J Mol Med (2013) 91:653–664
DOI 10.1007/s00109-013-1025-1

JMolMed

REVIEW

The role of amyloidogenic protein oligomerization in neurodegenerative disease

Gregor P. Lotz · Justin Legleiter

Received: 15 January 2013 / Revised: 20 February 2013 / Accepted: 12 March 2013 / Published online: 27 March 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract A common pathological hallmark in many neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and Huntington's disease, is the formation of fibrillar protein aggregates referred to as amyloids. The amyloidogenic aggregates were long thought to be toxic, but mounting evidence supports the notion that a variety of amyloid aggregate intermediates to fibril formation, termed oligomers, may in fact be the primary culprit leading to neuronal dysfunction and cell death. While amyloid formation is a complex, heterogeneous process, aggregates formed by diverse, diseases-related proteins share many conformational similarities, suggesting common toxic mechanisms among these diseases. Ideally, similar therapeutic strategies may be applicable. This review focuses on the potential role of amyloidogenic oligomers in neurodegenerative disease, highlighting some promising therapeutic strategies.

Keywords Amyloidogenic oligomers · Neurodegenerative disease · Alzheimer's disease · Parkinson's disease · Huntington's disease · Therapeutic strategies

Introduction

Most neurodegenerative diseases, including Alzheimer's disease(AD), Parkinson's diseases (PD), Huntington's disease (HD), amyloidoses, α1-antitrypsin deficiency, and the prion encephalopathies, are associated with extracellular or intracellular proteinaceous aggregates [1, 2]. These aggregates are predominately comprised of extended, β-sheet-rich fibril structures that share several biochemical/biophysical properties, commonly referred to as amyloids. Many factors, including mutations, increased concentration, environmental stresses, or aging, can trigger amyloid protein aggregation [3]. While there is no apparent correlation between amyloid-forming proteins in size or primary amino acid sequence, the common structural motif associated with protein deposits of each disease may indicate a conserved mechanism of pathogenesis associated with these phenotypically diverse diseases. While sometimes a specific mutation or dysfunctional process can be attributed to amyloid formation, the character and location of protein aggregates in vivo highly depends on the amyloids aberrant interaction with the proteome network.

Amyloid formation occurs via a variety of complex aggregation pathways. Misfolded or partially abnormal protein conformations initially form, leading to aberrant interactions and a heterogeneous mixture of intermediate aggregate structures such as dimers, a variety of oligomers, and protofibrils (Fig. 1) [4–6]. Protofibrils show amyloid-like structures with elongated, filament like morphologies and are late-stage intermediate precursors on the pathway to fibrillar amyloid formation. Using high resolution microscopic techniques, oligomers have been detected in multimeric states mostly with globular, spherical morphologies [7]. Oligomers vary in size, ranging from dimers and trimers to much larger aggregates [8–10]. A variety of aberrant structures and conformations are also observed that are off-pathway to amyloid formation, including annular aggregates of various size [11, 12].

While protein aggregation is a common feature of these diseases, the exact role of the aggregates and their relative importance is not fully elucidated. A common objection to the importance of protein deposition in these diseases is that often the number of plaques or inclusions does not correlate well with symptoms. There is evidence that fibrillar aggregates

G. P. Lotz (✉)
Novartis Institutes for Biomedical Research Novartis Pharma AG, Basel, CH 4002, Switzerland
e-mail: glotz54@gmail.com

J. Legleiter (✉)
The C. Eugene Bennett Department of Chemistry, Center for Neuroscience, Robert C. Byrd Health Sciences Center, West Virginia University, 217 Clark Hall, Morgantown, WV 26506, USA
e-mail: Justin.Legleiter@mail.wvu.edu

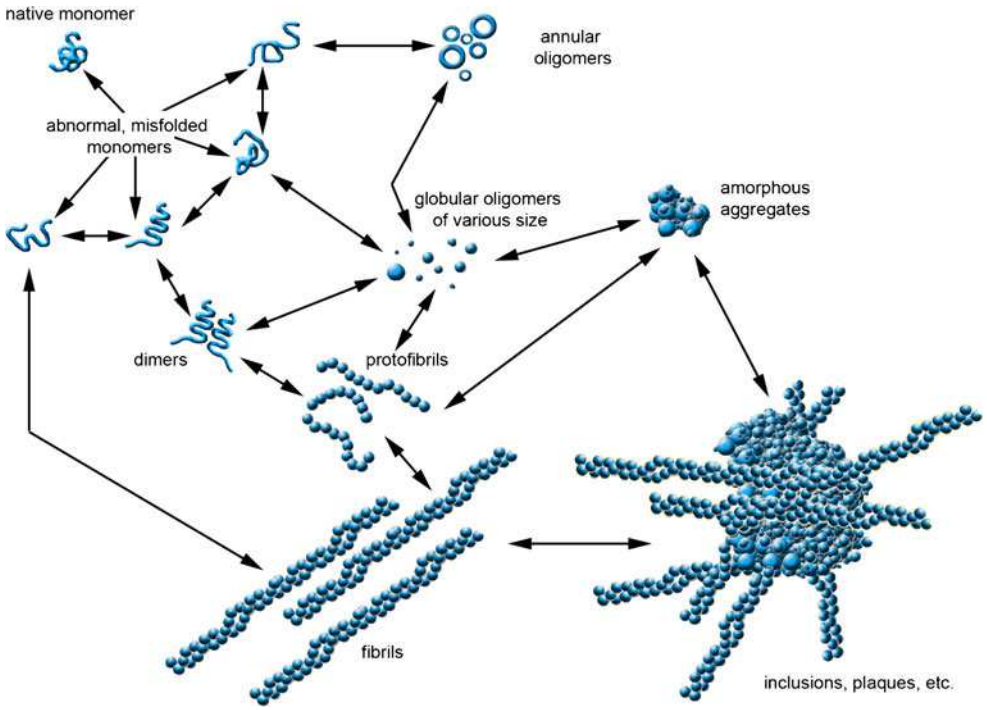

Fig. 1 Generic aggregation scheme for amyloid-forming proteins. Fibrillization can proceed via several potential pathways that can populate various intermediate aggregate states, including oligomers and protofibrils. These pathways are not necessarily mutually exclusive. Off-pathway aggregates, annular aggregates for example, may also form. These aggregates eventually accumulate to form the hallmark amyloid plaques or inclusions associated with each disease. For any given amyloid-forming protein, the aggregation process can vary considerably depending on protein properties, modifications, or environment

associated with neurodegenerative disease are toxic [13–18]; however, increasing evidence suggests that, rather than being pathogenic, fibrillar aggregates may be inert or potentially protective. For example, older individuals without clinical symptoms of AD often have amyloid aggregates present in the brain [19], and correlation between disease severity and Aβ neuritic plaque density is poor [20, 21]. However, the concentration of soluble forms of Aβ correlates much better with cognitive impairment [22–24]. In PD, neuropathological analyses suggest that Lewy body (inclusions containing mainly the protein α-synuclein) formation preferentially occurs in healthier neurons in comparison with adjacent cells that lack inclusions [25]. In HD, the relationship between amyloid-like inclusions and pathology is controversial because of several observations. Massive cellular degeneration is observed in the striatum along with the appearance of intracellular aggregates comprised of mutant huntingtin (mHtt) containing an expanded polyglutamine (polyQ) domain; however, the cerebral cortex displays moderate degeneration with typically a much larger number of aggregates [26]. Even within the striatum, medium spiny neurons are selectively lost in HD compared to large interneurons, but the interneurons typically have a larger aggregate load [27]. In cell culture models of mHtt toxicity, there is poor correlation between inclusion body formation and toxicity [28]. In a classic study using live cell imaging to track the fate of individual neurons in culture, cells expressing similar levels of mHtt were more likely to survive if inclusion bodies formed compared with cells in which mHtt remained diffuse [29]. While correlation between the inclusion body formation and cell survival suggests a protective role for sequestering mHtt species into larger aggregates, the exact nature of toxic species within diffuse mHtt is not fully understood. Without further analysis of the diffuse population of mHtt, the correlation with cell death does not necessarily lead to causation. Clarifying the controversial relationship between amyloid-like deposits with pathology in HD is difficult as most studies use only a small fragment of the entire Htt protein. While the precise fragment of mHtt that accumulate in HD are unknown, a recent study demonstrated that N-terminal fragments similar to exon1 are detected in knock-in mouse models of HD that express full-length Htt [30], and fragments slightly larger than exon1 have been detected in HD patients [31, 32], providing rationale for the use of exon1 as a model system. Collectively, these studies suggest that while some fibrils may possess toxic properties, another aggregate form may play a larger role in disease progression.

