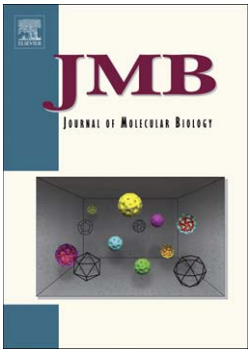

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Amyloidogenicity at a distance: how distal protein regions modulate aggregation in disease

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Abstract

The misfolding of proteins to form amyloid is a key pathological feature of several progressive, and currently incurable, diseases. A mechanistic understanding of the pathway from soluble, native protein to insoluble amyloid is crucial for therapeutic design and recent efforts have helped to elucidate the key molecular events that trigger protein misfolding. Generally, either global or local structural perturbations occur early in amyloidogenesis to expose aggregation-prone regions of the protein that can then self-associate to form toxic oligomers. Surprisingly, these initiating structural changes are often caused or influenced by protein regions distal to the classically amyloidogenic sequences. Understanding the importance of these distal regions in the pathogenic process has highlighted many remaining knowledge gaps regarding the precise molecular events that occur in classic aggregation pathways. In this review, we discuss how these distal regions can influence aggregation in disease and the recent technical and conceptual advances that have allowed this insight.

Introduction

Aggregation to amyloid is the process by which proteins misfold from their native and functional state to one that is amenable to self-association. This self-association generally occurs concomitantly with a global rearrangement to a β-sheet-rich structure, held tightly together through extensive hydrogen bonding, to form the repeating units of long polymeric fibres known as 'amyloid'. The common structural features of amyloid represent a stable state for a range of proteins of widely varying primary sequences. The presence and accumulation of amyloid fibrils is a classic hallmark of a range of amyloidoses that include the neurodegenerative diseases, Alzheimer's disease (AD) and Parkinson's disease (PD). This seminal observation led to an early focus of structural studies on end-stage amyloid fibrils, however, extensive research throughout the field has since implicated intermediate oligomers as a potential pathogenic species in these conditions. As such, the research emphasis has now shifted towards a structural characterisation of these pathogenic intermediates and how they arise from the native proteins.

Until recently, classic structural techniques had limited application to the study of the earliest events in protein misfolding, as these early events occur over rapid time scales, and involve small and highly dynamic conformational changes. This therefore limited any insight to low resolution structures of the early stage aggregation intermediates. As such, a structural understanding of the key molecular events that trigger aggregate nucleation has, for the most part, remained elusive. However, recent technological advances have facilitated more detailed examination of these phenomena. In particular, solid state NMR (ssNMR) and cryo electron microscopy (cryoEM) have been useful for determining the structural composition of fibrils, and theoretical techniques including molecular docking and dynamics simulations have allowed modelling of protein dynamics and interactions on very rapid time scales. In addition to the application of higher resolution experimental approaches, our understanding of the molecular basis of misfolding has been advanced by the recognition of the importance of regions distal to the classic amyloidogenic sequences in

defining the aggregation potential of a protein. Although often not involved in the core amyloid fibril β-structures, these regions have proven influential in triggering aggregation.

In this review, we will focus on recent advances that have been made in characterising the structural changes that occur during the earliest events in the misfolding pathway. In this context, we will revisit some classic examples of amyloid formation (including that of α-synuclein, β_2-microglobulin, apolipoprotein A-I, polyglutamine proteins, amyloid β and tau), to emphasise how the ability of each protein to aggregate can be initiated or enhanced by distal protein regions. These prototypical cases are redefining our view of the early molecular events in aggregation, whereby both global and local perturbations in structure beyond the amyloidogenic region itself appear to define pathological progression.

α-synuclein: aggregation triggered by global deprotection of amyloidogenic regions

α-synuclein (α-syn) is a 140 residue protein that is localised to presynaptic nerve terminals and is proposed to play a key role in neurotransmitter release, dopamine synthesis, vesicle trafficking and exocytosis [1]. α-syn amyloid was initially detected in Lewy Bodies, the pathologic hallmark of PD. Subsequently, α-syn aggregates were detected in a number of other neurodegenerative diseases, collectively known as synucleinopathies, which are characterised by α-syn aggregation and inclusion formation [1]. α-syn is an intrinsically disordered protein (IDP) comprised of three distinct regions: an N-terminus (residues 1-60) which contains four imperfect repeats of the conserved KTKEGV hexamer motif; the NAC domain (residues 61-95) which contains the highly amyloidogenic NAC region as well as three imperfect KTKEGV repeats; and the C-terminus (residues 96-140) which is highly acidic and proline-rich (Fig. 1A). The NAC domain is central to the ability of α-syn to form amyloid [2], however the N- and C- termini are critical modulators of nucleation, oligomer formation and end-stage fibril structure.

Two recent technical feats, harnessing micro electron diffraction (microED) and ssNMR, have provided high-resolution insights into the end-stage α-syn fibril structure [3,4]. MicroED revealed that short peptides, including part of the NAC domain, form similar steric-zipper protofilaments whereby the peptides pack in pairs to form in-register extended parallel β-sheets [3]. More recently, ssNMR studies provided a more complex model of the full-length α-syn fibril structure, whereby the NAC region forms the core of a complex β-serpentine arrangement [4]. Importantly, regions flanking the NAC domain are also incorporated into the amyloid core, which is comprised of the N-terminal domain residues 46-54 in addition to the NAC domain residues 63-96 (Fig. 1C) [4].

Given this important structural role of the N-terminus in the α-syn amyloid core, it is unsurprising that this region also plays a key role in modulating early steps of the aggregation pathway. The N-terminus can interact with cell membranes and, while this is likely critical for its physiological function, it also promotes a conformational change in the protein allowing it to sample a number of α-helical conformations (for example, an extended α-helix or broken α-helical states) (Fig. 1B) [5-9]. These membrane binding induced conformational changes appear to be both protective and detrimental during fibril formation. While it has been proposed that the membrane-bound α-helical states may inhibit fibril formation through stabilisation of non-amyloidogenic conformations [10], in *in vitro* studies membrane binding by the N-terminus was recently proposed to initiate aggregation by releasing the NAC domain [11]. This aggregation following N-terminal-membrane binding could however be explained by the correspondingly high local protein concentrations at the membrane [12]. Nevertheless, although the exact molecular contributions of the N-terminus towards aggregation are still undefined, the aggregation pathway for α-syn is clearly affected by the equilibrium between its free and membrane-bound state, the perturbation of which can trigger amyloid formation.

The C-terminal domain of α-syn has also been implicated in early events during the misfolding pathway. C-terminal truncation accelerates aggregation [13] by removing important long-range interactions between the C-terminus and the NAC domain that usually act to inhibit aggregation [14]. Changes in pH can also decrease these long-range interactions through charge neutralisation of the C-terminus upon protonation of its multiple aspartate and glutamate residues [15-18]. As such, under acidic conditions the aggregation-prone NAC domain is more highly exposed and α-syn can rapidly form fibrils [19]. Interestingly, immunotherapeutic strategies have uncovered an epitope in the C-terminus that is arguably a more efficacious target than the NAC domain [20] such that one C-terminus targeting antibody (PRX002, AFFiRiS AG) is currently undergoing testing in phase I clinical trials [21]. This strategy could become common amongst a number of proteins, with protective nanobodies discovered that bind non-amyloidogenic regions of both huntingtin [22,23] and lysozyme [24], highlighting these generally unexplored domains as important potential therapeutic targets.

The strength of the NAC-C-terminus intramolecular interaction is likely also decreased by disease-associated point mutations. The PD-linked A53T and H50Q α-syn mutations have similar native and intermediate structures to the wild-type protein, but display significantly increased aggregation kinetics [25,26]. Biophysical studies demonstrated that these mutants, along with an E46K mutant, are more rigid and solvent-inaccessible at their N- and C-termini [27], suggesting that they possess a higher propensity to form aggregation-prone intermediate structures. Of note, the misfolding of PD-linked mutants can occur concomitant with wild-type α-syn aggregation to form mixed species fibrils [28]. This is pertinent considering that the mutation-associated forms of PD are heterozygous.

Further, NMR and molecular dynamics (MD) simulations have suggested that residues 38-53 within the N-terminus have a high propensity to form a β-hairpin that is critical in initiating α-syn aggregation [29]. The formation of this hairpin is accelerated by the disease-associated A30P and

A53T mutations, whereas prevention of β-hairpin formation inhibits α-syn aggregation and toxicity [29,30] implicating it as a possible first step in the pathogenesis of PD. Binding of the N-terminus of α-syn to membranes can also reduce long-range intramolecular interactions within the protein, to free the C-terminus [31-33]. This process has been suggested to play a role in fibrilisation initiation [34]. However, it is unclear what effect the multimeric state of α-syn has on membrane binding and how this contributes to its further oligomerisation [35].

Together, these data indicate that the aggregation of α-syn through its central NAC domain is defined and modulated by contributions from its N- and C-termini. These flanking domains, and their intramolecular interactions, must therefore play a pivotal role in the equilibrium between native and aggregation-sensitive protein, however the nature of this equilibrium *in vivo* is largely unknown. This is clearly illustrated by the dual protective and detrimental roles that have been proposed for the binding of the N-terminus of α-syn to cell membranes. As most work to date has been performed *in vitro*, this example highlights a future need to understand the contributions of a cellular environment to the molecular mechanisms that underlie misfolding pathways.

β_2-Microglobulin: aggregation triggered by deprotection of amyloidogenic regions

β_2-Microglobulin (β2m) is a key component of the major histocompatibility complex class I (MHC I) where it aids in antigen presentation [36], and its aggregation and deposition as amyloid fibrils is the key pathological feature of dialysis-related amyloidosis [37]. The protein is 99 residues in length and forms an immunoglobulin fold comprising a seven-stranded anti-parallel β-sandwich (Fig. 2A,B) [38]. Owing in part to this small, single-domain architecture, a number of studies have effectively harnessed high-resolution techniques, including NMR, X-ray crystallography and hydrogen-deuterium (HD) exchange, to elucidate the many key structural deviations from the native-state that are critical in triggering β2m amyloid formation. β2m aggregation provides a well-characterised example of how highly localised changes, down to the isomerisation of an individual

residue, can propagate distally throughout a single domain to trigger the formation of a near-native, yet amyloidogenic-compatible structure.

In vitro, β2m aggregation occurs through a nucleation-dependent mechanism, and this nucleation step is dependent on the formation of a near-native folding intermediate (termed I_T) [39-41]. This folding intermediate has a *trans*-Pro32 backbone conformation distinct from that of the native state, which exists as a thermodynamically unfavourable *cis*-isomer [39-42]. The slow rate of conversion from the *trans*- to the c*is*-isomer results in a significant population of the I_T intermediate on the folding pathway under native conditions [41]. Importantly, studies of *trans*-locked β2m point mutants have demonstrated that the rearrangement of this X-Pro32 bond radiates throughout the protein to form a conformation that promotes amyloid nucleation [41,43]. While these structural rearrangements do not affect the overall protein fold, with low overall C_α r.m.s deviation between *cis*-wild-type and *trans*-locked mutants, major side-chain repacking of the hydrophobic core is observed in the *trans*-isomer [41,43]. The edge strands that protect the core hydrophobic regions have an altered conformation in *trans*-Pro32 monomers compared to the native *cis*-isomer. Interestingly, in the native state it is one of these edge strands, the D-strand, which forms contacts with the MHC I heavy chain [36]; HD exchange studies show that the D-strand is highly mobile in the unbound β2m monomer and that this dynamic nature correlates with an increased aggregation propensity [44,45]. Correspondingly, decreasing the mobility of this region through point mutations results in lower overall hydrophobic exposure and a subsequent decrease in aggregation kinetics [46]. In contrast, X-Pro32 isomerisation increases the dynamics of this strand causing exposure of the aggregation-prone hydrophobic core [45].

