initiate Synaptosomal Lipoperoxidation in a Sequence-Specific Fashion: Implications to Alzheimer's Disease," *Biochem. Biophys. Res. Commun.*, **200**(2), 710 (1994).

Butterfield, D. A., "Beta-Amyloid-Associated Free Fadical Oxidative Stress and Neurotoxicity: Implications for Alzheimer's Disease," *Chem. Res. Toxicol.*, **10**(5), 495 (1997).

Butterfield, D. A., S. M. Yatin, S. Varadarajan, and T. Koppal, "Amyloid Beta-Peptide-Associated Free Radical Oxidative Stress, Neurotoxicity, and Alzheimer's Disease," *Methods Enzymol.*, **309**, 746 (1999).

Buxbaum, J. D., M. Oishi, H. I. Chen, R. Pinkas-Kramarski, E. A. Jaffe, S. E. Gandy, and P. Greengard, "Cholinergic Agonists and Interleukin 1 Regulate Processing and Secretion of the Alzheimer Beta/A4 Amyloid Protein Precursor," *Proc. Natl. Acad. Sci. U.S.A.*, **89**(21), 10075 (1992).

Caporaso, G. L., S. E. Gandy, J. D. Buxbaum, T. V. Ramabhadran, and P. Greengard, "Protein Phosphorylation Regulates Secretion of Alzheimer Beta/A4 Amyloid Precursor Protein," *Proc. Natl. Acad. Sci. U.S.A.*, **89**(7), 3055 (1992).

Carr, D. B., A. Goate, D. Phil, and J. C. Morris, "Current Concepts in the Pathogenesis of Alzheimer's Disease," *Am. J. Med.*, **103**(3A), 3S (1997).

Carrell, R. W. and D. A. Lomas, "Conformational Disease [see comments]," *Lancet*, **350**(9071), 134 (1997).

Carrell, R. W. and B. Gooptu, "Conformational Changes and Disease-Serpins, Prions and Alzheimer's," *Curr. Opin. Struct. Biol.*, **8**(6), 799 (1998).

Chapman, P. F., G. L. White, M. W. Jones, D. Cooper-Blacketer, V. J. Marshall, M. Irizarry, L. Younkin, M. A. Good, T. V. Bliss, B. T. Hyman, S. G. Younkin, and K. K. Hsiao, "Impaired Synaptic Plasticity and Learning in Aged Amyloid Precursor Protein Transgenic Mice," *Nat. Neurosci.*, **2**(3), 271 (1999).

Chochina, S. V., N. A. Avdulov, U. Igbavboa, J. P. Cleary, E. O. O'Hare, and W. G. Wood, "Amyloid Beta-Peptide1-40 Increases Neuronal Membrane Fluidity: Role of Cholesterol and Brain Region," *J. Lipid. Res.*, **42**(8), 1292

(2001).

Choo-Smith, L. P., W. Garzon-Rodriguez, C. G. Glabe, and W. K. Surewicz, "Acceleration of Amyloid Fibril Formation by Specific Binding of Abeta- (1-40) Peptide to Ganglioside-Containing Membrane Vesicles," *J. Biol. Chem.*, **272**(37), 22987 (1997).

Chromy, B. A., R. J. Nowak, M. P. Lambert, K. L. Viola, L. Chang, P. T. Velasco, B. W. Jones, S. J. Fernandez, P. N. Lacor, P. Horowitz, C. E. Finch, G. A. Krafft, and W. L. Klein, "Self-Assembly of Abeta(1-42) into Globular Neurotoxins," *Biochemistry*, **42**(44), 12749 (2003).

Ciccotosto, G. D., D. Tew, C. C. Curtain, D. Smith, D. Carrington, C. L. Masters, A. I. Bush, R. A. Cherny, R. Cappai, and K. J. Barnham, "Enhanced Toxicity and Cellular Binding of a Modified Amyloid Beta Peptide with a Methionine to Valine Substitution," *J. Biol. Chem.*, **279**(41), 42528 (2004).

Cole, G. M., T. Morihara, G. P. Lim, F. S. Yang, A. Begum, and S. A. Frautschy, NSAID and Antioxidant Prevention of Alzheimer's Disease Lessons from In Vitro and Animal Models, In *Protective Strategies for Neurodegenerative Diseases*, p. 68 (2004).

Coles, M., W. Bicknell, A. A. Watson, D. P. Fairlie, and D. J. Craik, "Solution Structure of Amyloid Beta-Peptide(1-40) in a Water-Micelle Environment. Is the Membrane-Spanning Domain Where We Think It Is?," *Biochemistry*, **37**(31), 11064 (1998).

Corder, E. H., A. M. Saunders, W. J. Strittmatter, D. E. Schmechel, P. C. Gaskell, G. W. Small, A. D. Roses, J. L. Haines, and M. A. Pericak-Vance, "Gene Dose of Apolipoprotein E Type 4 Allele and the Risk of Alzheimer's Disease in Late Onset Families," *Science*, **261**(5123), 921 (1993).

Crescenzi, O., S. Tomaselli, R. Guerrini, S. Salvadori, A. M. D'Ursi, P. A. Temussi, and D. Picone, "Solution Structure of the Alzheimer Amyloid Beta-Peptide (1-42) in an Apolar Microenvironment. Similarity with a Virus Fusion Domain," *Eur. J. Biochem.*, **269**(22), 5642 (2002).

Cribbs, D. H., C. J. Pike, S. L. Weinstein, P. Velazquez, and C. W. Cotman, "All-D-enantiomers of Beta-Amyloid Exhibit Similar Biological Properties to All-L-Beta-Amyloids," *J. Biol. Chem.*, **272**(11), 7431 (1997).

Cummings, B. J., C. J. Pike, R. Shankle, and C. W. Cotman, "Beta-Amyloid Deposition and Other Measures of Neuropathology Predict Cognitive Status in Alzheimer's Disease," *Neurobiol. Aging.*, **17**(6), 921 (1996).

Czech, C., H. Forstl, F. Hentschel, U. Monning, C. Besthorn, C. Geiger-Kabisch, H. Sattel, C. Masters, and K. Beyreuther, "Apolipoprotein E-4 Gene Dose in Clinically Diagnosed Alzheimer's Disease: Prevalence, Plasma Cholesterol Levels and Cerebrovascular Change," *Eur. Arch. Psychiatry Clin. Neurosci.*, **243**(5), 291 (1994).

Datki, Z., A. Juhasz, M. Galfi, K. Soos, R. Papp, D. Zadori, and B. Penke, "Method for Measuring Neurotoxicity of Aggregating Polypeptides with the MTT Assay on Differentiated Neuroblastoma Cells," *Brain. Res. Bull.*, **62**(3), 223 (2003).

Datki, Z., R. Papp, D. Zadori, K. Soos, L. Fulop, A. Juhasz, G. Laskay, C. Hetenyi, E. Mihalik, M. Zarandi, and B. Penke, "*In vitro* model of Neurotoxicity of Abeta 1-42 and Neuroprotection by a Pentapeptide: Irreversible Events during the First Hour," *Neurobiol. Dis.*, **17**(3), 507 (2004).

Davis, J. B., H. F. McMurray, and D. Schubert, "The Amyloid Beta-Protein of Alzheimer's Disease is Chemotactic for Mononuclear Phagocytes," *Biochem. Biophys. Res. Commun.*, **189**(2), 1096 (1992).

Davis, J. B., "Oxidative Mechanisms in Beta-Amyloid Cytotoxicity," *Neurodegeneration*, **5**(4), 441 (1996).

De Felice, F. G., M. N. Vieira, L. M. Saraiva, J. D. Figueroa-Villar, J. Garcia-Abreu, R. Liu, L. Chang, W. L. Klein, and S. T. Ferreira, "Targeting the Neurotoxic Species in Alzheimer's Disease: Inhibitors of Abeta Oligomerization," *Faseb. J.*, **18**(12), 1366 (2004).

Del Mar Martinez-Senac, M., J. Villalain, and J. C. Gomez-Fernandez, "Structure of the Alzheimer Beta-Amyloid Peptide (25-35) and Its Interaction with Negatively Charged Phospholipid Vesicles," *Eur. J. Biochem.*, **265**(2), 744 (1999).

Delobette, S., A. Privat, and T. Maurice, "*In Vitro* Aggregation Facilities Beta-Amyloid Peptide-(25-35)-Induced Amnesia in the Rat," *Eur. J. Pharmacol.*, **319**(1), 1 (1997).

DeMattos, R. B., K. R. Bales, D. J. Cummins, J. C. Dodart, S. M. Paul, and D. M. Holtzman, "Peripheral Anti-A Beta Antibody Alters CNS and Plasma A Beta Clearance and Decreases Brain A Beta Burden in a Mouse Model of Alzheimer's Disease," *Proc. Natl. Acad. Sci. U.S.A.*, **98**(15), 8850 (2001).

Demeester, N., G. Baier, C. Enzinger, M. Goethals, J. Vandekerckhove, M. Rosseneu, and C. Labeur, "Apoptosis Induced in Neuronal Cells by C-Terminal Amyloid Beta-Fragments is Correlated with Their Aggregation Properties in Phospholipid Membranes," *Mol. Membr. Biol.*, **17**(4), 219 (2000).

Demeester, N., C. Mertens, H. Caster, M. Goethals, J. Vandekerckhove, M. Rosseneu, and C. Labeur, "Comparison of the Aggregation Properties, Secondary Structure and Apoptotic Effects of Wild-Type, Flemish and Dutch N-Terminally Truncated Amyloid Beta Peptides," *Eur. J. Neurosci.*, **13**(11), 2015 (2001).

Dobson, C. M., "Principles of Protein Folding, Misfolding and Aggregation," *Semin. Cell. Dev. Biol.*, **15**(1), 3 (2004).

Durham, R. A., C. A. Parker, M. R. Emmerling, C. L. Bisgaier, and L. C. Walker, "Effect of Age and Diet on the Expression of Beta-Amyloid 1-40 and 1-42 in the Brains of Apolipoprotein-E-Deficient Mice," *Neurobiol. Aging.*,

19, S281 (1998).