Another important aspect for toxic aggregate formation of mHtt are, besides the expanded polyQ domain, the flanking regions directly adjacent to the polyQ domain. Mutations of flanking serines 13 and 16 with either aspartate (phosphomimetic or SD) or alanine (phosphoresistant or SA) in a transgenic HD mice model expressing full-length mHtt, differentially resulted in alterations in pathogenicity [33]. SD mutations abolished selective neurodegeneration in mice with a reduction of inclusion formation, whereas the SA mutations preserved HD-like phenotype in mice and large inclusions were observed. Further, in vitro analysis of aggregation of Htt exon1 fragments containing either the SD or SA mutations demonstrated that the SD mutation retards aggregation while SA mutations accelerate

aggregation [33]. At first glance, this study would seem to be at odds with previous studies claiming a protective role for inclusion body formation; however, the SD mutations appear to promote a polymorphic aggregate form. Such an aggregation pathway may circumvent specific toxic species; therefore, negating the need to sequester diffuse Htt species. Further, in vitro aggregation studies of Htt exon1-like peptides containing phosphoryl-Ser residues at positions 13 and 16 further support reduced aggregation rates and atypical aggregate morphologies associated with these mutations [34].

While understanding of the exact toxic entity associated with protein aggregates in these diseases is still incomplete, concerns must be raised in regard to therapeutic strategies aimed at manipulating the aggregation pathway and/or clearing pre-existing protein deposits. For example, if an oligomeric aggregate is the most relevant toxic species for a particular disease, any strategies that remove fibrils in such a way that the pool of oligomers is augmented could very well be counterproductive. As some studies indicate that large inclusions may play a protective role by sequestering diffuse aggregates, the possibility that promoting the formation of large inclusions, while bypassing the formation of quickly sequestering oligomers, may have therapeutic benefit. In this regard, a compound that promotes inclusion formation in cellular models of HD and PD was shown to reduce cellular pathology, further supporting a potential protective role for inclusion formation [35]. Additional studies are required to further elucidate the complex relationship between protein aggregation and toxicity.

Several studies support the notion that small, diffusible oligomers might be the primary culprits in neuronal dysfunction and cell death. For example, soluble Aβ oligomers microinjected into rat hippocampus inhibit the late phase of long-term potentiation [36, 37]. Distinct toxic oligomers of Aβ have been identified, including ADDLs [38] and Aβ*56, a specific nonfibrillar, dodecameric Aβ assembly [39]. Aβ*56 caused memory deficits in hAPP mice (Tg2576) and when infused into the brains of NTG rats [39]. Furthermore, neurons with detection of low-molecular-weight conformational states of mHtt using specific antibodies strongly correlated with neuronal death [40], suggesting that monomers and possibly small oligomers may represent important toxic species. Interestingly, there is a correlation of mHtt aggregation and disease progression with polyQ length [41, 42], and it has been demonstrated that the formation of mHtt oligomers is polyQ length dependent [43, 44]. Importantly, oligomers of mHtt are detected in a variety of cellular and mouse models [45, 46]. Lentiviral infection of dopaminergic neurons in the substantia nigra of rats with mutated α-synuclein that forms oligomers were associated with significantly greater dopaminergic loss compared with fibril-forming mutants, demonstrating α-synuclein oligomer toxicity in vivo [47]. Further evidence of the "oxic oligomer" hypothesis is provided by the finding that a single monoclonal antibody recognizes a common conformational epitope found in several disease-associated oligomers, including Aβ, α-synuclein and polyQ-containing peptides [48]. Interestingly, pre-incubation of anti-oligomer antibodies with these oligomers blocks toxicity when applied to cultured cells, indicating that oligomeric structures formed by distinct disease proteins might confer toxicity through a similar mechanism [48].

Structural heterogeneity in oligomers and their formation/stability

Much is known concerning the structure of fibrils, as they share a common cross-β structure [49]; however, variability in the packing of intermolecular β-sheets can lead to distinct amyloid fibril morphologies, even for the same protein, giving rise to distinct fibril polymorphs [50]. Fibril polymorphs are observed for Aβ [51], calcitonin [52], amylin [53], insulin [54–56], and lysozyme [57]. Presumably, these polymorphic fibrils will form from distinct aggregate intermediates, adding to the heterogeneity of protein oligomers [58].

Compared to fibrils, the structural variety of oligomers is less well understood. Characterization is made difficult by oligomers typically occurring as metastable states that readily convert into more favorable conformations; therefore, structural analyses of oligomers often requires trapping these metastable intermediates [59]. There are Aβ oligomers comprised predominately of random coil [60], and there are Aβ oligomers that exhibit high β-sheet content [49, 59, 61]. Such findings suggest that oligomerization represent a polymorphic state. Antibodies specific for generic oligomeric epitopes have been developed for at least two distinct types of amyloid oligomers, classified as fibrillar and prefibrillar [58]. Aβ prefibrillar oligomers can further be classified using monoclonal antibodies into several more structural polymorphisms [16]. A factor that contributes to the formation of specific oligomers in vitro is variations in preparation protocols [58, 62]. However, oligomer heterogeneity can occur within the same sample for some proteins and preparations [43, 63]. In the case of expanded polyQ aggregation, associated with diseases like HD, adjacent flanking sequences have profound impact on the ability to form oligomeric aggregates [44, 64, 65]. The ability to form structurally distinct and varied oligomer species is important because different oligomer structures may exhibit different biological effects [58, 66, 67].

Polymorphic protein conformations and aggregates may play a particularly important role in prion diseases. Prion diseases are associated with the induced misfolding of a normal cellular form of the prion protein (PrP^{C}) by the presence of an infectious disease-related form of a prion protein (PrP^{Sc}) [68, 69]. Based on characteristic incubation periods and neuropathological profiles associated with serial

passages of PrP^{Sc} isolates from mice, there are distinct metastable strains of PrP^{Sc} [70]. The basis of this strain effect is likely a feature of the biochemical properties of PrP^{Sc}, which can be related to its ability to form polymorphic aggregate species. Studies on transmissible mink encephalopathy (TME) first indicated the strain phenomenon as a single isolate of TME resulted in two distinct laboratory strains [71], and these distinct phenotypes are related with differences in the protease-resistant core fragment size of PrP [72]. Since these initial observations, correlations between the biochemical properties of PrP^{Sc} and clinical phenotypes of a variety of prion diseases have been reported [73–75]. Several other amyloid-forming proteins may have prion-like infectious properties, including Aβ [76–78], tau [79], and α-synuclein [80, 81], and such a phenomenon appears to play a critical role in cell to cell translation of the disease state [82–85]. Several small molecules and cocktails of these molecules have been identified that appear to reverse the formation of polymorphic aggregates associated with prion-like capabilities [86, 87], providing a potential strategy to interfere with cell to cell transmission of disease.

Toxic mechanisms

Amyloid-associated diseases appear to predominantly result from toxic gains of function [88, 89]. Several hypotheses are under intensive investigation (Fig. 2). One prominent hypothesis is that amyloidogenic oligomeric aggregates contain exposed hydrophobic sites that facilitate aberrant interactions and sequestration of other proteins, impairing their function [1, 90]. Another hypothesis is that amyloidogenic oligomers exasperate general protein folding defects via direct interference with central protein quality control and clearance mechanisms [91–93]. A third hypothesis states that amyloidogenic oligomers compromise the integrity of lipid membranes [11]. While evidence supports all of these hypotheses, as will be discussed below, these mechanisms are not mutually exclusive and may act synchronistically.