Although a critical determinant of aggregation initiation, isomerisation of the X-Pro32 bond alone is insufficient for β2m nucleation under neutral pH [41]. Under these conditions, N-terminal residues alleviate the effects of Pro32 isomerisation to stabilise the β2m native state, with side chains of the

first six residues stabilising the BC-loop and backbone atoms interacting with the B-strand to lock the X-Pro32 in its *cis* conformation [42]. Without the stabilising N-terminus, the kinetic stability of the protein following Pro32 isomerisation decreases such that β2m can rapidly sample multiple amyloidogenic competent conformations, leading to an increased rate of aggregation [42]. As such, a β2m ΔN6 mutant found in dialysis-related amyloid deposits [47] has a significantly repacked hydrophobic core and a pH-dependent increase in backbone dynamics [42] (Fig. 2C). Further, it has been suggested that both ΔN6 and *trans*-locked mutants can interact with the wild-type monomer and induce its conformational change to a more aggregation-prone state in a prion-like fashion [42,48]. However, a recent report suggested that only fibrils, rather than monomers, can seed wild-type aggregation [49]. These results suggest that a nucleation mechanism rather than conformational conversion allows mutant β2m to trigger oligomerisation of the wild-type; the exact details of this mechanism still remain to be conclusively resolved.

Two crystal structures of dimeric β2m ΔN6 have helped to characterise the early events in β2m aggregation [50,51]. Interestingly, both structures show symmetrical domain-swapping between the monomers involving amyloidogenic regions within the central β-sheet [50,51]. Although the two structures disagree on which residues contribute to the switched 'hinge' region, both cases argue for an increased exposure of the amyloidogenic hinge such that it can interact between dimers [50,51]. This domain-swapping event may provide the structural basis for oligomer formation before structural rearrangement to β-rich amyloid (Fig. 2E, F) [51,52]. Interestingly, isomerisation of the X-Pro32 bond occurs concomitantly with a rotation of Phe30 towards the solvent face during these domain-swapping events [51,53]. Therefore, while multiple factors can contribute to the aggregation propensity of β2m, it appears that isomerisation of the X-Pro32 bond is a key and influential step that dramatically affects the propensity of the protein to aggregate.

The previous two examples demonstrate how small and large-scale conformational changes in flanking protein areas can lead to the exposure and self-association of aggregation-prone regions, allowing amyloid nuclei to form. Remarkably, small perturbations in regions located much more distal to the amyloidogenic 'hot spots' within a protein can also dramatically influence the structure and interactions of amyloid forming areas; one such case is that of apolipoprotein A-I (apoA-I).

ApoA-I: propagating structural perturbations initiate aggregation

Apolipoprotein A-I (apoA-I) plays a role in the sequestration and metabolism of lipids and is the major protein component of high-density lipoprotein (HDL) [54]. It is part of a family of apolipoproteins that have a high propensity to aggregate, largely due to the intrinsic hydrophobicity and structural flexibility that is required to sequester lipids [55-58]. In order to bind lipids, apoA-I must undergo a global conformational change involving an unfurling of the C-terminus through the central 'hinge region' (residues 121-142, Fig. 3A). Although highly ordered while sequestered in HDL, in its lipid-free form both the N- and C-termini of apoA-I have a high propensity for disorder [59]. Any shift in the equilibrium between the aggregation-prone monomer and the ordered lipid-bound dimer appears to have a great impact on the propensity of the protein to aggregate [60].

Aggregation occurs through rearrangement of N-terminal amyloidogenic regions to β-structures. In particular, within the N-terminus there are three key aggregation-prone regions (residues 14-22, 53-58 and 69-72) that are usually sterically protected by central packing within the overall α-helical structure (Fig. 3A) [61]. The aggregation of apoA-I depends on the inherent potential of these N-terminal regions to form of β-sheets and occurs primarily through their self-association [62]. As such, the majority of isolated *ex vivo* amyloid fibrils identified have been comprised of only the first N-terminal 80-100 residues [60]; a number of studies have suggested that cleavage of the C-terminus is necessary to release the aggregation-prone N-terminus, which subsequently aggregates to fibrils [63-66]. Recent biophysical analyses however have suggested that structural perturbations

through point mutations can promote full-length aggregation, first through the N-terminal aggregation hot spots, followed by alignment of the C-terminus within the fibril structure (Fig. 3B) [61].

Interestingly, mutations outside the fibrilogenic N-terminal domain have been implicated in the pathogenesis of familial apoA-I amyloidosis [54,67]. A recent study demonstrated that single residue mutations distant from the N-terminus can decrease the global thermodynamic stability of apoA-I and convert the α-helical arrangement of the amyloidogenic region to an overall β-zipper structure [68]. These distal mutations also enhanced the HD exchange rates of the structured N-terminal helical bundle such that the aggregation-prone 'hot spots' are deprotected [68]. Despite these long-ranging effects and the increased amyloidogenecity of the mutants, the overall change in global protein structural stability and molecular dynamics was mild compared to wild-type controls. As such, global structural destabilisation alone is not sufficient for the amyloidogenicity of apoA-I. This observation was supported by examination of a mutation that decreased the lipid-binding and overall stability of apoA-I, but had no effect on amyloidogenicity. The L159R mutation is located near a proteolytic cleavage site and induces local unfolding to release the C-terminus and allow the aggregation-prone N-terminus more conformational flexibility [68]. Similar effects have been observed for L174S and L178H mutants, which induce exposure of a proteolytic site within the residue 14-22 aggregation hot spot [69,70].

In the case of L159R however, the increased exposure of this cleavage site, in addition to charge contributions from the arginine, is believed to promote proteolytic cleavage *in vivo* and it is likely that this drastic unfolding saves the protein from aggregation by targeting it for cellular degradation (Fig. 3C). As such, although global perturbations in structure and stability are classically associated with an increased propensity for a protein to form amyloid, mere changes in global stability are

insufficient to induce aggregation. Instead, highly specific local changes in non-amyloidogenic regions of apoA-I dictate aggregate nucleation.

These structural insights into the aggregation propensity of apoA-I also revealed the potential importance of ensemble-based conformational allostery [71] to the amyloid field [68]. This paradigm suggests that proteins exist in a dynamic equilibrium of conformations, each with a defined probability of occurring. The non-stochastic nature of this equilibrium determines the time a protein spends sampling different conformations, and hence its activity and aggregation propensity. Interestingly, the state of this equilibrium is not only affected by perturbations proximal to amyloid-forming areas, but is a summation of local and global properties. This view challenges the classical idea of defined and static structural rearrangements as predictive of aggregation potential. Moreover, and importantly, this model suggests new possibilities for the design of therapeutics that may stabilise protective conformations or decrease the likelihood of sampling amyloidogenic intermediates by affecting the energetic barriers between such states. This is particularly pertinent in the study of IDPs such as α-syn; the function of this protein necessitates a lack of defined tertiary structure, allowing rapid sampling of multiple conformations. In such cases, intrinsic disorder has hindered classical structural techniques (particularly X-ray crystallography) in describing the conformational changes that occur to propagate aggregation. Instead, recent advances in ssNMR, MD simulations and single molecule fluorescence have helped to delineate the energetic landscape of the molecular ensemble [72-74]. Although we are only beginning to understand such ensembles *in vitro*, future work will need to assess how this translates to *in vivo* systems in the hopes of utilising this information for therapeutic benefit.

Polyglutamine proteins: distal protein domains nucleate aggregation

The family of polyglutamine (polyQ) proteins contains a number of examples wherein domains distal from the typical amyloidogenic sequences initiate aggregation nucleation. These nuclei

influence the amyloidogenic propensity of the rest of the protein and can act as seeds for amyloid growth. The neurodegenerative polyQ diseases are characterised by extended glutamine tracts in disease-associated proteins. These proteins display a polyQ-length dependent rate of amyloid-like fibril formation *in vitro*, a process that ultimately manifests as intranuclear protein inclusions in the neurons of patients [75-77]. PolyQ tracts have an inherent ability to form highly stable amyloid-like fibrils, a propensity that has been best characterised by the study of isolated polyQ peptides [76]. However, in the context of full-length or proteolytic fragments of disease-associated proteins, regions distal to the polyQ tract play a central role in both nucleating fibril assembly and forming key interactions in the aggregation pathway [78-82].

Regions flanking the polyQ tract in huntingtin (htt) exon-1, the most highly studied fragment of the 348 kDa htt protein, have opposing effects on the aggregation pathway. In both the monomeric and fibril structures, a proline-rich region downstream of the polyQ tract forms a polyproline II helical structure and random coil further towards the C-terminus [83-85]. The polyproline region has broadly suppressive effects on fibril formation [86,87] and these inhibitory effects are thought to occur due to propagation of the polyproline-helix II structure to a small number of glutamine residues within the end of the polyQ region (Fig. 4A) [88]. Interestingly, α-syn with the familial H50Q mutation has also been observed to have an increased population of polyproline II structure around the site of the mutation [89]. This structural motif appears to have opposing effects, promoting aggregation in α-syn. It is therefore likely that protein context defines the ability of a polyproline II structure to either promote or inhibit intermolecular interactions.

In contrast, the 17 N-terminal residues upstream of the polyQ tract (htt^{NT}) play a critical structural role in promoting both the nucleation and assembly of the polyQ-polyQ β-sheet fibril structure. Studies of synthesised polyQ peptides incorporating the htt^{NT}, as well as recombinant htt exon-1, support a model whereby htt^{NT} promotes fibril assembly by adopting an α-helical structure prone to

oligomerization [79,90]. This grouping of the htt^{NT} into α-helical tetrameric oligomers, likely through coil-coil interactions, drives the adoption of the highly stable end-stage polyQ fibril structure (Fig. 4A) [91-93]. Here, it is probable that the htt^{NT}-induced increase in the local concentration of polyQ tracts allows the protein to overcome the high-energetic barrier to the formation of the interdigitated polyQ fibril β-sheet structure [94]. This key role of the htt^{NT} in amyloid initiation is further supported by a number of studies demonstrating that the direct binding of chaperones to the htt^{NT} is critical to their ability to decrease the overall aggregation propensity of htt exon-1 [81,82,95]. Further, phosphorylation of Ser13 and Ser16 within the htt^{NT} reduces misfolding kinetics, resulting in aggregates of atypical morphologies [96] and attenuated htt toxicity in symptomatic Huntington's Disease mice [97,98]. It has been suggested that the phosphoserines interact with neighboring lysines to destabilise the aggregation-initiating htt^{NT} α-helix [96].