Dyrks, T., A. Weidemann, G. Multhaup, J. M. Salbaum, H. G. Lemaire, J. Kang, B. Muller-Hill, C. L. Masters, and K. Beyreuther, "Identification, Transmembrane Orientation and Biogenesis of the Amyloid A4 Precursor of Alzheimer's Disease," *Embo. J.*, **7**(4), 949 (1988).

El-Agnaf, O. M., D. J. Guthrie, D. M. Walsh, and G. B. Irvine, "The Influence of the Central Region Containing Residues 19-25 on the Aggregation Properties and Secondary Structure of Alzheimer's Beta-Amyloid Peptide," *Eur. J. Biochem.*, **256**(3), 560 (1998).

El-Agnaf, O. M., D. S. Mahil, B. P. Patel, and B. M. Austen, "Oligomerization and Toxicity of Beta-Amyloid-42 Implicated in Alzheimer's Disease," *Biochem. Biophys. Res. Commun.*, **273**(3), 1003 (2000).

Emmerling, M. R., K. Spiegel, and M. D. Watson, "Inhibiting the Formation of Classical C3-Convertase on the Alzheimer's Beta-Amyloid Peptide," *Immunopharmacology*, **38**(1-2), 101 (1997).

Esler, W. P., E. R. Stimson, J. R. Ghilardi, H. V. Vinters, J. P. Lee, P. W. Mantyh, and J. E. Maggio, "In Vitro Growth of Alzheimer's Disease Beta-Amyloid Plaques Displays First-Order Kinetics," *Biochemistry*, **35**(3), 749 (1996).

Evans, R. M., C. L. Emsley, S. Gao, A. Sahota, K. S. Hall, M. R. Farlow, and H. Hendrie, "Serum Cholesterol, APOE Genotype, and the Risk of Alzheimer's Disease: a Population-Based Study of African Americans," *Neurology*, **54**(1), 240 (2000).

Fassbender, K., M. Simons, C. Bergmann, M. Stroick, D. Lutjohann, P. Keller, H. Runz, S. Kuhl, T. Bertsch, K. von Bergmann, M. Hennerici, K. Beyreuther, and T. Hartmann, "Simvastatin Strongly Reduces Levels of Alzheimer's Disease Beta - Amyloid Peptides Abeta 42 and Abeta 40 in Vitro and In Vivo," *Proc. Natl. Acad. Sci. U.S.A.*, **98**(10), 5856 (2001).

Ferrari-DiLeo, G., D. C. Mash, and D. D. Flynn, "Attenuation of Muscarinic Receptor-G-Protein Interaction in Alzheimer Disease," *Mol. Chem. Neuropathol.*, **24**(1), 69 (1995).

Festy, F., L. Lins, G. Peranzi, J. N. Octave, R. Brasseur, and A. Thomas, "Is Aggregation of Beta-Amyloid Peptides a Mis-Functioning of a Current Interaction Process?," *Biochim. Biophys. Acta.*, **1546**(2), 356 (2001).

Fezoui, Y. and D. B. Teplow, "Kinetic Studies of Amyloid Beta-Protein Fibril Assembly. Differential Effects of Alpha-helix Stabilization," *J. Biol. Chem.*, **277**(40), 36948 (2002).

Fishman, C. E., S. L. White, C. A. DeLong, D. J. Cummins, W. H. Jordan, K. R. Bales, and S. M. Paul, "High Fat Diet Potentiates β-Amyloid Deposition in the APP V717F Transgenic Mouse Model of Alzheimer's Disease," *Soc. Neurosci.*, **25**, 1859 (1999).

Fraser, P. E., J. T. Nguyen, W. K. Surewicz, and D. A. Kirschner, "pH-Dependent Structural Transitions of Alzheimer Amyloid Peptides," *Biophys. J.*, **60**(5), 1190 (1991).

Fraser, P. E., D. R. McLachlan, W. K. Surewicz, C. A. Mizzen, A. D. Snow, J. T. Nguyen, and D. A. Kirschner, "Conformation and Fibrillogenesis of Alzheimer A Beta Peptides with Selected Substitution of Charged Residues," *J. Mol. Biol.*, **244**(1), 64 (1994).

Fraser, S. P., Y. H. Suh, and M. B. Djamgoz, "Ionic Effects of the Alzheimer's Disease Beta-Amyloid Precursor Protein and Its Metabolic Fragments," *Trends. Neurosci.*, **20**(2), 67 (1997).

Frears, E. R., D. J. Stephens, C. E. Walters, H. Davies, and B. M. Austen, "The Role of Cholesterol in the Biosynthesis of Beta-Amyloid," *Neuroreport*, **10**(8), 1699 (1999).

Gabuzda, D., J. Busciglio, and B. A. Yankner, "Inhibition of Beta-Amyloid Production by Activation of Protein Kinase C," *J. Neurochem.*, **61**(6), 2326 (1993).

Galbete, J. L., T. R. Martin, E. Peressini, P. Modena, R. Bianchi, and G. Forloni, "Cholesterol Decreases Secretion of the Secreted Form of Amyloid Precursor Protein by Interfering with Glycosylation in the Protein Secretory Pathway," *Biochem. J.*, **348** (2), 307 (2000).

Games, D., K. M. Khan, F. G. Soriano, P. S. Keim, D. L. Davis, K. Bryant, and I. Lieberburg, "Lack of Alzheimer Pathology after Beta-Amyloid Protein Injections in Rat Brain," *Neurobiol. Aging.*, **13**(5), 569 (1992).

Games, D., D. Adams, R. Alessandrini, R. Barbour, P. Berthelette, C. Blackwell, T. Carr, J. Clemens, T. Donaldson, F. Gillespie, "Alzheimer-Type Neuropathology in Transgenic Mice Overexpressing V717F Beta-Amyloid Precursor Protein," *Nature*, **373**(6514), 523 (1995).

Garzon-Rodriguez, W., M. Sepulveda-Becerra, S. Milton, and C. G. Glabe, "Soluble Amyloid Abeta-(1-40) Exists as a Stable Dimer at Low Concentrations," *J. Biol. Chem.*, **272**(34), 21037 (1997).

Gilman, A. G., "G Proteins: Transducers of Receptor-Generated Signals," *Annu. Rev. Biochem.*, **56**, 615 (1987).

Ginsberg, L., J. R. Atack, S. I. Rapoport, and N. L. Gershfeld, "Regional Specificity of Membrane Instability in Alzheimer's Disease Brain," *Brain Res.*, **615**(2), 355 (1993).

Giovannelli, L., C. Scali, M. S. Faussone-Pellegrini, G. Pepeu, and F. Casamenti, "Long-Term Changes in the Aggregation State and Toxic Effects of Beta-Amyloid Injected into the Rat Brain," *Neuroscience*, **87**(2), 349 (1998).

Golde, T. E. and C. B. Eckman, "Cholesterol Modulation as an Emerging Strategy for the Treatment of Alzheimer's Disease," *Drug Discov. Today*, **6**(20), 1049 (2001).

Good, T. A., "The Aggregation State Specific Role of Beta-Amyloid Peptide in Neurotoxicity Associated with

Alzheimer's Disease," In *Department of Chemical Engineering*. University of Wisconsin, Madison, p. 248 (1996).

Good, T. A., D. O. Smith, and R. M. Murphy, "Beta-Amyloid Peptide Blocks the Fast-Inactivating K^+ Current in Rat Hippocampal Neurons," *Biophys. J.*, **70**(1), 296 (1996).

Goodman, Y. and M. P. Mattson, "Secreted Forms of Beta-Amyloid Precursor Protein Protect Hippocampal Neurons Against Amyloid Beta-Peptide-Induced Oxidative Injury," *Exp. Neurol.*, **128**(1), 1 (1994a).

Goodman, Y. and M. P. Mattson, "Staurosporine and K-252 Compounds Protect Hippocampal Neurons Against Amyloid Beta-Peptide Toxicity and Oxidative Injury," *Brain Res*, **650**(1), 170 (1994b).

Gotz, J., F. Chen, J. van Dorpe, and R. M. Nitsch, "Formation of Neurofibrillary Tangles in P3011 Tau Transgenic Mice Induced by Abeta 42 Fibrils," *Science*, **293**(5534), 1491 (2001).

Graham, D. I., S. M. Gentleman, J. A. Nicoll, M. C. Royston, J. E. McKenzie, G. W. Roberts, R. E. Mrak, and W. S. Griffin, "Is There a Genetic Basis for the Deposition of Beta-Amyloid after Fatal Head Injury?," *Cell. Mol. Neurobiol.*, **19**(1), 19 (1999).

Gursky, O. and S. Aleshkov, "Temperature-Dependent Beta-Sheet Formation in Beta-Amyloid Abeta(1-40) Peptide in Water: Uncoupling Beta-Structure Folding from Aggregation," *Biochim. Biophys. Acta.*, **1476**(1), 93 (2000).

Haass, C., A. Y. Hung, M. G. Schlossmacher, T. Oltersdorf, D. B. Teplow, and D. J. Selkoe, "Normal Cellular Processing of the Beta-Amyloid Precursor Protein Results in the Secretion of the Amyloid Beta Peptide and Related Molecules," *Ann. N. Y. Acad. Sci.*, **695**, 109 (1993).

Hansson, H. A., J. Holmgren, and L. Svennerholm, "Ultrastructural Localization of Cell Membrane GM1 Ganglioside by Cholera Toxin," *Proc. Natl. Acad. Sci. U.S.A.*, **74**(9), 3782 (1977).

Hartley, D. M., D. M. Walsh, C. P. Ye, T. Diehl, S. Vasquez, P. M. Vassilev, D. B. Teplow, and D. J. Selkoe, "Protofibrillar Intermediates of Amyloid Beta-Protein Induce Acute Electrophysiological Changes and Progressive Neurotoxicity in Cortical Neurons," *J. Neurosci.*, **19**(20), 8876 (1999).

