Overview of Fibrillar and Oligomeric Assemblies of Amyloidogenic Proteins

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Chapter 1
Overview of Fibrillar and Oligomeric Assemblies of Amyloidogenic Proteins

Farid Rahimi and Gal Bitan

Abstract Aberrantly folded proteins are implicated in over 40 human diseases, including neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's, and Creutzfeldt–Jakob diseases; diseases of particular organs, including desmin-related cardiomyopathy or type-2 diabetes mellitus; and systemic diseases, such as senile systemic amyloidosis or light-chain amyloidosis. Although the proteins involved in each disease have unrelated sequences and dissimilar native structures, they all undergo conformational alterations and "misfold" to form fibrillar polymers characterized by a cross-β structure. Fibrillar assemblies build up progressively into intracellular or extracellular proteinaceous aggregates generating the pathognomonic amyloid-like lesions *in vivo*. Substantial evidence accumulated in the last decade suggest, that in many amyloid-related diseases, the lesions containing the protein aggregates are the end state of aberrant protein folding whereas the actual culprits causing the disease are soluble, non-fibrillar assemblies preceding the insoluble aggregates. The non-fibrillar protein assemblies are diverse and range from small, low-order oligomers to large assemblies, including spherical, annular, and protofibrillar species. Oligomeric species with different degrees of structural order are believed to mediate various pathogenic mechanisms that may lead to

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cellular dysfunction, cytotoxicity, and cell loss, eventuating in disease-specific degeneration. The particular pathologies thus are determined by the afflicted cell types, organs, systems, and the proteins involved. In many cases, the structure–function interrelationships amongst the various protein assemblies described *in vitro* are still elusive. Moreover, structural and mechanistic studies of amyloid proteins have been challenging due to the dynamic and metastable nature of the non-fibrillar oligomers and the non-crystalline nature of fibrillar protein aggregates. These factors have confounded the development and potential *in vivo* application of specific detection tools for non-fibrillar amyloid assemblies. Nevertheless, evidence suggests that non-fibrillar amyloid assemblies may share structural features and possibly common mechanisms of action as assessed *in vitro* or *in situ*. Deciphering these intricate structure–function correlations will help in understanding a complex array of pathogenic mechanisms, some of which may be common across different diseases albeit affecting different cell types or systems. This prefatory chapter aims to give an overview of historical definitions of amyloid along with a general discussion of fibrillar and non-fibrillar amyloid assemblies and their toxicity. The chapter also discusses some methodological challenges, which often are overlooked.

Keywords Amyloid, Cytotoxicity, Degeneration, Oligomer, Protein misfolding, Amyloid fibrils

1.1 Etymology of the Term "Amyloid"

The term "amyloid", in fact a misnomer, has been used in the context of histopathology since its neologism in 1838. Its first use then was by a German botanist, Matthias Schleiden (Schleiden 1838), who described amylaceous constituents of plant cell walls (reviewed in Kyle 2001; Steensma and Kyle 2007). Later in 1854, Rudolph Virchow, a German physician-scientist, used this term when examining the brain *corpora amylacea*, which stained pale blue upon treatment with iodine and violet when subsequently treated with sulfuric acid (Virchow 1854a, b) (reviewed in Kyle 2001; Sipe and Cohen 2000; Steensma and Kyle 2007). Because these staining characteristics are similar to those of starch, Virchow concluded that *corpora amylacea* were essentially cellulose and described the lesions as amyloid (i.e., starch-like). The term amyloid is derived from the Latin *amylum*, a transliteration of the Greek *amylon*, which was a term meaning "not ground at the mill" and referring to fine grains, especially starch (Steensma and Kyle 2007). At that time, the distinction between starch (in animals) and cellulose (in plants) was unclear (Sipe and Cohen 2000; Steensma and Kyle 2007).

In 1859, based on the high nitrogen content of amyloid lesions, Carl Friedreich and August Kekulé reported that the amyloid lesions contained albuminoid material and nothing chemically corresponding to *amylon* or cellulose (Friedreich and Kekulé 1859) (reviewed in Kyle 2001; Sipe and Cohen 2000; Steensma and Kyle 2007). They finally established the proteinaceous nature of amyloid lesions.

A century later, electron microscopic (EM) studies of human or animal amyloid lesions allowed observation of the fibrillar ultrastructure of amyloid (Cohen and Calkins 1959). Further progression of biochemical and biophysical techniques facilitated isolation of amyloid fibrils from tissue amyloid lesions in 1964 (Cohen and Calkins 1964) and their characteristic structure was determined by X-ray fiber diffraction in 1968 (Eanes and Glenner 1968). By then, the term amyloid had survived the test of time and was no longer a misnomer.

In the nineteenth and twentieth centuries, extensive studies have focused on deciphering the molecular and pathological mechanisms of protein misfolding, amyloid formation, amyloid-associated toxicity, and disease. This book provides a compendium of chapters describing these studies, with each chapter focusing on a particular protein, a particular disease, or a particular aspect of the relationship between protein misfolding and disease. Our opening chapter gives an overview of amyloids and different assemblies of amyloid proteins. The following chapter describes the pathologic lesions found in certain common amyloid diseases. All other chapters discuss structures of different amyloidogenic proteins and mechanisms mediated by these proteins involved in individual diseases. The final chapter outlines current therapeutic opportunities targeting these diseases and the associated amyloidogenic proteins.

1.2 Amyloid and Disease

To date, 27 human diseases are defined as classic amyloidoses (Westermark et al. 2007; Harrison et al. 2007; Sipe et al. 2010). These diseases are classified also as proteopathies (Walker et al. 2006), degenerative diseases (Dickson 2009), and conformational, protein-misfolding, protein-aggregation, or protein-deposition diseases (Surguchev and Surguchov 2010; Dobson 2004; Eisenberg et al. 2006). More than 40 human diseases collectively fall under the abovementioned classifications (Chiti and Dobson 2006). Of these, several neurodegenerative diseases, including Alzheimer's (AD) (Kril and Halliday 2001; Mayeux 2010; Aguzzi and O'Connor 2010; Lublin and Gandy 2010; Querfurth and LaFerla 2010), Parkinson's (PD) (Bagetta et al. 2010; Halliday and McCann 2010; Obeso et al. 2010; Pahwa and Lyons 2010; Postuma and Montplaisir 2009; Shulman 2010), Huntington's (HD) (Bauer and Nukina 2009; Cardoso 2009; Pfister and Zamore 2009; Rozas et al. 2010; Sassone et al. 2009), and prion diseases (Frost and Diamond 2010; Aguzzi and Calella 2009; Kupfer et al. 2009; Mallucci 2009; Sharma et al. 2009) are characterized pathognomonically by intracellular or extracellular microscopic lesions containing the proteinaceous amyloid aggregates. These diseases are characterized also by extensive neuron loss and atrophy in selected, vulnerable cerebral regions (Double et al. 2010), determining clinical presentations and outcomes. Amyloid-related diseases such as amyotrophic lateral sclerosis (ALS) (Cozzolino et al. 2008; Eisen 2009), non-neuropathic systemic diseases, e.g., light-chain and senile systemic amyloidoses (Comenzo 2006, 2007; Sanchorawala 2006), and other organ-specific diseases, such

as dialysis-related amyloidosis (Dember and Jaber 2006; Kiss et al. 2005; Yamamoto et al. 2009), hereditary renal amyloidosis (Hawkins 2003; Kissane 1973; Eshaghian et al. 2007; McCarthy and Kasper 1998), atrial amyloidosis (Eshaghian et al. 2007; McCarthy and Kasper 1998; Goette and Rocken 2004; Rocken et al. 2002; Benvenga and Facchiano 1995; Looi 1993), and type-2 diabetes mellitus (Hayden et al. 2005; Khemtemourian et al. 2008; Li and Holscher 2007; Scheuner and Kaufman 2008) also are characterized by extracellular deposition of aberrantly folded, insoluble amyloid proteins.

Although the proteins contributing to different amyloidoses may have dissimilar sequences or unrelated native tertiary structures, they all form insoluble amyloid fibrils, ultimately lose their soluble, functional states, and deposit as amyloid, or amyloid-like lesions (Sipe and Cohen 2000). Extracellularly deposited amyloid material can be distinguished from non-amyloid deposits by: (1) characteristic straight, unbranched fibrillar morphology; (2) a typical cross-β pattern, in which β-strands run perpendicularly to the fiber axis; and (3) characteristic tinctorial properties, particularly binding of the dyes Congo red and thioflavin S. The cross-β pattern consists of two characteristic fiber-diffraction signals located on axes perpendicular to one another, a sharp, intense meridian reflection (parallel with the fiber axis) at ~4.7–4.8 Å and an equatorial signal at ~10 Å (Sunde et al. 1997). Binding of Congo red gives rise to characteristic blue-green birefringence under polarized light (Harrison et al. 2007; Merlini and Westermark 2004; Westermark et al. 2007; Frid et al. 2007), and binding of thioflavin S results in a hyperchromic shift in thioflavin-S fluorescent emission spectrum compared with free thioflavin S (Khurana et al. 2005; LeVine 1999). In addition to their major, fibrillar proteinaceous component, amyloid deposits contain metal ions, glycosaminoglycans, serum amyloid P, apolipoprotein E, collagen, nucleic acids, and other components (Hirschfield and Hawkins 2003; Alexandrescu 2005; Ginsberg et al. 1999, 1998; Marcinkiewicz 2002; Liao et al. 2004; Kahn et al. 1999).

Besides extracellularly deposited amyloid lesions in amyloidoses, many different intranuclear/intracytoplasmic amyloid-like aggresomes (inclusion bodies) also have been associated with specific diseases. The term inclusion body or "inclusion" is used frequently in the context of protein misfolding and aggregation (Kopito 2000; Cruts et al. 2006). Inclusion bodies in the latter context are distinct from bacterial inclusion bodies. Aggresomes are inclusion bodies formed by retrograde transport of aggregated proteins on microtubules (Kopito 2000). They contain a major aggregated protein and are also enriched in various molecular chaperones (Kopito 2000). Bacterial inclusion bodies typically are highly enriched for a single protein species and isolation of bacterial inclusion bodies is usually the first step in purification of heterologous proteins recombinantly expressed in bacteria. Aggresomes share certain properties with amyloid fibrils but some of them do not meet all the characteristics required by the classic definition of amyloid (Westermark et al. 2007; Sipe et al. 2010). Biochemists and biophysicists formally call the latter "amyloid-like," "amyloid-related," or "amyloidogenic" proteins. For instance, in PD and HD, protein aggregates accumulate intracellularly, generating disease-

specific aggresomes—Lewy bodies and Huntington bodies, respectively. These structures have been excluded from the classification of amyloid by the Nomenclature Committee of the International Society of Amyloidosis (Westermark et al. 2007; Sipe et al. 2010) despite the fact that fibrils derived from the respective proteins, α-synuclein (Chap. 6) and polyglutamine-expanded huntingtin (Chap. 11), show all the characteristic features of amyloid (Conway et al. 2000a; Scherzinger et al. 1997; Chen et al. 2002; McGowan et al. 2000). A recent exception is the intracellular neurofibrillary tangles in AD. The main component of the neurofibrillary tangles is the microtubule-associated protein τ in a hyperphosphorylated form (Ihara et al. 1983; Joachim et al. 1987; Kosik et al. 1986; Nukina et al. 1987; Perl 2010; Steiner et al. 1990; Goedert et al. 1988). Despite their predominantly intracellular location, neurofibrillary tangles are now regarded as true amyloid (Sipe et al. 2010; Westermark et al. 2007) because of their fibrillar structure, cross-β X-ray diffraction pattern, and typical amyloid staining with Congo red (von Bergen et al. 2001; Giannetti et al. 2000; Berriman et al. 2003; Inouye et al. 2006).

1.3 Fibrillar Assemblies of Amyloid Proteins

In an Editorial in *Accounts of Chemical Research*, Ronald Wetzel once used the term "common threads" to allude to the common morphology of amyloid fibrils (Wetzel 2006). These fibrils revealed by transmission-electron microscopy (TEM) usually consist of 2–6 protofilaments each with diameter of 2–5 nm (Serpell et al. 2000). The protofilaments intertwine and form thread-like fibrils that are typically 7–13 nm wide (Serpell et al. 2000; Sunde and Blake 1997) or associate laterally to form long ribbons typically 2–5 nm thick and up to 30 nm wide (Bauer et al. 1995; Saiki et al. 2005). X-ray fiber-diffraction data indicate that in individual protofilaments the polypeptide chains are arranged in β-strands running perpendicular to the long axis of the fibril, forming the cross-β pattern (Sunde and Blake 1997).

The presence of highly organized and stable fibrillar deposits in affected organs in amyloid-related diseases long was viewed as a common causative link between aggregate formation and pathological symptoms. This had led to postulations such as the "amyloid cascade hypothesis" in the AD field. Originally, this hypothesis stated that deposition of amyloid β-protein (Aβ), the main component of amyloid plaques in AD-afflicted brains, was the cause of AD. This view was later reinforced by findings that Aβ-derived fibrils were neurotoxic (Pike et al. 1991; Lorenzo and Yankner 1994) and caused both membrane depolarization and alterations in the frequency of action potentials (Hartley et al. 1999). It was shown also that microinjection of fibrillar, but not soluble, Aβ into cerebral cortex of aged rhesus monkeys (*Macaca mulatta*) resulted in pathological events associated with AD, including profound neuronal loss, τ phosphorylation, and microglial proliferation (Geula et al. 1998). Similarly, monosialoganglioside GM1, a neuronal membrane component that is released from damaged neurons and is found in

higher levels in cerebrospinal fluid from patients with AD than from age-matched controls, was found to enhance formation of Aβ fibrils with cytotoxicity and cell affinity much stronger than those of Aβ fibrils formed in phosphate-buffered saline (Okada et al. 2007).

A similar fibril-centered hypothesis was thought to apply contextually to all amyloidoses. For example, cytotoxic effects have been reported for fibrillar prion protein (Novitskaya et al. 2006) and lysozyme (Gharibyan et al. 2007). Insulin aggregation has been associated with rare injection-related amyloidosis (Swift 2002). In addition, insulin aggregation has been studied *in vitro* by multiple groups as a convenient model of protein fibrillogenesis (Murali and Jayakumar 2005; Dzwolak et al. 2007, 2006; Grudzielanek et al. 2007a). Biophysical investigations of insulin fibrillogenesis have identified oligomeric populations with conformations distinct from those of natively folded insulin dimer and hexamer (Ahmad et al. 2005). In a recent study combining structural characterization and cytotoxicity experiments, Grudzielanek et al. found no toxicity for low-order insulin oligomers whereas substantial toxicity was measured for high-order, β-sheet-rich aggregates that displayed either fibrillar or amorphous morphology (Grudzielanek et al. 2007b). Other studies using primates and transgenic murine diabetes models have shown the importance of islet amyloid in the pathogenesis of type-2 diabetes. It was thought that amyloid fibrils preceded formation of islet amyloid deposits and that fibrils derived from islet amyloid polypeptide (IAPP) were likely toxic to β-cells, thereby causing islet dysfunction (Lorenzo et al. 1994). Similarly, deposition of islet amyloid was considered an early event in type-2 diabetes and its progressive accumulation as the cause for parenchymal mass reduction and dysfunction (Westermark and Wilander 1978; Clark et al. 1988). This was thought to lead to progressively deficient insulin secretion, reduced glucose tolerance, and eventual emergence of fasting hyperglycemia (Kahn et al. 1999). Studies in mice harboring the human IAPP transgene suggested that not only hyperglycemia was associated with the development of islet amyloid, but that amyloid contributed to generation of hyperglycemia due to loss of β-cells (Hoppener et al. 2000).

One of the factors responsible for fibril-induced cytotoxicity is thought to be the physicochemical compositions of the surface of amyloid fibrils (Yoshiike et al. 2007). Significant morphological variations exist among different fibrils derived from the same peptide or protein, e.g., calcitonin (Bauer et al. 1995), SH3 domain of phosphatidylinositol-3′-kinase (Jiménez et al. 1999), insulin (Jiménez et al. 2002), Aβ (Petkova et al. 2005; Paravastu et al. 2006; Wetzel et al. 2007), and IAPP (Goldsbury et al. 1997; Radovan et al. 2008). Even single-residue alterations have been shown to affect fibril structure profoundly. For example, the substitution of D23 by N in Aβ, linked to severe cerebral amyloid angiopathy in an Iowa kindred (Van Nostrand et al. 2001), causes formation of Aβ40 fibrils considerably faster than wild-type Aβ40 (Tycko et al. 2009). At the molecular level, D23N-Aβ40 fibrils are arranged predominantly in an anti-parallel array, in contrast to the in-register, parallel β-sheet structure commonly found in wild-type Aβ40 fibrils and most other amyloid fibrils (Tycko et al. 2009). Despite these differences in molecular arrange-

ment, the gross morphology, X-ray diffraction pattern, and dye-binding properties of amyloid fibrils appear to be universal amongst fibrillar structures of amyloid proteins.