Ataxin-3 is another example of a polyQ protein with a complex multi-step aggregation pathway, whereby the nucleating site of self-association is distal to the disease-instigating polyQ mutation. However, in comparison to the short htt exon-1, the ataxin-3 aggregation pathway is significantly more complex. This is in part due to the larger size of the protein, and the observation that aggregation is nucleated by the misfolding of an N-terminal and globular catalytic Josephin domain (JD), rather than a short peptide stretch such as htt^{NT} [78,99-101]. Pastore and colleagues initially identified the intrinsic aggregation capability of the isolated JD, which forms Thioflavin T-positive and short curvi-linear fibrils upon heating or after prolonged incubation [99]. Somewhat surprisingly however, it was observed that in the context of a full-length ataxin-3 variant with a pathological-length polyQ tract, it is JD misfolding and aggregation that predominates over direct polyQ-polyQ association [78]. Indeed, the first-stage of pathological ataxin-3 aggregation is JD-dependent, with the formation of short curvi-linear fibrils resembling that of the isolated JD [78]. What distinguishes pathologically-expanded ataxin-3 from non-expanded ataxin-3 and the isolated JD, is the presence of a second polyQ-dependent stage characterised by the formation of large aggregates with the

hallmark enhanced SDS-stability of the polyQ fibril structure [78]. Supporting observations of a two-stage ataxin-3 aggregation mechanism have been made following chaperone-modulated aggregation *in vivo* [101], lending weight to the importance of the misfolding of the distal JD to the disease mechanism. Interestingly, a comparable two-stage mechanism occurs *in vivo* upon fusion of polyglutamine tracts to the model CRABP protein [80].

Although both the JD-dependent and polyQ-dependent stages of ataxin-3 aggregation are often studied as two separate phenomena, expansion of the polyQ tract substantially increases the rate of ataxin-3 aggregation [78,100,102]. This mimics the polyQ length-dependence of aggregation reported for polyQ disease onset and demonstrates an interdependence between the two stages. Recent studies have begun to unravel how expansion of the distal polyQ tract modulates upstream JD structure to nucleate ataxin-3 self-association. HD exchange and mass spectrometry studies demonstrated that polyQ expansion increases the dynamics of helix-α4 within the JD without altering the overall global stability of the protein (Fig. 4B) [102,103]. In agreement with earlier MD simulations [104], the enhanced molecular mobility of this α-helix allows for an increase in the solvent exposure of internal amyloidogenic regions that form the core of first stage curvi-linear fibrils [102,103]. Further, alanine-scanning mutations in helix-α4 can limit the effect of polyQ expansion on its dynamic movement, reverting pathologically-expanded ataxin-3 to an aggregation rate of non-expanded ataxin-3 [103]. In this manner, aggregation can also be slowed by ubiquitin binding, which reduces the dynamics of the JD [105,106]. The mechanism by which an expanded polyQ tract allosterically modulates the dynamics of the distal JD is still to be determined, but draws significant parallels to the nucleation of the previously discussed mutations in apoA-I [107].

The ability of JD aggregation to override the inherent aggregation potential of the expanded polyQ tract is remarkably similar to the α-helical oligomerisation mechanism proposed for htt nucleation. In both cases, regions distal to the polyQ tract are required to nucleate fibril assembly, presumably

by increasing the local concentration of polyQ tracts to trigger a stochastic assembly mechanism [77]. This role of flanking domains in polyQ aggregation still remains somewhat surprising, but taken together likely highlights a mechanism to overcome the high energetic barrier presented by the interdigitated β-sheet packing of the polar glutamine side-chains observed in polyQ fibrils [94].

Aβ & tau: post-translational modifications as nucleation triggers

Aggregation-prone regions are often greatly affected by both proximal and distal post-translational modifications. This is exemplified by two proteins implicated in Alzheimer's Disease (AD): Aβ and tau. Aβ is a ~4kDa secreted peptide containing a central hydrophobic region that can self-associate as a steric zipper to form the core of amyloid plaques that are the key pathological feature of AD (Fig. 5A) [108]. Due to its intrinsically disordered nature, Aβ can rapidly sample multiple conformations, and its aggregation is subsequently highly sensitive to small chemical modifications. For example, oxidation of Met35 downstream of the central amyloidogenic hot spot decreases the rate of aggregation and has been suggested to play a role in AD-related oxidative damage [109]. MD simulations and NMR studies have found a decrease in β-structures in the C-terminal half of the peptide following oxidation of Met35 [110], likely influencing the intermediate structures sampled early in the aggregation process. Although it remains unclear exactly how these intermediates self-associate, a recent NMR study proposed that Met35 is responsible for forming a key contact between monomers in the mature amyloid [111]. Interestingly, a similar effect is observed for α-syn where oxidative stress-induced nitration of the C-terminus encourages a more globally extended conformation and nitrated intermediates can accelerate the fibrilisation rate of unmodified monomers [112,113].

Aβ itself is generated by multiple post-translational cleavage events in the amyloid precursor protein (APP), which is expressed in neuronal synapses and plays a key role in synapse formation and neuronal plasticity (Fig. 5A) [114]. APP is a highly conserved, integral membrane protein with a

large extracellular domain and cleavage sites for α-, β- and γ- secretases. β-secretase and γ-secretase processing of APP produces a 40 or 42-amino acid IDP ($A\beta_{40}$ or $A\beta_{42}$). Interestingly, Aβ aggregation is highly dependent on the two-residue difference between $A\beta_{40}$ and $A\beta_{42}$. Although their overall aggregation pathways are similar, $A\beta_{40}$ and $A\beta_{42}$ have significantly different self-association kinetics [115]. Replica-exchange molecular dynamics (REMD) simulations predict that the C-terminal two residues in $A\beta_{42}$ increase β-structures within the core amyloidogenic region through enhancing hydrophobic clustering, compared to $A\beta_{40}$ which has a decreased population of β-hairpin structures [116]. In addition, Aβ aggregation is also likely influenced by distal protein regions that can nucleate fibril growth in a manner analogous to that described for the polyQ diseases (see previous section). A range of hybrid peptides incorporating the N-terminal polyQ region and the C-terminus of the β-amyloid peptide (Aβ), have been studied to provide insight into the early oligomerisation events observed in Aβ fibril formation [117]. In the hybrid peptides, the $A\beta_{42}$ C-terminus acted in a similar manner to the htt^{NT} by forming an initial α-helical oligomeric structure that then promotes polyQ fibril formation. In contrast, $A\beta_{40}$ did not undergo α-helical oligomerisation and was unable to enhance the rate of polyQ fibril formation beyond the inherent propensity defined by the polyQ tract. This potentially reflects the decreased amyloidogenicity of the shorter $A\beta_{40}$ C-terminus observed in REMD simulations. Post-translational processing of APP to produce Aβ therefore has a marked influence on the rate of aggregation. Interestingly, some research suggests that both species can also inter-associate to form hybrid aggregates with substantially higher cellular toxicity [118]. Given the debated importance of early aggregation intermediates in amyloid disease toxicity (discussed in [119,120]), these studies reiterate the importance of gaining a greater structural understanding of the oligomerisation events that nucleate fibril growth.

In addition to Aβ plaques, another major hallmark of AD is the presence of neurofibrillary tangles (NFTs) that consist of aggregated tau protein. Under normal physiological conditions, tau is

responsible for neuronal microtubule assembly; tau binds tubulin via four microtubule binding regions (MBR) that form the amyloidogenic core of tau fibrils (Fig. 5B). Disruption of the tau-tubulin interaction appears to be key in the initiation of amyloid formation and in the pathogenesis of a number of neurodegenerative diseases collectively known as tauopathies [121]. Critically, tau is hyperphosphorylated in all tauopathies, and this hyperphosphorylation appears to precede amyloid formation [122] (Fig. 5B). MD simulations were used to determine the molecular basis of the hyperphosphorylation-induced aggregation of tau [123]. Phosphorylation enhances the formation of salt bridges between the negatively charged pThr231/pSer234 and neighbouring positively charged basic residues. This disrupts β-structures within tau such that it can no longer bind microtubules [124-126], while stabilising a salt bridge between pThr231 and Arg230 that competes with key salt bridges between tau and tubulin [127]. Interestingly, post-translational modification by acetylation also impairs the ability of tau to bind microtubules and concurrently increases its aggregation propensity [128].

Studies have suggested that hyperphosphorylated tau has more secondary structure than the intrinsically unfolded native state [129], and that these folded structures may increase the aggregation potential of the protein by favouring early intermediates in the aggregation pathway [122]. Specifically, phosphorylation stabilises an amyloidogenic α-helix between residues 236-243 [123,130]. A more recent study corroborated this theory, finding that phosphorylation at Ser235, Ser237 and Ser238 additionally stabilise a transient α-helix between residues 239-242, increasing the overall rate of fibril formation [127]. Interestingly, this intramolecular interaction is regulated by a *cis-trans* isomerisation of the Thr231-Pro232 bond and a *cis*-locked isomer is more resistant to dephosphorylation and is subsequently more aggregation-prone, in addition to being unable to promote microtubule assembly [131,132]. Hyperphosphorylation also promotes electrostatic repulsion between the amyloidogenic repeat region and the C-terminus, exposing the aggregation-prone residues and increasing rates of fibril growth [133-135]. The tau C-terminus is known to have

a protective effect on aggregation as its cleavage increases fibrilisation kinetics [136]. This region interacts with residues 321-375 within the microtubule-binding repeat region, potentially stabilising it or occluding it from aggregation-inducing intermolecular interactions [136]. Interestingly, C-terminally truncated tau, rather than the full-length protein, is released from neurons, indicating that this proteolytic processing may be a key event in the spacial progression of tauopathies [137].

Together, these cases illustrate how primary and secondary structural elements are insufficient to define the ability of a protein to aggregate. Contributions from chemical modifications and proteolytic cleavage, as well as the physical effects of the cellular milieu, can be definitive in the adoption of aggregation-prone structures.

Conclusions

Recent work discussed herein has highlighted the importance of regions distal to the classically implicated amyloidogenic domains in protein aggregation. However, considerable work remains in order to determine how the structural rearrangements described in this review translate to *in vivo* systems. Initial studies have begun to delineate the role of particular amyloidogenic intermediates within a cell, taking into account factors such as biomolecule interactions, the subcellular or extracellular environment and cellular crowding and physical effects [138-142]. However this area still requires extensive research, particularly focusing on the contributions from distal regions to these phenomena. The application of high-resolution structural techniques, including cryoEM and ssNMR, to examine the oligomeric species in these aggregation pathways should allow for even greater structural insight in the future. Specifically, the role and structure of key distal domains in these intermediate states will allow greater understanding of the extent of their role in protein folding. Future work will also need to elucidate the role of these regions in the pathological oligomeric intermediates and how they contribute to disease progression. Together, this knowledge will clarify potential strategies for therapeutics targeted to these regions.

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References

[1] E.H. Norris, B.I. Giasson, V.M.Y. Lee, Alpha-synuclein: normal function and role in neurodegenerative diseases, Curr. Top. Dev. Biol. 60 (2004) 17–54. doi:10.1016/S0070-2153(04)60002-0.

[2] B.I. Giasson, I.V. Murray, J.Q. Trojanowski, V.M. Lee, A hydrophobic stretch of 12 amino acid residues in the middle of alpha-synuclein is essential for filament assembly, J. Biol. Chem. 276 (2001) 2380–2386. doi:10.1074/jbc.M008919200.

[3] J.A. Rodriguez, M.I. Ivanova, M.R. Sawaya, D. Cascio, F.E. Reyes, D. Shi, et al., Structure of the toxic core of α-synuclein from invisible crystals, Nature. 525 (2015) 486–490. doi:10.1038/nature15368.