Sequestration and functional impairment of other cellular key proteins

Many cell regulatory proteins co-localize in pathological protein aggregates, suggesting that aggregation of disease proteins sequesters other important housekeeping proteins, disabling their key functions including transcription, translation, trafficking, redox-homeostasis, and cytoskeletal organization [1, 90]. For example, mutated polyQ oligomers aberrantly interact with other functional proteins with polyQ-rich domains, including the transcriptions-factors CBP, TBP, and Sp1, impairing their function [94, 95]. While this may explain the complexity associated with polyQ-mediated toxicity, it does not explain the aggregation and/or sequestration of other key cellular or disease proteins lacking these domains. A possible hint comes from α-synuclein which seems to preferentially exist in a metastable native conformation for functional flexibility; however, mutations or increased levels of α-synuclein lead to pathogenic self aggregation [96–100]. Interestingly, α-synuclein is also sequestered and colocalized with Aβ, tau, and Htt aggregates, suggesting that α-synuclein is particularly vulnerable to co-aggregation due to its metastable structure [101–103]. This hypothesis is supported by a recent quantitative proteomic study that analyzed the interactome of amyloidogenic oligomers in human cells, demonstrating that endogenous, metastable proteins are particularly vulnerable to sequestration by amyloidogenic oligomers [104]. Most of these proteins are large in size, unstructured, and show diverse functional flexibility to act as relay-proteins in cell regulation and signaling. Although this study used artificial amyloid-structured proteins, a range of prefibrillar aggregation intermediates are populated with potential toxic properties such as ANS-binding hydrophobic

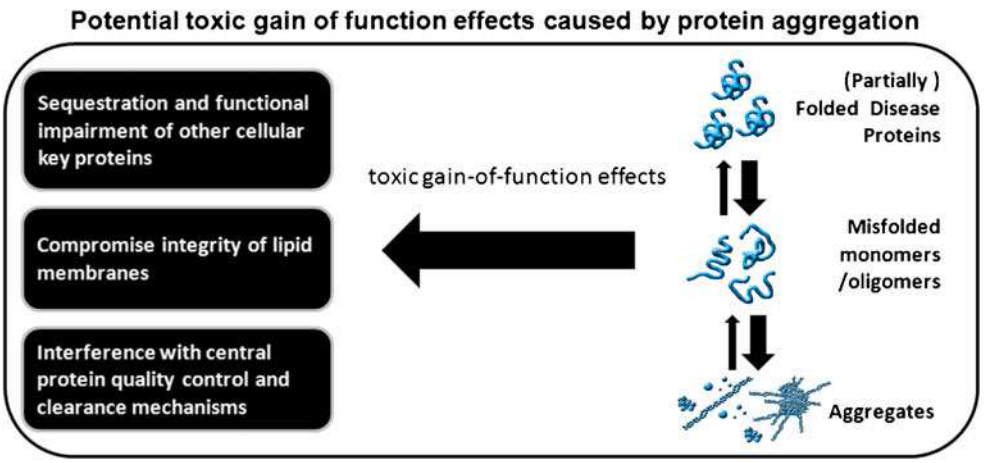

Fig. 2 Potential mechanisms for toxic gain of function associated with protein aggregates

surfaces and A11 anti-oligomer reactivity. These observations potentially explain the multi-factorial and severe toxicity associated with intracellular amyloidogenesis [105]. However, it still remains unclear whether widespread aberrant interactions with metastable proteins are engaged by protofibrils with flexible hydrophobic surfaces, unpaired backbone structures, or by advanced amyloid-structures with stable cross-β cores. It is also an open question whether oligomers formed on a non-amyloidogenic aggregation pathway show similar interaction and sequestration properties.

Interference with central protein quality control and clearance mechanisms

An interesting correlation appears to exist between age-dependent decline in protein quality control systems, the generally observed late-onset of protein-conformational brain diseases, and the sequestration of components of the degradation and protein quality control systems [91–93, 106, 107]. The irreversible co-localization of the proteasome, molecular chaperones and/or other components of protein quality control mechanisms indicate that, beyond simply responding to amyloidogenic protein, these systems become overwhelmed with age [106, 107]. Trapped quality control factors decrease efficient clearance, which is particularly relevant as overall capacity shrinks with aging. Resulting in a vicious cycle, the down-regulation of protein quality systems accelerates the onset of conformational diseases and has an age-dependent effect on protein homeostasis. For example, the expression of expanded polyQ protein results in the loss of function of a diverse set of metastable proteins [93]. Interestingly, these metastable proteins are not colocalized with the polyQ proteins suggesting that, rather than a direct interaction, misfolded polyQ proteins impact these proteins indirectly by impairing factors that stabilize protein homeostasis. Although this observation is in contrast to the direct toxic sequestration hypothesis, both gain of toxic function are not mutually exclusive and may act in parallel.

Evidence that multiple factors can cause toxicity is demonstrated in studies with molecular chaperones an important protein family to stabilize protein homeostasis. Oligomers can directly interact with molecular chaperones, impairs their function, and become sequestered into aggregates [108, 109], suggesting a direct toxic sequestration-based mechanism. However, decreased chaperone levels and reduced expression activity are observed in patients with neurodegenerative diseases due to HSF1 dysregulation, the major transcription factor for stress-inducible molecular chaperones [110, 111]. These studies indicate a transcriptional impairment of cellular quality control by misfolded proteins rather than an overloading/sequestration-based mechanism.

Additional cellular systems that preserve protein homeostasis are the autophagy and the ubiquitin-proteosome system (UPS), two important protein degradation systems [91]. Misfolded mHtt impairs cellular ubiquitin-proteosome activity in mouse models of HD prior to inclusion body formation [112], raising the possibility that amyloidogenic Htt oligomers in the cytosol interfere with ubiquitin-proteosome activity before localizing into inclusions. Failure in cargo recognition might be responsible for inefficient macroautophagy in HD cell and animal models [113].

Compromised integrity of lipid membranes

Based on experiments with model membranes, growing evidence supports the notion that absorption of amyloids modifies membrane properties and lipid order, affecting the activities of specific membrane proteins and possibly disrupting membrane integrity [5, 11, 114–116]. Oligomeric assemblies of several amyloid-forming proteins interact with phospholipid bilayers, modifying membrane structure and ion permeability [117]. While the underlying mechanism remains unclear, it appears that anionic lipid membranes can preferentially induce amyloid formation and toxic aggregation [117–119]. This is supported from observations in Alzheimer's disease patients where high level of anionic phospholipids in the neuronal membranes are detected [120]. Another membrane associated hypothesis concerning a potential toxic mechanism is that amyloid-forming proteins form unregulated, membrane-spanning pores or channels. A sub-population of early, ring-shaped protein oligomers that penetrate the cell membrane, resulting in non-specific pores, has been observed to form from a variety of amyloid-forming proteins [5, 11, 121]. For example, in vitro aggregation studies performed with Aβ, α-synuclein, ABri, ADan, serum amyloid A, and amylin demonstrated the formation of ion-channel-like structures that elicit single ion-channel currents [122]. However, the in vivo detection of such structures remains to be demonstrated, and the cellular defects associated with protein aggregation are so complex that it is difficult to attribute them to non-specific membrane dysfunction alone [105].

Targeting oligomers for therapeutic purposes

Modulation of amyloid aggregation has achieved beneficial therapeutic effects in some cellular and preclinical animal models of neurodegenerative diseases, suggesting such approaches may eventually become a medically significant strategy for treatments (Fig. 3). Here, we briefly discuss potential therapeutic strategies targeting the aggregation pathway to inhibit formation of toxic oligomeric aggregates

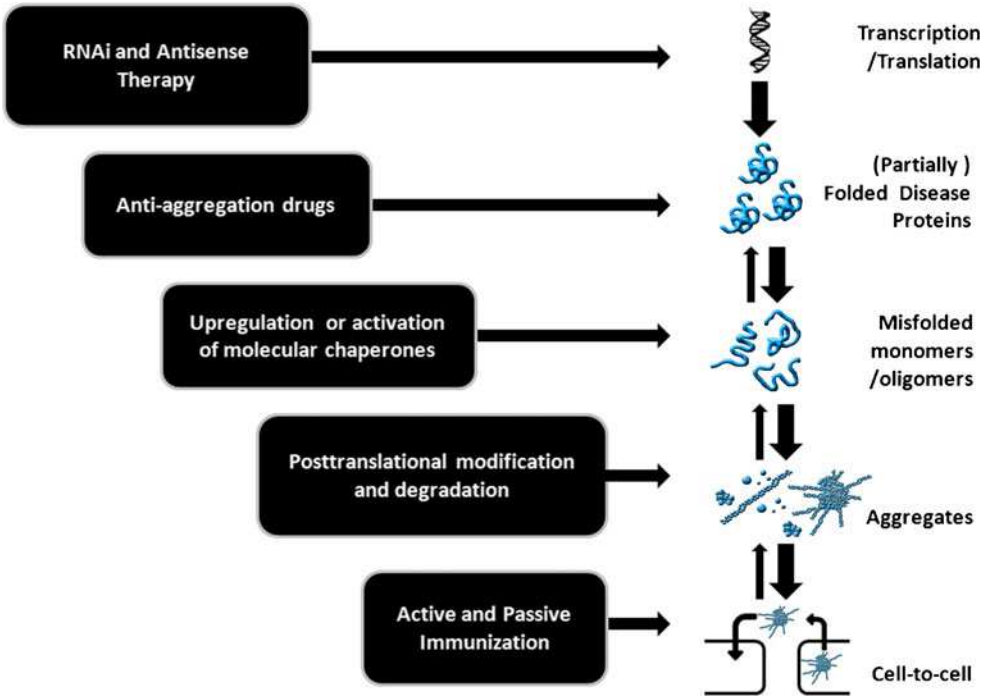

Fig. 3 Potential therapeutic strategies with specific targets indicated based on aggregation state