Hartmann, H., A. Eckert, and W. E. Muller, "Apolipoprotein E and Cholesterol Affect Neuronal Calcium Signalling: the Possible Relationship to Beta-Amyloid Neurotoxicity," *Biochem. Biophys. Res. Commun.*, **200**(3), 1185 (1994).

He, W. and C. J. Barrow, "The A Beta 3-Pyroglutamyl and 11-Pyroglutamyl Peptides Found in Senile Plaque Have Greater Beta-Sheet Forming and Aggregation Propensities In Vitro than Full-Length A Beta," *Biochemistry*, **38**(33), 10871 (1999).

Hensley, K., J. M. Carney, M. P. Mattson, M. Aksenova, M. Harris, J. F. Wu, R. A. Floyd, and D. A. Butterfield, "A Model for Beta-Amyloid Aggregation and Neurotoxicity Based on free Radical Generation by the Peptide: Relevance to Alzheimer Disease," *Proc. Nat. Acad. Sc. U.S.A.*, **91**(8), 3270 (1994).

Hertel, C., E. Terzi, N. Hauser, R. Jakob-Rotne, J. Seelig, and J. A. Kemp, "Inhibition of the Electrostatic Interaction Between Beta-Amyloid Peptide and Membranes Prevents Beta-Amyloid-Induced Toxicity," *Proc. Natl. Acad. Sci. U.S.A.*, **94**(17), 9412 (1997).

Hilbich, C., B. Kisters-Woike, J. Reed, C. L. Masters, and K. Beyreuther, "Aggregation and Secondary Structure of Synthetic Amyloid Beta A4 Peptides of Alzheimer's Disease," *J. Mol. Biol.*, **218**(1), 149 (1991).

Hirakura, Y., M. C. Lin, and B. L. Kagan, "Alzheimer Amyloid Abeta1-42 Channels: Effects of Solvent, pH, and Congo Red," *J. Neurosci. Res.*, **57**(4), 458 (1999).

Holcomb, L., M. N. Gordon, E. McGowan, X. Yu, S. Benkovic, P. Jantzen, K. Wright, I. Saad, R. Mueller, D. Morgan, S. Sanders, C. Zehr, K. O'Campo, J. Hardy, C. M. Prada, C. Eckman, S. Younkin, K. Hsiao, and K. Duff, "Accelerated Alzheimer-Type Phenotype in Transgenic Mice Carrying Both Mutant Amyloid Precursor Protein and Presenilin 1 Transgenes," *Nat. Med.*, **4**(1), 97 (1998).

Holscher, C., "Possible Causes of Alzheimer's Disease: Amyloid Fragments, Free Radicals, and Calcium Homeostasis," *Neurobiol. Dis.*, **5**(3), 129 (1998).

Hoshi, M., M. Sato, S. Matsumoto, A. Noguchi, K. Yasutake, N. Yoshida, and K. Sato, "Spherical Aggregates of Beta-Amyloid (Amylospheroid) Show High Neurotoxicity and Activate Tau Protein Kinase I/Glycogen Synthase Kinase-3beta," *Proc. Natl. Acad. Sci. U.S.A.*, **100**(11), 6370 (2003).

Howland, D. S., S. P. Trusko, M. J. Savage, A. G. Reaume, D. M. Lang, J. D. Hirsch, N. Maeda, R. Siman, B. D. Greenberg, R. W. Scott, and D. G. Flood, "Modulation of Secreted Beta-Amyloid Precursor Protein and Amyloid Beta-Peptide in Brain by Cholesterol," *J. Biol. Chem.*, **273**(26), 16576 (1998).

Howlett, D. R., K. H. Jennings, D. C. Lee, M. S. Clark, F. Brown, R. Wetzel, S. J. Wood, P. Camilleri, and G. W. Roberts, "Aggregation State and Neurotoxic Properties of Alzheimer Beta-Amyloid Peptide," *Neurodegeneration*, **4**(1), 23 (1995).

Hsia, A. Y., E. Masliah, L. McConlogue, G. Q. Yu, G. Tatsuno, K. Hu, D. Kholodenko, R. C. Malenka, R. A. Nicoll, and L. Mucke, "Plaque-Independent Disruption of Neural Circuits in Alzheimer's Disease Mouse Models," *Proc. Natl. Acad .Sci. U.S.A.*, **96**(6), 3228 (1999).

Hyman, B. T., G. W. van Horsen, A. R. Damasio, and C. L. Barnes, "Alzheimer's Disease: Cell-Specific Pathology Isolates the Hippocampal Formation," *Science*, **225**(4667), 1168 (1984).

Imbimbo, B. P., "The Potential Role of Non-Steroidal Anti-

inflammatory Drugs in Treating Alzheimer's Disease," *Expert Opinion on Investigational Drugs*, **13**(11), 1469 (2004).

Inouye, H., P. E. Fraser, and D. A. Kirschner, "Structure of Beta-Crystallite Assemblies Formed by Alzheimer Beta-Amyloid Protein Analogues: Analysis by X-Ray Diffraction," *Biophys. J.*, **64**(2), 502 (1993).

Iqbal, K., C. Alonso Adel, E. El-Akkad, C. X. Gong, N. Haque, S. Khatoon, J. J. Pei, H. Tanimukai, I. Tsujio, J. Z. Wang, and I. Grundke-Iqba, "Alzheimer Neurofibrillary Degeneration: Therapeutic Targets and High-Throughput Assays," *J. Mol. Neurosci.*, **20**(3), 425 (2003).

Ishiura, S., "Proteolytic Cleavage of the Alzheimer's Disease Amyloid A4 Precursor Protein," *J. Neurochem.*, **56**(2), 363 (1991).

Iversen, L. L., R. J. Mortishire-Smith, S. J. Pollack, and M. S. Shearman, "The Toxicity In Vitro of Beta-Amyloid Protein," *Biochem. J.*, **311**(1), 1 (1995).

Jacchieri, S. G., "Study of Alpha-Helix to Beta-Strand to Beta-Sheet Transitions in Amyloid: the Role of Segregated Hydrophobic Beta-Strands," *Biophys. Chem.*, **74**(1), 23 (1998).

Janus, C., J. Pearson, J. McLaurin, P. M. Mathews, Y. Jiang, S. D. Schmidt, M. A. Chishti, P. Horne, D. Heslin, J. French, H. T. Mount, R. A. Nixon, M. Mercken, C. Bergeron, P. E. Fraser, P. St George-Hyslop, and D. Westaway, "A Beta Peptide Immunization Reduces Behavioural Impairment and Plaques in a Model of Alzheimer's Disease," *Nature*, **408**(6815), 979 (2000).

Jarrett, J. T. and P. T. Lansbury, Jr., "Amyloid Fibril Formation Requires a Chemically Discriminating Nucleation Event: Studies of an Amyloidogenic Sequence from the Bacterial Protein OsmB," *Biochemistry*, **31**(49), 12345 (1992).

Jarrett, J. T., E. P. Berger, and P. T. Lansbury, Jr., "The Carboxy Terminus of the Beta Amyloid Protein is Critical for the Seeding of Amyloid Formation: Implications for the Pathogenesis of Alzheimer's Disease," *Biochemistry*, **32**(18), 4693 (1993).

Jarvik, G. P., E. M. Wijsman, W. A. Kukull, G. D. Schellenberg, C. Yu, and E. B. Larson, "Interactions of Apolipoprotein E Genotype, Total Cholesterol Level, Age, and Sex in Prediction of Alzheimer's Disease: A Case-Control Study," *Neurology*, **45**(6), 1092 (1995).

Ji, S. R., Y. Wu, and S. F. Sui, "Study of the Correlation of Secondary Structure of Beta-Amyloid Peptide (Abeta40) with the Hydrophobic Exposure Under Different Conditions," *Gen. Physiol. Biophys.*, **21**(4), 415 (2002).

Johnson-Wood, K., M. Lee, R. Motter, K. Hu, G. Gordon, R. Barbour, K. Khan, M. Gordon, H. Tan, D. Games, I. Lieberburg, D. Schenk, P. Seubert, and L. McConlogue, "Amyloid Precursor Protein Processing and A Beta42 Deposition in a Transgenic Mouse Model of Alzheimer Disease," *Proc. Natl. Acad. Sci. U.S.A.*, **94**(4), 1550 (1997).

Jonsson, K. O., H. L. Hedin, and C. J. Fowler, "Investigation into the Rapid Effects of 17 Beta-Estradiol and Neuroactive Steroids Upon Beta-Amyloid(25-35)-Induced Activation of Phosphoinositide-Specific Phospholipase C in Human Platelets," *Methods Find Exp Clin Pharmacol*, **22**(8), 615 (2000).

Kakio, A., S. I. Nishimoto, K. Yanagisawa, Y. Kozutsumi, and K. Matsuzaki, "Cholesterol-Dependent Formation of GM1 Ganglioside-Bound Amyloid Beta-Protein, an Endogenous Seed for Alzheimer Amyloid," *J. Biol. Chem.*, **276**(27), 24985 (2001).

Kakio, A., S. Nishimoto, K. Yanagisawa, Y. Kozutsumi, and K. Matsuzaki, "Interactions of Amyloid Beta-Protein with Various Gangliosides in Raft-Like Membranes: Importance of GM1 Ganglioside-Bound form as an Endogenous Seed for Alzheimer Amyloid," *Biochemistry*, **41**(23), 7385 (2002).

Kang, J., H. G. Lemaire, A. Unterbeck, J. M. Salbaum, C. L. Masters, K. H. Grzeschik, G. Multhaup, K. Beyreuther, and B. Muller-Hill, "The Precursor of Alzheimer's Disease Amyloid A4 Protein Resembles a Cell-Surface Receptor," *Nature*, **325**(6106), 733 (1987).

Kawooya, J. K., T. L. Emmons, P. A. Gonzalez-DeWhitt, M. C. Camp, and S. C. D'Andrea, "Electrophoretic Mobility of Alzheimer's Amyloid-Beta Peptides in Urea-Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis," *Anal. Biochem.*, **323**(1), 103 (2003).