[4] M.D. Tuttle, G. Comellas, A.J. Nieuwkoop, D.J. Covell, D.A. Berthold, K.D. Kloepper, et al., Solid-state NMR structure of a pathogenic fibril of full-length human α-synuclein, Nat. Struct. Mol. Biol. 23 (2016) 409–415. doi:10.1038/nsmb.3194.

[5] R.J. Perrin, W.S. Woods, D.F. Clayton, J.M. George, Interaction of human alpha-Synuclein and Parkinson's disease variants with phospholipids. Structural analysis using site-directed mutagenesis, J. Biol. Chem. 275 (2000) 34393–34398. doi:10.1074/jbc.M004851200.

[6] D. Eliezer, E. Kutluay, R. Bussell, G. Browne, Conformational properties of alpha-synuclein in its free and lipid-associated states, J. Mol. Biol. 307 (2001) 1061–1073. doi:10.1006/jmbi.2001.4538.

[7] M. Zhu, J. Li, A.L. Fink, The association of alpha-synuclein with membranes affects bilayer structure, stability, and fibril formation, J. Biol. Chem. 278 (2003) 40186–40197.

doi:10.1074/jbc.M305326200.

[8] H.-J. Lee, C. Choi, S.-J. Lee, Membrane-bound alpha-synuclein has a high aggregation propensity and the ability to seed the aggregation of the cytosolic form, J. Biol. Chem. 277 (2002) 671–678. doi:10.1074/jbc.M107045200.

[9] K. Beyer, Mechanistic aspects of Parkinson's disease: alpha-synuclein and the biomembrane, Cell Biochem. Biophys. 47 (2007) 285–299.

[10] L. Fonseca-Ornelas, S.E. Eisbach, M. Paulat, K. Giller, C.O. Fernandez, T.F. Outeiro, et al., Small molecule-mediated stabilization of vesicle-associated helical α-synuclein inhibits pathogenic misfolding and aggregation, Nat Commun. 5 (2014) 5857. doi:10.1038/ncomms6857.

[11] D. Ysselstein, M. Joshi, V. Mishra, A.M. Griggs, J.M. Asiago, G.P. McCabe, et al., Effects of impaired membrane interactions on α-synuclein aggregation and neurotoxicity, Neurobiol. Dis. 79 (2015) 150–163. doi:10.1016/j.nbd.2015.04.007.

[12] C. Galvagnion, A.K. Buell, G. Meisl, T.C.T. Michaels, M. Vendruscolo, T.P.J. Knowles, et al., Lipid vesicles trigger α-synuclein aggregation by stimulating primary nucleation, Nat. Chem. Biol. 11 (2015) 229–234. doi:10.1038/nchembio.1750.

[13] R.A. Crowther, R. Jakes, M.G. Spillantini, M. Goedert, Synthetic filaments assembled from C-terminally truncated alpha-synuclein, FEBS Lett. 436 (1998) 309–312.

[14] C.W. Bertoncini, Y.-S. Jung, C.O. Fernandez, W. Hoyer, C. Griesinger, T.M. Jovin, et al., Release of long-range tertiary interactions potentiates aggregation of natively unstructured alpha-synuclein, Proc Natl Acad Sci USA. 102 (2005) 1430–1435. doi:10.1073/pnas.0407146102.

[15] K.-P. Wu, D.S. Weinstock, C. Narayanan, R.M. Levy, J. Baum, Structural reorganization of alpha-synuclein at low pH observed by NMR and REMD simulations, J. Mol. Biol. 391 (2009) 784–796. doi:10.1016/j.jmb.2009.06.063.

[16] S. McClendon, C.C. Rospigliosi, D. Eliezer, Charge neutralization and collapse of the C-

terminal tail of alpha-synuclein at low pH, Protein Science. 18 (2009) 1531–1540. doi:10.1002/pro.149.

[17] M.A. Saraiva, C.D. Jorge, H. Santos, A.L. Maçanita, Earliest events in α-synuclein fibrillation probed with the fluorescence of intrinsic tyrosines, J. Photochem. Photobiol. B, Biol. 154 (2016) 16–23. doi:10.1016/j.jphotobiol.2015.11.006.

[18] S. Park, J. Yoon, S. Jang, K. Lee, S. Shin, The role of the acidic domain of α-synuclein in amyloid fibril formation: a molecular dynamics study, J. Biomol. Struct. Dyn. 34 (2016) 376–383. doi:10.1080/07391102.2015.1033016.

[19] S.J. Wood, J. Wypych, S. Steavenson, J.C. Louis, M. Citron, A.L. Biere, alpha-synuclein fibrillogenesis is nucleation-dependent. Implications for the pathogenesis of Parkinson's disease, J. Biol. Chem. 274 (1999) 19509–19512.

[20] E. Valera, B. Spencer, E. Masliah, Immunotherapeutic Approaches Targeting Amyloid-β, α-Synuclein, and Tau for the Treatment of Neurodegenerative Disorders, Neurotherapeutics. 13 (2016) 179–189. doi:10.1007/s13311-015-0397-z.

[21] D. Games, E. Valera, B. Spencer, E. Rockenstein, M. Mante, A. Adame, et al., Reducing C-terminal-truncated alpha-synuclein by immunotherapy attenuates neurodegeneration and propagation in Parkinson's disease-like models, J. Neurosci. 34 (2014) 9441–9454. doi:10.1523/JNEUROSCI.5314-13.2014.

[22] J.M. Lecerf, T.L. Shirley, Q. Zhu, A. Kazantsev, P. Amersdorfer, D.E. Housman, et al., Human single-chain Fv intrabodies counteract in situ huntingtin aggregation in cellular models of Huntington's disease, Proc Natl Acad Sci USA. 98 (2001) 4764–4769. doi:10.1073/pnas.071058398.

[23] E. De Genst, D.Y. Chirgadze, F.A.C. Klein, D.C. Butler, D. Matak-Vinković, Y. Trottier, et al., Structure of a single-chain Fv bound to the 17 N-terminal residues of huntingtin provides insights into pathogenic amyloid formation and suppression, J. Mol. Biol. 427 (2015) 2166–2178. doi:10.1016/j.jmb.2015.03.021.

[24] E. De Genst, P.-H. Chan, E. Pardon, S.-T.D. Hsu, J.R. Kumita, J. Christodoulou, et al., A nanobody binding to non-amyloidogenic regions of the protein human lysozyme enhances partial unfolding but inhibits amyloid fibril formation, J Phys Chem B. 117 (2013) 13245–13258. doi:10.1021/jp403425z.

[25] K.A. Conway, J.D. Harper, P.T. Lansbury, Accelerated in vitro fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease, Nat. Med. 4 (1998) 1318–1320. doi:10.1038/3311.

[26] D.F. Lázaro, E.F. Rodrigues, R. Langohr, H. Shahpasandzadeh, T. Ribeiro, P. Guerreiro, et al., Systematic comparison of the effects of alpha-synuclein mutations on its oligomerization and aggregation, PLoS Genet. 10 (2014) e1004741. doi:10.1371/journal.pgen.1004741.

[27] S. Sahay, D. Ghosh, S. Dwivedi, A. Anoop, G.M. Mohite, M. Kombrabail, et al., Familial Parkinson disease-associated mutations alter the site-specific microenvironment and dynamics of α-synuclein, J. Biol. Chem. 290 (2015) 7804–7822. doi:10.1074/jbc.M114.598607.

[28] A. Giese, B. Bader, J. Bieschke, G. Schaffar, S. Odoy, P.J. Kahle, et al., Single particle detection and characterization of synuclein co-aggregation, Biochem. Biophys. Res. Commun. 333 (2005) 1202–1210. doi:10.1016/j.bbrc.2005.06.025.

[29] E.A. Mirecka, H. Shaykhalishahi, A. Gauhar, Sequestration of a b-Hairpin for Control of a-Synuclein Aggregation, Angew Chem Int …. (2014).

[30] H. Yu, W. Han, W. Ma, K. Schulten, Transient β-hairpin formation in α-synuclein monomer revealed by coarse-grained molecular dynamics simulation, J Chem Phys. 143 (2015) 243142. doi:10.1063/1.4936910.

[31] C.R. Bodner, C.M. Dobson, A. Bax, Multiple tight phospholipid-binding modes of alpha-synuclein revealed by solution NMR spectroscopy, J. Mol. Biol. 390 (2009) 775–790. doi:10.1016/j.jmb.2009.05.066.

[32] C.R. Bodner, A.S. Maltsev, C.M. Dobson, A. Bax, Differential phospholipid binding of alpha-synuclein variants implicated in Parkinson's disease revealed by solution NMR spectroscopy, Biochemistry. 49 (2010) 862–871. doi:10.1021/bi901723p.

[33] T. Bartels, L.S. Ahlstrom, A. Leftin, F. Kamp, C. Haass, M.F. Brown, et al., The N-terminus of the intrinsically disordered protein α-synuclein triggers membrane binding and helix folding, Biophys. J. 99 (2010) 2116–2124. doi:10.1016/j.bpj.2010.06.035.

[34] T. Högen, J. Levin, F. Schmidt, M. Caruana, N. Vassallo, H. Kretzschmar, et al., Two different binding modes of α-synuclein to lipid vesicles depending on its aggregation state, Biophys. J. 102 (2012) 1646–1655. doi:10.1016/j.bpj.2012.01.059.

[35] U. Dettmer, D. Selkoe, T. Bartels, New insights into cellular α-synuclein homeostasis in health and disease, Curr. Opin. Neurobiol. 36 (2016) 15–22. doi:10.1016/j.conb.2015.07.007.

[36] E.Y. Jones, J. Tormo, S.W. Reid, D.I. Stuart, Recognition surfaces of MHC class I, Immunol. Rev. 163 (1998) 121–128.

[37] F. Gejyo, T. Yamada, S. Odani, Y. Nakagawa, M. Arakawa, T. Kunitomo, et al., A new form of amyloid protein associated with chronic hemodialysis was identified as beta 2-microglobulin, Biochem. Biophys. Res. Commun. 129 (1985) 701–706.

[38] J.W. Becker, G.N. Reeke, Three-dimensional structure of beta 2-microglobulin, Proc Natl Acad Sci USA. 82 (1985) 4225–4229.

[39] F. Chiti, E. De Lorenzi, S. Grossi, P. Mangione, S. Giorgetti, G. Caccialanza, et al., A partially structured species of beta 2-microglobulin is significantly populated under physiological conditions and involved in fibrillogenesis, J. Biol. Chem. 276 (2001) 46714–46721. doi:10.1074/jbc.M107040200.

[40] A. Kameda, M. Hoshino, T. Higurashi, S. Takahashi, H. Naiki, Y. Goto, Nuclear magnetic resonance characterization of the refolding intermediate of beta2-microglobulin trapped by non-native prolyl peptide bond, J. Mol. Biol. 348 (2005) 383–397.

doi:10.1016/j.jmb.2005.02.050.

[41] T.R. Jahn, M.J. Parker, S.W. Homans, S.E. Radford, Amyloid formation under physiological conditions proceeds via a native-like folding intermediate, Nat. Struct. Mol. Biol. 13 (2006) 195–201. doi:10.1038/nsmb1058.

[42] T. Eichner, A.P. Kalverda, G.S. Thompson, S.W. Homans, S.E. Radford, Conformational conversion during amyloid formation at atomic resolution, Mol. Cell. 41 (2011) 161–172. doi:10.1016/j.molcel.2010.11.028.