Lowering disease protein level by RNAi and antisense therapy

The manifestation of polyQ diseases has been reversed by blocking the mutant protein expression in conditional mice models [123, 124], suggesting that the reduction of the causative amyloidogenic protein level provides a promising aggregation and disease-modifying strategy. Two oligonucleotide approaches based on RNAi and antisense oligonucleotides are under intensive investigation to intervene directly with the production of amyloid-proteins [125]. Challenges to both approaches are the selective and potent suppression of the causative proteins and local delivery of the oligonucleotides into the brain [125]. For example, current oligonucleotide-based approaches in HD models are not selective enough to target the mutant alleles with CAG repeat expansions. On the upside, viral delivery of inhibitory shRNA into striatum of HD transgenic mice models resulted in robust, but not complete suppression, of mutant and wild-type Htt mRNAs and strikingly rescued most aspects of pathology [126]. These are important findings because partially silencing nonallele-specific Htt might be sufficient to avoid side-effects and to improve HD pathology despite significant suppression of wild-type Htt. To optimize delivery of oligonucleotides into the brain, preclinical studies in animal models of HD have confirmed that direct CNS administration in saline or in a viral package is a promising approach to silence the mutant protein and ameliorate neuropathology [125]. Taken together, selective and potent oligonucleotides combined with sufficient delivery into the brain and the development of quantitative assays to evaluate and monitor target activity are key for translational success. Successful proof-of-concept studies in patients would have far reaching implication for the treatment of protein-conformational disease.

Anti-aggregation drugs

Another promising approach to target protein aggregation is the identification of small molecules that inhibit and/or reverse the formation of toxic oligomers. Several compounds that directly affect aggregation have been identified [109, 127–131]. Some stabilize native conformations, others prevent abnormal β-sheet formation (β-sheet breaker) or interact directly with mature fibrillar structure resulting in their destabilization and disassembly. However, most of these compounds have been demonstrated to be ineffective in vivo. This disappointing outcome might be due to lack of quantitative methodologies to detect toxic oligomers in vivo leading to insufficient pharmacodynamic read-out on the right oligomeric target. Another explanation might be that some of the formed aggregates are the wrong targets and are not associated with the disease pathway. A less specific and less selective compound methylene blue modulates aggregation of Aβ and has shown promising results in a Phase II clinical trial for Alzheimer's disease [132]. Arguably, the most promising results for effective small molecules treatment of amyloid aggregation have been obtained for transthyretin familial amyloid polyneuropathy, a rare but

fatal neurodegenerative disorder characterized by progressive sensory, motor and autonomic impairment [130, 133]. Pathogenic mutations in the transthyretin (TTR) protein lead to destabilization of its tetrameric structure and subsequent formation of amyloid aggregates [130]. Tafamidis (Pfizer), a small-molecule inhibitor, binds selectively to TTR and stabilizes the tetrameric structure, reducing pathology and significantly slowing down disease progression in patients. It has already been approved by the European Commission for the treatment of the disease, and it is currently under review by the FDA [133].

Posttranslational modification and clearance

Another interesting therapeutic approach is to target post-translational modification of amyloids needed for degradation. Modified sites by acetylation, phosphorylation, sumoylation, ubiquitation, and palmitoylation are detected in several disease proteins and seem to play an important role in protein aggregation. Therapeutic modulation of these sites may avoid aggregate formation and may increase degradation of toxic proteins. For example, acetylation of mHtt facilitates its trafficking to autophagosomes and improves clearance by macroautophagy [134]. The CREB binding protein (CBP), a histone acetyl-transferase, increases mHtt acetylation [135], whereas HDAC1, a histone deacetylase, strongly decreases its acetylation. Therefore, targeting acetylation may be an interesting mechanism that promotes degradation of mHtt by autophagy. Similar approaches might be interesting for other diseases. In AD, the abnormal acetylation and phosphorylation of tau protein accelerates its aggregation [135–137] leading to loss of its function and gain of toxic functions. Interfering with these posttranslational modifications might ameliorate disease progression.

Upregulation and activation of molecular chaperones

Most amyloidogenic aggregates also sequester molecular chaperones, a protein family that assist in folding, re-folding, stabilization and processing of client proteins including misfolded proteins in neurodegenerative diseases. Molecular chaperone modulation has achieved remarkable therapeutic effects in some cellular and preclinical animal models of protein-conformational diseases [109], raising hope for chaperone-based therapeutic strategies. Today, the best characterized drugs inhibit Hsp90/HSPD function and target its ATP-binding domain. Blocking ATP hydrolysis causes degradation of client proteins but also induces the cell-protective cellular stress response, which includes general chaperone expression [131, 138]. Both strategies are being considered for treating neurodegenerative diseases. For example, HSPD inhibition protects against α-synuclein and other amyloidogenic protein toxicity [139, 140] via stress response and increased levels of other chaperones including Hsp70 and Hsp40 members (HSPA8 and DNAJB1) [138, 140]. Also, mHtt protein is a Hsp90 client, and ATP-site inhibitors disrupt the HSPD-mHtt interaction, inducing mHtt clearance via the UPS [141]. However, drugs that target chaperone core sites, such as the ATP-binding pocket, could cause undesirable side-effects, narrow the therapeutic window, and limit translation into the clinic. Thus, other interesting chaperone targets are worthy of consideration. The chaperonins, small Hsps, Hsp70, and Hsp40 members are all effective in inhibiting protein aggregation and ameliorate pathology [108, 142, 143]. For example, the Hsp40 members, DNAJB1 and DNAJB6, inhibit the aggregation and toxicity of mHtt [108, 144]. DNAJA1 overexpression facilitates the degradation of Tau, and DNAJB1 inhibits Tau aggregation [145–147]. As a result, specifically targeting Hsp40s appears to be a promising strategy to intervene therapeutically in protein-conformational disorders.

Active and passive immunization

Active and passive immunization strategies have been successfully used to prevent amyloid aggregation in several neurodegenerative disease models, including AD, PD and prion diseases [123]. For example, AD transgenic mice were actively immunized against Aβ(1–42) by administration of synthetic peptides [124]. These mice did not develop Aβ aggregates and showed reduced pathology [124–126]. Passive immunization has also been successfully applied by injection of antibodies against Aβ leading to reduced aggregate load and pathology [127]. This promising result suggests that applied antibodies can cross the blood/brain barrier and bind to amyloids. Thus, both therapeutic strategies, passive and active immunization, have entered clinical trials to remove extracellular cerebral Aβ amyloid in patients with AD [128, 129]. Although 6 % of the patients in these trials developed severe cerebral inflammation, a significant reduction in amyloid burden was detected and a decline on cognitive malfunction on follow up studies of 30 patients observed. Although these results give hope that immunotherapy is a useful strategy to treat amyloidogenic diseases, more investigation is necessary as recent clinical trials using Aβ-targeting monoclonal antibodies (mAbs) have revealed (bapineuzumab—Wyeth and Elan; solanezumab—Eli Lilly; gantenerumab—Roche, etc.). For instance, the application of bapineuzumab initially showed a positive response in apoE non-carriers, but subsequent trials in apoE4 carriers were halted due to lack of effect [148, 149]. Passive immunization trials with solanezumab have been promising (LY2062430; Ely Lilly) (www.clinicaltrials.gov). Although solanezumab also failed to meet its primary and functional end points, pooled data from phase III clinical trials demonstrated a small,

statistically significant decrease in cognitive decline (Galimberti et al. 2013). As trials for both of these drugs were performed with patients in late stages of AD, it may have been too late to effectively intervene in the disease via passive immunization. However, non-invasive and quantitative imaging methods of aggregates using improved specific PET ligands have been developed, allowing for better and earlier detection of aggregate species [131]. In combination with the development of selective and potent conformation-specific antibodies and optimized brain uptake, e.g. via transcytotic target receptors [130], antibodies administration at premanifest disease stages might be the potential therapeutic strategy to trigger pharmacodynamic Parkinson's disease changes on amyloidogenic protein aggregates that translates into meaningful effects on protein fate and downstream neurophysiological and clinical effects. Notably, an anti-Aβ antibody was also shown to alter CNS levels of Aβ when administered peripherally without crossing the blood/brain barrier, leading the peripheral sink hypothesis that states that circulating antibodies sequester Aβ, resulting in an increased efflux of Aβ from the CNS [132].