Morphological differences in amyloid fibrils are governed also by conditions used for fibril preparation. For example, EM and nuclear magnetic resonance (NMR) enable visualization of polymorphic structure of Aβ40 fibrils prepared under agitated or quiescent conditions (Petkova et al. 2005). Seeding experiments may facilitate detailed structural characterization of amyloid fibrils developing *in vivo* and elucidate the controversial role of fibrils (Hardy and Selkoe 2002) in human amyloidoses. To study fibril polymorphism *in vivo* and based on the ability of preformed amyloid fibrils to propagate their structures through seeded growth *in vitro* (Petkova et al. 2005), fibrils extracted from AD brain tissue were used to seed growth of synthetic Aβ40 fibrils (Paravastu et al. 2009). This allowed Paravastu et al. to deduce putative structures of fibrils extracted from AD brain by recapitulating these structures using seeded fibrillar growth of synthetic Aβ40. Paravastu et al. showed that fibrils grown after being seeded with material extracted from two separate AD patients' brains had predominantly the same two fibril structures (Paravastu et al. 2009). These predominant fibril structures differed from the two previously described, purely synthetic Aβ40 fibril structures (Paravastu et al. 2008; Petkova et al. 2006), indicating that seeded growth combined with structural studies may determine the molecular structures of fibrils developing in AD brain or in fibrils involved in other amyloid diseases *in vivo*. The results described above suggest that each amyloid protein potentially forms a spectrum of structurally distinct fibrils, and that kinetic and microenvironmental factors determine which of these alternatives predominate under given circumstances, which can differ considerably *in vitro* and *in vivo*.

Direct correlation between specific molecular organization and fibril toxicity may be important where pathogenic mechanisms of sporadic and genetic forms of amyloid diseases are studied. For example, some genetic cases of AD (Taddei et al. 1998; Miyoshi 2009; Moro et al. 2010; McDonald et al. 2010) and PD (Dawson 2007; Gasser 2009; Inzelberg and Polyniki 2010; Schiesling et al. 2008; Houlden et al. 2001) have an earlier onset and a faster progression than sporadic forms of these diseases suggesting potentially different underlying molecular mechanisms. Studies similar to those of Paravastu et al. could be extended to compare fibril structures of Aβ in sporadic *versus* genetic, early-onset forms of AD. Such studies will potentially delineate correlations between protein structure and disease severity or progression at the molecular level. Aging-induced spontaneous chemical modifications, such as amino-acid racemization or amino-acid isomerization—e.g., involving aspartate and asparagine residues—may affect Aβ production, polymerization, and clearance, potentially playing a pivotal role in the pathogenesis of sporadic and genetic forms of AD (Moro et al. 2010). Therefore, studies linking fibril morphology with aging-induced posttranslational protein modifications in AD may unravel correlations between fibril structure and pathogenesis. This example is potentially applicable and relevant to other amyloidoses, for example PD.

1.4 Non-fibrillar Assemblies of Amyloid Proteins

Contrary to the original amyloid cascade hypothesis (Hardy and Higgins 1992), substantial evidence suggests that fibrillar aggregates are the end state of aberrant protein folding and eventuate as potentially protective sinks for the cytotoxic, oligomeric, non-fibrillar protein assemblies. The transient, non-fibrillar assemblies likely are the actual culprits. These assemblies are believed to initiate the pathogenic mechanisms that lead to cellular dysfunction, cell loss, loss of functional tissue, and disease-specific regional or organ-specific atrophy (Kirkitadze et al. 2002; Gurlo et al. 2010; Haataja et al. 2008; Luibl et al. 2006; Meier et al. 2006).

Amyloid β-protein (Aβ), the causative agent in AD, is considered an archetypal amyloidogenic protein. The multitude and variety of structural, functional, and pathophysiological studies of Aβ exemplify the complexity of research findings covering non-fibrillar assemblies of amyloidogenic proteins. Extensive biophysical studies in the Aβ field have led to functional and structural descriptions of non-fibrillar and pre-fibrillar Aβ assemblies. For example, the discovery of Aβ protofibrils (Walsh et al. 1997; Harper et al. 1997) and other toxic non-fibrillar Aβ assemblies, including low-order oligomers, Aβ-derived diffusible ligands, and paranuclei (reviewed in Rahimi et al. 2008) have led to a paradigm shift (Kirkitadze et al. 2002; Haass and Selkoe 2007; Glabe 2006; Glabe and Kayed 2006) in AD research, challenging the original, fibril-centered, amyloid cascade hypothesis (Hardy and Higgins 1992). An updated version of the hypothesis presented a decade after the original one (Hardy and Selkoe 2002) emphasizes that early, pre-fibrillar Aβ assemblies or Aβ assemblies unrelated to fibrils are the primary cytotoxins in AD pathogenesis leading to synaptic dysfunction and neuron loss (Sakono and Zako 2010; Gong et al. 2003; Klein 2002a; Cleary et al. 2005; Lambert et al. 1998). This paradigm shift and the centrality of non-fibrillar Aβ assemblies in AD research have led to a search for similar non-fibrillar protein assemblies in other amyloid-related diseases. To date, at least 40 different proteins have been identified as causative agents of amyloidoses (Bellotti et al. 2007; Chiti and Dobson 2006). In most cases, including prion proteins (Simoneau et al. 2007, also discussed in Chaps. 9 and 10), transthyretin (Sorgjerd et al. 2008 and Chap. 13), α-synuclein (van Rooijen et al. 2010 and Chap. 6), apolipoprotein C-II (Ryan et al. 2008), τ (Peterson et al. 2008; Sahara et al. 2008; Kayed et al. 2009 and Chap. 5), superoxide dismutase (Cozzolino et al. 2009 and Chap. 8), polyglutamine-expanded proteins (Legleiter et al. 2010 and Chap. 11), and islet amyloid polypeptide (Haataja et al. 2008 and Chap. 7), non-fibrillar protein assemblies have been found and shown to exert adverse biological effects similar to those of non-fibrillar Aβ oligomers (Kirkitadze et al. 2002; Caughey and Lansbury 2003; Ferreira et al. 2007; Glabe 2006; Jellinger 2009; Kitamura and Kubota 2010; Sakono and Zako 2010; Roychaudhuri et al. 2009 and Chaps. 3, 4, 5).

Before the focus in the amyloid field shifted from fibrils to non-fibrillar assemblies, it was known that despite sequence dissimilarity among amyloidogenic proteins, amyloid fibrils were largely similar in the core regions (Eisenberg

et al. 2006; Serpell 2000). The realization that the non-fibrillar oligomeric structures may be the proximate disease-causing agents in the amyloidoses related to these proteins raised the question of whether oligomeric structures were also similar. High-resolution microscopic studies of oligomeric structures, mostly by TEM and AFM, have demonstrated that in most cases the morphologies observed were spherical, annular, or protofibrillar (worm-like). Despite morphological similarities, studies have demonstrated that small structural changes may have a large impact on the oligomer populations formed by the same protein (Bitan et al. 2003a, b, c).

Protofibrils, the penultimate precursors of fibrillar assemblies, are curvilinear, fibril-like structures of 4–8 nm diameter, ≤200 nm length (Walsh et al. 1997), and may have an axial twisting periodicity of 20 nm (Hartley et al. 1999). They have been described as spherical beads of 2–5 nm diameter arranged as beaded chains in linear, curvilinear, or annular arrangements in studies originally reporting them (Harper et al. 1997; Walsh et al. 1999, 1997). The annular protofibrils have been the predominant structures found in several studies (Caughey and Lansbury 2003; Lashuel et al. 2002a, b; Ding et al. 2002; Kayed et al. 2009). However, as discussed elsewhere (Bitan et al. 2005), it is important to note that in many cases the term protofibril has been used even though the morphologies of the assemblies under study were distinct from those originally defined as protofibrils. It is also important to distinguish between protofibrils and protofilaments, which are the constituent units of mature fibrils (Serpell et al. 2000; Teplow 1998).

One of the most-studied amyloidogenic proteins is α-synuclein (Chap. 6). α-Synuclein, first characterized in zebra finch (*Taeniopygia guttata*) (George et al. 1995) (under the UniProt accession number Q91448, the organism described is *Serinus canaria* (Island canary) or *Fringilla canaria*), was thought to be important in neural plasticity during vertebrate development. The exact function of α-synuclein still is not clear though it is thought to be part of the proteasomal system (reviewed in Layfield et al. 2003; Betarbet et al. 2005), vesicle trafficking and endocytosis (Varkey et al. 2010), and/or SNARE complex assembly (Burré et al. 2010). α-Synuclein has been shown to form α-helical structures when interacting with artificial (Jao et al. 2008; Trexler and Rhoades 2009; Georgieva et al. 2008) or biological membranes (Kim et al. 2006). As discussed earlier, α-synuclein is the predominant component in Lewy bodies, the pathological hallmarks in PD brains. It has been implicated also in other degenerative disorders (synucleinopathies), including dementia with Lewy bodies and multiple-system atrophy (Ian et al. 2001; Jellinger 2009; Chiti and Dobson 2006). Similar to Aβ, α-synuclein belongs to a growing family of "intrinsically disordered" proteins (Tompa 2002; Dyson and Wright 2005), a characteristic that perhaps renders these proteins more prone to undergoing amyloidogenic assembly because of their structural instability. Mutant α-synuclein alloforms linked to familial PD were found to oligomerize faster than the wild-type protein, whereas the rate of fibril formation did not correlate with the presence of disease-causing mutations (Conway et al. 2000b). Non-fibrillar assemblies of both wild-type and mutant α-synuclein included spherical oligomers, protofibrillar structures, and most abundantly, annular protofibrils (Ding et al. 2002; Lashuel et al. 2002b). The latter morphology suggested that the

mechanism whereby α-synuclein induces toxicity is pore formation in cell membranes. In agreement with this idea, protofibrillar α-synuclein was found to permeabilize synthetic vesicles (Volles et al. 2001). Interestingly, this effect was increased by the familial PD-linked mutants A30P and A53T (Volles and Lansbury 2002), but not by the mutant E46K (Fredenburg et al. 2007). Thus, although pore formation may be involved in α-synuclein-induced toxicity, other mechanisms also have been implicated, but these are not understood well (Takeda et al. 2006).

IAPP aggregation is thought to cause type-2 diabetes. IAPP is a 37-residue peptide hormone produced in pancreatic β-cells and co-secreted with insulin. Early stages of type-2 diabetes are characterized by insulin resistance followed by increased insulin and IAPP secretion. Elevated IAPP levels lead to its assembly into toxic, soluble oligomers and insoluble aggregates (Marzban et al. 2003). Oligomeric and protofibrillar IAPP were shown to interact with synthetic membranes (Anguiano et al. 2002), a characteristic that decreases with further aggregation, providing a clue for the mechanism of IAPP toxicity (Porat et al. 2003). Similar to α-synuclein, interaction with biological membranes may induce a transient α-helical conformation in IAPP, presumably facilitating penetration of the oligomers into the membrane resulting in solute leakage across the membrane (Jayasinghe and Langen 2005; Knight et al. 2006). Strong evidence for the cytotoxic role of IAPP oligomers in type-2 diabetes was given in a study in which rifampicin, an inhibitor of IAPP fibril, but not oligomer, formation, did not protect pancreatic β-cells against apoptosis induced by either exogenous or endogenously expressed IAPP (Meier et al. 2006). More recent data have suggested that *in vivo*, toxic IAPP oligomers are formed intracellularly and therefore, oligomer-specific antibodies do not prevent cell death *in vitro* or *in vivo* (Lin et al. 2007).

1.4.1 Analytical Challenges in Studies of Amyloid Protein Oligomers

Structural studies of oligomers of amyloidogenic proteins have been challenging because these assemblies typically are metastable and comprise heterogeneous mixtures of species. Immunological insights have been obtained by Glabe and co-workers, who developed antibodies that bound specifically to oligomers but not to the monomeric or fibrillar forms of proteins of unrelated sequences (Kayed et al. 2003). The first polyclonal antibody, A11, and similar antibodies developed in follow-up studies (Kayed and Glabe 2006; Georganopoulou et al. 2005; Lafaye et al. 2009), showed remarkable ability to bind to oligomers formed by proteins as diverse as Aβ, α-synuclein, IAPP, lysozyme, insulin, polyglutamine, and prion fragments (Kayed et al. 2003). In a recent study, iterative immunization of aged beagles with an aggregated Aβ preparation (Head et al. 2008) was shown to produce antibodies specific for monomeric, non-fibrillar, or fibrillar Aβ42 preparations (Vasilevko et al. 2010). However, dot-blotting results in this study were not conclusive enough to designate the canine antibodies as purely oligomer-specific anti-Aβ

antibodies because some degree of cross-reactivity (50% by densitometry) was evident and the results were not complemented by structural studies of Aβ preparations used for antibody-specificity assays.

As discussed above, recent studies by Paravastu et al. (2009) showed that the dominant structure of Aβ fibrils grown by Aβ fibril seeds derived from AD-afflicted brains differed from that in fibrils derived from purely synthetic Aβ40, suggesting that fibrillization conditions *in vitro*, and by inference, oligomerization conditions, differ from conditions *in vivo*. Findings based on the above studies by Paravastu et al. (Paravastu et al. 2009) and others (Petkova et al. 2005; Inaba et al. 2005; Lee et al. 2007; Kayed et al. 2009; Nekooki-Machida et al. 2009) argue against the idea, based on immunoreactivity data, TEM, and AFM studies, that non-fibrillar or pre-fibrillar amyloid assemblies are structurally similar. Although *in vitro* studies provide valuable insight into the structure and activity of non-fibrillar amyloid assemblies, these studies must be interpreted carefully because: (1) the conditions *in vivo* differ from those *in vitro* due to the complexity of cellular and tissue milieus; (2) mutations, amino-acid substitutions, or amino-acid modifications can result in different oligomer populations, different levels of oligomer toxicity or different fibrillar structures with different toxic properties (Bitan et al. 2003b; Yoshiike et al. 2007; Hung et al. 2008); and (3) fibrils grown in the presence of monosialoganglioside GM1 released from damaged neurons are more toxic than those prepared in buffer alone (Okada et al. 2007). Conclusively, non-fibrillar amyloid structures and compositions *in vivo* likely differ, at least to some degree, from those produced, analyzed, and studied *in vitro*. Some of the confounding factors in these cases involve post-extraction or post-analysis sample handling (e.g., freeze–thaw cycles, transportation, etc.). For examples, it was initially shown that Aβ dimers isolated from human brain tissue inhibited long-term potentiation (LTP), enhanced long-term depression, and reduced dendritic spine density in rodent hippocampal neurons (Shankar et al. 2008). However, these toxic activities were ascribed later to Aβ protofibrils, which formed readily from covalently stabilized Aβ dimers (O'Nuallain et al. 2010). These data suggest that by the time the activity of a certain Aβ preparation is measured, potentially inert Aβ species (e.g., dimers) may have converted to toxic species (e.g., protofibrils).

The same argument may apply to studies whereby non-fibrillar amyloid assemblies were extracted and studied *in vitro* (Shankar et al. 2008, 2007; Paleologou et al. 2009; Klucken et al. 2006; Sharon et al. 2003; Lesné et al. 2006; Head et al. 2010). Many extraction procedures use detergents, such as sodium dodecyl sulfate (SDS), which are known to disrupt the structure of non-fibrillar amyloid assemblies (Bitan et al. 2005; Hepler et al. 2006). Although electrophoretic separation of proteins in the presence of SDS (SDS–PAGE) generally is an excellent analytical method, the effect of SDS on all proteins is not equivalent (Gudiksen et al. 2006). Different proteins, different conformations of the same protein (Leffers et al. 2004), or truncated versions of certain proteins (Kawooya et al. 2003) may not bind stoichiometric amounts of SDS. In addition, in certain cases, SDS can induce or stabilize secondary or quaternary structures rather than denaturing them (Leffers et al. 2004; Montserret et al. 2000; Yamamoto et al. 2004). Further, SDS may cause

dissociation of some protein assemblies or conversely induce protein self-association, depending on the specific protein studied (Yamamoto et al. 2004; Rangachari et al. 2007, 2006; Piening et al. 2006). For example, Aβ42-derived "globulomers" are oligomeric species produced by incubating Aβ42 in the presence of 0.2% SDS (Barghorn et al. 2005). Apparent electrophoretic fractionation of monomeric or oligomeric components in a protein mixture does not necessarily indicate existence of such components prior to SDS treatment. Examples of this shortcoming of SDS–PAGE have been reported in its applications to studies of Aβ (Bitan et al. 2005; Hepler et al. 2006) and α-synuclein (Moussa et al. 2004). A recent example is a study of Aβ40 dimers stabilized by an intermolecular disulfide bridge, which showed the same SDS–PAGE profile before and after formation of β-sheet-rich protofibrils (O'Nuallain et al. 2010).