[43] C.M. Eakin, A.J. Berman, A.D. Miranker, A native to amyloidogenic transition regulated by a backbone trigger, Nat. Struct. Mol. Biol. 13 (2006) 202–208. doi:10.1038/nsmb1068.

[44] J.P. Hodkinson, T.R. Jahn, S.E. Radford, A.E. Ashcroft, HDX-ESI-MS reveals enhanced conformational dynamics of the amyloidogenic protein beta(2)-microglobulin upon release from the MHC-1, J. Am. Soc. Mass Spectrom. 20 (2009) 278–286. doi:10.1016/j.jasms.2008.10.005.

[45] E. Rennella, A. Corazza, F. Fogolari, P. Viglino, S. Giorgetti, M. Stoppini, et al., Equilibrium unfolding thermodynamics of beta2-microglobulin analyzed through native-state H/D exchange, Biophys. J. 96 (2009) 169–179. doi:10.1529/biophysj.108.142448.

[46] C. Santambrogio, S. Ricagno, M. Colombo, A. Barbiroli, F. Bonomi, V. Bellotti, et al., DE-loop mutations affect beta2 microglobulin stability, oligomerization, and the low-pH unfolded form, Protein Science. 19 (2010) 1386–1394. doi:10.1002/pro.419.

[47] V. Bellotti, M. Stoppini, P. Mangione, M. Sunde, C. Robinson, L. Asti, et al., Beta2-microglobulin can be refolded into a native state from ex vivo amyloid fibrils, Eur. J. Biochem. 258 (1998) 61–67.

[48] V. Torbeev, M.-O. Ebert, J. Dolenc, D. Hilvert, Substitution of proline32 by α-methylproline preorganizes β2-microglobulin for oligomerization but not for aggregation into amyloids, J. Am. Chem. Soc. 137 (2015) 2524–2535. doi:10.1021/ja510109p.

[49] A. Natalello, P.P. Mangione, S. Giorgetti, R. Porcari, L. Marchese, I. Zorzoli, et al., Co-

fibrillogenesis of Wild-type and D76N β2-Microglobulin: THE CRUCIAL ROLE OF FIBRILLAR SEEDS, J. Biol. Chem. 291 (2016) 9678–9689. doi:10.1074/jbc.M116.720573.

[50] C. Liu, M.R. Sawaya, D. Eisenberg, β2-microglobulin forms three-dimensional domain-swapped amyloid fibrils with disulfide linkages, Nat. Struct. Mol. Biol. 18 (2011) 49–55. doi:10.1038/nsmb.1948.

[51] K. Domanska, S. Vanderhaegen, V. Srinivasan, E. Pardon, F. Dupeux, J.A. Marquez, et al., Atomic structure of a nanobody-trapped domain-swapped dimer of an amyloidogenic beta2-microglobulin variant, Proc. Natl. Acad. Sci. U.S.a. 108 (2011) 1314–1319. doi:10.1073/pnas.1008560108.

[52] Z. Hall, C. Schmidt, A. Politis, Uncovering the Early Assembly Mechanism for Amyloidogenic β2-Microglobulin Using Cross-linking and Native Mass Spectrometry, J. Biol. Chem. 291 (2016) 4626–4637. doi:10.1074/jbc.M115.691063.

[53] M.F. Calabrese, C.M. Eakin, J.M. Wang, A.D. Miranker, A regulatable switch mediates self-association in an immunoglobulin fold, Nat. Struct. Mol. Biol. 15 (2008) 965–971. doi:10.1038/nsmb.1483.

[54] P.G. Frank, Y.L. Marcel, Apolipoprotein A-I: structure-function relationships, J. Lipid Res. 41 (2000) 853–872.

[55] V. Narayanaswami, S.S.W. Szeto, R.O. Ryan, Lipid Association-induced N- and C-terminal Domain Reorganization in Human Apolipoprotein E3, J. Biol. Chem. 276 (2001) 37853–37860.

[56] V. Raussens, C.A. Fisher, E. Goormaghtigh, R.O. Ryan, J.-M. Ruysschaert, The Low Density Lipoprotein Receptor Active Conformation of Apolipoprotein E HELIX ORGANIZATION IN N-TERMINAL DOMAIN-PHOSPHOLIPID DISC PARTICLES, J. Biol. Chem. 273 (1998) 25825–25830. doi:10.1074/jbc.273.40.25825.

[57] D.M. Hatters, G.J. Howlett, The structural basis for amyloid formation by plasma

apolipoproteins: a review, Eur Biophys J. 31 (2002) 2–8. doi:10.1007/s002490100172.

[58] B. Lu, J.A. Morrow, K.H. Weisgraber, Conformational reorganization of the four-helix bundle of human apolipoprotein E in binding to phospholipid, J. Biol. Chem. 275 (2000) 20775–20781. doi:10.1074/jbc.M003508200.

[59] P. Mendoza-Espinosa, D. Montalvan-Sorrosa, V. García-González, A. Moreno, R. Castillo, J. Mas-Oliva, Microenvironmentally controlled secondary structure motifs of apolipoprotein A-I derived peptides, Mol. Cell. Biochem. 393 (2014) 99–109. doi:10.1007/s11010-014-2050-2.

[60] A. Arciello, R. Piccoli, D.M. Monti, Apolipoprotein A-I: the dual face of a protein, FEBS Lett. 590 (2016) 4171–4179. doi:10.1002/1873-3468.12468.

[61] M. Das, X. Mei, S. Jayaraman, D. Atkinson, O. Gursky, Amyloidogenic mutations in human apolipoprotein A-I are not necessarily destabilizing - a common mechanism of apolipoprotein A-I misfolding in familial amyloidosis and atherosclerosis, Febs J. 281 (2014) 2525–2542. doi:10.1111/febs.12809.

[62] J. Petrlova, A. Bhattacherjee, W. Boomsma, S. Wallin, J.O. Lagerstedt, A. Irbäck, Conformational and aggregation properties of the 1–93 fragment of apolipoprotein A-I, Protein Science. 23 (2014) 1559–1571. doi:10.1002/pro.2534.

[63] S. Raimondi, F. Guglielmi, S. Giorgetti, S. Di Gaetano, A. Arciello, D.M. Monti, et al., Effects of the known pathogenic mutations on the aggregation pathway of the amyloidogenic peptide of apolipoprotein A-I, J. Mol. Biol. 407 (2011) 465–476. doi:10.1016/j.jmb.2011.01.044.

[64] A. Arciello, N. De Marco, R. Del Giudice, F. Guglielmi, P. Pucci, A. Relini, et al., Insights into the fate of the N-terminal amyloidogenic polypeptide of ApoA-I in cultured target cells, J. Cell. Mol. Med. 15 (2011) 2652–2663. doi:10.1111/j.1582-4934.2011.01271.x.

[65] E. Adachi, H. Nakajima, C. Mizuguchi, P. Dhanasekaran, H. Kawashima, K. Nagao, et

al., Dual role of an N-terminal amyloidogenic mutation in apolipoprotein A-I: destabilization of helix bundle and enhancement of fibril formation, J. Biol. Chem. 288 (2013) 2848–2856. doi:10.1074/jbc.M112.428052.

[66] J.O. Lagerstedt, G. Cavigiolio, L.M. Roberts, H.-S. Hong, L.-W. Jin, P.G. Fitzgerald, et al., Mapping the structural transition in an amyloidogenic apolipoprotein A-I, Biochemistry. 46 (2007) 9693–9699. doi:10.1021/bi7005493.

[67] M.G. Sorci-Thomas, M.J. Thomas, The Effects of Altered Apolipoprotein A-I Structure on Plasma HDL Concentration, Trends in Cardiovascular Medicine. 12 (2002) 121–128. doi:10.1016/S1050-1738(01)00163-3.

[68] M. Das, C.J. Wilson, X. Mei, T.E. Wales, J.R. Engen, O. Gursky, Structural Stability and Local Dynamics in Disease-Causing Mutants of Human Apolipoprotein A-I: What Makes the Protein Amyloidogenic? J. Mol. Biol. 428 (2016) 449–462. doi:10.1016/j.jmb.2015.10.029.

[69] R. Del Giudice, A. Arciello, F. Itri, A. Merlino, M. Monti, M. Buonanno, et al., Protein conformational perturbations in hereditary amyloidosis: Differential impact of single point mutations in ApoAI amyloidogenic variants, Biochim. Biophys. Acta. 1860 (2016) 434–444. doi:10.1016/j.bbagen.2015.10.019.

[70] J. Petrlova, T. Duong, M.C. Cochran, A. Axelsson, M. Mörgelin, L.M. Roberts, et al., The fibrillogenic L178H variant of apolipoprotein A-I forms helical fibrils, J. Lipid Res. 53 (2012) 390–398. doi:10.1194/jlr.M020883.

[71] H.N. Motlagh, J.O. Wrabl, J. Li, V.J. Hilser, The ensemble nature of allostery, Nature. 508 (2014) 331–339. doi:10.1038/nature13001.

[72] A.J. Trexler, E. Rhoades, Function and dysfunction of α-synuclein: probing conformational changes and aggregation by single molecule fluorescence, Mol. Neurobiol. 47 (2013) 622–631. doi:10.1007/s12035-012-8338-x.

[73] A. Nath, M. Sammalkorpi, D.C. DeWitt, A.J. Trexler, S. Elbaum-Garfinkle, C.S. O'Hern,

et al., The conformational ensembles of α-synuclein and tau: combining single-molecule FRET and simulations, Biophys. J. 103 (2012) 1940–1949. doi:10.1016/j.bpj.2012.09.032.

[74] M. Schwalbe, V. Ozenne, S. Bibow, M. Jaremko, L. Jaremko, M. Gajda, et al., Predictive Atomic Resolution Descriptions of Intrinsically Disordered hTau40 and α-Synuclein in Solution from NMR and Small Angle Scattering, Structure. 22 (2014) 238–249. doi:10.1016/j.str.2013.10.020.

[75] Trinucleotide repeats: mechanisms and pathophysiology, 1 (2000) 281–328. doi:10.1146/annurev.genom.1.1.281.

[76] S. Chen, V. Berthelier, J.B. Hamilton, B. O'Nuallain, R. Wetzel, Amyloid-like features of polyglutamine aggregates and their assembly kinetics, Biochemistry. 41 (2002) 7391–7399. doi:10.1021/bi011772q.

[77] R. Wetzel, Physical chemistry of polyglutamine: intriguing tales of a monotonous sequence, J. Mol. Biol. 421 (2012) 466–490. doi:10.1016/j.jmb.2012.01.030.

[78] A.M. Ellisdon, B. Thomas, S.P. Bottomley, The two-stage pathway of ataxin-3 fibrillogenesis involves a polyglutamine-independent step, J. Biol. Chem. 281 (2006) 16888–16896. doi:10.1074/jbc.M601470200.

[79] A.K. Thakur, M. Jayaraman, R. Mishra, M. Thakur, V.M. Chellgren, I.-J.L. Byeon, et al., Polyglutamine disruption of the huntingtin exon 1 N terminus triggers a complex aggregation mechanism, Nat. Struct. Mol. Biol. 16 (2009) 380–389. doi:10.1038/nsmb.1570.

[80] Z. Ignatova, A.K. Thakur, R. Wetzel, L.M. Gierasch, In-cell aggregation of a polyglutamine-containing chimera is a multistep process initiated by the flanking sequence, J. Biol. Chem. 282 (2007) 36736–36743. doi:10.1074/jbc.M703682200.