Another alternative immunization approach, beside Aβ-targeting antibodies in AD, is the development of antibodies generated against targets involved in the APP processing. Here, the most prominent target is BACE-1 and its inhibition leads to decreased Aβ level and improved clinical symptoms in vivo [150]. A recent study has shown that a coding mutation (A673T) in the APP gene protects against AD [151]. Interestingly, this substitution is adjacent to BACE-1 cleavage site in APP, and results in an approximately 40 % reduction in the formation of amyloidogenic peptides in vitro [151]. The strong protective effect of the A673T substitution against Alzheimer's disease provides proof of principle for the hypothesis that reducing the BACE-1 cleavage of APP and therefore, lowering the level of amyloidogenic Aβ may protect against the disease.

Finally, evidence is growing that several misfolded protein pathologies can spread and cause non-cell autonomous damage [133, 134]. If this hypothesis can be confirmed in patients, active and passive immunization might be an effective therapeutic strategy to halt disease progression and to modify these fatal diseases.

Concluding remarks

Drugs targeting protein aggregation in the CNS have many challenges in common with other CNS drugs, most importantly, getting across the blood/brain barrier, proof of efficacy, safety and tolerability. Neurodegenerative diseases generally show slow progression rates and therefore age-dependent changes in protein quality control mechanisms have to be taken into account when selecting patients for proof-of-concept studies. Exposures of therapeutic antibodies or small molecules in brain should suffice to trigger pharmacodynamic Parkinson's disease changes on the misfolded protein that translate into meaningful effects on its fate, be it stabilization, functional recovery, or clearance, and its downstream neurophysiological and clinical effects.

Acknowledgments JL is supported by the National Science Foundation (Grant 1054211), and the Alzheimer's Association (NIRG-11-203834).

Disclosure The authors declare that they have no conflict of interests.

References

1. Chiti F, Dobson CM (2006) Protein misfolding, functional amyloid, and human disease. Annual Review of Biochemistry 333–366
2. Crowther DC (2002) Familial conformational diseases and dementias. Hum Mutat 20:1–14
3. Ross CA, Margolis RL (2005) Neurogenetics: insights into degenerative diseases and approaches to schizophrenia. Clin Neurosci Res 5:3–14
4. Platt GW, Radford SE (2009) Glimpses of the molecular mechanisms of beta(2)-microglobulin fibril formation in vitro: aggregation on a complex energy landscape. FEBS Lett 583:2623–2629
5. 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
6. Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat Rev Mol Cell Biol 8:101–112
7. Kowalewski T, Holtzman DM (1999) In situ atomic force microscopy study of Alzheimer's β-amyloid peptide on different substrates: new insights into mechanism of β-sheet formation. Proc Natl Acad Sci U S A 96:3688–3693
8. Fandrich M (2012) Oligomeric intermediates in amyloid formation: structure determination and mechanisms of toxicity. J Mol Biol 421:427–440
9. Broersen K, Rousseau F, Schymkowitz J (2010) The culprit behind amyloid beta peptide related neurotoxicity in Alzheimer's disease: oligomer size or conformation? Alzheimers Res Ther 2:12
10. Sakono M, Zako T (2010) Amyloid oligomers: formation and toxicity of A beta oligomers. FEBS J 277:1348–1358
11. Lashuel HA, Lansbury PT (2006) Are amyloid diseases caused by protein aggregates that mimic bacterial pore-forming toxins? Q Rev Biophys 39:167–201
12. 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
13. Lorenzo A, Yankner BA (1994) Beta-amyloid neurotoxicity requires fibril formation and is inhibited by Congo Red. Proc Natl Acad Sci U S A 91:12243–12247
14. Pike CJ, Burdick D, Walencewicz AJ, Glabe CG, Cotman CW (1993) Neurodegeneration induced by beta-amyloid peptides in vitro—the role of peptide assembly state. J Neurosci 13:1676–1687
15. Pike CJ, Walencewicz AJ, Glabe CG, Cotman CW (1991) In vitro aging of beta-amyloid protein causes peptide aggregation and neurotoxicity. Brain Res 563:311–314

16. Kayed R, Canto I, Breydo L, Rasool S, Lukacsovich T, Wu J, Albay R, Pensalfini A, Yeung S, Head E et al (2010) Conformation dependent monoclonal antibodies distinguish different replicating strains or conformers of prefibrillar Aβ oligomers. Mol Neurodegner 5:57
17. 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
18. 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
19. Katzman R, Terry R, DeTeresa R, Brown T, Davies P, Fuld P, Renbing X, Peck A (1988) Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous neocortical plaques. Ann Neurol 23:138–144
20. Terry RD, Peck A, DeTeresa R, Schechter R, Horoupian DS (1981) Some morphometric aspects of the brain in senile dementia of the Alzheimer type. Ann Neurol 10:184–192
21. Braak H, Braak E (1991) Morphological changes in the human cerebral cortex in dementia. J Hirnforsch 32:277–282
22. Lue LF, Kuo YM, Roher AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE, Rogers J (1999) Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am J Pathol 155:853–862
23. McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, Bush AI, Masters CL (1999) Soluble pool of Aβ amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. Ann Neurol 46:860–866
24. Naslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P, Buxbaum JD (2000) Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. JAMA-J Am Med Assoc 283:1571–1577
25. Tompkins MM, Hill WD (1997) Contribution of somal Lewy bodies to neuronal death. Brain Res 775:24–29
26. Gutekunst CA, Li SH, Yi H, Mulroy JS, Kuemmerle S, Jones R, Rye D, Ferrante RJ, Hersch SM, Li XJ (1999) Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. J Neurosci 19:2522–2534
27. Kuemmerle S, Gutekunst CA, Klein AM, Li XJ, Li SH, Beal MF, Hersch SM, Ferrante RJ (1999) Huntingtin aggregates may not predict neuronal death in Huntington's disease. Ann Neurol 46:842–849
28. Saudou F, Finkbeiner S, Devys D, Greenberg ME (1998) Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. Cell 95:55–66
29. Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S (2004) Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature 431:805–810
30. Landles C, Sathasivam K, Weiss A, Woodman B, Moffitt H, Finkbeiner S, Sun B, Gafni J, Ellerby LM, Trottier Y, Richards WG, Osmand A, Paganetti P, Bates GP (2010) Proteolysis of mutant huntingtin produces an exon 1 fragment that accumulates as an aggregated protein in neuronal nuclei in Huntington Disease. J Biol Chem 285:8808–8823
31. Lunkes A, Mandel JL (1998) A cellular model that recapitulates major pathogenic steps of Huntington's disease. Hum Mol Genet 7:1355–1361
32. Ratovitski T, Gucek M, Jiang H, Chighladze E, Waldron E, D'Ambola J, Hou Z, Liang Y, Poirier MA, Hirschhorn RR, Graham R, Hayden MR, Cole RN, Ross CA (2009) Mutant huntingtin N-terminal fragments of specific size mediate aggregation and toxicity in neuronal cells. J Biol Chem 284:10855–10867
33. Gu X, Greiner ER, Mishra R, Kodali R, Osmand A, Finkbeiner S, Steffan JS, Thompson LM, Wetzel R, Yang XW (2009) Serines 13 and 16 are critical determinants of full-length human mutant huntingtin induced disease pathogenesis in HD Mice. Neuron 64:828–840
34. Mishra R, Hoop CL, Kodali R, Sahoo B, van der Wel PCA, Wetzel R (2012) Serine phosphorylation suppresses huntingtin amyloid accumulation by altering protein aggregation properties. J Mol Biol 424:1–14
35. Bodner RA, Outeiro TF, Altmann S, Maxwell MM, Cho SH, Hyman BT, McLean PJ, Young AB, Housman DE, Kazantsev AG (2006) Pharmacological promotion of inclusion formation: a therapeutic approach for Huntington's and Parkinson's diseases. Proc Nat Acad Sci U S A 103:4246–4251
36. Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416:535–539
37. Cleary JP, Walsh DM, Hofmeister J, 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
38. Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL et al (1998) Diffusible, nonfibrillar ligands derived from Aβ1–42 are potent central nervous system neurotoxins. Proc Nat Acad Sci U S A 95:6448–6453
39. Lesné S, MT K, 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
40. Miller J, Arrasate M, Brooks E, Libeu CP, Legleiter J, Hatters D, Curtis J, Cheung K, Krishnan P, Mitra S et al (2012) Identifying polyglutamine protein species in situ that best predict neurodegeneration. Nat Chem Biol 7(12):925–934
41. Snell RG, Macmillan JC, Cheadle JP, Fenton I, Lazarou LP, Davies P, Macdonald ME, Gusella JF, Harper PS, Shaw DJ (1993) Relationship between trinucleotide repeat expansion and phenotypic variation in Huntingtons disease. Nat Genet 4:393–397
42. Penney JB, Vonsattel JP, MacDonald ME, Gusella JF, Myers RH (1997) CAG repeat number governs the development rate of pathology in Huntington's disease. Ann Neurol 41:689–692
43. 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
44. Wetzel R (2012) Physical chemistry of polyglutamine: intriguing tales of a monotonous sequence. J Mol Biol 421:466–490
45. Marcellin D, Abramowski D, Young D, Richter J, Weiss A, Marcel A, Maassen J, Kauffmann M, Bibel M, Shimshek DR et al (2012) Fragments of HdhQ150 mutant huntingtin form a soluble oligomer pool that declines with aggregate deposition upon aging. PLoS One 7:e44457
46. Olshina MA, Angley LM, Ramdzan YM, Tang JW, Bailey MF, Hill AF, Hatters DM (2010) Tracking mutant huntingtin aggregation kinetics in cells reveals three major populations that include an invariant oligomer pool. J Biol Chem 285:21807–21816
47. Winner B, Jappelli R, Maji SK, Desplats PA, Boyer L, Aigner S, Hetzer C, Loher T, Vilar M, Campioni S et al (2011) In vivo demonstration that α-synuclein oligomers are toxic. Proc Nat Acad Sci U S A 108:4194–4199
48. Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe C (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 300:486–489
49. Habicht G, Haupt C, Friedrich RP, Hortschansky P, Sachse C, Meinhardt J, Wieligmann K, Gellermann GP, Brodhun M, Gotz J