Because of the structural instability of amyloidogenic protein oligomers and the abovementioned analytical artifacts, studies reporting on structural properties of amyloidogenic proteins based on SDS–PAGE findings must be interpreted cautiously. This is particularly relevant to those studies, which have reported characterization of antibodies specific for oligomeric assemblies of amyloidogenic proteins relying on SDS–PAGE and western blotting. Recently, an elaborate study using ultrathin array tomography and immunofluorescence showed that senile plaques in brains of a murine model of AD are surrounded by "haloes of oligomeric Aβ" (Koffie et al. 2009) based on immunoreactivity of an antibody (NAB61), which apparently was reactive to oligomeric Aβ assemblies fractionated by SDS–PAGE (Lee et al. 2006). The original paper, which described this antibody, reported that NAB61 also recognized synthetic Aβ fibrils (Lee et al. 2006). Considering these caveats, one may question the major conclusions drawn by Koffie et al. because of the use of an antibody that was claimed to be specific for SDS–PAGE-fractionated oligomeric Aβ but was also cross-reactive with fibrillar Aβ assemblies. Similar cross-reactivity was apparent in antibodies that were produced and characterized after iterative immunization of beagles (Vasilevko et al. 2010) with an aggregated Aβ preparation (Head et al. 2008). Caveats regarding binding specificity of reagents ostensibly recognizing non-fibrillar amyloid assemblies also are relevant to aptamers. Aptamers are short ribo- or single-stranded deoxyribo-oligonucleotides used as specific molecular recognition tools in research, diagnostics, and therapy. Recently, we have found that aptamers bind fibrillar assemblies of amyloid proteins avidly yet non-specifically (Rahimi et al. 2009). Despite the fact that our aptamers were selected using covalently stabilized oligomeric preparations of Aβ40, they were found to bind not only Aβ-derived fibrillar structures, but also fibrils of other amyloid proteins (Rahimi et al. 2009). Similar high affinity for fibrils was observed using aptamers selected by multiple rounds of enrichment and two non-enriched, "naïve" RNA libraries demonstrating that fibril binding was a general phenomenon rather than a characteristic of particular RNA sequences (Rahimi et al. 2009). Comparable findings were observed previously with aptamers selected against fibrillar and non-fibrillar β_2-microglobulin (Bunka et al. 2007). Moreover, nucleic acids have been shown to enhance formation of amyloid fibrils and interactions between amyloid-forming peptides and nucleic acids have been shown to cause

formation of combined protein–nucleic-acid fibrils (Braun et al. 2011). These findings suggest that non-specific reactivity with oligonucleotides may be a universal property of amyloid proteins (Rahimi and Bitan 2010; Rahimi et al. 2009; Braun et al. 2011; Ylera et al. 2002; Bunka et al. 2007). Therefore, aptamers developed against non-fibrillar amyloid assemblies or those claimed to be specific for non- or pre-fibrillar amyloid assemblies must be tested for specificity for fibrillar assemblies of amyloid proteins. This is particularly applicable to studies reporting aptamers "specific" for monomeric or oligomeric Aβ (Takahashi et al. 2009) or α-synuclein (Tsukakoshi et al. 2010). Further delineation of specific mechanisms governing these interactions requires additional studies and will be important in interpretation of structure–function relationships and for designing reagents that recognize non-fibrillar amyloid assemblies specifically or potentially block amyloid-related toxicity. A relevant recent News article in *Nature* (Ledford 2010) has highlighted similar challenges researchers are facing in studying diseases with complex mechanisms and outlined some of the complexities and controversies involved in studies linking the prion protein and Aβ in AD research.

1.5 Non-fibrillar and Fibrillar Assemblies of Disease-Unrelated Proteins

The milieu of a polypeptide chain may cause it to adopt a multitude of conformations, or interconvert among many, in a wide temporal range (Dobson 2001; Dzwolak et al. 2007; Frieden 2007; Guijarro et al. 1998; Gursky and Aleshkov 2000; Stefani and Dobson 2003; Kelly 1998; Cruz et al. 2005; De Felice et al. 2004). This complexity is more relevant *in vivo* where interactions amongst proteins and interactions between proteins and other cellular components govern various cellular functional processes (Canale et al. 2006; Kitamura and Kubota 2010; Stefani and Dobson 2003; Zhang et al. 2004). Conformational heterogeneity renders the study of amyloidogenic proteins particularly difficult due to the transient nature of the adopted conformations, which populate closely related minima in the thermodynamic energy landscape (Miller et al. 2010). Besides disease-associated amyloid-forming proteins and proteins that naturally form non-pathological, functional amyloid-like fibrils (reviewed in Chiti and Dobson 2006) (see also Maji et al. 2009a), disease-unrelated proteins (Stefani and Dobson 2003) and artificially designed peptides (Fezoui et al. 2000; Wang et al. 2007; Kammerer and Steinmetz 2006) were shown to form amyloid under particular non-native conditions. The first proteins shown to form amyloid fibrils were reported by (Guijarro et al. 1998 and Litvinovich et al. 1998). The src-homology 3 (SH3) domain of bovine phosphatidyl inositol 3-kinase (PI3K), an 85-residue, β-structured protein, was shown to form amyloid fibrils slowly under acidic conditions (Guijarro et al. 1998). Thenceforth, the disease-unrelated SH3 domain has served as an excellent model system for studies examining structural properties of amyloid fibrils and molecular mechanisms of amyloid formation (Jiménez et al. 1999; Zurdo et al. 2001a, b; Carulla et al. 2005). It was found that the

initial protein aggregates were relatively dynamic and flexible to allow particular interactions guiding formation of the highly ordered fibrils (Polverino de Laureto et al. 2003).

After Litvinovich et al. demonstrated formation of amyloid-like fibrils by self-association of a murine fibronectin type-III module (Litvinovich et al. 1998), others reported that similar conversions in a number of disease-unrelated proteins could be induced *in vitro* by a deliberate, rational choice of experimental conditions (Chiti et al. 2001, 1999; Stefani and Dobson 2003). Formation of fibrils from full-length proteins occurs under solution conditions that partially or completely disrupt the native structure of the protein but do not completely break hydrogen bonds (Chiti et al. 2001). On the other hand, in the aggregation of unstructured proteins, e.g., Aβ, partially structured conformers have been shown to be necessary for fibril formation (Fezoui and Teplow 2002; Kirkitadze et al. 2001; Maji et al. 2005). It was shown that proteins with as few as four residues, and amino-acid homopolymers unable to fold into stable globular structures, form fibrils readily (Stefani and Dobson 2003; Tjernberg et al. 2002; Lopez De La Paz et al. 2002). Therefore, it has been suggested that the ability to form amyloid fibrils could be a generic property of polypeptide chains (Stefani and Dobson 2003).

In contrast to the hypothesis that adoption of amyloid or amyloid-like conformation is a generic property of the polypeptide backbone with only a minor contribution by the amino-acid side-chains (Dobson 2001), Maji et al. argued that side-chain interactions are essential in the aggregation process (Maji et al. 2009b) as demonstrated in fibril-related crystal structures (Nelson et al. 2005; Nelson and Eisenberg 2006; Sawaya et al. 2007), in studies showing the sequence-specific nature of amyloid aggregation (Tjernberg et al. 2002; Margittai and Langen 2006; Zanuy and Nussinov 2003), and by the scale of amino-acid aggregation propensities determined experimentally, ranging from aggregation-prone hydrophobic residues to aggregation-interfering, charged side-chains (Fernandez-Escamilla et al. 2004; Tartaglia et al. 2008). These studies suggest that under non-physiological conditions, including acidic pH, extremes of protein concentration, or addition of aprotic solvents (Guijarro et al. 1998; Chiti et al. 1999; Polverino de Laureto et al. 2003; Marcon et al. 2005), the influence of side-chains in the aggregation process can be altered, eventually driving the protein of interest into amyloid fibrils (Maji et al. 2009b).

1.6 Studying the Toxicity of Non-fibrillar Amyloid Assemblies

One of the main pathogenic mechanisms (Jellinger 2010) underlying many neurodegenerative diseases is abnormal protein dynamics and protein misfolding (Skovronsky et al. 2006; Herczenik and Gebbink 2008) accompanied by an imbalance between protein production and degradation, proteasomal/autophagy impairment, and dysfunction or mutation of molecular chaperones (Jellinger 2009, 2010). Oxidative stress in the form of reactive oxygen/nitrogen species, free radical formation, and lipid peroxidation also is involved in protein-misfolding diseases

(Butterfield et al. 2010; Kahle et al. 2009; Sesti et al. 2010; Ahmad et al. 2009). Oxidative stress goes hand-in-hand with inflammatory mechanisms and production of cytokines and chemokines in the disease-affected tissues (Ahmad et al. 2009; Lee et al. 2009; Lucin and Wyss-Coray 2009; Sugama et al. 2009; Tansey and Goldberg 2010; Sokolova et al. 2009; Shepherd et al. 2006). Mitochondrial dysfunction, DNA damage, disruption of ion homeostasis, and impaired bioenergetics coincide with oxidative stress and inflammatory conditions (Jellinger 2009, 2010). All these pathogenic mechanisms, inter-related in complex cycles, lead to cellular dysfunction, apoptosis, and/or necrosis. In the central nervous system, depending on the cell populations affected, these pathogenic mechanisms lead to emergence of specific or mixed disease phenotypes and complex clinical presentations and outcomes (Dickson 2009; Boeve 2007; Murray et al. 2005; Pittock and Lucchinetti 2007; Lansbury and Lashuel 2006).

Numerous experimental approaches have facilitated study of cytotoxic mechanisms of non-fibrillar assemblies of amyloidogenic proteins. *In vitro* experiments using cell culture and tissue slices along with biophysical studies have been performed to examine the toxic mechanisms of non-fibrillar amyloid assemblies using recombinant, synthetic, cell-, or tissue-derived variants of the amyloidogenic proteins. As discussed above, a concern in these experimental setups is that only a small proportion of the artificial assemblies may closely resemble non-fibrillar assemblies occurring *in vivo*. Other experimental approaches include the use of animal models, such as insect (Botella et al. 2009; Cowan et al. 2010; Iijima and Iijima-Ando 2008; Iijima-Ando and Iijima 2010; Khurana 2008; Lu 2009; Lu and Vogel 2009; Park et al. 2009; van Ham et al. 2009), *Caenorhabditis elegans* (Johnson et al. 2010), *Brachydanio rerio* (Sager et al. 2010; Ingham 2009; Malaga-Trillo and Sempou 2009), murine (Ashe and Zahs 2010; Dawson et al. 2010; Elder et al. 2010; Guyenet et al. 2010; Park et al. 2010; Taylor et al. 2010), rat (Flood et al. 2009), canine (Barsoum et al. 2000; Green and Tolwani 1999; Lossi et al. 2005; Vasilevko and Head 2009; Woodruff-Pak 2008), and simian models (Yang et al. 2008; Wang and Qin 2006; Qin et al. 2006; Walker 1997) to assess various aspects of etiology and pathogenesis, including genetics, behavior, system functions, or nutritional and therapeutic applications.

Toxicity mechanisms of non-fibrillar amyloid assemblies in various diseases are discussed in detail in individual chapters of this book. Here we highlight a few examples of non-fibrillar amyloid assemblies and their associated toxicity mechanisms. In one prominent example, synthetic Aβ oligomers derived from cells transfected with amyloid precursor protein (Podlisny et al. 1995) were shown to disrupt LTP in hippocampal tissue slices and *in vivo* (Townsend et al. 2006; Walsh et al. 2002), impair the memory of a complex pre-learned behavior (Cleary et al. 2005), memory consolidation, and synaptic remodeling causing loss of functional synapses in rats (Freir et al. 2011).

Another type of oligomer studied extensively is Aβ-derived diffusible ligands (ADDLs), which are synthetic Aβ42-derived species formed in the presence of apoJ (Oda et al. 1995), in F-12 media (Klein 2002b), or in phosphate-buffered saline (De Felice et al. 2008) as small globules 3–8 nm in diameter (Chromy et al. 2003) in

polydisperse mixtures of 150–1,000-kDa complexes (Hepler et al. 2006). ADDLs have been shown to be highly neurotoxic (Lambert et al. 1998; Xia et al. 1997), inhibit LTP (Lambert et al. 1998), promote oxidative stress and increased $[Ca^{2+}]_i$ (De Felice et al. 2007), induce τ phosphorylation (De Felice et al. 2008), and enhance interleukin-1β, inducible nitric oxide synthase (iNOS), nitric oxide, and tumor-necrosis-factor-α expression in astrocytes (White et al. 2005). Recently, it has been shown that ADDLs are sequestered into, and seed, new amyloid plaques in the brains of a murine AD model (Gaspar et al. 2010). However, the underlying mechanisms of this observation require further studies.

In neurodegenerative diseases characterized by intraneuronal α-synuclein deposition, even modest α-synuclein elevations can be toxic, though the precise mechanisms underlying synaptotoxicity in these diseases are unclear. Recently, a quantitative model system was used to evaluate the time-course and localization of evolving α-synuclein-induced pathologic events using cultured neurons isolated from brains of transgenic mice overexpressing fluorescently labeled human α-synuclein (Scott et al. 2010). Transgenic α-synuclein was shown to be altered pathologically over time while overexpressing neurons showed enlarged synaptic vesicles and striking deficits in neurotransmitter release (Scott et al. 2010), a phenotype characteristic of animal models lacking critical presynaptic proteins (Abeliovich et al. 2000; Chandra et al. 2004, 2005). In this model, Scott et al. showed that several endogenous presynaptic proteins were undetectable in a subset of transgenic synaptic boutons, suggesting that such diminutions triggered the overall synaptic pathology due to increased α-synuclein levels (Scott et al. 2010). Similar alterations in levels of synaptic proteins were retrospectively observed in human pathologic brains (Mukaetova-Ladinska et al. 2009; Bertrand et al. 2003), highlighting potential relevance to human disease.

Another toxic mechanism proposed for non-fibrillar assemblies of amyloid proteins is their pore- or channel-forming capacity that may lead to membrane leakage and increased $[Ca^{2+}]_i$ (Lashuel and Lansbury 2006; Lashuel et al. 2002a). In lipid bilayers *in vitro*, Aβ was shown to form uniform pore-like structures (Lin et al. 2001; Quist et al. 2005). These are thought to serve as Ca^{2+} channels and thus have been hypothesized to cause excitotoxicity and mediate Aβ-induced neurotoxicity in AD (Arispe et al. 1993b, a). Reports of various models including artificial phospholipid membrane bilayers, excised neuronal membrane patches, whole-cell patch-clamp experiments, and phospholipid vesicles support a channel-forming property of Aβ (Lin et al. 2001; Arispe et al. 1993b; Kawahara et al. 1997; Kawahara and Kuroda 2000; Sanderson et al. 1997; Rhee et al. 1998; Hirakura et al. 1999; Lin et al. 1999; Bhatia et al. 2000; Kourie et al. 2001; Kagan et al. 2002; Lin and Kagan 2002; Bahadi et al. 2003; Alarcon et al. 2006) and α-synuclein (Adamczyk and Strosznajder 2006; Di Pasquale et al. 2010; Kim et al. 2009; Tsigelny et al. 2007; Zakharov et al. 2007; Feng et al. 2010). Imaging techniques (Lin et al. 2001, 1999; Rhee et al. 1998; Bhatia et al. 2000), electrophysiological experiments (Arispe et al. 1993b; Kawahara et al. 1997; Sanderson et al. 1997; Rhee et al. 1998; Hirakura et al. 1999; Bhatia et al. 2000; Kourie et al. 2001; Bahadi et al. 2003; Alarcon et al. 2006), or cation-sensitive dyes (Bhatia et al. 2000; Jelinek and Sheynis 2010) were

used to assess channel-like properties of Aβ. However, other studies have reported general disruption of the plasma membrane homeostasis without channel formation (Sokolov et al. 2006; Demuro et al. 2005; Kayed et al. 2004).