[81] S. Tam, C. Spiess, W. Auyeung, L. Joachimiak, B. Chen, M.A. Poirier, et al., The chaperonin TRiC blocks a huntingtin sequence element that promotes the conformational

switch to aggregation, Nat. Struct. Mol. Biol. 16 (2009) 1279–1285. doi:10.1038/nsmb.1700.

[82] E. Monsellier, V. Redeker, G. Ruiz-Arlandis, L. Bousset, R. Melki, Molecular interaction between the chaperone Hsc70 and the N-terminal flank of huntingtin exon 1 modulates aggregation, J. Biol. Chem. 290 (2015) 2560–2576. doi:10.1074/jbc.M114.603332.

[83] C.W. Bugg, J.M. Isas, T. Fischer, P.H. Patterson, R. Langen, Structural features and domain organization of huntingtin fibrils, J. Biol. Chem. 287 (2012) 31739–31746. doi:10.1074/jbc.M112.353839.

[84] C.L. Hoop, H.-K. Lin, K. Kar, Z. Hou, M.A. Poirier, R. Wetzel, et al., Polyglutamine amyloid core boundaries and flanking domain dynamics in huntingtin fragment fibrils determined by solid-state nuclear magnetic resonance, Biochemistry. 53 (2014) 6653–6666. doi:10.1021/bi501010q.

[85] J.M. Isas, R. Langen, A.B. Siemer, Solid-State Nuclear Magnetic Resonance on the Static and Dynamic Domains of Huntingtin Exon-1 Fibrils, Biochemistry. 54 (2015) 3942–3949. doi:10.1021/acs.biochem.5b00281.

[86] B. Hollenbach, E. Scherzinger, K. Schweiger, R. Lurz, H. Lehrach, E.E. Wanker, Aggregation of truncated GST-HD exon 1 fusion proteins containing normal range and expanded glutamine repeats, Philos. Trans. R. Soc. Lond., B, Biol. Sci. 354 (1999) 991–994. doi:10.1098/rstb.1999.0450.

[87] A. Bhattacharyya, A.K. Thakur, V.M. Chellgren, G. Thiagarajan, A.D. Williams, B.W. Chellgren, et al., Oligoproline Effects on Polyglutamine Conformation and Aggregation, J. Mol. Biol. 355 (2006) 524–535. doi:10.1016/j.jmb.2005.10.053.

[88] G. Darnell, J.P.R.O. Orgel, R. Pahl, S.C. Meredith, Flanking polyproline sequences inhibit beta-sheet structure in polyglutamine segments by inducing PPII-like helix structure, J. Mol. Biol. 374 (2007) 688–704. doi:10.1016/j.jmb.2007.09.023.

[89] R. Porcari, C. Proukakis, C.A. Waudby, B. Bolognesi, P.P. Mangione, J.F.S. Paton, et al.,

The H50Q Mutation Induces a 10-fold Decrease in the Solubility of α-Synuclein, J. Biol. Chem. 290 (2015) 2395–2404. doi:10.1074/jbc.M114.610527.

[90] N.W. Kelley, X. Huang, S. Tam, C. Spiess, J. Frydman, V.S. Pande, The Predicted Structure of the Headpiece of the Huntingtin Protein and Its Implications on Huntingtin Aggregation, J. Mol. Biol. 388 (2009) 919–927. doi:10.1016/j.jmb.2009.01.032.

[91] M. Jayaraman, R. Kodali, B. Sahoo, A.K. Thakur, A. Mayasundari, R. Mishra, et al., Slow amyloid nucleation via α-helix-rich oligomeric intermediates in short polyglutamine-containing huntingtin fragments, J. Mol. Biol. 415 (2012) 881–899. doi:10.1016/j.jmb.2011.12.010.

[92] J.R. Arndt, S.G. Kondalaji, M.M. Maurer, A. Parker, J. Legleiter, S.J. Valentine, Huntingtin N-Terminal Monomeric and Multimeric Structures Destabilized by Covalent Modification of Heteroatomic Residues, Biochemistry. 54 (2015) 4285–4296. doi:10.1021/acs.biochem.5b00478.

[93] V.N. Sivanandam, M. Jayaraman, C.L. Hoop, R. Kodali, R. Wetzel, P.C.A. van der Wel, The Aggregation-Enhancing Huntingtin N-Terminus Is Helical in Amyloid Fibrils, Journal of the …. 133 (2011) 4558–4566. doi:10.1021/ja110715f.

[94] C.L. Hoop, H.-K. Lin, K. Kar, G. Magyarfalvi, J.M. Lamley, J.C. Boatz, et al., Huntingtin exon 1 fibrils feature an interdigitated β-hairpin-based polyglutamine core, Proc. Natl. Acad. Sci. U.S.a. 113 (2016) 1546–1551. doi:10.1073/pnas.1521933113.

[95] K.R. Choudhury, N.P. Bhattacharyya, Chaperone protein HYPK interacts with the first 17 amino acid region of Huntingtin and modulates mutant HTT-mediated aggregation and cytotoxicity, Biochem. Biophys. Res. Commun. 456 (2015) 66–73. doi:10.1016/j.bbrc.2014.11.035.

[96] R. Mishra, C.L. Hoop, R. Kodali, B. Sahoo, P.C.A. van der Wel, R. Wetzel, Serine Phosphorylation Suppresses Huntingtin Amyloid Accumulation by Altering Protein Aggregation Properties, J. Mol. Biol. 424 (2012) 1–14. doi:10.1016/j.jmb.2012.09.011.

[97] X. Gu, E.R. Greiner, R. Mishra, R. Kodali, A. Osmand, S. Finkbeiner, et al., Serines 13 and 16 are critical determinants of full-length human mutant huntingtin induced disease pathogenesis in HD mice, Neuron. 64 (2009) 828–840. doi:10.1016/j.neuron.2009.11.020.

[98] A. Di Pardo, V. Maglione, M. Alpaugh, M. Horkey, R.S. Atwal, J. Sassone, et al., Ganglioside GM1 induces phosphorylation of mutant huntingtin and restores normal motor behavior in Huntington disease mice, Proc. Natl. Acad. Sci. U.S.a. 109 (2012) 3528–3533. doi:10.1073/pnas.1114502109.

[99] L. Masino, G. Nicastro, R.P. Menon, F. Dal Piaz, L. Calder, A. Pastore, Characterization of the structure and the amyloidogenic properties of the Josephin domain of the polyglutamine-containing protein ataxin-3, J. Mol. Biol. 344 (2004) 1021–1035. doi:10.1016/j.jmb.2004.09.065.

[100] A.M. Ellisdon, M.C. Pearce, S.P. Bottomley, Mechanisms of ataxin-3 misfolding and fibril formation: kinetic analysis of a disease-associated polyglutamine protein, J. Mol. Biol. 368 (2007) 595–605. doi:10.1016/j.jmb.2007.02.058.

[101] M. Cushman-Nick, N.M. Bonini, J. Shorter, Hsp104 suppresses polyglutamine-induced degeneration post onset in a drosophila MJD/SCA3 model, PLoS Genet. 9 (2013) e1003781. doi:10.1371/journal.pgen.1003781.

[102] C.A. Scarff, B. Almeida, J. Fraga, S. Macedo-Ribeiro, S.E. Radford, A.E. Ashcroft, Examination of Ataxin-3 (atx-3) Aggregation by Structural Mass Spectrometry Techniques: A Rationale for Expedited Aggregation upon Polyglutamine (polyQ) Expansion, Mol. Cell Proteomics. 14 (2015) 1241–1253. doi:10.1074/mcp.M114.044610.

[103] C.J. Lupton, D.L. Steer, P.L. Wintrode, S.P. Bottomley, V.A. Hughes, A.M. Ellisdon, Enhanced molecular mobility of ordinarily structured regions drives polyglutamine disease, J. Biol. Chem. 290 (2015) 24190–24200. doi:10.1074/jbc.M115.659532.

[104] M.A. Deriu, G. Grasso, G. Licandro, A. Danani, D. Gallo, J.A. Tuszynski, et al.,

Investigation of the Josephin Domain protein-protein interaction by molecular dynamics, PLoS ONE. 9 (2014) e108677. doi:10.1371/journal.pone.0108677.

[105] G. Nicastro, L. Masino, V. Esposito, R.P. Menon, A. De Simone, F. Fraternali, et al., Josephin domain of ataxin-3 contains two distinct ubiquitin-binding sites, Biopolymers. 91 (2009) 1203–1214. doi:10.1002/bip.21210.

[106] L. Masino, G. Nicastro, L. Calder, M. Vendruscolo, A. Pastore, Functional interactions as a survival strategy against abnormal aggregation, Faseb J. 25 (2011) 45–54. doi:10.1096/fj.10-161208.

[107] M. Das, O. Gursky, Amyloid-Forming Properties of Human Apolipoproteins: Sequence Analyses and Structural Insights, Adv. Exp. Med. Biol. 855 (2015) 175–211. doi:10.1007/978-3-319-17344-3_8.

[108] M.R. Sawaya, S. Sambashivan, R. Nelson, M.I. Ivanova, S.A. Sievers, M.I. Apostol, et al., Atomic structures of amyloid cross-β spines reveal varied steric zippers, Nature. 447 (2007) 453–457. doi:10.1038/nature05695.

[109] L. Hou, H. Shao, Y. Zhang, H. Li, N.K. Menon, E.B. Neuhaus, et al., Solution NMR Studies of the Aβ(1−40) and Aβ(1−42) Peptides Establish that the Met35 Oxidation State Affects the Mechanism of Amyloid Formation, J. Am. Chem. Soc. 126 (2004) 1992–2005. doi:10.1021/ja036813f.

[110] A.M. Brown, J.A. Lemkul, N. Schaum, D.R. Bevan, Simulations of monomeric amyloid β-peptide (1–40) with varying solution conditions and oxidation state of Met35: Implications for aggregation, Archives of Biochemistry and Biophysics. 545 (2014) 44–52. doi:10.1016/j.abb.2014.01.002.

[111] M.T. Colvin, R. Silvers, Q.Z. Ni, T.V. Can, I. Sergeyev, M. Rosay, et al., Atomic Resolution Structure of Monomorphic Aβ42 Amyloid Fibrils, Journal of the …. 138 (2016) 9663–9674. doi:10.1021/jacs.6b05129.

[112] V.N. Uversky, G. Yamin, L.A. Munishkina, M.A. Karymov, I.S. Millett, S. Doniach, et

al., Effects of nitration on the structure and aggregation of alpha-synuclein, Brain Res. Mol. Brain Res. 134 (2005) 84–102. doi:10.1016/j.molbrainres.2004.11.014.

[113] R. Hodara, E.H. Norris, B.I. Giasson, A.J. Mishizen-Eberz, D.R. Lynch, V.M.Y. Lee, et al., Functional consequences of alpha-synuclein tyrosine nitration: diminished binding to lipid vesicles and increased fibril formation, J. Biol. Chem. 279 (2004) 47746–47753. doi:10.1074/jbc.M408906200.

[114] H. Zheng, E.H. Koo, Biology and pathophysiology of the amyloid precursor protein, Molecular Neurodegeneration 2011 6:1. 6 (2011) 27. doi:10.1186/1750-1326-6-27.

[115] S.W. Snyder, U.S. Ladror, W.S. Wade, G.T. Wang, L.W. Barrett, E.D. Matayoshi, et al., Amyloid-beta aggregation: selective inhibition of aggregation in mixtures of amyloid with different chain lengths, Biophys. J. 67 (1994) 1216–1228. doi:10.1016/S0006-3495(94)80591-0.