et al (2007) Directed selection of a conformational antibody domain that prevents mature amyloid fibril formation by stabilizing A beta protofibrils. Proc Nat Acad Sci U S A 104:19232–19237
50. Kodali R, Wetzel R (2007) Polymorphism in the intermediates and products of amyloid assembly. Curr Opin Struc Biol 17:48–57
51. Kodali R, Williams AD, Chemuru S, Wetzel R (2010) A beta(1–40) forms five distinct amyloid structures whose beta-sheet contents and fibril stabilities are correlated. J Mol Biol 401:503–517
52. Bauer HH, Aebi U, Haner M, Hermann R, Muller M, Arvinte T, Merkle HP (1995) Architecture and polymorphism of fibrillar supramolecular assemblies produced by in-vitro aggregation of human calcitonin. J Struct Biol 115:1–15
53. Goldsbury CS, Cooper GJS, Goldie KN, Muller SA, Saafi EL, Gruijters WTM, Misur MP (1997) Polymorphic fibrillar assembly of human amylin. J Struct Biol 119:17–27
54. Jimenez JL, Nettleton EJ, Bouchard M, Robinson CV, Dobson CM, Saibil HR (2002) The protofilament structure of insulin amyloid fibrils. Proc Nat Acad Sci U S A 99:9196–9201
55. Bouchard M, Zurdo J, Nettleton EJ, Dobson CM, Robinson CV (2000) Formation of insulin amyloid fibrils followed by FTIR simultaneously with CD and electron microscopy. Protein Sci 9:1960–1967
56. Dzwolak W, Smirnovas V, Jansen R, Winter R (2004) Insulin forms amyloid in a strain-dependent manner: an FT-IR spectroscopic study. Protein Sci 13:1927–1932
57. Chamberlain AK, MacPhee CE, Zurdo J, Morozova-Roche LA, Hill HAO, Dobson CM, Davis J (2000) Ultrastructural organization of amyloid fibrils by atomic force microscopy. Biophys J 79:3282–3293
58. Glabe CG (2008) Structural classification of toxic amyloid oligomers. J Biol Chem 283:29639–29643
59. Chimon S, Shaibat MA, Jones CR, Calero DC, Aizezi B, Ishii Y (2007) Evidence of fibril-like beta-sheet structures in a neurotoxic amyloid intermediate of Alzheimer's beta-amyloid. Nat Struct Mol Biol 14:1157–1164
60. Sandberg A, Luheshi L, Sollvander S, de Barros T, Macao B, Knowles T, Biverstal H, Lendel C, Ekholm-Petterson F, Dubnovitsky A et al (2010) Stabilization of neurotoxic Alzheimer amyloid-beta oligomers by protein engineering. Proc Natl Acad Sci U S A 107:15595–15600
61. Eckert A, Hauptmann S, Scherping I, Meinhardt J, Rhein V, Drose S, Brandt U, Fandrich M, Muller W, Gotz J (2008) Oligomeric and fibrillar species of beta-amyloid (A beta 42) both impair mitochondrial function in P301L tau transgenic mice. J Mol Med-JMM 86:1255–1267
62. Stine WB, Dahlgren KN, Krafft GA, LaDu MJ (2003) In vitro characterization of conditions for amyloid-beta peptide oligomerization and fibrillogenesis. J Biol Chem 278:11612–11622
63. Orte A, Birkett NR, Clarke RW, Devlin GL, Dobson CM, Klenerman D (2008) Direct characterization of amyloidogenic oligomers by single-molecule fluorescence. Proc Nat Acad Sci U S A 105:14424–14429
64. Jayaraman M, Kodali R, Sahoo B, Thakur AK, Mayasundari A, Mishra R, Peterson CB, Wetzel R (2012) Slow amyloid nucleation via alpha-helix-rich oligomeric intermediates in short polyglutamine-containing huntingtin fragments. J Mol Biol 415:881–899
65. Thakur AK, Jayaraman M, Mishra R, Thakur M, Chellgren VM, Byeon I-JL, Anjum DH, Kodali R, Creamer TP, Conway JF et al (2009) Polyglutamine disruption of the huntingtin exon 1 N terminus triggers a complex aggregation mechanism. Nat Struct Mol Biol 16:380–389
66. Campioni S, Mannini B, Zampagni M, Pensalfini A, Parrini C, Evangelisti E, Relini A, Stefani M, Dobson C, Cecchi C et al (2010) A causative link between the structure of aberrant protein oligomers and their toxicity. Nat Chem Biol 6:140–147
67. Glabe CG, Kayed R (2006) Common structure and toxic function of amyloid oligomers implies a common mechanism of pathogenesis. Neurology 66:S74–S78
68. Caughey BW, Dong A, Bhat KS, Ernst D, Hayes SF, Caughey WS (1991) Secondary structure analysis of the scrapie-associated protein PrP 27–30 in water by infrared spectroscopy. Biochemistry 30:7672–7680
69. Pan K-M, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D, Mehlhorn I, Huang Z, Fletterick RJ, Cohen FE et al (1993) Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. Proc Natl Acad Sci U S A 90:10962–10966
70. Bruce ME (2003) TSE strain variation. Brit Med Bull 66:99–108
71. Bessen RA, Marsh RF (1992) Identification of 2 biologically distinct strains of transmissible mink encephalopathy in hamsters. J Gen Virol 73:329–334
72. Bessen RA, Marsh RF (1994) Distinct PrP properties suggest the molecular-basis of strain variation in transmissible mink encephalopathy. J Virol 68:7859–7868
73. Bruce ME (2003) TSE strain variation: an investigation into prion disease diversity. Brit Med Bull 66:99–108
74. González L, Martin S, Begara-McGorum I, Hunter N, Houston F, Simmons M, Jeffrey M (2002) Effects of agent strain and host genotype on PrP accumulation in the brain of sheep naturally and experimentally affected with scrapie. J Comp Pathol 126:17–29
75. Tanaka M, Collins SR, Toyama BH, Weissman JS (2006) The physical basis of how prion conformations determine strain phenotypes. Nature 442:585–589
76. Kane MD, Lipinski WJ, Callahan MJ, Bian F, Durham RA, Schwarz RD, Roher AE, Walker LC (2000) Evidence for seeding of beta-amyloid by intracerebral infusion of Alzheimer brain extracts in beta-amyloid precursor protein-transgenic mice. J Neurosci 20:3606–3611
77. Meyer-Luehmann M, Coomaraswamy J, Bolmont T, Kaeser S, Schaefer C, Kilger E, Neuenschwander A, Abramowski D, Frey P, Jaton AL et al (2006) Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. Science 313:1781–1784
78. Stohr J, Watts JC, Mensinger ZL, Oehler A, Grillo SK, DeArmond SJ, Prusiner SB, Giles K (2012) Purified and synthetic Alzheimer's amyloid beta (Aβ) prions. Proc Natl Acad Sci U S A 109:11025–11030
79. Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A, Fraser G, Stalder AK, Beibel M, Staufenbiel M et al (2009) Transmission and spreading of tauopathy in transgenic mouse brain. Nat Cell Biol 11:909–U325
80. Mougenot A-L, Nicot S, Bencsik A, Morignat E, Verchere J, Lakhdar L, Legastelois S, Baron T (2012) Prion-like acceleration of a synucleinopathy in a transgenic mouse model. Neurobiol Aging 33:2225–2228
81. Luk KC, Kehm VM, Zhang B, O'Brien P, Trojanowski JQ, Lee VMY (2012) Intracerebral inoculation of pathological alpha-synuclein initiates a rapidly progressive neurodegenerative alpha-synucleinopathy in mice. J Exp Med 209:975–986
82. Braak H, Del Tredici K (2011) Alzheimer's pathogenesis: is there neuron-to-neuron propagation? Acta Neuropathol 121:589–595
83. Braak H, Rub U, Gai WP, Del Tredici K (2003) Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. J Neural Transm 110:517–536
84. Ravits J, Paul P, Jorg C (2007) Focality of upper and lower motor neuron degeneration at the clinical onset of ALS. Neurology 68:1571–1575
85. Vonsattel JPG, DiFiglia M (1998) Huntington disease. J Neuropath Exp Neurol 57:369–384