Kayed, R., E. Head, J. L. Thompson, T. M. McIntire, S. C. Milton, C. W. Cotman, and C. G. Glabe, "Common Structure of Soluble Amyloid Oligomers Implies Common Mechanism of Pathogenesis," *Science*, **300**(5618), 486 (2003).

Kelly, J. W., "The Alternative Conformations of Amyloidogenic Proteins and Their Multi-Step Assembly Pathways," *Curr. Opin. Struct. Biol.*, **8**(1), 101 (1998a).

Kelly, J. W., "The Environmental Dependency of Protein Folding Best Explains Prion and Amyloid Diseases," *Proc. Natl. Acad. Sci. U.S.A.*, **95**(3), 930 (1998b).

Kheterpal, I., S. Zhou, K. D. Cook, and R. Wetzel, "Abeta Amyloid Fibrils Possess a Core Structure Highly Resistant to Hydrogen Exchange," *Proc. Natl. Acad. Sci. U.S.A.*, **97**(25), 13597 (2000).

Kheterpal, I., A. Williams, C. Murphy, B. Bledsoe, and R. Wetzel, "Structural Features of the Abeta Amyloid Fibril Elucidated by Limited Proteolysis," *Biochemistry*, **40**(39), 11757 (2001).

Kheterpal, I., H. A. Lashuel, D. M. Hartley, T. Walz, P. T. Lansbury, Jr., and R. Wetzel, "Abeta Protofibrils Possess a Stable Core Structure Resistant to Hydrogen Exchange," *Biochemistry*, **42**(48), 14092 (2003a).

Kheterpal, I., R. Wetzel, and K. D. Cook, "Enhanced Correction Methods for Hydrogen Exchange-Mass

Spectrometric Studies of Amyloid Fibrils," *Protein. Sci.*, **12**(3), 635 (2003b).

Kilsdonk, E. P., P. G. Yancey, G. W. Stoudt, F. W. Bangerter, W. J. Johnson, M. C. Phillips, and G. H. Rothblat, "Cellular Cholesterol Efflux Mediated by Cyclodextrins," *J. Biol. Chem.*, **270**(29), 17250 (1995).

Kitaguchi, N., Y. Takahashi, Y. Tokushima, S. Shiojiri, and H. Ito, "Novel Precursor of Alzheimer's Disease Amyloid Protein Shows Protease Inhibitory Activity," *Nature*, **331**(6156), 530 (1988).

Klein, I., L. Moore, and I. Pastan, "Effect of Liposomes Containing Cholesterol on Adenylate Cyclase Activity of Cultured Mammalian Fibroblasts," *Biochim. Biophys. Acta.*, **506**(1), 42 (1978).

Klein, U., G. Gimpl, and F. Fahrenholz, "Alteration of the Myometrial Plasma Membrane Cholesterol Content with Beta-Cyclodextrin Modulates the Binding Affinity of the Oxytocin Receptor," *Biochemistry*, **34**(42), 13784 (1995).

Klein, W. L., G. A. Krafft, and C. E. Finch, "Targeting Small Abeta Oligomers: the Solution to an Alzheimer's Disease Conundrum?" *Trends Neurosci.*, **24**(4), 219 (2001).

Klein, W. L., W. B. Stine, Jr., and D. B. Teplow, "Small Assemblies of Unmodified Amyloid Beta-Protein are the Proximate Neurotoxin in Alzheimer's Disease," *Neurobiol. Aging.*, **25**(5), 569 (2004).

Kohno, T., K. Kobayashi, T. Maeda, K. Sato, and A. Takashima, "Three-Dimensional Structures of the Amyloid Beta Peptide (25-35) in Membrane-Mimicking Environment," *Biochemistry*, **35**(50), 16094 (1996).

Kourie, J. I., C. L. Henry, and P. Farrelly, "Diversity of Amyloid Beta Protein Fragment [1-40]-Formed Channels," *Cell. Mol. Neurobiol.*, **21**(3), 255 (2001).

Kowalska, A., D. Pruchnik-Wolinska, J. Florczak, R. Modestowicz, J. Szczech, W. Kozubski, G. Rossa, and M. Wender, "Genetic Study of Familial Cases of Alzheimer's Disease," *Acta. Biochim. Pol.*, **51**(1), 245 (2004).

Kremer, J. J., M. M. Pallitto, D. J. Sklansky, and R. M. Murphy, "Correlation of Beta-Amyloid Aggregate Size and Hydrophobicity with Decreased Bilayer Fluidity of Model Membranes," *Biochemistry*, **39**(33), 10309 (2000).

LaFerla, F. M., B. T. Tinkle, C. J. Bieberich, C. C. Haudenschild, and G. Jay, "The Alzheimer's A Beta Peptide Induces Neurodegeneration and Apoptotic Cell Death in Transgenic Mice," *Nat. Genet.*, **9**(1), 21 (1995).

Lambert, M. P., A. K. Barlow, B. A. Chromy, C. Edwards, R. Freed, M. Liosatos, T. E. Morgan, I. Rozovsky, B. Trommer, K. L. Viola, P. Wals, C. Zhang, C. E. Finch, G. A. Krafft, and W. L. Klein, "Diffusible, Nonfibrillar Ligands Derived from Abeta1-42 are Potent Central Nervous System Neurotoxins," *Proc. Natl. Acad. Sci. U.S.A.*, **95**(11), 6448 (1998).

Lansbury, P. T., Jr., "Evolution of Amyloid: What Normal Protein Folding May Tell Us About Fibrillogenesis and Disease," *Proc. Natl. Acad. Sci. U.S.A.*, **96**(7), 3342 (1999).

Le Prince, G., "Alterations of Glial Fibrillary Acidic Protein mRNA Level in the Aging Brain and in Seneile Dementia of the Alzheimer Type," *Neuroscience Lett.*, **151**,71 (1993).

Lendon, C. L., A. Martinez, I. M. Behrens, K. S. Kosik, L. Madrigal, J. Norton, R. Neuman, A. Myers, F. Busfield, M. Wragg, M. Arcos, J. C. Arango Viana, J. Ossa, A. Ruiz, A. M. Goate, and F. Lopera, "E280A PS-1 Mutation Causes Alzheimer's Disease but Age of Onset is not Modified by ApoE Alleles," *Hum. Mutat.*, **10**(3), 186 (1997).

Lewis, J., D. W. Dickson, W. L. Lin, L. Chisholm, A. Corral, G. Jones, S. H. Yen, N. Sahara, L. Skipper, D. Yager, C. Eckman, J. Hardy, M. Hutton, and E. McGowan, "Enhanced Neurofibrillary Degeneration in Transgenic Mice Expressing Mutant Tau and APP," *Science*, **293**(5534), 1487 (2001).

Li, W. Y., D. L. Czilli, and L. K. Simmons, "Neuronal Membrane Conductance Activated by Amyloid Beta Peptide: Importance of Peptide Conformation," *Brain Res.*, **682**(1-2), 207 (1995).

Li, L., S. Zeigler, R. J. Lindsey, and K. Fukuchi, "Effects of an Atherogenic Diet on Amyloidosis in Transgenic Mice Overexpressing the C-Terminal Portion of β-Amyloid Precursor Protein," *Soc. Neurosci.*, **25**, 1859 (1999).

Lim, G. P., F. Yang, T. Chu, P. Chen, W. Beech, B. Teter, T. Tran, O. Ubeda, K. H. Ashe, S. A. Frautschy, and G. M. Cole, "Ibuprofen Suppresses Plaque Pathology and Inflammation in a Mouse Model for Alzheimer's Disease," *J. Neurosci.*, **20**(15), 5709 (2000).

Lin, H., Y. J. Zhu, and R. Lal, "Amyloid Beta Protein (1-40) Forms Calcium-Permeable, Zn^{2+}-Sensitive Channel in Reconstituted Lipid Vesicles," *Biochemistry*, **38**(34), 11189 (1999).

Linder, M. E. and A. G. Gilman, "G Proteins," *Sci. Am.*, **267**(1), 56 (1992).

Lockhart, B. P., C. Benicourt, J. L. Junien, and A. Privat, "Inhibitors of Free Radical Formation Fail to Attenuate Direct Beta-Amyloid (25-35) Peptide-Mediated Neurotoxicity in Rat Hippocampal Cultures," *J. Neurosci. Res.*, **39**(4), 494 (1994).

Lomakin, A., D. S. Chung, G. B. Benedek, D. A. Kirschner, and D. B. Teplow, "On the Nucleation and Growth of Amyloid Beta-Protein Fibrils: Detection of Nuclei and Quantitation of Rate Constants," *Proc. Natl. Acad. Sci. U.S.A.*, **93**(3), 1125 (1996).

Lynn, D. G. and S. C. Meredith, "Review: Model Peptides and the Physicochemical Approach to Beta-Amyloids," *J. Struct. Biol.*, **130**(2-3), 153 (2000).

Mansfield, S. L., D. A. Jayawickrama, J. S. Timmons, and C. K. Larive, "Measurement of Peptide Aggregation with Pulsed-Field Gradient Nuclear Magnetic Resonance Spectroscopy," *Biochim. Biophys. Acta.*, **1382**(2), 257 (1998).

Mark, R. J., K. Hensley, D. A. Butterfield, and M. P. Mattson, "Amyloid Beta-Peptide Impairs Ion-Motive ATPase Activities: Evidence for a Role in Loss of Neuronal Ca^{2+} Homeostasis and Cell Death," *J. Neurosci.*, **15**(9), 6239 (1995).

Markesbery, W. R., "Oxidative Stress Hypothesis in Alzheimer's Disease," *Free Radic. Biol. Med.*, **23**(1), 134 (1997).

Martins, R. N., P. J. Robinson, J. O. Chleboun, K. Beyreuther, and C. L. Masters, "The Molecular Pathology of Amyloid Deposition in Alzheimer's Disease," *Mol Neurobiol*, **5**(2-4), 389 (1991).

Marx, H. H., "The Eilemma of Effectiveness and Economic Value of Alternative Medicine," *Gesundheitswesen*, **59**(5), 297 (1997).