[116] K.A. Ball, A.H. Phillips, D.E. Wemmer, T. Head-Gordon, Differences in β-strand populations of monomeric Aβ40 and Aβ42, Biophys. J. 104 (2013) 2714–2724. doi:10.1016/j.bpj.2013.04.056.

[117] P. Misra, R. Kodali, S. Chemuru, K. Kar, R. Wetzel, Rapid α-oligomer formation mediated by the Aβ C terminus initiates an amyloid assembly pathway, Nat Commun. 7 (2016) 12419. doi:10.1038/ncomms12419.

[118] C.-C. Chang, J.C. Althaus, C.J.L. Carruthers, M.A. Sutton, D.G. Steel, A. Gafni, Synergistic interactions between Alzheimer's Aβ40 and Aβ42 on the surface of primary neurons revealed by single molecule microscopy, PLoS ONE. 8 (2013) e82139. doi:10.1371/journal.pone.0082139.

[119] R. Kayed, E. Head, J.L. Thompson, T.M. McIntire, S.C. Milton, C.W. Cotman, et al., Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis, Science. 300 (2003) 486–489. doi:10.1126/science.1079469.

[120] C. Haass, D.J. Selkoe, Soluble protein oligomers in neurodegeneration: lessons from the

Alzheimer's amyloid beta-peptide, Nat. Rev. Mol. Cell Biol. 8 (2007) 101–112. doi:10.1038/nrm2101.

[121] F. Liu, B. Li, E.-J. Tung, I. Grundke-Iqbal, K. Iqbal, C.-X. Gong, Site-specific effects of tau phosphorylation on its microtubule assembly activity and self-aggregation, Eur. J. Neurosci. 26 (2007) 3429–3436. doi:10.1111/j.1460-9568.2007.05955.x.

[122] M.G. Spillantini, M. Goedert, Tau pathology and neurodegeneration, The Lancet Neurology. 12 (2013) 609–622. doi:10.1016/S1474-4422(13)70090-5.

[123] A.J. Lyons, N.S. Gandhi, R.L. Mancera, Molecular dynamics simulation of the phosphorylation-induced conformational changes of a tau peptide fragment, Proteins. 82 (2014) 1907–1923. doi:10.1002/prot.24544.

[124] G.T. Bramblett, M. Goedert, R. Jakes, S.E. Merrick, J.Q. Trojanowski, V.M.Y. Lee, Abnormal tau phosphorylation at Ser396 in alzheimer's disease recapitulates development and contributes to reduced microtubule binding, Neuron. 10 (1993) 1089–1099. doi:10.1016/0896-6273(93)90057-X.

[125] J. Eidenmüller, T. Fath, A. Hellwig, J. Reed, E. Sontag, R. Brandt, Structural and functional implications of tau hyperphosphorylation: information from phosphorylation-mimicking mutated tau proteins, Biochemistry. 39 (2000) 13166–13175.

[126] V.N. Uversky, A.L. Fink, Conformational constraints for amyloid fibrillation: the importance of being unfolded, Biochim. Biophys. Acta. 1698 (2004) 131–153. doi:10.1016/j.bbapap.2003.12.008.

[127] M. Schwalbe, H. Kadavath, J. Biernat, V. Ozenne, M. Blackledge, E. Mandelkow, et al., Structural Impact of Tau Phosphorylation at Threonine 231, Structure. 23 (2015) 1448–1458. doi:10.1016/j.str.2015.06.002.

[128] T.J. Cohen, J.L. Guo, D.E. Hurtado, L.K. Kwong, I.P. Mills, J.Q. Trojanowski, et al., The acetylation of tau inhibits its function and promotes pathological tau aggregation, Nat Commun. 2 (2011) 252. doi:10.1038/ncomms1255.

[129] S. Jeganathan, M. von Bergen, E.-M. Mandelkow, E. Mandelkow, The natively unfolded character of tau and its aggregation to Alzheimer-like paired helical filaments, Biochemistry. 47 (2008) 10526–10539. doi:10.1021/bi800783d.

[130] N. Sibille, I. Huvent, C. Fauquant, D. Verdegem, L. Amniai, A. Leroy, et al., Structural characterization by nuclear magnetic resonance of the impact of phosphorylation in the proline-rich region of the disordered Tau protein, Proteins. 80 (2012) 454–462. doi:10.1002/prot.23210.

[131] K. Nakamura, A. Greenwood, L. Binder, E.H. Bigio, S. Denial, L. Nicholson, et al., Proline isomer-specific antibodies reveal the early pathogenic tau conformation in Alzheimer's disease, Cell. 149 (2012) 232–244. doi:10.1016/j.cell.2012.02.016.

[132] A. Barman, D. Hamelberg, Loss of intramolecular electrostatic interactions and limited conformational ensemble may promote self-association of cis-tau peptide, Proteins. 83 (2015) 436–444. doi:10.1002/prot.24740.

[133] L. Xu, J. Zheng, M. Margittai, R. Nussinov, B. Ma, How Does Hyperphopsphorylation Promote Tau Aggregation and Modulate Filament Structure and Stability? ACS Chem Neurosci. 7 (2016) 565–575. doi:10.1021/acschemneuro.5b00294.

[134] A. Abraha, N. Ghoshal, T.C. Gamblin, V. Cryns, R.W. Berry, J. Kuret, et al., C-terminal inhibition of tau assembly in vitro and in Alzheimer's disease, J. Cell. Sci. 113 Pt 21 (2000) 3737–3745.

[135] C. Haase, J.T. Stieler, T. Arendt, M. Holzer, Pseudophosphorylation of tau protein alters its ability for self-aggregation, J. Neurochem. 88 (2004) 1509–1520.

[136] R.W. Berry, A. Abraha, S. Lagalwar, N. LaPointe, T.C. Gamblin, V.L. Cryns, et al., Inhibition of tau polymerization by its carboxy-terminal caspase cleavage fragment, Biochemistry. 42 (2003) 8325–8331. doi:10.1021/bi027348m.

[137] D. Kanmert, A. Cantlon, C.R. Muratore, M. Jin, T.T. O'Malley, G. Lee, et al., C-Terminally Truncated Forms of Tau, But Not Full-Length Tau or Its C-Terminal

Fragments, Are Released from Neurons Independently of Cell Death, J. Neurosci. 35 (2015) 10851–10865. doi:10.1523/JNEUROSCI.0387-15.2015.

[138] A. Ben-Zvi, E.A. Miller, R.I. Morimoto, Collapse of proteostasis represents an early molecular event in Caenorhabditis elegans aging, Proc. Natl. Acad. Sci. U.S.a. 106 (2009) 14914–14919. doi:10.1073/pnas.0902882106.

[139] G.S. Kaminski Schierle, S. van de Linde, M. Erdelyi, E.K. Esbjörner, T. Klein, E. Rees, et al., In situ measurements of the formation and morphology of intracellular β-amyloid fibrils by super-resolution fluorescence imaging, J. Am. Chem. Soc. 133 (2011) 12902–12905. doi:10.1021/ja201651w.

[140] E.K. McGuire, M. Motskin, B. Bolognesi, S.D. Bergin, T.P.J. Knowles, J. Skepper, et al., Selenium-enhanced electron microscopic imaging of different aggregate forms of a segment of the amyloid β peptide in cells, ACS Nano. 6 (2012) 4740–4747. doi:10.1021/nn204859e.

[141] J. Ries, V. Udayar, A. Soragni, S. Hornemann, K.P.R. Nilsson, R. Riek, et al., Superresolution imaging of amyloid fibrils with binding-activated probes, ACS Chem Neurosci. 4 (2013) 1057–1061. doi:10.1021/cn400091m.

[142] D. Pinotsi, C.H. Michel, A.K. Buell, R.F. Laine, P. Mahou, C.M. Dobson, et al., Nanoscopic insights into seeding mechanisms and toxicity of α-synuclein species in neurons, Proc Natl Acad Sci USA. 113 (2016) 3815–3819. doi:10.1073/pnas.1516546113.

[143] T.S. Ulmer, A. Bax, N.B. Cole, R.L. Nussbaum, Structure and dynamics of micelle-bound human alpha-synuclein, J. Biol. Chem. 280 (2005) 9595–9603. doi:10.1074/jbc.M411805200.

[144] O.Y. Borbulevych, P. Do, B.M. Baker, Structures of native and affinity-enhanced WT1 epitopes bound to HLA-A*0201: implications for WT1-based cancer therapeutics, Mol. Immunol. 47 (2010) 2519–2524. doi:10.1016/j.molimm.2010.06.005.

[145] C.H. Trinh, D.P. Smith, A.P. Kalverda, S.E.V. Phillips, S.E. Radford, Crystal structure of monomeric human beta-2-microglobulin reveals clues to its amyloidogenic properties, Proc Natl Acad Sci USA. 99 (2002) 9771–9776. doi:10.1073/pnas.152337399.

[146] S. Vanderhaegen, M. Fislage, K. Domanska, W. Versées, E. Pardon, V. Bellotti, et al., Structure of an early native-like intermediate of β2-microglobulin amyloidogenesis, Protein Science. 22 (2013) 1349–1357. doi:10.1002/pro.2321.

[147] M.T. Mazhab-Jafari, C.B. Marshall, M.J. Smith, G.M.C. Gasmi-Seabrook, P.B. Stathopulos, F. Inagaki, et al., Oncogenic and RASopathy-associated K-RAS mutations relieve membrane-dependent occlusion of the effector-binding site, Proc. Natl. Acad. Sci. U.S.a. 112 (2015) 6625–6630. doi:10.1073/pnas.1419895112.

[148] X. Mei, D. Atkinson, Crystal structure of C-terminal truncated apolipoprotein A-I reveals the assembly of high density lipoprotein (HDL) by dimerization, J. Biol. Chem. 286 (2011) 38570–38582. doi:10.1074/jbc.M111.260422.

[149] M.W. Kim, Y. Chelliah, S.W. Kim, Z. Otwinowski, I. Bezprozvanny, Secondary structure of Huntingtin amino-terminal region, Structure. 17 (2009) 1205–1212. doi:10.1016/j.str.2009.08.002.

[150] S.D. Weeks, K.C. Grasty, L. Hernandez-Cuebas, P.J. Loll, Crystal structure of a Josephin-ubiquitin complex: evolutionary restraints on ataxin-3 deubiquitinating activity, J. Biol. Chem. 286 (2011) 4555–4565. doi:10.1074/jbc.M110.177360.

[151] J. Rossjohn, R. Cappai, S.C. Feil, A. Henry, W.J. McKinstry, D. Galatis, et al., Crystal structure of the N-terminal, growth factor-like domain of Alzheimer amyloid precursor protein, Nat. Struct. Biol. 6 (1999) 327–331. doi:10.1038/7562.

[152] K.J. Barnham, W.J. McKinstry, G. Multhaup, D. Galatis, C.J. Morton, C.C. Curtain, et al., Structure of the Alzheimer's disease amyloid precursor protein copper binding domain. A regulator of neuronal copper homeostasis, J. Biol. Chem. 278 (2003) 17401–17407. doi:10.1074/jbc.M300629200.

[153] Y. Wang, Y. Ha, The X-ray structure of an antiparallel dimer of the human amyloid precursor protein E2 domain, Mol. Cell. 15 (2004) 343–353. doi:10.1016/j.molcel.2004.06.037.