86. Duennwald ML, Shorter J (2010) Countering amyloid polymorphism and drug resistance with minimal drug cocktails. Prion 4:244–251
87. Roberts BE, Duennwald ML, Wang H, Chung C, Lopreiato NP, Sweeny EA, Knight MN, Shorter J (2009) A synergistic small-molecule combination directly eradicates diverse prion strain structures. Nat Chem Biol 5:936–946
88. Stefani M, Dobson CM (2003) Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. J Mol Med-JMM 81:678–699
89. Winklhofer KF, Tatzelt J, Haass C (2008) The two faces of protein misfolding: gain- and loss-of-function in neurodegenerative diseases. EMBO J 27:336–349
90. Bolognesi B, Kumita JR, Barros TP, Esbjorner EK, Luheshi LM, Crowther DC, Wilson MR, Dobson CM, Favrin G, Yerbury J (2010) ANS binding reveals common features of cytotoxic amyloid species. ACS Chem Biol 5:735–740
91. Balch WE, Morimoto RI, Dillin A, Kelly JW (2008) Adapting proteostasis for disease intervention. Science 319:916–919
92. Bence NF, Sampat RM, Kopito RR (2001) Impairment of the ubiquitin-proteasome system by protein aggregation. Science 292:1552–1555
93. Gidalevitz T, Ben-Zvi A, Ho KH, Brignull HR, Morimoto RI (2006) Progressive disruption of cellular protein folding in models of polyglutaminediseases. Science 311:1471–1474
94. Chen-Plotkin AS, Sadri-Vakill G, Yohrling GJ, Bravernan MW, Berin CL, Glajch KE, DiRocco DP, Farrella LA, Krainc D, Gines S et al (2006) Decreased association of the transcription factor Sp1 with genes downregulated in Huntington's disease. Neurobiol Dis 22:233–241
95. Hands SL, Wyttenbach A (2010) Neurotoxic protein oligomerisation associated with polyglutamine diseases. Acta Neuropathol 120:419–437
96. Chartier-Harlin MC, Kachergus J, Roumier C, Mouroux V, Douay X, Lincoln S, Levecque C, Larvor L, Andrieux J, Hulihan M et al (2004) alpha-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet 364:1167–1169
97. Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, Przuntek H, Epplen JT, Schols L, Riess O (1998) Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Nat Genet 18:106–108
98. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R et al (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276:2045–2047
99. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R et al (2003) alpha-synuclein locus triplication causes Parkinson's disease. Science 302:841–841
100. Zarranz J, Alegre J, Gomez-Esteban JC, Lezcano E, Ros R, Ampuero I, Vidal L, Hoenicka J, Rodriguez O, Atares B et al (2004) The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann Neurol 55:164–173
101. Charles V, Mezey E, Reddy PH, Dehejia A, Young TA, Polymeropoulos MH, Brownstein MJ, Tagle DA (2000) Alpha-synuclein immunoreactivity of huntingtin polyglutamine aggregates in striatum and cortex of Huntington's disease patients and transgenic mouse models. Neurosci Lett 289:29–32
102. Fujishiro H, Tsuboi Y, Lin W, Uchikado H, Dickson D (2008) Co-localization of tau and alpha-synuclein in the olfactory bulb in Alzheimer's disease with amygdala Lewy bodies. Acta Neuropathol 116:17–24
103. Masliah E, Rockenstein E, Veinbergs I, Sagara Y, Mallory M, Hashimoto M, Mucke L (2001) beta-Amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. Proc Natl Acad Sci USA 98:12245–12250
104. Olzscha H, Schermann S, Woerner A, Pinkert S, Hecht M, Tartaglia G, Vendruscolo M, Hayer-Hartl M, Hartl F, Vabulas R (2011) Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. Cell 144:67–78
105. Vabulas R, Hartl F (2011) Aberrant protein interactions in amyloid disease. Cell Cycle 10:1512–1513
106. Calderwood SK, Murshid A, Prince T (2009) The shock of aging: molecular chaperones and the heat shock response in longevity and aging—a mini-review. Gerontology 55:550–558
107. Koga H, Kaushik S, Cuervo AM (2011) Protein homeostasis and aging: the importance of exquisite quality control. Ageing Res Rev 10:205–215
108. Lotz GP, Legleiter J, Aron R, Mitchell EJ, Huang SY, Ng CP, Glabe C, Thompson LM, Muchowski PJ (2010) Hsp70 and Hsp40 functionally interact with soluble mutant huntingtin oligomers in a classic ATP-dependent reaction cycle. J Biol Chem 285:38183–38193
109. Muchowski PJ, Wacker JL (2005) Modulation of neurodegeneration by molecular chaperones. Nat Rev Neurosci 6:11–22
110. Chafekar SM, Duennwald ML (2012) Impaired heat shock response in cells expressing full-length polyglutamine-expanded huntingtin. PLoS One 7:e37929
111. Labbadia J, Cunliffe H, Weiss A, Katsyuba E, Sathasivam K, Seredenina T, Woodman B, Moussaoui S, Frentzel S, Luthi-Carter R et al (2011) Altered chromatin architecture underlies progressive impairment of the heat shock response in mouse models of Huntington disease. J Clin Invest 121:3306–3319
112. Bennett EJ, Shaler TA, Woodman B, Ryu KY, Zaitseva TS, Becker CH, Bates GP, Schulman H, Kopito RR (2007) Global changes to the ubiquitin system in Huntington's disease. Nature 448:704–U711
113. Martinez-Vicente M, Talloczy Z, Wong E, Tang G, Koga H, Kaushik S, de Vries R, Arias E, Harris S, Sulzer D et al (2010) Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease. Nat Neurosci 13:567–U574
114. Kazlauskaite J, Sanghera N, Sylvester I, Venien-Bryan C, Pinheiro TJT (2003) Structural changes of the prion protein in lipid membranes leading to aggregation and fibrillization. Biochemistry 42:3295–3304
115. Legleiter J, Fryer JD, Holtzman DM, Kowalewski T (2011) The modulating effect of mechanical changes in lipid bilayers caused by ApoE-containing lipoproteins on a beta induced membrane disruption. ACS Chem Neurol 2:588–599
116. Porat Y, Kolusheva S, Jelinek R, Gazit E (2003) The human islet amyloid polypeptide forms transient membrane-active prefibrillar assemblies. Biochemistry 42:10971–10977
117. Stefani M (2007) Generic cell dysfunction in neurodegenerative disorders: role of surfaces in early protein misfolding, aggregation, and aggregate cytotoxicity. Neuroscientist 13:519–531
118. Yates EA, Cucco EM, Legleiter J (2011) Point mutations in A beta induce polymorphic aggregates at liquid/solid interfaces. ACS Chem Neurol 2:294–307
119. Zhao HX, Tuominen EKJ, Kinnunen PKJ (2004) Formation of amyloid fibers triggered by phosphatidylserine-containing membranes. Biochemistry 43:10302–10307
120. Wells K, Farooqui AA, Liss L, Horrocks LA (1995) Neural membrane phospholipids in Alzheimer disease. Neurochem Res 20:1329–1333
121. Kagan BL, Azimov R, Azimova R (2004) Amyloid peptide channels. J Membr Biol 202:1–10
122. Quist A, Doudevski L, 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 Nat Acad Sci U S A 102:10427–10432
123. Yamamoto A, Lucas J, Hen R (2000) Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. Cell 101:57–66