Masdeu, J. C., J. L. Zubieta, and J. Arbizu, "Neuroimaging as a Marker of the Onset and Progression of Alzheimer's Disease," *J. Neurol. Sci.*, **236**, 55 (2005).

Masliah, E., A. Sisk, M. Mallory, L. Mucke, D. Schenk, and D. Games, "Comparison of Neurodegenerative Pathology in Transgenic Mice Overexpressing V717F Beta-Amyloid Precursor Protein and Alzheimer's Disease," *J. Neurosci.*, **16**(18), 5795 (1996).

Mason, R. P., W. J. Shoemaker, L. Shajenko, and L. G. Herbette, "X-Ray Diffraction Analysis of Brain Lipid Membrane Structure in Alzheimer's Disease and Beta-Amyloid Peptide Interactions," *Ann. N. Y. Acad. Sci.*, **695**, 54 (1993).

Mason, R. P., R. F. Jacob, M. F. Walter, P. E. Mason, N. A. Avdulov, S. V. Chochina, U. Igbavboa, and W. G. Wood, "Distribution and Fluidizing Action of Soluble and Aggregated Amyloid Beta-Peptide in Rat Synaptic Plasma Membranes," *J. Biol. Chem.*, **274**(26), 18801 (1999).

Matsuzaki, K. and C. Horikiri, "Interactions of Amyloid Beta-Peptide (1-40) with Ganglioside-Containing Membranes," *Biochemistry*, **38**(13), 4137 (1999).

Mattson, M. P., B. Cheng, A. R. Culwell, F. S. Esch, I. Lieberburg, and R. E. Rydel, "Evidence for Excitoprotective and Intraneuronal Calcium-Regulating Roles for Secreted Forms of the Beta-Amyloid Precursor Protein," *Neuron*, **10**(2), 243 (1993).

Mattson, M. P. and Y. Goodman, "Different Amyloidogenic Peptides Share a Similar Mechanism of Neurotoxicity Involving Reactive Oxygen Species and Calcium," *Brain Res.*, **676**(1), 219 (1995).

McLaurin, J., T. Franklin, P. E. Fraser, and A. Chakrabartty, "Structural Transitions Associated with the Interaction of Alzheimer Beta-Amyloid Peptides with Gangliosides," *J. Biol. Chem.*, **273**(8), 4506 (1998).

Meda, L., P. Baron, and G. Scarlato, "Glial Activation in Alzheimer's Disease: the Role of Abeta and Its Associated Proteins," *Neurobiol. Aging.*, **22**(6), 885 (2001).

Michaelis, M. L., S. Ansar, Y. Chen, E. R. Reiff, K. I. Seyb, R. H. Himes, K. L. Audus, and G. I. Georg, "{Beta}-Amyloid-Induced Neurodegeneration and Protection by Structurally Diverse Microtubule-Stabilizing Agents," *J. Pharmacol. Exp. Ther.*, **312**(2), 659 (2005).

Mirzabekov, T., M. C. Lin, W. L. Yuan, P. J. Marshall, M. Carman, K. Tomaselli, I. Lieberburg, and B. L. Kagan, "Channel Formation in Planar Lipid Bilayers by a Neurotoxic Fragment of the Beta-Amyloid Peptide," *Biochem. Biophys. Res. Commun.*, **202**(2), 1142 (1994).

Mitchell, D. C., M. Straume, J. L. Miller, and B. J. Litman, "Modulation of Metarhodopsin Formation by Cholesterol-Induced Ordering of Bilayer Lipids," *Biochemistry*, **29**(39), 9143 (1990).

Miyata, T., R. Inagi, Y. Iida, M. Sato, N. Yamada, O. Oda, K. Maeda, and H. Seo, "Involvement of Beta 2-Microglobulin Modified with Advanced Glycation End Products in the Pathogenesis of Hemodialysis-Associated Amyloidosis. Induction of Human Monocyte Chemotaxis and Macrophage Secretion of Tumor Necrosis Factor-Alpha and Interleukin-1," *J. Clin. Invest.*, **93**(2), 521 (1994).

Mizuno, T., M. Nakata, H. Naiki, M. Michikawa, R. Wang, C. Haass, and K. Yanagisawa, "Cholesterol-Dependent Generation of a Seeding Amyloid Beta-Protein in Cell Culture," *J. Biol. Chem.*, **274**(21), 15110 (1999).

Molnar, Z., P. Kovacs, I. Laczko, K. Soos, L. Fulop, B. Penke, and I. Lengyel, "Enhanced G-Protein Activation by a Mixture of Abeta(25-35), Abeta(1-40/42) and Zinc," *J. Neurochem.*, **89**(5), 1215 (2004).

Morgan, D., D. M. Diamond, P. E. Gottschall, K. E. Ugen, C. Dickey, J. Hardy, K. Duff, P. Jantzen, G. DiCarlo, D. Wilcock, K. Connor, J. Hatcher, C. Hope, M. Gordon, and G. W. Arendash, "A Beta Peptide Vaccination Prevents Memory Loss in an Animal Model of Alzheimer's Disease," *Nature*, **408**(6815), 982 (2000).

Muller, W. E., S. Koch, A. Eckert, H. Hartmann, and K. Scheuer, "Beta-Amyloid Peptide Decreases Membrane Fluidity," *Brain Res.*, **674**(1), 133 (1995).

Muller, W. E., G. P. Eckert, K. Scheuer, N. J. Cairns, A. Maras, and W. F. Gattaz, "Effects of Beta-Amyloid Peptides on the Fluidity of Membranes from Frontal and Parietal Lobes of Human Brain. High Potencies of A Beta 1- 42 and A Beta 1-43," *Amyloid*, **5**(1), 10 (1998).

Muller, W. E., C. Kirsch, and G. P. Eckert, "Membrane-Disordering Effects of Beta-Amyloid Peptides," *Biochem. Soc. Trans.*, **29**(Pt 4), 617 (2001).

Murphy, R. M., "Peptide Aggregation in Neurodegenerative Disease," *Annu. Rev. Biomed. Eng.*, **4**, 155 (2002).

Nagele, R. G., J. Wegiel, V. Venkataraman, H. Imaki, and K. C. Wang, "Contribution of Glial Cells to the Development of Amyloid Plaques in Alzheimer's Disease," *Neurobiol. Aging.*, **25**(5), 663 (2004).

Naidu, A., D. Quon, and B. Cordell, "Beta-Amyloid Peptide Produced In Vitro is Degraded by Proteinases Released by

Cultured Cells," *J. Biol. Chem.*, **270**(3), 1369 (1995).

Naiki, H. and K. Nakakuki, "First-Order Kinetic Model of Alzheimer's Beta-Amyloid Fibril Extension In Vitro," *Lab. Invest.*, **74**(2), 374 (1996).

Neufeld, E. B., A. M. Cooney, J. Pitha, E. A. Dawidowicz, N. K. Dwyer, P. G. Pentchev, and E. J. Blanchette-Mackie, "Intracellular Trafficking of Cholesterol Monitored with a Cyclodextrin," *J. Biol. Chem.*, **271**(35), 21604 (1996).

Nishimoto, I., T. Okamoto, Y. Matsuura, S. Takahashi, Y. Murayama, and E. Ogata, "Alzheimer Amyloid Protein Precursor Complexes with Brain GTP-Binding Protein G(o)," *Nature*, **362**(6415), 75 (1993).

Nitsch, R. M., S. A. Farber, J. H. Growdon, and R. J. Wurtman, "Release of Amyloid Beta-Protein Precursor Derivatives by Electrical Depolarization of Rat Hippocampal Slices," *Proc. Natl. Acad. Sci. U.S.A.*, **90**(11), 5191 (1993).

Nitta, A., T. Fukuta, T. Hasegawa, and T. Nabeshima, "Continuous Infusion of Beta-Amyloid Protein into the Rat Cerebral Ventricle Induces Learning Impairment and Neuronal and Morphological Degeneration," *Jpn. J. Pharmacol.*, **73**(1), 51 (1997).

Nordstedt, C., J. Naslund, L. O. Tjernberg, A. R. Karlstrom, J. Thyberg, and L. Terenius, "The Alzheimer A Beta Peptide Develops Protease Resistance in Association with Its Polymerization into Fibrils," *J. Biol. Chem.*, **269**(49), 30773 (1994).

Notkola, I. L., R. Sulkava, J. Pekkanen, T. Erkinjuntti, C. Ehnholm, P. Kivinen, J. Tuomilehto, and A. Nissinen, "Serum Total Cholesterol, Apolipoprotein E Epsilon 4 Allele, and Alzheimer's Disease," *Neuroepidemiology*, **17**(1), 14 (1998).

Okamoto, T., S. Takeda, Y. Murayama, E. Ogata, and I. Nishimoto, "Ligand-Dependent G Protein Coupling Function of Amyloid Transmembrane Precursor," *J. Biol. Chem.*, **270**(9), 4205 (1995).

Pallitto, M. M., J. Ghanta, P. Heinzelman, L. L. Kiessling, and R. M. Murphy, "Recognition Sequence Design for Peptidyl Modulators of Beta-Amyloid Aggregation and Toxicity," *Biochemistry*, **38**(12), 3570 (1999).

Pappolla, M. A., Y. J. Chyan, R. A. Omar, K. Hsiao, G. Perry, M. A. Smith, and P. Bozner, "Evidence of Oxidative Stress and In Vivo Neurotoxicity of Beta-Amyloid in a Transgenic Mouse Model of Alzheimer's Disease: A Chronic Oxidative Paradigm for Testing Antioxidant Therapies In Vivo," *Am. J. Pathol.*, **152**(4), 871 (1998).

Pereira, C., M. S. Santos, and C. Oliveira, "Involvement of Oxidative Stress on the Impairment of Energy Metabolism Induced by A Beta Peptides on PC12 Cells: Protection by Antioxidants," *Neurobiol. Dis.*, **6**(3), 209 (1999).

Petkova, A. T., G. Buntkowsky, F. Dyda, R. D. Leapman, W. M. Yau, and R. Tycko, "Solid State NMR Reveals a pH-Dependent Antiparallel Beta-Sheet Registry in Fibrils Formed by a Beta-Amyloid Peptide," *J. Mol. Biol.*, **335**(1), 247 (2004).