It has been shown that directed expression of the molecular chaperone, Hsp70, one of numerous molecular chaperones that guide the correct folding of polypeptides, prevented dopaminergic neuronal loss associated with α-synuclein in a *Drosophila* model of PD and that interference with endogenous chaperone activity accelerated α-synuclein toxicity (Auluck et al. 2002). This work, and similar approaches in polyglutamine-related disorders (Warrick et al. 1999; Opal and Zoghbi 2002), indicate that such diseases are indeed disorders of protein folding, suggesting that activation of chaperones and other compensatory mechanisms, such as the ubiquitin–proteasome system, potentially can decrease accumulation of misfolded proteins or enhance their clearance.

In contrast to fibrils of disease-causing amyloidogenic proteins (discussed above), those formed by disease-unrelated proteins do not cause cytotoxicity in cell-culture experiments. For example, fibrils formed by an artificially designed α-helix-turn-α-helix (ααα) peptide displayed no neurotoxicity, even though they were morphologically indistinguishable from Aβ and IAPP fibrils, which were toxic (Fezoui et al. 2000). However, the pre-fibrillar assemblies of PI3K-SH3 and HypF-N were shown to be highly toxic to PC12 cells and murine fibroblasts *in vitro* (Bucciantini et al. 2004). The extent of cellular injury caused by the cytotoxic oligomers was comparable to that by Aβ42 oligomers, whereas the corresponding fibrils of both PI3K-SH3 and HypF-N were benign.

Early pre-fibrillar HypF-N assemblies were shown to permeabilize artificial phospholipid membranes more efficiently than mature fibrils, suggesting that this disease-unrelated protein shared toxic properties with non-fibrillar assemblies of peptides and proteins involved in pathology (Relini et al. 2004). Further investigation of the cellular effects of HypF-N oligomers revealed that they entered the cytoplasm and caused an acute rise in levels of reactive oxygen species and $[Ca^{2+}]_i$, leading to cell death (Bucciantini et al. 2004). In a study in which murine fibroblasts or endothelial cells were treated with pre-fibrillar HypF-N assemblies, the two cell types underwent two different death mechanisms—fibroblasts exposed for 24 h to 10 μM HypF-N oligomers underwent necrosis, whereas endothelial cells treated similarly underwent apoptosis (Bucciantini et al. 2005). A similar study comparing cytotoxic effects of pre-fibrillar and fibrillar HypF-N assemblies using a panel of normal and pathological cell-lines showed that cells were variably affected by the same amount of pre-fibrillar aggregates, whereas mature fibrils showed little or no toxicity (Cecchi et al. 2006). Recently, it has been shown that microinjection into rat brain nucleus basalis magnocellularis of non-fibrillar assemblies of PI3K-SH3 or HypF-N, but not the corresponding mature fibrils, compromised neuronal viability dose-dependently (Baglioni et al. 2006). Taken together, these data clearly demonstrate that the non-fibrillar assemblies of disease-unrelated proteins are highly toxic whereas most of the corresponding mature fibrils are not (Baglioni et al. 2006). The toxic effects of the oligomers may arise when these assemblies assume a "misfolded" conformation, which may expose hydrophobic residues that are natively buried within the core

structure. These exposed hydrophobic sequences are aggregation-prone and may interact with membranes and other cellular constituents modifying their structural/functional homeostasis. Interestingly, two types of stable, pre-fibrillar oligomers of HypF-N, which display similar morphologic and tinctorial properties, were shown to differ in their cytotoxic effects (Campioni et al. 2010). The differences in the packing of hydrophobic interactions between adjacent protein molecules in the oligomers determined the ability of the two oligomeric assemblies to cause cellular dysfunction and toxicity. Thus, a lower degree of hydrophobic packing within the oligomer core structure was found to correlate with a higher ability to penetrate the cell membrane and cause Ca^{2+} influx (Campioni et al. 2010).

1.7 Conclusions

Since the discovery and definition of amyloid lesions, intensive research has led to accumulation of data elucidating the pathogenic mechanisms of protein-misfolding diseases. Initially, pathogenic and toxic primacy was given to fibrillar forms of amyloidogenic proteins as these structures were found to be the major pathological hallmarks in neurodegenerative diseases. As discussed previously, earlier studies attributing toxicity to amyloid fibrils may have found this effect because of the inadvertent use of immature amyloid fibrils or equilibrium mixtures of oligomers and fibrils, which are cytotoxic, rather than pure preparations of mature amyloid fibrils, which often are not (Aksenov et al. 1996; Martins et al. 2008). Importantly, as our understanding of the devastating neurodegenerative and protein-misfolding diseases has been growing, an alternative paradigm has emerged. This paradigm postulates that non-fibrillar protein assemblies rather than mature amyloidogenic fibrils likely are the key neurotoxins responsible for most of the pathogenic mechanisms in protein-misfolding and neurodegenerative diseases. Accordingly, oligomeric species are thought to mediate diverse but interrelated pathogenic mechanisms that may lead to cytotoxicity and cell loss eventuating in organic and systemic involvement. This interrelation may lead to self-promoting and -propagating pathogenic cycles that worsen with age and chronicity. For instance, mechanisms associated with protein-misfolding may cause other events, such as inflammation and oxidative stress, which in turn aggravate misfolding. Overall, it is postulated that the non-fibrillar amyloidogenic proteins are “on path” to fibrillogenesis. The resulting protein fibrils are thought to be the end-stage sinks for the toxic non-fibrillar species. Fibrillar assemblies accumulate progressively into intracellular or extracellular proteinaceous amyloid aggregates generating the disease-specific lesions *in vivo*.

Global research efforts have established a framework for understanding the fundamentals of protein assembly and misfolding. A remaining challenge is to assess how these fundamental structural principles are linked to cellular and tissue microenvironments during progression of disease. Many experimental conditions have been used to study the structure and function of non-fibrillar assemblies; however, due to methodological limitations, regeneration and scrutiny of the actual *in vivo* milieus

and conditions in which protein assembly, oligomerization, fibrillization, and deposition occur are difficult. Similarly, it is extremely difficult to assess all the possible interactions these assemblies may have with various cellular components and organelles in the course of pathogenesis. A multitude of detrimental mechanisms, including disruption of cellular metabolism, deregulation of synapse structure and function, membrane damage, ionic imbalance, oxidative/inflammatory stress, apoptosis, and other cytotoxic effects, have been shown to be mediated by non-fibrillar assemblies of amyloidogenic proteins, emphasizing that a single therapeutic approach likely will be insufficient to prevent or treat the progression of diseases involving protein misfolding. Involvement of complex pathogenic mechanisms in these diseases calls for multifaceted rational diagnostic and therapeutic approaches that could potentially target not only a single assembly or a single mechanism but a multitude of assemblies or mechanisms. Agents that arrest the self-assembly process at the earliest stages or divert the process into formation of non-toxic species likely have the highest chance of success preventing and treating amyloid-related diseases because they inhibit formation and/or toxicity of both initial toxic oligomers and later aggregates.

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References

Abeliovich A, Schmitz Y, Farinas I, Choi-Lundberg D, Ho WH, Castillo PE, Shinsky N, Verdugo JM, Armanini M, Ryan A, Hynes M, Phillips H, Sulzer D, Rosenthal A (2000) Mice lacking α-synuclein display functional deficits in the nigrostriatal dopamine system. Neuron 25:239–252

Adamczyk A, Strosznajder JB (2006) α-Synuclein potentiates Ca^{2+} influx through voltage-dependent Ca^{2+} channels. Neuroreport 17:1883–1886

Aguzzi A, Calella AM (2009) Prions: protein aggregation and infectious diseases. Physiol Rev 89:1105–1152

Aguzzi A, O'Connor T (2010) Protein aggregation diseases: pathogenicity and therapeutic perspectives. Nat Rev Drug Discov 9:237–248

Ahmad A, Uversky VN, Hong D, Fink AL (2005) Early events in the fibrillation of monomeric insulin. J Biol Chem 280:42669–42675

Ahmad R, Rasheed Z, Ahsan H (2009) Biochemical and cellular toxicology of peroxynitrite: implications in cell death and autoimmune phenomenon. Immunopharmacol Immunotoxicol 31:388–396

Aksenov MY, Aksenova MV, Butterfield DA, Hensley K, Vigo-Pelfrey C, Carney JM (1996) Glutamine synthetase-induced enhancement of β-amyloid peptide Aβ (1–40) neurotoxicity accompanied by abrogation of fibril formation and Aβ fragmentation. J Neurochem 66: 2050–2056

Alarcon JM, Brito JA, Hermosilla T, Atwater I, Mears D, Rojas E (2006) Ion channel formation by Alzheimer's disease amyloid β-peptide (Aβ40) in unilamellar liposomes is determined by anionic phospholipids. Peptides 27:95–104

Alexandrescu AT (2005) Amyloid accomplices and enforcers. Protein Sci 14:1–12

Anguiano M, Nowak RJ, Lansbury PT Jr (2002) Protofibrillar islet amyloid polypeptide permeabilizes synthetic vesicles by a pore-like mechanism that may be relevant to type II diabetes. Biochemistry 41:11338–11343

Arispe N, Pollard HB, Rojas E (1993a) Giant multilevel cation channels formed by Alzheimer disease amyloid β-protein [AβP-(1–40)] in bilayer membranes. Proc Natl Acad Sci USA 90:10573–10577

Arispe N, Rojas E, Pollard HB (1993b) Alzheimer disease amyloid β protein forms calcium channels in bilayer membranes: blockade by tromethamine and aluminum. Proc Natl Acad Sci USA 90:567–571

Ashe KH, Zahs KR (2010) Probing the biology of Alzheimer's disease in mice. Neuron 66:631–645

Auluck PK, Chan HY, Trojanowski JQ, Lee VM, Bonini NM (2002) Chaperone suppression of α-synuclein toxicity in a *Drosophila* model for Parkinson's disease. Science 295:865–868

Bagetta V, Ghiglieri V, Sgobio C, Calabresi P, Picconi B (2010) Synaptic dysfunction in Parkinson's disease. Biochem Soc Trans 38:493–497

Baglioni S, Casamenti F, Bucciantini M, Luheshi LM, Taddei N, Chiti F, Dobson CM, Stefani M (2006) Prefibrillar amyloid aggregates could be generic toxins in higher organisms. J Neurosci 26:8160–8167

Bahadi R, Farrelly PV, Kenna BL, Curtain CC, Masters CL, Cappai R, Barnham KJ, Kourie JI (2003) Cu^{2+}-induced modification of the kinetics of Aβ(1–42) channels. Am J Physiol Cell Physiol 285:C873–C880

Barghorn S, Nimmrich V, Striebinger A, Krantz C, Keller P, Janson B, Bahr M, Schmidt M, Bitner RS, Harlan J, Barlow E, Ebert U, Hillen H (2005) Globular amyloid β-peptide oligomer—a homogenous and stable neuropathological protein in Alzheimer's disease. J Neurochem 95:834–847

Barsoum SC, Callahan HM, Robinson K, Chang PL (2000) Canine models for human genetic neurodegenerative diseases. Prog Neuropsychopharmacol Biol Psychiatry 24:811–823

Bauer PO, Nukina N (2009) The pathogenic mechanisms of polyglutamine diseases and current therapeutic strategies. J Neurochem 110:1737–1765

Bauer HH, Aebi U, Haner M, Hermann R, Muller M, Merkle HP (1995) Architecture and polymorphism of fibrillar supramolecular assemblies produced by *in vitro* aggregation of human calcitonin. J Struct Biol 115:1–15

Bellotti V, Nuvolone M, Giorgetti S, Obici L, Palladini G, Russo P, Lavatelli F, Perfetti V, Merlini G (2007) The workings of the amyloid diseases. Ann Med 39:200–207

Benvenga S, Facchiano A (1995) Atrial natriuretic peptide and amyloidosis. J Intern Med 237:525–526

Berriman J, Serpell LC, Oberg KA, Fink AL, Goedert M, Crowther RA (2003) Tau filaments from human brain and from *in vitro* assembly of recombinant protein show cross-β structure. Proc Natl Acad Sci USA 100:9034–9038

Bertrand E, Lechowicz W, Lewandowska E, Szpak GM, Dymecki J, Kosno-Kruszewska E, Wierzba-Bobrowicz T (2003) Degenerative axonal changes in the hippocampus and amygdala in Parkinson's disease. Folia Neuropathol 41:197–207

Betarbet R, Sherer TB, Greenamyre JT (2005) Ubiquitin–proteasome system and Parkinson's diseases. Exp Neurol 191(Suppl 1):S17–S27

Bhatia R, Lin H, Lal R (2000) Fresh and globular amyloid β protein (1–42) induces rapid cellular degeneration: evidence for AβP channel-mediated cellular toxicity. FASEB J 14:1233–1243

Bitan G, Kirkitadze MD, Lomakin A, Vollers SS, Benedek GB, Teplow DB (2003a) Amyloid β-protein (Aβ) assembly: Aβ40 and Aβ42 oligomerize through distinct pathways. Proc Natl Acad Sci USA 100:330–335

Bitan G, Tarus B, Vollers SS, Lashuel HA, Condron MM, Straub JE, Teplow DB (2003b) A molecular switch in amyloid assembly: Met35 and amyloid β-protein oligomerization. J Am Chem Soc 125:15359–15365

Bitan G, Vollers SS, Teplow DB (2003c) Elucidation of primary structure elements controlling early amyloid β-protein oligomerization. J Biol Chem 278:34882–34889

Bitan G, Fradinger EA, Spring SM, Teplow DB (2005) Neurotoxic protein oligomers—what you see is not always what you get. Amyloid 12:88–95

Boeve BF (2007) Links between frontotemporal lobar degeneration, corticobasal degeneration, progressive supranuclear palsy, and amyotrophic lateral sclerosis. Alzheimer Dis Assoc Disord 21:S31–S38

Botella JA, Bayersdorfer F, Gmeiner F, Schneuwly S (2009) Modelling Parkinson's disease in *Drosophila*. Neuromolecular Med 11:268–280

Braun S, Humphreys C, Fraser E, Brancale A, Bochtler M, Dale TC (2011) Amyloid-associated nucleic acid hybridisation. PLoS One 6:e19125

Bucciantini M, Calloni G, Chiti F, Formigli L, Nosi D, Dobson CM, Stefani M (2004) Prefibrillar amyloid protein aggregates share common features of cytotoxicity. J Biol Chem 279: 31374–31382

Bucciantini M, Rigacci S, Berti A, Pieri L, Cecchi C, Nosi D, Formigli L, Chiti F, Stefani M (2005) Patterns of cell death triggered in two different cell lines by HypF-N prefibrillar aggregates. FASEB J 19:437–439

Bunka DH, Mantle BJ, Morten IJ, Tennent GA, Radford SE, Stockley PG (2007) Production and characterization of RNA aptamers specific for amyloid fibril epitopes. J Biol Chem 282:34500–34509

Burré J, Sharma M, Tsetsenis T, Buchman V, Etherton MR, Sudhof TC (2010) α-Synuclein promotes SNARE-complex assembly *in vivo* and *in vitro*. Science 329:1663–1667

Butterfield DA, Bader Lange ML, Sultana R (2010) Involvements of the lipid peroxidation product, HNE, in the pathogenesis and progression of Alzheimer's disease. Biochim Biophys Acta 1801:924–929

Campioni S, Mannini B, Zampagni M, Pensalfini A, Parrini C, Evangelisti E, Relini A, Stefani M, Dobson CM, Cecchi C, Chiti F (2010) A causative link between the structure of aberrant protein oligomers and their toxicity. Nat Chem Biol 6:140–147

Canale C, Torrassa S, Rispoli P, Relini A, Rolandi R, Bucciantini M, Stefani M, Gliozzi A (2006) Natively folded HypF-N and its early amyloid aggregates interact with phospholipid monolayers and destabilize supported phospholipid bilayers. Biophys J 91:4575–4588

Cardoso F (2009) Huntington disease and other choreas. Neurol Clin 27:719–736, vi

Carulla N, Caddy GL, Hall DR, Zurdo J, Gairi M, Feliz M, Giralt E, Robinson CV, Dobson CM (2005) Molecular recycling within amyloid fibrils. Nature 436:554–558

Caughey B, Lansbury PT (2003) Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders. Annu Rev Neurosci 26:267–298

Cecchi C, Pensalfini A, Baglioni S, Fiorillo C, Caporale R, Formigli L, Liguri G, Stefani M (2006) Differing molecular mechanisms appear to underlie early toxicity of prefibrillar HypF-N aggregates to different cell types. FEBS J 273:2206–2222