124. Zu T, Duvick L, Kaytor M, Berlinger M, Zoghbi H, Clark H, Orr H (2004) Recovery from polyglutamine-induced neurodegeneration in conditional SCA1 transgenic mice. J Neurosci 24:8853–8861
125. Sah DWY, Aronin N (2011) Oligonucleotide therapeutic approaches for Huntington disease. J Clin Invest 121:500–507
126. Boudreau RL, McBride JL, Martins I, Shen SH, Xing Y, Carter BJ, Davidson BL (2009) Nonallele-specific silencing of mutant and wild-type huntingtin demonstrates therapeutic efficacy in Huntington's disease mice. Mol Ther 17:1053–1063
127. Cohen F, Kelly J (2003) Therapeutic approaches to protein-misfolding diseases. Nature 426:905–909
128. Estrada L, Soto C (2006) Inhibition of protein misfolding and aggregation by small rationally-designed peptides. Curr Pharm Des 12:2557–2567
129. De Lorenzi E, Giorgetti S, Grossi S, Merlini G, Caccialanza G, Bellotti V (2004) Pharmaceutical strategies against amyloidosis: old and new drugs in targeting a "protein misfolding disease". Curr Med Chem 11:1065–1084
130. Johnson SM, Connelly S, Fearns C, Powers ET, Kelly JW (2012) The transthyretin amyloidoses: from delineating the molecular mechanism of aggregation linked to pathology to a regulatory-agency-approved drug. J Mol Biol 421:185–203
131. Neckers L, Workman P (2012) Hsp90 molecular chaperone inhibitors: are we there yet? Clin Cancer Res 18:64–76
132. Gura T (2008) Hope in Alzheimer's fight emerges from unexpected places. Nat Med 14:894–894
133. de Lartigue J (2012) Tafamidis for transthyretin amyloidosis. Drugs Today 48:331–337
134. Arrasate M, Finkbeiner S (2012) Protein aggregates in Huntington's disease. Exp Neurol 238:1–11
135. Steffan J, Bodai L, Pallos J, Poelman M, McCampbell A, Apostol B, Kazantsev A, Schmidt E, Zhu Y, Greenwald M et al (2001) Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in *Drosophila*. Nature 413:739–743
136. Iqbal K, Liu F, Gong C, Grundke-Iqbal I (2010) Tau in Alzheimer Disease and related tauopathies. Curr Alzheimer Res 7:656–664
137. Min S, Cho S, Zhou Y, Schroeder S, Haroutunian V, Seeley W, Huang E, Shen Y, Masliah E, Mukherjee C et al (2010) Acetylation of tau inhibits its degradation and contributes to tauopathy. Neuron 67:953–966
138. Soga S, Akinaga S, Shiotsu Y (2013) Hsp90 inhibitors as anticancer agents, from basic discoveries to clinical development. Curr Pharm Design 19
139. Auluck PK, Meulener MC, Bonini NM (2005) Mechanisms of suppression of alpha-synuclein neurotoxicity by geldanamycin in *Drosophila*. J Biol Chem 280:2873–2878
140. Waza M, Adachi H, Katsuno M, Minamiyama M, Sang C, Tanaka F, Inukai A, Doyu M, Sobue G (2005) 17-AAG, an Hsp90 inhibitor, ameliorates polyglutamine-mediated motor neuron degeneration. Nat Med 11:1088–1095
141. Baldo B, Weiss A, Parker CN, Bibel M, Paganetti P, Kaupmann K (2012) A Screen for enhancers of clearance identifies huntingtin as a heat shock protein 90 (Hsp90) client protein. J Biol Chem 287:1406–1414
142. Kalia S, Kalia L, McLean P (2010) Molecular chaperones as rational drug targets for Parkinson's disease therapeutics. CNS Neurol Disord-DR 9:741–753
143. Voisine C, Pedersen J, Morimoto R (2010) Chaperone networks: tipping the balance in protein folding diseases. Neurobiol Dis 40:12–20
144. Hageman J, van Waarde M, Zylicz A, Walerych D, Kampinga HH (2011) The diverse members of the mammalian HSP70 machine show distinct chaperone-like activities. Biochem J 435:127–142
145. Abisambra JF, Jinwal UK, Suntharalingam A, Arulselvam K, Brady S, Cockman M, Jin Y, Zhang B, Dickey CA (2012) DnaJA1 antagonizes constitutive Hsp70-mediated stabilization of tau. J Mol Biol 421:653–661
146. Fewell SW, Smith CM, Lyon MA, Dumitrescu TP, Wipf P, Day BW, Brodsky JL (2004) Small molecule modulators of endogenous and co-chaperone-stimulated Hsp70 ATPase activity. J Biol Chem 279:51131–51140
147. Sahara N, Maeda S, Yoshiike Y, Mizoroki T, Yamashita S, Murayama M, Park JM, Saito Y, Murayama S, Takashima A (2007) Molecular chaperone-mediated Tau protein metabolism counteracts the formation of granular Tau oligomers in human brain. J Neurosci Res 85:3098–3108
148. Galimberti D, Scarpini E (2011) Disease-modifying treatments for Alzheimer's disease. Ther Adv Neurol Disord 4:203–216
149. Gerald Z, Ockert W (2013) Alzheimer's disease market: hope deferred. Nat Rev Drug Discov 12:19–20
150. Atwal JK, Chen Y, Chiu C, Mortensen DL, Meilandt WJ, Liu Y, Heise CE, Hoyte K, Luk W, Lu Y, Peng K, Wu P, Rouge L, Zhang Y, Lazarus RA, Scearce-Levie K, Wang W, Wu Y, Tessier-Lavigne M, Watts RJ (2011) A therapeutic antibody targeting BACE1 inhibits amyloid-β production in vivo. Sci Transl Med 3:84ra43
151. Jonsson T, Atwal JK, Steinberg S, Snaedal J, Jonsson PV, Bjornsson S, Stefansson H, Sulem P, Gudbjartsson D, Maloney J, Hoyte K, Gustafson A, Liu Y, Lu Y, Bhangale T, Graham RR, Huttenlocher J, Bjornsdottir G, Andreassen OA, Jonsson EG, Palotie A, Behrens TW, Magnusson OT, Kong A, Thorsteinsdottir U, Watts RJ, Stefansson K (2012) A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. Nature 488:96–99