Petrella, J. R., R. E. Coleman, and P. M. Doraiswamy, "Neuroimaging and Early Diagnosis of Alzheimer Disease: a Look to the Future," *Radiology*, **226**(2), 315 (2003).

Pike, C. J., D. Burdick, A. J. Walencewicz, C. G. Glabe, and C. W. Cotman, "Neurodegeneration Induced by Beta-Amyloid Peptides In Vitro: the Role of Peptide Assembly State," *J. Neurosci.*, **13**(4), 1676 (1993).

Plant, L. D., J. P. Boyle, I. F. Smith, C. Peers, and H. A. Pearson, "The Production of Amyloid Beta Peptide is a Critical Requirement for the Viability of Central Neurons," *J. Neurosci.*, **23**(13), 5531 (2003).

Pollard, H. B., N. Arispe, and E. Rojas, "Ion Channel Hypothesis for Alzheimer Amyloid Peptide Neurotoxicity," *Cell Mol. Neurobiol.*, **15**(5), 513 (1995).

Racchi, M., R. Baetta, N. Salvietti, P. Ianna, G. Franceschini, R. Paoletti, R. Fumagalli, S. Govoni, M. Trabucchi, and M. Soma, "Secretory Processing of Amyloid Precursor Protein is Inhibited by Increase in Cellular Cholesterol Content," *Biochem. J.*, **322**(3), 893 (1997).

Refolo, L. M., B. Malester, J. LaFrancois, T. Bryant-Thomas, R. Wang, G. S. Tint, K. Sambamurti, K. Duff, and M. A. Pappolla, "Hypercholesterolemia Accelerates the Alzheimer's Amyloid Pathology in a Transgenic Mouse Model," *Neurobiol. Dis.*, **7**(4), 321 (2000).

Refolo, L. M., M. A. Pappolla, J. LaFrancois, B. Malester, S. D. Schmidt, T. Thomas-Bryant, G. S. Tint, R. Wang, M. Mercken, S. S. Petanceska, and K. E. Duff, "A Cholesterol-Lowering Drug Reduces Beta-Amyloid Pathology in a Transgenic Mouse Model of Alzheimer's Disease," *Neurobiol. Dis.*, **8**(5), 890 (2001).

Rhee, S. K., A. P. Quist, and R. Lal, "Amyloid Beta Protein-(1-42) forms Calcium-Permeable, Zn^{2+}-Sensitive Channel," *J. Biol. Chem.*, **273**(22), 13379 (1998).

Rogaev, E. I., R. Sherrington, E. A. Rogaeva, G. Levesque, M. Ikeda, Y. Liang, H. Chi, C. Lin, K. Holman, and T. Tsuda, "Familial Alzheimer's Disease in Kindreds with Missense Mutations in a Gene on Chromosome 1 Related to the Alzheimer's Disease Type 3 Gene," *Nature*, **376**(6543), 775 (1995).

Rogers, J., N. R. Cooper, S. Webster, J. Schultz, P. L. McGeer, S. D. Styren, W. H. Civin, L. Brachova, B. Bradt, P. Ward, "Complement Activation by Beta-Amyloid in Alzheimer Disease," *Proc. Natl. Acad. Sci. U.S.A.*, **89**(21), 10016 (1992).

Roher, A. E., M. O. Chaney, Y. M. Kuo, S. D. Webster, W. B. Stine, L. J. Haverkamp, A. S. Woods, R. J. Cotter, J. M. Tuohy, G. A. Krafft, B. S. Bonnell, and M. R. Emmerling, "Morphology and Toxicity of Abeta-(1-42) Dimer Derived from Neuritic and Vascular Amyloid Deposits of Alzheimer's Disease," *J. Biol. Chem.*, **271**(34), 20631 (1996).

Ross, C. A. and M. A. Poirier, "Protein Aggregation and Neurodegenerative Disease," *Nat. Med.*, 10 Suppl, S10 (2004).

Roth, G. S., J. A. Joseph, and R. P. Mason, "Membrane Alterations as Causes of Impaired Signal Transduction in Alzheimer's Disease and Aging," *Trends Neurosci.*, **18**(5), 203 (1995).

Rymer, D. L. and T. A. Good, "The Role of G Protein Activation in the Toxicity of Amyloidogenic Abeta- (1-40), Abeta-(25-35), and Bovine Calcitonin," *J. Biol. Chem.*, **276**(4), 2523 (2001).

Sadowski, M., J. Pankiewicz, H. Scholtzova, J. A. Ripellino, Y. Li, S. D. Schmidt, P. M. Mathews, J. D. Fryer, D. M. Holtzman, E. M. Sigurdsson, and T. Wisniewski, "A Synthetic Peptide Blocking the Apolipoprotein E/Beta-Amyloid Binding Mitigates Beta-Amyloid Toxicity and Fibril Formation In Vitro and Reduces Beta-Amyloid Plaques in Transgenic Mice," *Am. J. Pathol.*, **165**(3), 937 (2004).

Saunders, A. M., W. J. Strittmatter, D. Schmechel, P. H. George-Hyslop, M. A. Pericak-Vance, S. H. Joo, B. L. Rosi, J. F. Gusella, D. R. Crapper-MacLachlan, and M. J. Alberts, "Association of Apolipoprotein E Allele Epsilon 4 with Late-Onset Familial and Sporadic Alzheimer's Disease," *Neurology*, **43**(8), 1467 (1993).

Saunders, A. M., "Gene Identification in Alzheimer's Disease," *Pharmacogenomics*, **2**(3), 239 (2001).

Schellenberg, G. D., "Genetic Dissection of Alzheimer Disease, a Heterogeneous Disorder," *Proc. Natl. Acad. Sci. U.S.A.*, **92**(19), 8552 (1995).

Schmechel, D. E., A. M. Saunders, W. J. Strittmatter, B. J. Crain, C. M. Hulette, S. H. Joo, M. A. Pericak-Vance, D. Goldgaber, and A. D. Roses, "Increased Amyloid Beta-Peptide Deposition in Cerebral Cortex as a Consequence of Apolipoprotein E Genotype in Late-Onset Alzheimer Disease," *Proc. Natl. Acad. Sci. U.S.A.*, **90**(20), 9649 (1993).

Schnecko, A., K. Witte, J. Bohl, T. Ohm, and B. Lemmer, "Adenylyl Cyclase Activity in Alzheimer's Disease Brain: Stimulatory and Inhibitory Signal Transduction Pathways are Differently Affected," *Brain Res.*, **644**(2), 291 (1994).

Schroeder, F., J. R. Jefferson, A. B. Kier, J. Knittel, T. J. Scallen, W. G. Wood, and I. Hapala, "Membrane Cholesterol Dynamics: Cholesterol Domains and Kinetic Pools," *Proc. Soc. Exp. Biol. Med.*, **196**(3), 235 (1991).

Schuster, D., A. Rajendran, S. W. Hui, T. Nicotera, T. Srikrishnan, and M. L. Kruzel, "Protective Effect of Colostrinin on Neuroblastoma cell Survival is Due to Reduced Aggregation of Beta-Amyloid," *Neuropeptides*, (2005).

Seelig, J., R. Lehrmann, and E. Terzi, "Domain Formation Induced by Lipid-Ion and Lipid-Peptide Interactions," *Mol. Membr. Biol.*, **12**(1), 51 (1995).

Seilheimer, B., B. Bohrmann, L. Bondolfi, F. Muller, D. Stuber, and H. Dobeli, "The Toxicity of the Alzheimer's Beta-Amyloid Peptide Correlates with a Distinct Fiber Morphology," *J. Struct. Biol.*, **119**(1), 59 (1997).

Selkoe, D. J., M. B. Podlisny, C. L. Joachim, E. A. Vickers, G. Lee, L. C. Fritz, and T. Oltersdorf, "Beta-Amyloid Precursor Protein of Alzheimer Disease Occurs as 110- to 135-Kilodalton Membrane-Associated Proteins in Neural and Nonneural Tissues," *Proc. Natl. Acad. Sci. U.S.A.*, **85**(19), 7341 (1988).

Selkoe, D. J., "Biochemistry of Altered Brain Proteins in Alzheimer's Disease," *Annu. Rev. Neurosci.*, **12**, 463 (1989).

Selkoe, D. J., "Amyloid Protein and Alzheimer's Disease," *Sci. Am.*, **265**(5), 68 (1991).

Selkoe, D. J., "Amyloid Beta-Protein and the Genetics of Alzheimer's Disease," *J. Biol. Chem.*, **271**(31), 18295 (1996).

Selkoe, D. J., "Alzheimer's Disease: Genotypes, Phenotypes, and Treatments," *Science*, **275**(5300), 630 (1997).

Serpell, L. C., "Alzheimer's Amyloid Fibrils: Structure and Assembly," *Biochim. Biophys. Acta.*, **1502**(1), 16 (2000).

Shen, C. L. and R. M. Murphy, "Solvent Effects on Self-Assembly of Beta-Amyloid Peptide," *Biophys. J.*, **69**(2), 640 (1995).

Shie, F. S., R. C. LeBoeuf, J. B. Leverenz, and L. W. Jin, "Effects of Cholesterol Feeding on Histopathologic Hallmarks of Alzheimer's Disease in β-Amyloid Precursor Protein (APP) Transgenic Mice," *Soc. Neurosci.*, **25**, 1859 (1999).

Shin, R. W., K. Ogino, A. Kondo, T. C. Saido, J. Q. Trojanowski, T. Kitamoto, and J. Tateishi, "Amyloid Beta-Protein (Abeta) 1-40 but not Abeta1-42 Contributes to the Experimental Formation of Alzheimer Disease Amyloid Fibrils in Rat Brain," *J. Neurosci.*, **17**(21), 8187 (1997).

Simmons, L. K., P. C. May, K. J. Tomaselli, R. E. Rydel, K. S. Fuson, E. F. Brigham, S. Wright, I. Lieberburg, G. W. Becker, and D. N. Brems, "Secondary Structure of Amyloid Beta Peptide Correlates with Neurotoxic Activity In Vitro," *Mol. Pharmacol.*, **45**(3), 373 (1994).