Chandra S, Fornai F, Kwon HB, Yazdani U, Atasoy D, Liu X, Hammer RE, Battaglia G, German DC, Castillo PE, Sudhof TC (2004) Double-knockout mice for α- and β-synucleins: effect on synaptic functions. Proc Natl Acad Sci USA 101:14966–14971

Chandra S, Gallardo G, Fernandez-Chacon R, Schluter OM, Sudhof TC (2005) α-Synuclein cooperates with CSPα in preventing neurodegeneration. Cell 123:383–396

Chen S, Berthelier V, Hamilton JB, O'Nuallain B, Wetzel R (2002) Amyloid-like features of polyglutamine aggregates and their assembly kinetics. Biochemistry 41:7391–7399

Chiti F, Dobson CM (2006) Protein misfolding, functional amyloid, and human disease. Annu Rev Biochem 75:333–366

Chiti F, Webster P, Taddei N, Clark A, Stefani M, Ramponi G, Dobson CM (1999) Designing conditions for *in vitro* formation of amyloid protofilaments and fibrils. Proc Natl Acad Sci USA 96:3590–3594

Chiti F, Bucciantini M, Capanni C, Taddei N, Dobson CM, Stefani M (2001) Solution conditions can promote formation of either amyloid protofilaments or mature fibrils from the HypF *N*-terminal domain. Protein Sci 10:2541–2547

Chromy BA, Nowak RJ, Lambert MP, Viola KL, Chang L, Velasco PT, Jones BW, Fernandez SJ, Lacor PN, Horowitz P, Finch CE, Krafft GA, Klein WL (2003) Self-assembly of Aβ(1–42) into globular neurotoxins. Biochemistry 42:12749–12760

Clark A, Wells CA, Buley ID, Cruickshank JK, Vanhegan RI, Matthews DR, Cooper GJ, Holman RR, Turner RC (1988) Islet amyloid, increased A-cells, reduced B-cells and exocrine fibrosis: quantitative changes in the pancreas in type 2 diabetes. Diabetes Res 9:151–159

Cleary JP, Walsh DM, Hofmeister JJ, Shankar GM, Kuskowski MA, Selkoe DJ, Ashe KH (2005) Natural oligomers of the amyloid-β protein specifically disrupt cognitive function. Nat Neurosci 8:79–84

Cohen AS, Calkins E (1959) Electron microscopic observations on a fibrous component in amyloid of diverse origins. Nature 183:1202–1203

Cohen AS, Calkins E (1964) The isolation of amyloid fibrils and a study of the effect of collagenase and hyaluronidase. J Cell Biol 21:481–486

Comenzo RL (2006) Systemic immunoglobulin light-chain amyloidosis. Clin Lymphoma Myeloma 7:182–185

Comenzo RL (2007) Managing systemic light-chain amyloidosis. J Natl Compr Canc Netw 5:179–187

Conway KA, Harper JD, Lansbury PT Jr (2000a) Fibrils formed *in vitro* from α-synuclein and two mutant forms linked to Parkinson's disease are typical amyloid. Biochemistry 39:2552–2563

Conway KA, Lee SJ, Rochet JC, Ding TT, Williamson RE, Lansbury PT Jr (2000b) Acceleration of oligomerization, not fibrillization, is a shared property of both α-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. Proc Natl Acad Sci USA 97:571–576

Cowan CM, Chee F, Shepherd D, Mudher A (2010) Disruption of neuronal function by soluble hyperphosphorylated tau in a *Drosophila* model of tauopathy. Biochem Soc Trans 38:564–570

Cozzolino M, Ferri A, Carri MT (2008) Amyotrophic lateral sclerosis: from current developments in the laboratory to clinical implications. Antioxid Redox Signal 10:405–443

Cozzolino M, Pesaresi MG, Amori I, Crosio C, Ferri A, Nencini M, Carri MT (2009) Oligomerization of mutant SOD1 in mitochondria of motoneuronal cells drives mitochondrial damage and cell toxicity. Antioxid Redox Signal 11:1547–1558

Cruts M, Gijselinck I, van der Zee J, Engelborghs S, Wils H, Pirici D, Rademakers R, Vandenberghe R, Dermaut B, Martin JJ, van Duijn C, Peeters K, Sciot R, Santens P, De Pooter T, Mattheijssens M, Van den Broeck M, Cuijt I, Vennekens K, De Deyn PP, Kumar-Singh S, Van Broeckhoven C (2006) Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. Nature 442:920–924

Cruz L, Urbanc B, Borreguero JM, Lazo ND, Teplow DB, Stanley HE (2005) Solvent and mutation effects on the nucleation of amyloid β-protein folding. Proc Natl Acad Sci USA 102:18258–18263

Dawson TM (2007) Unraveling the role of defective genes in Parkinson's disease. Parkinsonism Relat Disord 13(Suppl 3):S248–S249

Dawson TM, Ko HS, Dawson VL (2010) Genetic animal models of Parkinson's disease. Neuron 66:646–661

De Felice FG, Vieira MN, Meirelles MN, Morozova-Roche LA, Dobson CM, Ferreira ST (2004) Formation of amyloid aggregates from human lysozyme and its disease-associated variants using hydrostatic pressure. FASEB J 18:1099–1101

De Felice FG, Velasco PT, Lambert MP, Viola K, Fernandez SJ, Ferreira ST, Klein WL (2007) Aβ oligomers induce neuronal oxidative stress through an *N*-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. J Biol Chem 282:11590–11601

De Felice FG, Wu D, Lambert MP, Fernandez SJ, Velasco PT, Lacor PN, Bigio EH, Jerecic J, Acton PJ, Shughrue PJ, Chen-Dodson E, Kinney GG, Klein WL (2008) Alzheimer's disease-type neuronal tau hyperphosphorylation induced by Aβ oligomers. Neurobiol Aging 29:1334–1347

Dember LM, Jaber BL (2006) Dialysis-related amyloidosis: late finding or hidden epidemic? Semin Dial 19:105–109

Demuro A, Mina E, Kayed R, Milton SC, Parker I, Glabe CG (2005) Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. J Biol Chem 280:17294–17300

Di Pasquale E, Fantini J, Chahinian H, Maresca M, Taieb N, Yahi N (2010) Altered ion channel formation by the Parkinson's-disease-linked E46K mutant of α-synuclein is corrected by GM3 but not by GM1 gangliosides. J Mol Biol 397:202–218

Dickson DW (2009) Neuropathology of non-Alzheimer degenerative disorders. Int J Clin Exp Pathol 3:1–23

Ding TT, Lee SJ, Rochet JC, Lansbury PT Jr (2002) Annular α-synuclein protofibrils are produced when spherical protofibrils are incubated in solution or bound to brain-derived membranes. Biochemistry 41:10209–10217

Dobson CM (2001) The structural basis of protein folding and its links with human disease. Philos Trans R Soc Lond B Biol Sci 356:133–145

Dobson CM (2004) Principles of protein folding, misfolding and aggregation. Semin Cell Dev Biol 15:3–16

Double KL, Reyes S, Werry EL, Halliday GM (2010) Selective cell death in neurodegeneration: why are some neurons spared in vulnerable regions? Prog Neurobiol 92:316–329

Dyson HJ, Wright PE (2005) Intrinsically unstructured proteins and their functions. Nat Rev Mol Cell Biol 6:197–208

Dzwolak W, Loksztejn A, Smirnovas V (2006) New insights into the self-assembly of insulin amyloid fibrils: an H–D exchange FT-IR study. Biochemistry 45:8143–8151

Dzwolak W, Loksztejn A, Galinska-Rakoczy A, Adachi R, Goto Y, Rupnicki L (2007) Conformational indeterminism in protein misfolding: chiral amplification on amyloidogenic pathway of insulin. J Am Chem Soc 129:7517–7522

Eanes ED, Glenner GG (1968) X-ray diffraction studies on amyloid filaments. J Histochem Cytochem 16:673–677

Eisen A (2009) Amyotrophic lateral sclerosis: a 40-year personal perspective. J Clin Neurosci 16:505–512

Eisenberg D, Nelson R, Sawaya MR, Balbirnie M, Sambashivan S, Ivanova MI, Madsen AO, Riekel C (2006) The structural biology of protein aggregation diseases: fundamental questions and some answers. Acc Chem Res 39:568–575

Elder GA, Gama Sosa MA, De Gasperi R (2010) Transgenic mouse models of Alzheimer's disease. Mt Sinai J Med 77:69–81

Eshaghian S, Kaul S, Shah PK (2007) Cardiac amyloidosis: new insights into diagnosis and management. Rev Cardiovasc Med 8:189–199

Feng LR, Federoff HJ, Vicini S, Maguire-Zeiss KA (2010) α-Synuclein mediates alterations in membrane conductance: a potential role for α-synuclein oligomers in cell vulnerability. Eur J Neurosci 32:10–17

Fernandez-Escamilla AM, Rousseau F, Schymkowitz J, Serrano L (2004) Prediction of sequence-dependent and mutational effects on the aggregation of peptides and proteins. Nat Biotechnol 22:1302–1306

Ferreira ST, Vieira MN, De Felice FG (2007) Soluble protein oligomers as emerging toxins in Alzheimer's and other amyloid diseases. IUBMB Life 59:332–345

Fezoui Y, Teplow DB (2002) Kinetic studies of amyloid β-protein fibril assembly. Differential effects of α-helix stabilization. J Biol Chem 277:36948–36954

Fezoui Y, Hartley DM, Walsh DM, Selkoe DJ, Osterhout JJ, Teplow DB (2000) A *de novo* designed helix-turn-helix peptide forms nontoxic amyloid fibrils. Nat Struct Biol 7:1095–1099

Flood DG, Lin YG, Lang DM, Trusko SP, Hirsch JD, Savage MJ, Scott RW, Howland DS (2009) A transgenic rat model of Alzheimer's disease with extracellular Aβ deposition. Neurobiol Aging 30:1078–1090

Fredenburg RA, Rospigliosi C, Meray RK, Kessler JC, Lashuel HA, Eliezer D, Lansbury PT Jr (2007) The impact of the E46K mutation on the properties of α-synuclein in its monomeric and oligomeric states. Biochemistry 46:7107–7118

Freir DB, Fedriani R, Scully D, Smith IM, Selkoe DJ, Walsh DM, Regan CM (2011) Aβ oligomers inhibit synapse remodelling necessary for memory consolidation. Neurobiol Aging 32: 2211–2218

Frid P, Anisimov SV, Popovic N (2007) Congo red and protein aggregation in neurodegenerative diseases. Brain Res Rev 53:135–160

Frieden C (2007) Protein aggregation processes: in search of the mechanism. Protein Sci 16:2334–2344

Friedreich N, Kekulé FA (1859) Zur Amyloidfrage. Virchows Arch Pathol Anat Physiol XVI:50–65

Frost B, Diamond MI (2010) Prion-like mechanisms in neurodegenerative diseases. Nat Rev Neurosci 11:155–159

Gaspar RC, Villarreal SA, Bowles N, Hepler RW, Joyce JG, Shughrue PJ (2010) Oligomers of β-amyloid are sequestered into and seed new plaques in the brains of an AD mouse model. Exp Neurol 223:394–400

Gasser T (2009) Molecular pathogenesis of Parkinson disease: insights from genetic studies. Expert Rev Mol Med 11:e22

Georganopoulou DG, Chang L, Nam JM, Thaxton CS, Mufson EJ, Klein WL, Mirkin CA (2005) Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease. Proc Natl Acad Sci USA 102:2273–2276

George JM, Jin H, Woods WS, Clayton DF (1995) Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. Neuron 15:361–372

Georgieva ER, Ramlall TF, Borbat PP, Freed JH, Eliezer D (2008) Membrane-bound α-synuclein forms an extended helix: long-distance pulsed ESR measurements using vesicles, bicelles, and rodlike micelles. J Am Chem Soc 130:12856–12857

Geula C, Wu CK, Saroff D, Lorenzo A, Yuan M, Yankner BA (1998) Aging renders the brain vulnerable to amyloid β-protein neurotoxicity. Nat Med 4:827–831

Gharibyan AL, Zamotin V, Yanamandra K, Moskaleva OS, Margulis BA, Kostanyan IA, Morozova-Roche LA (2007) Lysozyme amyloid oligomers and fibrils induce cellular death *via* different apoptotic/necrotic pathways. J Mol Biol 365:1337–1349

Giannetti AM, Lindwall G, Chau MF, Radeke MJ, Feinstein SC, Kohlstaedt LA (2000) Fibers of tau fragments, but not full length tau, exhibit a cross β-structure: implications for the formation of paired helical filaments. Protein Sci 9:2427–2435

Ginsberg SD, Galvin JE, Chiu TS, Lee VM, Masliah E, Trojanowski JQ (1998) RNA sequestration to pathological lesions of neurodegenerative diseases. Acta Neuropathol 96:487–494

Ginsberg SD, Crino PB, Hemby SE, Weingarten JA, Lee VM, Eberwine JH, Trojanowski JQ (1999) Predominance of neuronal mRNAs in individual Alzheimer's disease senile plaques. Ann Neurol 45:174–181

Glabe CG (2006) Common mechanisms of amyloid oligomer pathogenesis in degenerative disease. Neurobiol Aging 27:570–575

Glabe CG, Kayed R (2006) Common structure and toxic function of amyloid oligomers implies a common mechanism of pathogenesis. Neurology 66:S74–S78

Goedert M, Wischik CM, Crowther RA, Walker JE, Klug A (1988) Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau. Proc Natl Acad Sci USA 85:4051–4055

Goette A, Rocken C (2004) Atrial amyloidosis and atrial fibrillation: a gender-dependent "arrhythmogenic substrate"? Eur Heart J 25:1185–1186

Goldsbury CS, Cooper GJ, Goldie KN, Muller SA, Saafi EL, Gruijters WT, Misur MP, Engel A, Aebi U, Kistler J (1997) Polymorphic fibrillar assembly of human amylin. J Struct Biol 119:17–27

Gong Y, Chang L, Viola KL, Lacor PN, Lambert MP, Finch CE, Krafft GA, Klein WL (2003) Alzheimer's disease-affected brain: presence of oligomeric Aβ ligands (ADDLs) suggests a molecular basis for reversible memory loss. Proc Natl Acad Sci USA 100:10417–10422

Green SL, Tolwani RJ (1999) Animal models for motor neuron disease. Lab Anim Sci 49:480–487

Grudzielanek S, Smirnovas V, Winter R (2007a) The effects of various membrane physical-chemical properties on the aggregation kinetics of insulin. Chem Phys Lipids 149:28–39

Grudzielanek S, Velkova A, Shukla A, Smirnovas V, Tatarek-Nossol M, Rehage H, Kapurniotu A, Winter R (2007b) Cytotoxicity of insulin within its self-assembly and amyloidogenic pathways. J Mol Biol 370:372–384

Gudiksen KL, Gitlin I, Whitesides GM (2006) Differentiation of proteins based on characteristic patterns of association and denaturation in solutions of SDS. Proc Natl Acad Sci USA 103:7968–7972

Guijarro JI, Sunde M, Jones JA, Campbell ID, Dobson CM (1998) Amyloid fibril formation by an SH3 domain. Proc Natl Acad Sci USA 95:4224–4228

Gurlo T, Ryazantsev S, Huang CJ, Yeh MW, Reber HA, Hines OJ, O'Brien TD, Glabe CG, Butler PC (2010) Evidence for proteotoxicity in β cells in type 2 diabetes: toxic islet amyloid polypeptide oligomers form intracellularly in the secretory pathway. Am J Pathol 176:861–869

Gursky O, Aleshkov S (2000) Temperature-dependent β-sheet formation in β-amyloid Aβ(1–40) peptide in water: uncoupling β-structure folding from aggregation. Biochim Biophys Acta 1476:93–102

Guyenet SJ, Furrer SA, Damian VM, Baughan TD, La Spada AR, Garden GA (2010) A simple composite phenotype scoring system for evaluating mouse models of cerebellar ataxia. J Vis Exp 39:1787. doi:10.3791/1787

Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β-peptide. Nat Rev Mol Cell Biol 8:101–112

Haataja L, Gurlo T, Huang CJ, Butler PC (2008) Islet amyloid in type 2 diabetes, and the toxic oligomer hypothesis. Endocr Rev 29:303–316

Halliday GM, McCann H (2010) The progression of pathology in Parkinson's disease. Ann N Y Acad Sci 1184:188–195

Hardy JA, Higgins GA (1992) Alzheimer's disease: the amyloid cascade hypothesis. Science 256:184–185

Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297:353–356

Harper JD, Wong SS, Lieber CM, Lansbury PT (1997) Observation of metastable Aβ amyloid protofibrils by atomic force microscopy. Chem Biol 4:119–125

Harrison RS, Sharpe PC, Singh Y, Fairlie DP (2007) Amyloid peptides and proteins in review. Rev Physiol Biochem Pharmacol 159:1–77

Hartley DM, Walsh DM, Ye CP, Diehl T, Vasquez S, Vassilev PM, Teplow DB, Selkoe DJ (1999) Protofibrillar intermediates of amyloid β-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. J Neurosci 19:8876–8884

Hawkins PN (2003) Hereditary systemic amyloidosis with renal involvement. J Nephrol 16:443–448

Hayden MR, Tyagi SC, Kerklo MM, Nicolls MR (2005) Type 2 diabetes mellitus as a conformational disease. JOP 6:287–302

Head E, Pop V, Vasilevko V, Hill M, Saing T, Sarsoza F, Nistor M, Christie LA, Milton S, Glabe C, Barrett E, Cribbs D (2008) A two-year study with fibrillar β-amyloid (Aβ) immunization in aged canines: effects on cognitive function and brain Aβ. J Neurosci 28:3555–3566

Head E, Pop V, Sarsoza F, Kayed R, Beckett TL, Studzinski CM, Tomic JL, Glabe CG, Murphy MP (2010) Amyloid β-peptide and oligomers in the brain and cerebrospinal fluid of aged canines. J Alzheimers Dis 20:637–646

Hepler RW, Grimm KM, Nahas DD, Breese R, Dodson EC, Acton P, Keller PM, Yeager M, Wang H, Shughrue P, Kinney G, Joyce JG (2006) Solution state characterization of amyloid β-derived diffusible ligands. Biochemistry 45:15157–15167

Herczenik E, Gebbink MF (2008) Molecular and cellular aspects of protein misfolding and disease. FASEB J 22:2115–2133

Hirakura Y, Lin MC, Kagan BL (1999) Alzheimer amyloid Aβ1–42 channels: effects of solvent, pH, and Congo Red. J Neurosci Res 57:458–466

Hirschfield GM, Hawkins PN (2003) Amyloidosis: new strategies for treatment. Int J Biochem Cell Biol 35:1608–1613

Hoppener JW, Ahren B, Lips CJ (2000) Islet amyloid and type 2 diabetes mellitus. N Engl J Med 343:411–419

Houlden H, Crook R, Dolan RJ, McLaughlin J, Revesz T, Hardy J (2001) A novel presenilin mutation (M233V) causing very early onset Alzheimer's disease with Lewy bodies. Neurosci Lett 313:93–95

Hung LW, Ciccotosto GD, Giannakis E, Tew DJ, Perez K, Masters CL, Cappai R, Wade JD, Barnham KJ (2008) Amyloid-β peptide (Aβ) neurotoxicity is modulated by the rate of peptide aggregation: Aβ dimers and trimers correlate with neurotoxicity. J Neurosci 28:11950–11958

Ian VJM, Virginia MYL, John QT (2001) Synucleinopathies: a pathological and molecular review. Clin Neurosci Res 1:445–455

Ihara Y, Abraham C, Selkoe DJ (1983) Antibodies to paired helical filaments in Alzheimer's disease do not recognize normal brain proteins. Nature 304:727–730

Iijima K, Iijima-Ando K (2008) *Drosophila* models of Alzheimer's amyloidosis: the challenge of dissecting the complex mechanisms of toxicity of amyloid-β42. J Alzheimers Dis 15:523–540

Iijima-Ando K, Iijima K (2010) Transgenic *Drosophila* models of Alzheimer's disease and tauopathies. Brain Struct Funct 214:245–262

Inaba S, Okada T, Konakahara T, Kodaka M (2005) Specific binding of amyloid-β-protein to IMR-32 neuroblastoma cell membrane. J Pept Res 65:485–490

Ingham PW (2009) The power of the zebrafish for disease analysis. Hum Mol Genet 18:R107–R112

Inouye H, Sharma D, Goux WJ, Kirschner DA (2006) Structure of core domain of fibril-forming PHF/Tau fragments. Biophys J 90:1774–1789

Inzelberg R, Polyniki A (2010) Are genetic and sporadic Parkinson's disease patients equally susceptible to develop dementia? J Neurol Sci 289:23–26

Jao CC, Hegde BG, Chen J, Haworth IS, Langen R (2008) Structure of membrane-bound α-synuclein from site-directed spin labeling and computational refinement. Proc Natl Acad Sci USA 105:19666–19671

Jayasinghe SA, Langen R (2005) Lipid membranes modulate the structure of islet amyloid polypeptide. Biochemistry 44:12113–12119

Jelinek R, Sheynis T (2010) Amyloid–membrane interactions: experimental approaches and techniques. Curr Protein Pept Sci 11:372–384

Jellinger KA (2009) Recent advances in our understanding of neurodegeneration. J Neural Transm 116:1111–1162

Jellinger KA (2010) Basic mechanisms of neurodegeneration: a critical update. J Cell Mol Med 14:457–487

Jiménez JL, Guijarro JI, Orlova E, Zurdo J, Dobson CM, Sunde M, Saibil HR (1999) Cryo-electron microscopy structure of an SH3 amyloid fibril and model of the molecular packing. EMBO J 18:815–821

Jiménez JL, Nettleton EJ, Bouchard M, Robinson CV, Dobson CM, Saibil HR (2002) The protofilament structure of insulin amyloid fibrils. Proc Natl Acad Sci USA 99:9196–9201

Joachim CL, Morris JH, Kosik KS, Selkoe DJ (1987) Tau antisera recognize neurofibrillary tangles in a range of neurodegenerative disorders. Ann Neurol 22:514–520

Johnson JR, Jenn RC, Barclay JW, Burgoyne RD, Morgan A (2010) *Caenorhabditis elegans*: a useful tool to decipher neurodegenerative pathways. Biochem Soc Trans 38:559–563

Kagan BL, Hirakura Y, Azimov R, Azimova R, Lin MC (2002) The channel hypothesis of Alzheimer's disease: current status. Peptides 23:1311–1315

Kahle PJ, Waak J, Gasser T (2009) DJ-1 and prevention of oxidative stress in Parkinson's disease and other age-related disorders. Free Radic Biol Med 47:1354–1361

Kahn SE, Andrikopoulos S, Verchere CB (1999) Islet amyloid: a long-recognized but underappreciated pathological feature of type 2 diabetes. Diabetes 48:241–253

Kammerer RA, Steinmetz MO (2006) *De novo* design of a two-stranded coiled-coil switch peptide. J Struct Biol 155:146–153

Kawahara M, Kuroda Y (2000) Molecular mechanism of neurodegeneration induced by Alzheimer's β-amyloid protein: channel formation and disruption of calcium homeostasis. Brain Res Bull 53:389–397

Kawahara M, Arispe N, Kuroda Y, Rojas E (1997) Alzheimer's disease amyloid β-protein forms Zn^{2+}-sensitive, cation-selective channels across excised membrane patches from hypothalamic neurons. Biophys J 73:67–75

Kawooya JK, Emmons TL, Gonzalez-DeWhitt PA, Camp MC, D'Andrea SC (2003) Electrophoretic mobility of Alzheimer's amyloid-β peptides in urea-sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Anal Biochem 323:103–113

Kayed R, Glabe CG (2006) Conformation-dependent anti-amyloid oligomer antibodies. Methods Enzymol 413:326–344

Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 300:486–489

Kayed R, Sokolov Y, Edmonds B, McIntire TM, Milton SC, Hall JE, Glabe CG (2004) Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. J Biol Chem 279:46363–46366

Kayed R, Pensalfini A, Margol L, Sokolov Y, Sarsoza F, Head E, Hall J, Glabe C (2009) Annular protofibrils are a structurally and functionally distinct type of amyloid oligomer. J Biol Chem 284:4230–4237

Kelly JW (1998) The alternative conformations of amyloidogenic proteins and their multi-step assembly pathways. Curr Opin Struct Biol 8:101–106

Khemtemourian L, Killian JA, Hoppener JW, Engel MF (2008) Recent insights in islet amyloid polypeptide-induced membrane disruption and its role in β-cell death in type 2 diabetes mellitus. Exp Diabetes Res 2008:421287

Khurana V (2008) Modeling tauopathy in the fruit fly *Drosophila melanogaster*. J Alzheimers Dis 15:541–553

Khurana R, Coleman C, Ionescu-Zanetti C, Carter SA, Krishna V, Grover RK, Roy R, Singh S (2005) Mechanism of thioflavin T binding to amyloid fibrils. J Struct Biol 151:229–238

Kim YS, Laurine E, Woods W, Lee SJ (2006) A novel mechanism of interaction between α-synuclein and biological membranes. J Mol Biol 360:386–397

Kim HY, Cho MK, Kumar A, Maier E, Siebenhaar C, Becker S, Fernandez CO, Lashuel HA, Benz R, Lange A, Zweckstetter M (2009) Structural properties of pore-forming oligomers of α-synuclein. J Am Chem Soc 131:17482–17489

Kirkitadze MD, Condron MM, Teplow DB (2001) Identification and characterization of key kinetic intermediates in amyloid β-protein fibrillogenesis. J Mol Biol 312:1103–1119

Kirkitadze MD, Bitan G, Teplow DB (2002) Paradigm shifts in Alzheimer's disease and other neurodegenerative disorders: the emerging role of oligomeric assemblies. J Neurosci Res 69:567–577

Kiss E, Keusch G, Zanetti M, Jung T, Schwarz A, Schocke M, Jaschke W, Czermak BV (2005) Dialysis-related amyloidosis revisited. AJR Am J Roentgenol 185:1460–1467

Kissane JM (1973) Hereditary disorders of the kidney. II. Hereditary nephropathies. Perspect Pediatr Pathol 1:147–187

Kitamura A, Kubota H (2010) Amyloid oligomers: dynamics and toxicity in the cytosol and nucleus. FEBS J 277:1369–1379

Klein WL (2002a) ADDLs & protofibrils—the missing links? Neurobiol Aging 23:231–235

Klein WL (2002b) Aβ toxicity in Alzheimer's disease: globular oligomers (ADDLs) as new vaccine and drug targets. Neurochem Int 41:345–352

Klucken J, Ingelsson M, Shin Y, Irizarry MC, Hedley-Whyte ET, Frosch M, Growdon J, McLean P, Hyman BT (2006) Clinical and biochemical correlates of insoluble α-synuclein in dementia with Lewy bodies. Acta Neuropathol 111:101–108

Knight JD, Hebda JA, Miranker AD (2006) Conserved and cooperative assembly of membrane-bound α-helical states of islet amyloid polypeptide. Biochemistry 45:9496–9508

Koffie RM, Meyer-Luehmann M, Hashimoto T, Adams KW, Mielke ML, Garcia-Alloza M, Micheva KD, Smith SJ, Kim ML, Lee VM, Hyman BT, Spires-Jones TL (2009) Oligomeric amyloid β associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. Proc Natl Acad Sci USA 106:4012–4017

Kopito RR (2000) Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol 10:524–530

Kosik KS, Joachim CL, Selkoe DJ (1986) Microtubule-associated protein tau (τ) is a major antigenic component of paired helical filaments in Alzheimer disease. Proc Natl Acad Sci USA 83:4044–4048

Kourie JI, Henry CL, Farrelly P (2001) Diversity of amyloid β protein fragment [1–40]-formed channels. Cell Mol Neurobiol 21:255–284

Kril JJ, Halliday GM (2001) Alzheimer's disease: its diagnosis and pathogenesis. Int Rev Neurobiol 48:167–217

Kupfer L, Hinrichs W, Groschup MH (2009) Prion protein misfolding. Curr Mol Med 9:826–835

Kyle RA (2001) Amyloidosis: a convoluted story. Br J Haematol 114:529–538

Lafaye P, Achour I, England P, Duyckaerts C, Rougeon F (2009) Single-domain antibodies recognize selectively small oligomeric forms of amyloid β, prevent Aβ-induced neurotoxicity and inhibit fibril formation. Mol Immunol 46:695–704

Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, Klein WL (1998) Diffusible, nonfibrillar ligands derived from Aβ1–42 are potent central nervous system neurotoxins. Proc Natl Acad Sci USA 95:6448–6453

Lansbury PT, Lashuel HA (2006) A century-old debate on protein aggregation and neurodegeneration enters the clinic. Nature 443:774–779

Lashuel HA, Lansbury PT Jr (2006) Are amyloid diseases caused by protein aggregates that mimic bacterial pore-forming toxins? Q Rev Biophys 39:167–201

Lashuel HA, Hartley D, Petre BM, Walz T, Lansbury PT Jr (2002a) Neurodegenerative disease: amyloid pores from pathogenic mutations. Nature 418:291

Lashuel HA, Petre BM, Wall J, Simon M, Nowak RJ, Walz T, Lansbury PT Jr (2002b) α-Synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils. J Mol Biol 322:1089–1102

Layfield R, Cavey JR, Lowe J (2003) Role of ubiquitin-mediated proteolysis in the pathogenesis of neurodegenerative disorders. Ageing Res Rev 2:343–356

Ledford H (2010) Key Alzheimer's findings questioned. Nature 466:1031

Lee EB, Leng LZ, Zhang B, Kwong L, Trojanowski JQ, Abel T, Lee VM (2006) Targeting amyloid-β peptide (Aβ) oligomers by passive immunization with a conformation-selective monoclonal antibody improves learning and memory in Aβ precursor protein (APP) transgenic mice. J Biol Chem 281:4292–4299

Lee S, Fernandez EJ, Good TA (2007) Role of aggregation conditions in structure, stability, and toxicity of intermediates in the Aβ fibril formation pathway. Protein Sci 16:723–732

Lee JK, Tran T, Tansey MG (2009) Neuroinflammation in Parkinson's disease. J Neuroimmune Pharmacol 4:419–429

Leffers KW, Schell J, Jansen K, Lucassen R, Kaimann T, Nagel-Steger L, Tatzelt J, Riesner D (2004) The structural transition of the prion protein into its pathogenic conformation is induced by unmasking hydrophobic sites. J Mol Biol 344:839–853

Legleiter J, Mitchell E, Lotz GP, Sapp E, Ng C, DiFiglia M, Thompson LM, Muchowski PJ (2010) Mutant huntingtin fragments form oligomers in a polyglutamine length-dependent manner *in vitro* and *in vivo*. J Biol Chem 285:14777–14790

Lesné S, Koh MT, Kotilinek L, Kayed R, Glabe CG, Yang A, Gallagher M, Ashe KH (2006) A specific amyloid-β protein assembly in the brain impairs memory. Nature 440:352–357

LeVine H 3rd (1999) Quantification of β-sheet amyloid fibril structures with thioflavin T. Methods Enzymol 309:274–284

Li L, Holscher C (2007) Common pathological processes in Alzheimer disease and type 2 diabetes: a review. Brain Res Rev 56:384–402

Liao L, Cheng D, Wang J, Duong DM, Losik TG, Gearing M, Rees HD, Lah JJ, Levey AI, Peng J (2004) Proteomic characterization of postmortem amyloid plaques isolated by laser capture microdissection. J Biol Chem 279:37061–37068

Lin MC, Kagan BL (2002) Electrophysiologic properties of channels induced by Aβ25–35 in planar lipid bilayers. Peptides 23:1215–1228

Lin H, Zhu YJ, Lal R (1999) Amyloid β protein (1–40) forms calcium-permeable, Zn^{2+}-sensitive channel in reconstituted lipid vesicles. Biochemistry 38:11189–11196

Lin H, Bhatia R, Lal R (2001) Amyloid β protein forms ion channels: implications for Alzheimer's disease pathophysiology. FASEB J 15:2433–2444

Lin CY, Gurlo T, Kayed R, Butler AE, Haataja L, Glabe CG, Butler PC (2007) Toxic human islet amyloid polypeptide (h-IAPP) oligomers are intracellular, and vaccination to induce anti-toxic oligomer antibodies does not prevent h-IAPP-induced β-cell apoptosis in h-IAPP transgenic mice. Diabetes 56:1324–1332

Litvinovich SV, Brew SA, Aota S, Akiyama SK, Haudenschild C, Ingham KC (1998) Formation of amyloid-like fibrils by self-association of a partially unfolded fibronectin type III module. J Mol Biol 280:245–258

Looi LM (1993) Isolated atrial amyloidosis: a clinicopathologic study indicating increased prevalence in chronic heart disease. Hum Pathol 24:602–607