Simon, M. I., M. P. Strathmann, and N. Gautam, "Diversity of G Proteins in Signal Transduction," *Science*, **252**(5007), 802 (1991).

Simons, M., P. Keller, B. De Strooper, K. Beyreuther, C. G. Dotti, and K. Simons, "Cholesterol Depletion Inhibits the Generation of Beta-Amyloid in Hippocampal Neurons," *Proc. Natl. Acad. Sci. U.S.A.*, **95**(11), 6460 (1998).

Singh, I. N., G. Sorrentino, D. S. Sitar, and J. N. Kanfer, "(-) Nicotine Inhibits the Activations of Phospholipases A2 and D by Amyloid Beta Peptide," *Brain Res.*, **800**(2), 275 (1998).

Sinha, A. K., S. J. Shattil, and R. W. Colman, "Cyclic AMP Metabolism in Cholesterol-Rich Platelets," *J. Biol. Chem.*,

252(10), 3310 (1977).

Small, D. H., S. S. Mok, and J. C. Bornstein, "Alzheimer's Disease and Abeta Toxicity: from Top to Bottom," *Nat. Rev. Neurosci.*, **2**(8), 595 (2001).

Snyder, S. W., U. S. Ladror, W. S. Wade, G. T. Wang, L. W. Barrett, E. D. Matayoshi, H. J. Huffaker, G. A. Krafft, and T. F. Holzman, "Amyloid-Beta Aggregation: Selective Inhibition of Aggregation in Mixtures of Amyloid with Different Chain Lengths," *Biophys. J.*, **67**(3), 1216 (1994).

Soomets, U., R. Mahlapuu, R. Tehranian, J. Jarvet, E. Karelson, M. Zilmer, K. Iverfeldt, M. Zorko, A. Graslund, and U. Langel, "Regulation of GTPase and Adenylate Cyclase Activity by Amyloid Beta-Peptide and Its Fragments in Rat Brain Tissue," *Brain Res.*, **850**(1-2), 179 (1999).

Soreghan, B., J. Kosmoski, and C. Glabe, "Surfactant Properties of Alzheimer's A Beta Peptides and the Mechanism of Amyloid Aggregation," *J. Biol. Chem.*, **269**(46), 28551 (1994).

Soto, C., E. M. Castano, B. Frangione, and N. C. Inestrosa, "The Alpha-Helical to Beta-Strand Transition in the Amino-Terminal Fragment of the Amyloid Beta-Peptide Modulates Amyloid Formation," *J. Biol. Chem.*, **270**(7), 3063 (1995).

Soto, C., "Alzheimer's and Prion Disease as Disorders of Protein Conformation: Implications for the Design of Novel Therapeutic Approaches," *J. Mol. Med.*, **77**(5), 412 (1999).

Stein-Behrens, B., K. Adams, M. Yeh, and R. Sapolsky, "Failure of Beta-Amyloid Protein Fragment 25-35 to Cause Hippocampal Damage in the Rat," *Neurobiol. Aging*, **13**(5), 577 (1992).

Sticht, H., P. Bayer, D. Willbold, S. Dames, C. Hilbich, K. Beyreuther, R. W. Frank, and P. Rosch, "Structure of Amyloid A4-(1-40)-Peptide of Alzheimer's Disease," *Eur. J. Biochem.*, **233**(1), 293 (1995).

Stine, W. B., Jr., K. N. Dahlgren, G. A. Krafft, and M. J. LaDu, "In Vitro Characterization of Conditions for Amyloid-Beta Peptide Oligomerization and Fibrillogenesis," *J. Biol. Chem.*, **278**(13), 11612 (2003).

Sung, Y. J., C. L. Cheng, C. S. Chen, H. B. Huang, F. L. Huang, P. C. Wu, M. S. Shiao, and H. J. Tsay, "Distinct Mechanisms Account for Beta-Amyloid Toxicity in PC12 and Differentiated PC12 Neuronal Cells," *J. Biomed. Sci.*, **10**(4), 379 (2003).

Szabo, Z., E. Klement, K. Jost, M. Zarandi, K. Soos, and B. Penke, "An FT-IR Study of the Beta-Amyloid Conformation: Standardization of Aggregation Grade," *Biochem. Biophys. Res. Commun.*, **265**(2), 297 (1999).

Talafous, J., K. J. Marcinowski, G. Klopman, and M. G. Zagorski, "Solution Structure of Residues 1-28 of the Amyloid Beta-Peptide," *Biochemistry*, **33**(25), 7788 (1994).

Tandon, A. and P. Fraser, "The Presenilins," *Genome Biol.*, **3**(11), Reviews3014 (2002).

Tashima, Y., R. Oe, S. Lee, G. Sugihara, E. J. Chambers, M. Takahashi, and T. Yamada, "The Effect of Cholesterol and Monosialoganglioside (GM1) on the Release and Aggregation of Amyloid Beta-Peptide from Liposomes Prepared From Brain Membrane-Llike Lipids," *J. Biol. Chem.*, **279**(17), 17587 (2004).

Taubes, G., "Misfolding the Way to Disease," *Science*, **271**(5255), 1493 (1996).

Taylor, J. P., J. Hardy, and K. H. Fischbeck, "Toxic Proteins in Neurodegenerative Disease," *Science*, **296**(5575), 1991 (2002).

Terry, R. D. and R. Katzman, "Senile Dementia of the Alzheimer Type," *Ann. Neurol.*, **14**(5), 497 (1983).

Terzi, E., G. Holzemann, and J. Seelig, "Alzheimer Beta-Amyloid Peptide 25-35: Electrostatic Interactions with Phospholipid Membranes," *Biochemistry*, **33**(23), 7434 (1994).

Terzi, E., G. Holzemann, and J. Seelig, "Self-Association of Beta-Amyloid Peptide (1-40) in Solution and Binding to Lipid Membranes," *J. Mol. Biol.*, **252**(5), 633 (1995).

Terzi, E., G. Holzemann, and J. Seelig, "Interaction of Alzheimer Beta-Amyloid Peptide(1-40) with Lipid Membranes," *Biochemistry*, **36**(48), 14845 (1997).

Tettamanti, G. and L. Riboni, "Gangliosides and Modulation of the Function of Neural Cells," *Adv. Lipid. Res.*, **25**, 235 (1993).

Tjernberg, L. O., D. J. Callaway, A. Tjernberg, S. Hahne, C. Lilliehook, L. Terenius, J. Thyberg, and C. Nordstedt, "A Molecular Model of Alzheimer Amyloid Beta-Peptide Fibril Formation," *J. Biol. Chem.*, **274**(18), 12619 (1999).

Tjernberg, L. O., J. Naslund, F. Lindqvist, J. Johansson, A. R. Karlstrom, J. Thyberg, L. Terenius, and C. Nordstedt, "Arrest of Beta-Amyloid Fibril Formation by a Pentapeptide Ligand," *J. Biol. Chem.*, **271**(15), 8545 (1996).

Tomiyama, T., S. Asano, Y. Suwa, T. Morita, K. Kataoka, H. Mori, and N. Endo, "Rifampicin Prevents the Aggregation and Neurotoxicity of Amyloid Beta Protein In Vitro," *Biochem. Biophys. Res. Commun.*, **204**(1), 76 (1994).

Tomski, S. J. and R. M. Murphy, "Kinetics of Aggregation of Synthetic Beta-Amyloid Peptide," *Arch. Biochem. Biophys.*, **294**(2), 630 (1992).

Uversky, V. N. and A. L. Fink, "Conformational Constraints for Amyloid Fibrillation: the Importance of Being Unfolded," *Biochim. Biophys. Acta.*, **1698**(2), 131 (2004).

Varadarajan, S., S. Yatin, M. Aksenova, and D. A. Butterfield, "Review: Alzheimer's Amyloid Beta-Peptide-Associated free Radical Oxidative Stress and Neurotoxicity [In Process Citation]," *J. Struct. Biol.*, **130**(2-3), 184 (2000).

Vaudry, D., C. Cottet-Rousselle, M. Basille, A. Falluel-Morel, A. Fournier, H. Vaudry, and B. J. Gonzalez, "Pituitary

Adenylate Cyclase-Activating Polypeptide Inhibits Caspase-3 Activity but does not Protect Cerebellar Granule Neurons Against Beta-Amyloid (25-35)-Induced Apoptosis," *Regul. Pept.*, **123**(1-3), 43 (2004).

Villemagne, V. L., C. C. Rowe, S. Macfarlane, K. E. Novakovic, and C. L. Masters, "Imaginem Oblivionis: the Prospects of Neuroimaging for Early Detection of Alzheimer's Disease," *J. Clin. Neurosci.*, **12**(3), 221 (2005).

Vitek, M. P., K. Bhattacharya, J. M. Glendening, E. Stopa, H. Vlassara, R. Bucala, K. Manogue, and A. Cerami, "Advanced Glycation End Products Contribute to Amyloidosis in Alzheimer Disease," *Proc. Natl. Acad. Sci. U.S.A.*, **91**(11), 4766 (1994).

Wakabayashi, M., T. Okada, Y. Kozutsumi, and K. Matsuzaki, "GM1 Ganglioside-Mediated Accumulation of Amyloid Beta-Protein on Cell Membranes," *Biochem. Biophys. Res. Commun.*, **328**(4), 1019 (2005).

Walsh, D. M., A. Lomakin, G. B. Benedek, M. M. Condron, and D. B. Teplow, "Amyloid Beta-Protein Fibrillogenesis. Detection of a Protofibrillar Intermediate," *J. Biol. Chem.*, **272**(35), 22364 (1997).

Walsh, D. M., D. M. Hartley, Y. Kusumoto, Y. Fezoui, M. M. Condron, A. Lomakin, G. B. Benedek, D. J. Selkoe, and D. B. Teplow, "Amyloid Beta-Protein Fibrillogenesis. Structure and Biological Activity of Protofibrillar Intermediates," *J. Biol. Chem.*, **274**(36), 25945 (1999).

Walsh, D. M., I. Klyubin, J. V. Fadeeva, W. K. Cullen, R. Anwyl, M. S. Wolfe, M. J. Rowan, and D. J. Selkoe, "Naturally Secreted Oligomers of Amyloid Beta Protein Potently Inhibit Hippocampal Long-Term Potentiation In Vivo," *Nature*, **416**(6880), 535 (2002).

Wang, S. S., D. L. Rymer, and T. A. Good, "Reduction in Cholesterol and Sialic Acid Content Protects Cells from the Toxic Effects of Beta -Amyloid Peptides," *J. Biol. Chem.*, **276**(45), 42027 (2001).

Wang, S. S., A. Becerra-Arteaga, and T. A. Good, "Development of a Novel Diffusion-Based Method to Estimate the Size of the Aggregated Abeta Species Responsible for Neurotoxicity," *Biotechnol. Bioeng.*, **80**(1), 50 (2002).

Wang, S. S., V. Kazantzi, and T. A. Good, "A Kinetic Analysis of the Mechanism of Beta-Amyloid Induced G Protein Activation," *J. Theor. Biol.*, **221**(2), 269 (2003a).

Wang, S. S., S. A. Tobler, T. A. Good, and E. J. Fernandez, "Hydrogen Exchange-Mass Spectrometry Analysis of Beta-Amyloid Peptide Structure," *Biochemistry*, **42**(31), 9507 (2003b).

Wang, S. S., T. A. Good, and D. L. Rymer, "The Influence of Phospholipid Membranes on Bovine Calcitonin Peptide's Secondary Structure and Induced Neurotoxic Effects," *Int. J. Biochem. Cell. Biol.*, **37**(8), 1656 (2005a).

Wang, S. S., T. A. Good, and D. L. Rymer, "The Influence of Phospholipid Membranes on Bovine Calcitonin Secondary Structure and Amyloid Formation," *Protein. Sci.*, **14**(6), 1419 (2005b).

Wang, S. S.-S., Y. T. Chen, P. H. Chen, and K. N. Liu, "A Kinetic Study on the Aggregation Behavior of Beta-Amyloid Peptides in Different Initial Solvent Environments," *Biochem. Eng. J.*, (2005c).

Ward, R. V., K. H. Jennings, R. Jepras, W. Neville, D. E. Owen, J. Hawkins, G. Christie, J. B. Davis, A. George, E. H. Karran, and D. R. Howlett, "Fractionation and Characterization of Oligomeric, Protofibrillar and Fibrillar forms of Beta-Amyloid Peptide," *Biochem. J.*, **348**(1), 137 (2000).

Webster, S., B. Bonnell, and J. Rogers, "Charge-Based Binding of Complement Component C1q to the Alzheimer Amyloid Beta-Peptide," *Am. J. Pathol.*, **150**(5), 1531 (1997).

Wegiel, J., K. C. Wang, M. Tarnawski, and B. Lach, "Microglia Cells are the Driving Force in Fibrillar Plaque Formation, Whereas Astrocytes are a Leading Factor in Plague Degradation," *Acta. Neuropathol. (Berl)*, **100**(4), 356 (2000).

Weiss, J. H., C. J. Pike, and C. W. Cotman, "Ca^{2+} Channel Blockers Attenuate Beta-Amyloid Peptide Toxicity to Cortical Neurons in Culture," *J. Neurochem.*, **62**(1), 372 (1994).

Weldon, D. T., S. D. Rogers, J. R. Ghilardi, M. P. Finke, J. P. Cleary, E. O'Hare, W. P. Esler, J. E. Maggio, and P. W. Mantyh, "Fibrillar Beta-Amyloid Induces Microglial Phagocytosis, Expression of Inducible Nitric Oxide Synthase, and Loss of a Select Population of Neurons in the Rat CNS In Vivo," *J. Neurosci.*, **18**(6), 2161 (1998).

Whitson, J. S., M. P. Mims, W. J. Strittmatter, T. Yamaki, J. D. Morrisett, and S. H. Appel, "Attenuation of the Neurotoxic Effect of A Beta Amyloid Peptide by Apolipoprotein E," *Biochem. Biophys. Res. Commun.*, **199**(1), 163 (1994).

Whittemore, N. A., R. Mishra, I. Kheterpal, A. D. Williams, R. Wetzel, and E. H. Serpersu, "Hydrogen-Deuterium (H/D) Exchange Mapping of Abeta 1-40 Amyloid Fibril Secondary Structure Using Nuclear Magnetic Resonance Spectroscopy," *Biochemistry*, **44**(11), 4434 (2005).

Wiegandt, H., S. Kanda, K. Inoue, K. Utsumi, and S. Nojima, "Studies on the Cell Association of Exogenous Glycolipids," *Adv. Exp. Med. Biol.*, **152**, 343 (1982).

Williams, A. D., M. Sega, M. Chen, I. Kheterpal, M. Geva, V. Berthelier, D. T. Kaleta, K. D. Cook, and R. Wetzel, "Structural Properties of Abeta Protofibrils Stabilized by a Small Molecule," *Proc. Natl. Acad. Sci. U.S.A.*, **102**(20), 7115 (2005).

Wood, S. J., R. Wetzel, J. D. Martin, and M. R. Hurle, "Prolines and Amyloidogenicity in Fragments of the Alzheimer's Peptide Beta/A4," *Biochemistry*, **34**(3), 724

(1995).

Wood, W. G., G. P. Eckert, U. Igbavboa, and W. E. Muller, "Amyloid Beta-Protein Interactions with Membranes and Cholesterol: Causes or Casualties of Alzheimer's Disease," *Biochimica Et Biophysica Acta-Biomembranes*, **1610**(2), 281 (2003).

Xu, J., S. Chen, S. H. Ahmed, H. Chen, G. Ku, M. P. Goldberg, and C. Y. Hsu, "Amyloid-Beta Peptides are Cytotoxic to Oligodendrocytes," *J. Neurosci.*, **21**(1), RC118 (2001).

Yamamoto, N., U. Igbabvoa, Y. Shimada, Y. Ohno-Iwashita, M. Kobayashi, W. G. Wood, S. C. Fujita, and K. Yanagisawa, "Accelerated Abeta Aggregation in the Presence of GM1-Ganglioside-Accumulated Synaptosomes of Aged ApoE4-Knock-in Mouse Brain," *FEBS Lett.*, **569**(1-3), 135 (2004).

Yan, Q., J. H. Zhang, H. T. Liu, S. Babu-Khan, R. Vassar, A. L. Biere, M. Citron, and G. Landreth, "Anti-Inflammatory Drug Therapy Alters Beta-Amyloid Processing and Deposition in an Animal Model of Alzheimer's Disease," *J. Neuroscience*, **23**(20), 7504 (2003).

Yanagisawa, K., A. Odaka, N. Suzuki, and Y. Ihara, "GM1 Ganglioside-Bound Amyloid Beta-Protein (A Beta): A Possible form of Preamyloid in Alzheimer's Disease [see comments]," *Nat. Med.*, **1**(10), 1062 (1995).

Yanagisawa, K. A. Asami-Odaka, N. Suzuki, and Y. Ihara, "Fractionation of Amyloid Beta-Protein (A Beta) in Alzheimer's Disease and Down's Syndrome Brains. Presence of Membrane-Bound A Beta," *Ann. N. Y. Acad. Sci.*, **786**,184 (1996).

Yanagisawa, K. and Y. Ihara, "GM1 Ganglioside-Bound Amyloid Beta-Protein in Alzheimer's Disease Brain," *Neurobiol. Aging*, 19(1 Suppl), S65 (1998).

Yankner, B. A., L. I. Benowitz, L. Villa-Komaroff, and R. L. Neve, "Transfection of PC12 Cells with the Human GAP-43 Gene: Effects on Neurite Outgrowth and Regeneration," *Brain Res. Mol. Brain Res.*, **7**(1), 39 (1990).

Yatin, S. M., M. Aksenov, and D. A. Butterfield, "The Antioxidant Vitamin E Modulates Amyloid Beta-Peptide-Induced Creatine Kinase Activity Inhibition and Increased Protein Oxidation: Implications for the Free Radical Hypothesis of Alzheimer's Disease," *Neurochem. Res.*, **24**(3), 427 (1999a).

Yatin, S. M., S. Varadarajan, C. D. Link, and D. A. Butterfield, "In Vitro and In Vivo Oxidative Stress Associated with Alzheimer's Amyloid Beta-Peptide (1-42)," *Neurobiol. Aging*, **20**(3), 325 (1999b).

Yeagle, P. L., "Modulation of Membrane Function by Cholesterol," *Biochimie*, **73**(10), 1303 (1991).

Zambrzycka, A., R. P. Strosznajder, and J. B. Strosznajder, "Aggregated Beta Amyloid Peptide 1-40 Decreases Ca^{2+}- and Cholinergic Receptor-Mediated Phosphoinositide Degradation by Alteration of Membrane and Cytosolic Phospholipase C in Brain Cortex," *Neurochem. Res.*, **25**(2), 189 (2000).

Zekanowski, C., D. Religa, C. Graff, S. Filipek, and J. Kuznicki, "Genetic Aspects of Alzheimer's Disease," *Acta. Neurobiol. Exp. (Wars)*, **64**(1), 19 (2004).

Zhang, C., H. E. Qiu, G. A. Krafft, and W. L. Klein, "Protein Kinase C and F-Actin are Essential for Stimulation of Neuronal FAK Tyrosine Phosphorylation by G-proteins and Amyloid Beta Protein," *FEBS Lett.*, **386**(2-3), 185 (1996).

Zhang, Q., E. T. Powers, J. Nieva, M. E. Huff, M. A. Dendle, J. Bieschke, C. G. Glabe, A. Eschenmoser, P. Wentworth, Jr., R. A. Lerner, and J. W. Kelly, "Metabolite-Initiated Protein Misfolding May Trigger Alzheimer's Disease," *Proc. Natl. Acad. Sci. U.S.A.*, **101**(14), 4752 (2004).