Lopez De La Paz M, Goldie K, Zurdo J, Lacroix E, Dobson CM, Hoenger A, Serrano L (2002) *De novo* designed peptide-based amyloid fibrils. Proc Natl Acad Sci USA 99:16052–16057

Lorenzo A, Yankner BA (1994) β-Amyloid neurotoxicity requires fibril formation and is inhibited by Congo red. Proc Natl Acad Sci USA 91:12243–12247

Lorenzo A, Razzaboni B, Weir GC, Yankner BA (1994) Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. Nature 368:756–760

Lossi L, Cantile C, Tamagno I, Merighi A (2005) Apoptosis in the mammalian CNS: lessons from animal models. Vet J 170:52–66

Lu B (2009) Recent advances in using *Drosophila* to model neurodegenerative diseases. Apoptosis 14:1008–1020

Lu B, Vogel H (2009) *Drosophila* models of neurodegenerative diseases. Annu Rev Pathol 4:315–342

Lublin AL, Gandy S (2010) Amyloid-β oligomers: possible roles as key neurotoxins in Alzheimer's disease. Mt Sinai J Med 77:43–49

Lucin KM, Wyss-Coray T (2009) Immune activation in brain aging and neurodegeneration: too much or too little? Neuron 64:110–122

Luibl V, Isas JM, Kayed R, Glabe CG, Langen R, Chen J (2006) Drusen deposits associated with aging and age-related macular degeneration contain nonfibrillar amyloid oligomers. J Clin Invest 116:378–385

Maji SK, Amsden JJ, Rothschild KJ, Condron MM, Teplow DB (2005) Conformational dynamics of amyloid β-protein assembly probed using intrinsic fluorescence. Biochemistry 44:13365–13376

Maji SK, Perrin MH, Sawaya MR, Jessberger S, Vadodaria K, Rissman RA, Singru PS, Nilsson KP, Simon R, Schubert D, Eisenberg D, Rivier J, Sawchenko P, Vale W, Riek R (2009a) Functional amyloids as natural storage of peptide hormones in pituitary secretory granules. Science 325:328–332

Maji SK, Wang L, Greenwald J, Riek R (2009b) Structure–activity relationship of amyloid fibrils. FEBS Lett 583:2610–2617

Malaga-Trillo E, Sempou E (2009) PrPs: proteins with a purpose: lessons from the zebrafish. Prion 3:129–133

Mallucci GR (2009) Prion neurodegeneration: starts and stops at the synapse. Prion 3:195–201

Marcinkiewicz M (2002) βAPP and furin mRNA concentrates in immature senile plaques in the brain of Alzheimer patients. J Neuropathol Exp Neurol 61:815–829

Marcon G, Plakoutsi G, Canale C, Relini A, Taddei N, Dobson CM, Ramponi G, Chiti F (2005) Amyloid formation from HypF-N under conditions in which the protein is initially in its native state. J Mol Biol 347:323–335

Margittai M, Langen R (2006) Side chain-dependent stacking modulates tau filament structure. J Biol Chem 281:37820–37827

Martins IC, Kuperstein I, Wilkinson H, Maes E, Vanbrabant M, Jonckheere W, Van Gelder P, Hartmann D, D'Hooge R, De Strooper B, Schymkowitz J, Rousseau F (2008) Lipids revert inert Aβ amyloid fibrils to neurotoxic protofibrils that affect learning in mice. EMBO J 27:224–233

Marzban L, Park K, Verchere CB (2003) Islet amyloid polypeptide and type 2 diabetes. Exp Gerontol 38:347–351

Mayeux R (2010) Clinical practice. Early Alzheimer's disease. N Engl J Med 362:2194–2201

McCarthy RE 3rd, Kasper EK (1998) A review of the amyloidoses that infiltrate the heart. Clin Cardiol 21:547–552

McDonald RJ, Craig LA, Hong NS (2010) The etiology of age-related dementia is more complicated than we think. Behav Brain Res 214:3–11

McGowan DP, van Roon-Mom W, Holloway H, Bates GP, Mangiarini L, Cooper GJS, Faull RLM, Snell RG (2000) Amyloid-like inclusions in Huntington's disease. Neuroscience 100:677–680

Meier JJ, Kayed R, Lin CY, Gurlo T, Haataja L, Jayasinghe S, Langen R, Glabe CG, Butler PC (2006) Inhibition of human IAPP fibril formation does not prevent β-cell death: evidence for distinct actions of oligomers and fibrils of human IAPP. Am J Physiol Endocrinol Metab 291:E1317–E1324

Merlini G, Westermark P (2004) The systemic amyloidoses: clearer understanding of the molecular mechanisms offers hope for more effective therapies. J Intern Med 255:159–178

Miller Y, Ma B, Nussinov R (2010) Polymorphism in Alzheimer Aβ amyloid organization reflects conformational selection in a rugged energy landscape. Chem Rev 110:4820–4838

Miyoshi K (2009) What is 'early onset dementia'? Psychogeriatrics 9:67–72

Montserret R, McLeish MJ, Bockmann A, Geourjon C, Penin F (2000) Involvement of electrostatic interactions in the mechanism of peptide folding induced by sodium dodecyl sulfate binding. Biochemistry 39:8362–8373

Moro ML, Collins MJ, Cappellini E (2010) Alzheimer's disease and amyloid β-peptide deposition in the brain: a matter of 'aging'? Biochem Soc Trans 38:539–544

Moussa CE, Wersinger C, Rusnak M, Tomita Y, Sidhu A (2004) Abnormal migration of human wild-type α-synuclein upon gel electrophoresis. Neurosci Lett 371:239–243

Mukaetova-Ladinska EB, Xuereb JH, Garcia-Sierra F, Hurt J, Gertz HJ, Hills R, Brayne C, Huppert FA, Paykel ES, McGee MA, Jakes R, Honer WG, Harrington CR, Wischik CM (2009) Lewy body variant of Alzheimer's disease: selective neocortical loss of t-SNARE proteins and loss of MAP2 and α-synuclein in medial temporal lobe. ScientificWorldJournal 9:1463–1475

Murali J, Jayakumar R (2005) Spectroscopic studies on native and protofibrillar insulin. J Struct Biol 150:180–189

Murray B, Lynch T, Farrell M (2005) Clinicopathological features of the tauopathies. Biochem Soc Trans 33:595–599

Nekooki-Machida Y, Kurosawa M, Nukina N, Ito K, Oda T, Tanaka M (2009) Distinct conformations of *in vitro* and *in vivo* amyloids of huntingtin-exon1 show different cytotoxicity. Proc Natl Acad Sci USA 106:9679–9684

Nelson R, Eisenberg D (2006) Recent atomic models of amyloid fibril structure. Curr Opin Struct Biol 16:260–265

Nelson R, Sawaya MR, Balbirnie M, Madsen AO, Riekel C, Grothe R, Eisenberg D (2005) Structure of the cross-β spine of amyloid-like fibrils. Nature 435:773–778

Novitskaya V, Bocharova OV, Bronstein I, Baskakov IV (2006) Amyloid fibrils of mammalian prion protein are highly toxic to cultured cells and primary neurons. J Biol Chem 281:13828–13836

Nukina N, Kosik KS, Selkoe DJ (1987) Recognition of Alzheimer paired helical filaments by monoclonal neurofilament antibodies is due to crossreaction with tau protein. Proc Natl Acad Sci USA 84:3415–3419

O'Nuallain B, Freir DB, Nicoll AJ, Risse E, Ferguson N, Herron CE, Collinge J, Walsh DM (2010) Amyloid β-protein dimers rapidly form stable synaptotoxic protofibrils. J Neurosci 30:14411–14419

Obeso JA, Rodriguez-Oroz MC, Goetz CG, Marin C, Kordower JH, Rodriguez M, Hirsch EC, Farrer M, Schapira AH, Halliday G (2010) Missing pieces in the Parkinson's disease puzzle. Nat Med 16:653–661

Oda T, Wals P, Osterburg HH, Johnson SA, Pasinetti GM, Morgan TE, Rozovsky I, Stine WB, Snyder SW, Holzman TF et al (1995) Clusterin (apoJ) alters the aggregation of amyloid β-peptide (Aβ1–42) and forms slowly sedimenting Aβ complexes that cause oxidative stress. Exp Neurol 136:22–31

Okada T, Wakabayashi M, Ikeda K, Matsuzaki K (2007) Formation of toxic fibrils of Alzheimer's amyloid β-protein-(1–40) by monosialoganglioside GM1, a neuronal membrane component. J Mol Biol 371:481–489

Opal P, Zoghbi HY (2002) The role of chaperones in polyglutamine disease. Trends Mol Med 8:232–236

Pahwa R, Lyons KE (2010) Early diagnosis of Parkinson's disease: recommendations from diagnostic clinical guidelines. Am J Manag Care 16(Suppl Implications):S94–S99

Paleologou KE, Kragh CL, Mann DM, Salem SA, Al-Shami R, Allsop D, Hassan AH, Jensen PH, El-Agnaf OM (2009) Detection of elevated levels of soluble α-synuclein oligomers in post-mortem brain extracts from patients with dementia with Lewy bodies. Brain 132:1093–1101

Paravastu AK, Petkova AT, Tycko R (2006) Polymorphic fibril formation by residues 10–40 of the Alzheimer's β-amyloid peptide. Biophys J 90:4618–4629

Paravastu AK, Leapman RD, Yau WM, Tycko R (2008) Molecular structural basis for polymorphism in Alzheimer's β-amyloid fibrils. Proc Natl Acad Sci USA 105:18349–18354

Paravastu AK, Qahwash I, Leapman RD, Meredith SC, Tycko R (2009) Seeded growth of β-amyloid fibrils from Alzheimer's brain-derived fibrils produces a distinct fibril structure. Proc Natl Acad Sci USA 106:7443–7448

Park J, Kim Y, Chung J (2009) Mitochondrial dysfunction and Parkinson's disease genes: insights from *Drosophila*. Dis Model Mech 2:336–340

Park GH, Kariya S, Monani UR (2010) Spinal muscular atrophy: new and emerging insights from model mice. Curr Neurol Neurosci Rep 10:108–117

Perl DP (2010) Neuropathology of Alzheimer's disease. Mt Sinai J Med 77:32–42

Peterson DW, Zhou H, Dahlquist FW, Lew J (2008) A soluble oligomer of tau associated with fiber formation analyzed by NMR. Biochemistry 47:7393–7404

Petkova AT, Leapman RD, Guo Z, Yau WM, Mattson MP, Tycko R (2005) Self-propagating, molecular-level polymorphism in Alzheimer's β-amyloid fibrils. Science 307:262–265

Petkova AT, Yau WM, Tycko R (2006) Experimental constraints on quaternary structure in Alzheimer's β-amyloid fibrils. Biochemistry 45:498–512

Pfister EL, Zamore PD (2009) Huntington's disease: silencing a brutal killer. Exp Neurol 220:226–229

Piening N, Weber P, Hogen T, Beekes M, Kretzschmar H, Giese A (2006) Photo-induced cross-linking of prion protein oligomers and prions. Amyloid 13:67–77

Pike CJ, Walencewicz AJ, Glabe CG, Cotman CW (1991) *In vitro* aging of β-amyloid protein causes peptide aggregation and neurotoxicity. Brain Res 563:311–314

Pittock SJ, Lucchinetti CF (2007) The pathology of MS: new insights and potential clinical applications. Neurologist 13:45–56

Podlisny MB, Ostaszewski BL, Squazzo SL, Koo EH, Rydell RE, Teplow DB, Selkoe DJ (1995) Aggregation of secreted amyloid β-protein into sodium dodecyl sulfate-stable oligomers in cell culture. J Biol Chem 270:9564–9570

Polverino de Laureto P, Taddei N, Frare E, Capanni C, Costantini S, Zurdo J, Chiti F, Dobson CM, Fontana A (2003) Protein aggregation and amyloid fibril formation by an SH3 domain probed by limited proteolysis. J Mol Biol 334:129–141

Porat Y, Kolusheva S, Jelinek R, Gazit E (2003) The human islet amyloid polypeptide forms transient membrane-active prefibrillar assemblies. Biochemistry 42:10971–10977

Postuma RB, Montplaisir J (2009) Predicting Parkinson's disease—why, when, and how? Parkinsonism Relat Disord 15(Suppl 3):S105–S109

Qin W, Chachich M, Lane M, Roth G, Bryant M, de Cabo R, Ottinger MA, Mattison J, Ingram D, Gandy S, Pasinetti GM (2006) Calorie restriction attenuates Alzheimer's disease type brain amyloidosis in Squirrel monkeys (*Saimiri sciureus*). J Alzheimers Dis 10:417–422

Querfurth HW, LaFerla FM (2010) Alzheimer's disease. N Engl J Med 362:329–344

Quist A, Doudevski I, Lin H, Azimova R, Ng D, Frangione B, Kagan B, Ghiso J, Lal R (2005) Amyloid ion channels: a common structural link for protein-misfolding disease. Proc Natl Acad Sci USA 102:10427–10432

Radovan D, Smirnovas V, Winter R (2008) Effect of pressure on islet amyloid polypeptide aggregation: revealing the polymorphic nature of the fibrillation process. Biochemistry 47:6352–6360

Rahimi F, Bitan G (2010) Selection of aptamers for amyloid β-protein, the causative agent of Alzheimer's disease. J Vis Exp 39:1955. doi:10.3791/1955

Rahimi F, Shanmugam A, Bitan G (2008) Structure–function relationships of pre-fibrillar protein assemblies in Alzheimer's disease and related disorders. Curr Alzheimer Res 5:319–341

Rahimi F, Murakami K, Summers JL, Chen CH, Bitan G (2009) RNA aptamers generated against oligomeric Aβ40 recognize common amyloid aptatopes with low specificity but high sensitivity. PLoS One 4:e7694

Rangachari V, Reed DK, Moore BD, Rosenberry TL (2006) Secondary structure and interfacial aggregation of amyloid-β(1–40) on sodium dodecyl sulfate micelles. Biochemistry 45:8639–8648

Rangachari V, Moore BD, Reed DK, Sonoda LK, Bridges AW, Conboy E, Hartigan D, Rosenberry TL (2007) Amyloid-β(1–42) rapidly forms protofibrils and oligomers by distinct pathways in low concentrations of sodium dodecylsulfate. Biochemistry 46:12451–12462

Relini A, Torrassa S, Rolandi R, Gliozzi A, Rosano C, Canale C, Bolognesi M, Plakoutsi G, Bucciantini M, Chiti F, Stefani M (2004) Monitoring the process of HypF fibrillization and liposome permeabilization by protofibrils. J Mol Biol 338:943–957

Rhee SK, Quist AP, Lal R (1998) Amyloid β protein-(1–42) forms calcium-permeable, Zn^{2+}-sensitive channel. J Biol Chem 273:13379–13382

Rocken C, Peters B, Juenemann G, Saeger W, Klein HU, Huth C, Roessner A, Goette A (2002) Atrial amyloidosis: an arrhythmogenic substrate for persistent atrial fibrillation. Circulation 106:2091–2097

Roychaudhuri R, Yang M, Hoshi MM, Teplow DB (2009) Amyloid β-protein assembly and Alzheimer disease. J Biol Chem 284:4749–4753

Rozas JL, Gomez-Sanchez L, Tomas-Zapico C, Lucas JJ, Fernandez-Chacon R (2010) Presynaptic dysfunction in Huntington's disease. Biochem Soc Trans 38:488–492

Ryan TM, Howlett GJ, Bailey MF (2008) Fluorescence detection of a lipid-induced tetrameric intermediate in amyloid fibril formation by apolipoprotein C-II. J Biol Chem 283:35118–35128

Sager JJ, Bai Q, Burton EA (2010) Transgenic zebrafish models of neurodegenerative diseases. Brain Struct Funct 214:285–302

Sahara N, Maeda S, Takashima A (2008) Tau oligomerization: a role for tau aggregation intermediates linked to neurodegeneration. Curr Alzheimer Res 5:591–598

Saiki M, Honda S, Kawasaki K, Zhou D, Kaito A, Konakahara T, Morii H (2005) Higher-order molecular packing in amyloid-like fibrils constructed with linear arrangements of hydrophobic and hydrogen-bonding side-chains. J Mol Biol 348:983–998

Sakono M, Zako T (2010) Amyloid oligomers: formation and toxicity of Aβ oligomers. FEBS J 277:1348–1358

Sanchorawala V (2006) Light-chain (AL) amyloidosis: diagnosis and treatment. Clin J Am Soc Nephrol 1:1331–1341

Sanderson KL, Butler L, Ingram VM (1997) Aggregates of a β-amyloid peptide are required to induce calcium currents in neuron-like human teratocarcinoma cells: relation to Alzheimer's disease. Brain Res 744:7–14

Sassone J, Colciago C, Cislaghi G, Silani V, Ciammola A (2009) Huntington's disease: the current state of research with peripheral tissues. Exp Neurol 219:385–397

Sawaya MR, Sambashivan S, Nelson R, Ivanova MI, Sievers SA, Apostol MI, Thompson MJ, Balbirnie M, Wiltzius JJ, McFarlane HT, Madsen AO, Riekel C, Eisenberg D (2007) Atomic structures of amyloid cross-β spines reveal varied steric zippers. Nature 447:453–457

Scherzinger E, Lurz R, Turmaine M, Mangiarini L, Hollenbach B, Hasenbank R, Bates GP, Davies SW, Lehrach H, Wanker EE (1997) Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates *in vitro* and *in vivo*. Cell 90:549–558

Scheuner D, Kaufman RJ (2008) The unfolded protein response: a pathway that links insulin demand with β-cell failure and diabetes. Endocr Rev 29:317–333

Schiesling C, Kieper N, Seidel K, Kruger R (2008) Review: familial Parkinson's disease—genetics, clinical phenotype and neuropathology in relation to the common sporadic form of the disease. Neuropathol Appl Neurobiol 34:255–271

Schleiden MJ (1838) Beiträge zur phytogenesis. Arch Anat Physiol Wiss Med 13:137–176

Scott DA, Tabarean I, Tang Y, Cartier A, Masliah E, Roy S (2010) A pathologic cascade leading to synaptic dysfunction in α-synuclein-induced neurodegeneration. J Neurosci 30:8083–8095

Serpell LC (2000) Alzheimer's amyloid fibrils: structure and assembly. Biochim Biophys Acta 1502:16–30

Serpell LC, Sunde M, Benson MD, Tennent GA, Pepys MB, Fraser PE (2000) The protofilament substructure of amyloid fibrils. J Mol Biol 300:1033–1039

Sesti F, Liu S, Cai SQ (2010) Oxidation of potassium channels by ROS: a general mechanism of aging and neurodegeneration? Trends Cell Biol 20:45–51

Shankar GM, Bloodgood BL, Townsend M, Walsh DM, Selkoe DJ, Sabatini BL (2007) Natural oligomers of the Alzheimer amyloid-β protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. J Neurosci 27:2866–2875

Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ (2008) Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med 14:837–842

Sharma S, Mukherjee M, Kedage V, Muttigi MS, Rao A, Rao S (2009) Sporadic Creutzfeldt-Jakob disease—a review. Int J Neurosci 119:1981–1994

Sharon R, Bar-Joseph I, Frosch MP, Walsh DM, Hamilton JA, Selkoe DJ (2003) The formation of highly soluble oligomers of α-synuclein is regulated by fatty acids and enhanced in Parkinson's disease. Neuron 37:583–595

Shepherd CE, Goyette J, Utter V, Rahimi F, Yang Z, Geczy CL, Halliday GM (2006) Inflammatory S100A9 and S100A12 proteins in Alzheimer's disease. Neurobiol Aging 27:1554–1563

Shulman LM (2010) Understanding disability in Parkinson's disease. Mov Disord 25 (Suppl 1):S131–S135

Simoneau S, Rezaei H, Sales N, Kaiser-Schulz G, Lefebvre-Roque M, Vidal C, Fournier JG, Comte J, Wopfner F, Grosclaude J, Schatzl H, Lasmezas CI (2007) *In vitro* and *in vivo* neurotoxicity of prion protein oligomers. PLoS Pathog 3:e125

Sipe JD, Cohen AS (2000) Review: history of the amyloid fibril. J Struct Biol 130:88–98

Sipe JD, Benson MD, Buxbaum JN, Ikeda S, Merlini G, Saraiva MJ, Westermark P (2010) Amyloid fibril protein nomenclature: 2010 recommendations from the nomenclature committee of the International Society of Amyloidosis. Amyloid 17:101–104

Skovronsky DM, Lee VM, Trojanowski JQ (2006) Neurodegenerative diseases: new concepts of pathogenesis and their therapeutic implications. Annu Rev Pathol 1:151–170

Sokolov Y, Kozak JA, Kayed R, Chanturiya A, Glabe C, Hall JE (2006) Soluble amyloid oligomers increase bilayer conductance by altering dielectric structure. J Gen Physiol 128:637–647

Sokolova A, Hill MD, Rahimi F, Warden LA, Halliday GM, Shepherd CE (2009) Monocyte chemoattractant protein-1 plays a dominant role in the chronic inflammation observed in Alzheimer's disease. Brain Pathol 19:392–398

Sorgjerd K, Klingstedt T, Lindgren M, Kagedal K, Hammarstrom P (2008) Prefibrillar transthyretin oligomers and cold stored native tetrameric transthyretin are cytotoxic in cell culture. Biochem Biophys Res Commun 377:1072–1078

Steensma DP, Kyle RA (2007) A history of the kidney in plasma cell disorders. Contrib Nephrol 153:5–24

Stefani M, Dobson CM (2003) Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. J Mol Med 81:678–699

Steiner B, Mandelkow EM, Biernat J, Gustke N, Meyer HE, Schmidt B, Mieskes G, Soling HD, Drechsel D, Kirschner MW et al (1990) Phosphorylation of microtubule-associated protein tau: identification of the site for Ca^{2+}-calmodulin dependent kinase and relationship with tau phosphorylation in Alzheimer tangles. EMBO J 9:3539–3544

Sugama S, Takenouchi T, Cho BP, Joh TH, Hashimoto M, Kitani H (2009) Possible roles of microglial cells for neurotoxicity in clinical neurodegenerative diseases and experimental animal models. Inflamm Allergy Drug Targets 8:277–284

Sunde M, Blake C (1997) The structure of amyloid fibrils by electron microscopy and X-ray diffraction. Adv Protein Chem 50:123–159

Sunde M, Serpell LC, Bartlam M, Fraser PE, Pepys MB, Blake CC (1997) Common core structure of amyloid fibrils by synchrotron X-ray diffraction. J Mol Biol 273:729–739

Surguchev A, Surguchov A (2010) Conformational diseases: looking into the eyes. Brain Res Bull 81:12–24

Swift B (2002) Examination of insulin injection sites: an unexpected finding of localized amyloidosis. Diabet Med 19:881–882

Taddei K, Kwok JB, Kril JJ, Halliday GM, Creasey H, Hallupp M, Fisher C, Brooks WS, Chung C, Andrews C, Masters CL, Schofield PR, Martins RN (1998) Two novel presenilin-1 mutations (Ser169Leu and Pro436Gln) associated with very early onset Alzheimer's disease. Neuroreport 9:3335–3339

Takahashi T, Tada K, Mihara H (2009) RNA aptamers selected against amyloid β-peptide (Aβ) inhibit the aggregation of Aβ. Mol Biosyst 5:986–991

Takeda A, Hasegawa T, Matsuzaki-Kobayashi M, Sugeno N, Kikuchi A, Itoyama Y, Furukawa K (2006) Mechanisms of neuronal death in synucleinopathy. J Biomed Biotechnol 2006:19365

Tansey MG, Goldberg MS (2010) Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. Neurobiol Dis 37:510–518

Tartaglia GG, Pawar AP, Campioni S, Dobson CM, Chiti F, Vendruscolo M (2008) Prediction of aggregation-prone regions in structured proteins. J Mol Biol 380:425–436

Taylor TN, Greene JG, Miller GW (2010) Behavioral phenotyping of mouse models of Parkinson's disease. Behav Brain Res 211:1–10

Teplow DB (1998) Structural and kinetic features of amyloid β-protein fibrillogenesis. Amyloid 5:121–142

Tjernberg L, Hosia W, Bark N, Thyberg J, Johansson J (2002) Charge attraction and β propensity are necessary for amyloid fibril formation from tetrapeptides. J Biol Chem 277:43243–43246

Tompa P (2002) Intrinsically unstructured proteins. Trends Biochem Sci 27:527–533

Townsend M, Shankar GM, Mehta T, Walsh DM, Selkoe DJ (2006) Effects of secreted oligomers of amyloid β-protein on hippocampal synaptic plasticity: a potent role for trimers. J Physiol 572:477–492

Trexler AJ, Rhoades E (2009) α-Synuclein binds large unilamellar vesicles as an extended helix. Biochemistry 48:2304–2306

Tsigelny IF, Bar-On P, Sharikov Y, Crews L, Hashimoto M, Miller MA, Keller SH, Platoshyn O, Yuan JX, Masliah E (2007) Dynamics of α-synuclein aggregation and inhibition of pore-like oligomer development by β-synuclein. FEBS J 274:1862–1877

Tsukakoshi K, Harada R, Sode K, Ikebukuro K (2010) Screening of DNA aptamer which binds to α-synuclein. Biotechnol Lett 32:643–648

Tycko R, Sciarretta KL, Orgel JP, Meredith SC (2009) Evidence for novel β-sheet structures in Iowa mutant β-amyloid fibrils. Biochemistry 48:6072–6084

van Ham TJ, Breitling R, Swertz MA, Nollen EA (2009) Neurodegenerative diseases: lessons from genome-wide screens in small model organisms. EMBO Mol Med 1:360–370

Van Nostrand WE, Melchor JP, Cho HS, Greenberg SM, Rebeck GW (2001) Pathogenic effects of D23N Iowa mutant amyloid β-protein. J Biol Chem 276:32860–32866

van Rooijen BD, Claessens MM, Subramaniam V (2010) Membrane interactions of oligomeric α-synuclein: potential role in Parkinson's disease. Curr Protein Pept Sci 11:334–342

Varkey J, Isas JM, Mizuno N, Jensen MB, Bhatia VK, Jao CC, Petrlova J, Voss JC, Stamou DG, Steven AC, Langen R (2010) Membrane curvature induction and tubulation are common features of synucleins and apolipoproteins. J Biol Chem 285:32486–32493

Vasilevko V, Head E (2009) Immunotherapy in a natural model of Aβ pathogenesis: the aging beagle. CNS Neurol Disord Drug Targets 8:98–113

Vasilevko V, Pop V, Kim HJ, Saing T, Glabe CC, Milton S, Barrett EG, Cotman CW, Cribbs DH, Head E (2010) Linear and conformation specific antibodies in aged beagles after prolonged vaccination with aggregated Aβ. Neurobiol Dis 39:301–310

Virchow R (1854a) Über den Gang der amyloiden degeneration. Archiv für pathologische Anatomie und Physiologie und für klinische Medicin 8:364–368

Virchow R (1854b) Ueber eine im Gehirn und Rückenmark des Menschen aufgefundene Substanz mit der chemsichen Reaction der Cellulose. Virchows Arch 6:135–138

Volles MJ, Lansbury PT Jr (2002) Vesicle permeabilization by protofibrillar α-synuclein is sensitive to Parkinson's disease-linked mutations and occurs by a pore-like mechanism. Biochemistry 41:4595–4602

Volles MJ, Lee SJ, Rochet JC, Shtilerman MD, Ding TT, Kessler JC, Lansbury PT Jr (2001) Vesicle permeabilization by protofibrillar α-synuclein: implications for the pathogenesis and treatment of Parkinson's disease. Biochemistry 40:7812–7819

von Bergen M, Barghorn S, Li L, Marx A, Biernat J, Mandelkow EM, Mandelkow E (2001) Mutations of tau protein in frontotemporal dementia promote aggregation of paired helical filaments by enhancing local β-structure. J Biol Chem 276:48165–48174

Walker LC (1997) Animal models of cerebral β-amyloid angiopathy. Brain Res Brain Res Rev 25:70–84

Walker LC, Levine H 3rd, Mattson MP, Jucker M (2006) Inducible proteopathies. Trends Neurosci 29:438–443

Walsh DM, Lomakin A, Benedek GB, Condron MM, Teplow DB (1997) Amyloid β-protein fibrillogenesis. Detection of a protofibrillar intermediate. J Biol Chem 272:22364–22372

Walsh DM, Hartley DM, Kusumoto Y, Fezoui Y, Condron MM, Lomakin A, Benedek GB, Selkoe DJ, Teplow DB (1999) Amyloid β-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. J Biol Chem 274:25945–25952

Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ (2002) Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation *in vivo*. Nature 416:535–539

Wang LH, Qin ZH (2006) Animal models of Huntington's disease: implications in uncovering pathogenic mechanisms and developing therapies. Acta Pharmacol Sin 27:1287–1302

Wang C, Huang L, Wang L, Hong Y, Sha Y (2007) One-dimensional self-assembly of a rational designed β-structure peptide. Biopolymers 86:23–31

Warrick JM, Chan HY, Gray-Board GL, Chai Y, Paulson HL, Bonini NM (1999) Suppression of polyglutamine-mediated neurodegeneration in *Drosophila* by the molecular chaperone HSP70. Nat Genet 23:425–428

Westermark P, Wilander E (1978) The influence of amyloid deposits on the islet volume in maturity onset diabetes mellitus. Diabetologia 15:417–421

Westermark P, Benson MD, Buxbaum JN, Cohen AS, Frangione B, Ikeda S, Masters CL, Merlini G, Saraiva MJ, Sipe JD (2007) A primer of amyloid nomenclature. Amyloid 14:179–183

Wetzel R (2006) Amyloid fibrils—common threads in the natural history of proteins. Acc Chem Res 39:567

Wetzel R, Shivaprasad S, Williams AD (2007) Plasticity of amyloid fibrils. Biochemistry 46:1–10

White JA, Manelli AM, Holmberg KH, Van Eldik LJ, Ladu MJ (2005) Differential effects of oligomeric and fibrillar amyloid-β1–42 on astrocyte-mediated inflammation. Neurobiol Dis 18:459–465

Woodruff-Pak DS (2008) Animal models of Alzheimer's disease: therapeutic implications. J Alzheimers Dis 15:507–521

Xia W, Zhang J, Kholodenko D, Citron M, Podlisny MB, Teplow DB, Haass C, Seubert P, Koo EH, Selkoe DJ (1997) Enhanced production and oligomerization of the 42-residue amyloid β-protein by Chinese hamster ovary cells stably expressing mutant presenilins. J Biol Chem 272:7977–7982

Yamamoto S, Hasegawa K, Yamaguchi I, Tsutsumi S, Kardos J, Goto Y, Gejyo F, Naiki H (2004) Low concentrations of sodium dodecyl sulfate induce the extension of β2-microglobulin-related amyloid fibrils at a neutral pH. Biochemistry 43:11075–11082

Yamamoto S, Kazama JJ, Narita I, Naiki H, Gejyo F (2009) Recent progress in understanding dialysis-related amyloidosis. Bone 45(Suppl 1):S39–S42

Yang SH, Cheng PH, Banta H, Piotrowska-Nitsche K, Yang JJ, Cheng EC, Snyder B, Larkin K, Liu J, Orkin J, Fang ZH, Smith Y, Bachevalier J, Zola SM, Li SH, Li XJ, Chan AW (2008) Towards a transgenic model of Huntington's disease in a non-human primate. Nature 453:921–924

Ylera F, Lurz R, Erdmann VA, Furste JP (2002) Selection of RNA aptamers to the Alzheimer's disease amyloid peptide. Biochem Biophys Res Commun 290:1583–1588

Yoshiike Y, Akagi T, Takashima A (2007) Surface structure of amyloid-β fibrils contributes to cytotoxicity. Biochemistry 46:9805–9812

Zakharov SD, Hulleman JD, Dutseva EA, Antonenko YN, Rochet JC, Cramer WA (2007) Helical α-synuclein forms highly conductive ion channels. Biochemistry 46:14369–14379

Zanuy D, Nussinov R (2003) The sequence dependence of fiber organization. A comparative molecular dynamics study of the islet amyloid polypeptide segments 22–27 and 22–29. J Mol Biol 329:565–584

Zhang Q, Powers ET, Nieva J, Huff ME, Dendle MA, Bieschke J, Glabe CG, Eschenmoser A, Wentworth P Jr, Lerner RA, Kelly JW (2004) Metabolite-initiated protein misfolding may trigger Alzheimer's disease. Proc Natl Acad Sci USA 101:4752–4757

Zurdo J, Guijarro JI, Dobson CM (2001a) Preparation and characterization of purified amyloid fibrils. J Am Chem Soc 123:8141–8142

Zurdo J, Guijarro JI, Jimenez JL, Saibil HR, Dobson CM (2001b) Dependence on solution conditions of aggregation and amyloid formation by an SH3 domain. J Mol Biol 311:325–340