[154] O. Crescenzi, S. Tomaselli, R. Guerrini, S. Salvadori, A.M. D'Ursi, P.A. Temussi, et al., Solution structure of the Alzheimer amyloid beta-peptide (1-42) in an apolar microenvironment. Similarity with a virus fusion domain, Eur. J. Biochem. 269 (2002) 5642–5648.

[155] M. Coles, W. Bicknell, A.A. Watson, D.P. Fairlie, D.J. Craik, Solution structure of amyloid beta-peptide(1-40) in a water-micelle environment. Is the membrane-spanning domain where we think it is? Biochemistry. 37 (1998) 11064–11077. doi:10.1021/bi972979f.

Figures legends

Fig 1. Aggregation triggered by global deprotection of amyloidogenic regions. (A) α-syn is an IDP comprised of an N-terminus, the amyloidogenic NAC domain and a C-terminal tail that forms inhibitory interactions with the NAC domain (upper left). The primary sequence of α-syn is shown (lower left), highlighting the amyloidogenic NAC domain (red), residues 46-54 that form part of the core of pre-amyloid fibrils (light blue) and PD-associated mutations (A30P, E46K, H50Q, A53T, dark blue). (B) α-syn acquires additional secondary structure upon interaction with the cell membrane (PDB ID 1XQ8 [143]), and (C) the equilibrium between the unstructured and partially-structured forms is believed to be pivotal in formation of amyloid fibrils (PDB ID 2N0A [4]) (right).

Fig. 2. Aggregation triggered by deprotection of amyloidogenic regions. (A) β2m binds MHC I (green) to aid in antigen presentation (PDB ID 3MYJ [144]). (B) Monomeric β2m (PDB ID 1LDS [145]) is β-sheet-rich and adopts an IgG-like fold. (C) Conformational rearrangements occur upon truncation of the N-terminal six residues (ΔN6, PDB ID 2XKU [42]) to increase rates of aggregation. (D) Isomerisation of the X-Pro32 bond to a *trans* state to form the aggregation intermediate, I_T (PDB ID 4KDT [146]) induces a global conformational change to expose the central hydrophobic region (lower middle). Further amyloid formation has been proposed to occur by two alternate domain-swap theories, involving: (E) exchange of the D-E loop (dark purple, PDB ID 3LOW [50]) and oligomer growth by runaway domain swapping; or (F) exchange of the F-G loop (light purple, PDB ID 2X89 [51]) and oligomer growth by dimer addition (right). The primary sequence of β2m is shown (lower left), highlighting the amyloidogenic region (red), N-terminal six residues (light blue), Pro32 (dark blue) and two loop regions proposed as the domain-swapped regions in alternate hypotheses (light and dark purple).

Fig. 3. Propagating structural perturbations initiate aggregation. (A) ApoA-I forms helical dimers to bind lipids (PDB ID 2MSC [147]) (upper left). Dimerisation from lipid-free apoA-I involves unfurling of the N-terminal helical bundle through conformational change within the hinge region (light blue) (PDB ID 3R2P, monomer model from [148]) (upper middle). (B) Aggregation occurs first through rearrangement of N-terminal amyloidogenic regions to β-structures, which then propagate to downstream regions (right). This structural propagation is facilitated by disease-associated point mutations. (C) Proteolysis downstream of Leu159 targets the protein for cellular degradation. The primary sequence of apoA-I is shown, highlighting amyloidogenic residues 14-22, 53-58, 69-72 and 227-232 (red), the hinge region (light blue) and Leu159 (dark blue).

Fig 4. Distal protein domains nucleate aggregation. (A) Aggregation of htt exon-1 (PDB ID 3IOW [149]) occurs firstly through self-association of the helical htt^{NT} (upper left), followed by aggregation of the extended polyQ region (right) to form β-rich aggregates. The primary sequence of htt exon-1 is shown (lower left), highlighting the htt^{NT} (dark blue), polyQ tract (red) and polyP region (light blue). (B) Ataxin-3 aggregation occurs through JD self-association (PDB ID 3O65 [150]), whereby helix-α4 (dark blue) movement and self-association forms the JD core (upper left). Expansion of the polyQ tract (red) increases the dynamics of helix-α4, increasing the rate of JD-dependent fibrilisation and allowing polyQ-dependent aggregation (upper right). The primary sequence of ataxin-3 is shown (lower middle), highlighting helix-α4 (residues 76-89) and polyQ tract.

Fig. 5. Post-translational modifications as nucleation triggers. (A) Proteolytic processing of APP (PDB IDs 1MWP [151], 1OWT [152], 3NYL [153], 1IYT [154]) by β and γ secretases (upper left) releases Aβ (centre, upper PDB ID 1BA4 [155], lower PDB ID 1IYT) which has a high propensity to aggregate to fibrils (right, PDB ID 5KK3 [111]) and amyloid, dependent on the length of its C-terminus (light blue) and oxidation of Met35 (dark blue). The primary sequence of Aβ is shown

(lower centre), highlighting the amyloidogenic region (red), Met35 and C-terminal extension for $A\beta_{42}$. (B) Under normal physiological conditions, tau binds tubulin through its microtubule binding regions (MBR, green) to maintain the structure of microtubules (left). Upon phosphorylation, aggregation-prone residues (red) within the MBRs are freed from inhibitory interactions with the C-terminus, allowing tau aggregation (right). The rate of aggregation is further increased by N-terminal and C-terminal proteolytic cleavage. The primary sequence of tau is shown (lower centre), highlighting the amyloidogenic residues.

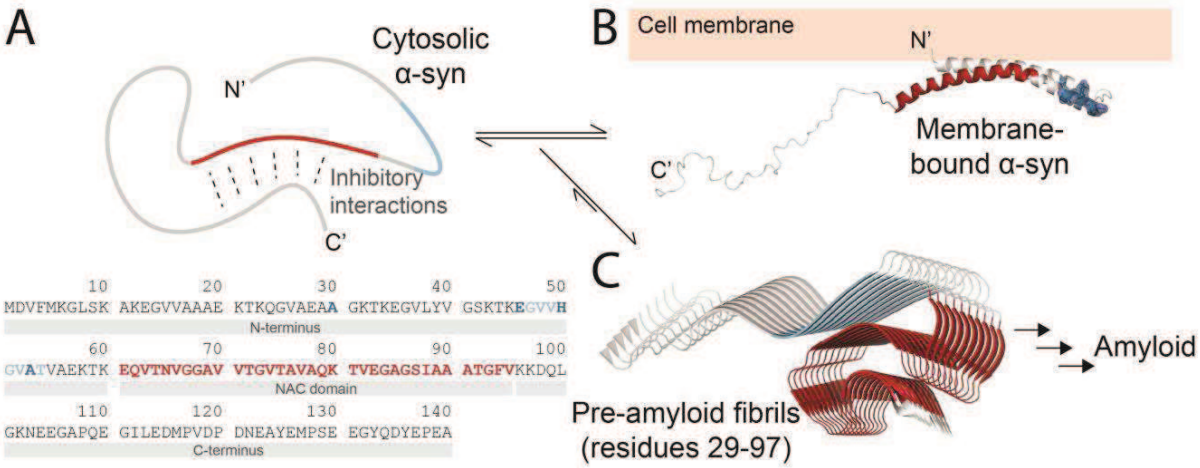


Figure 1

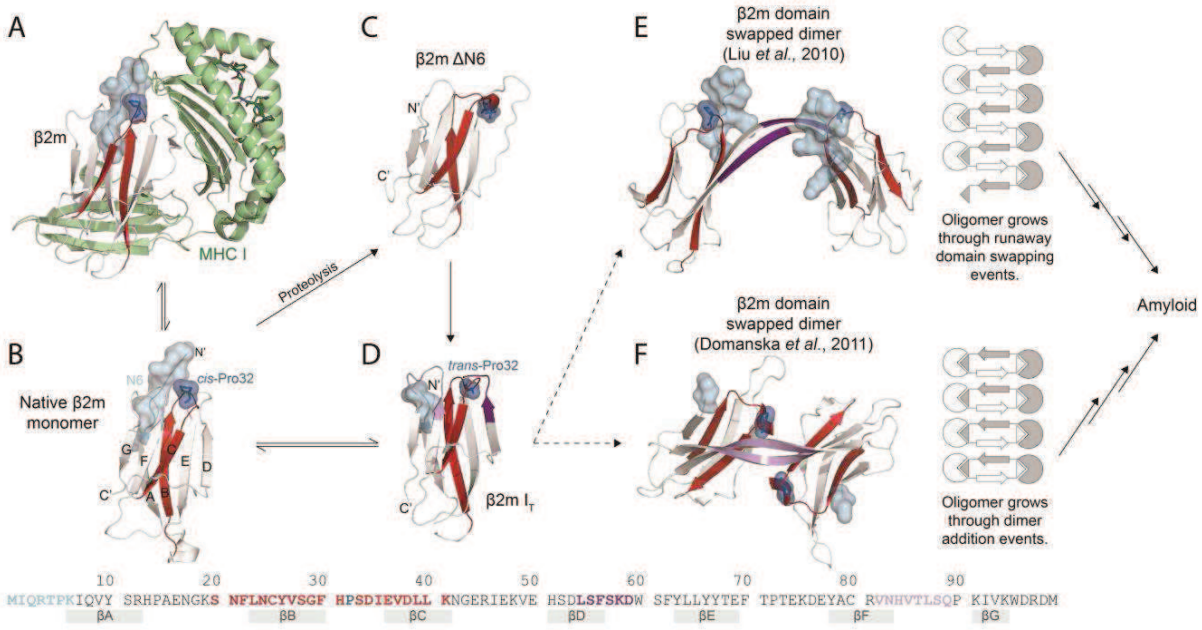


Figure 2

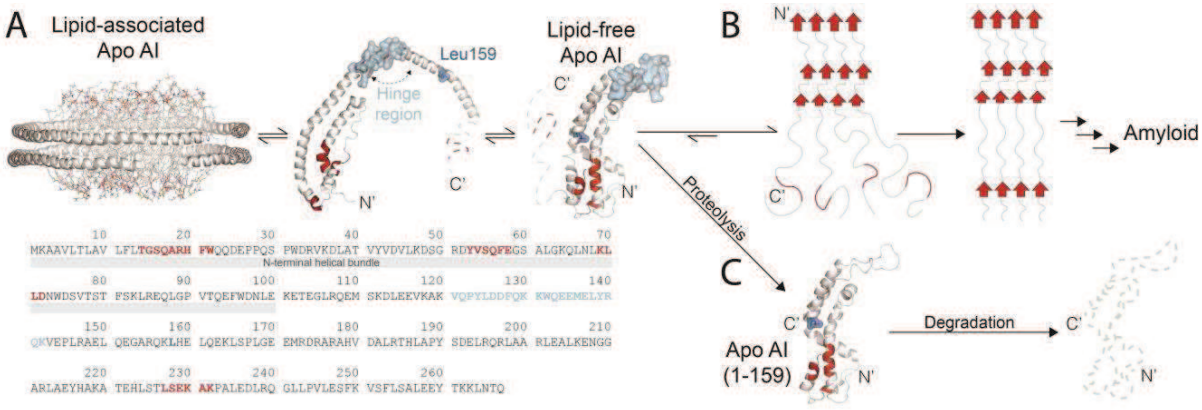


Figure 3

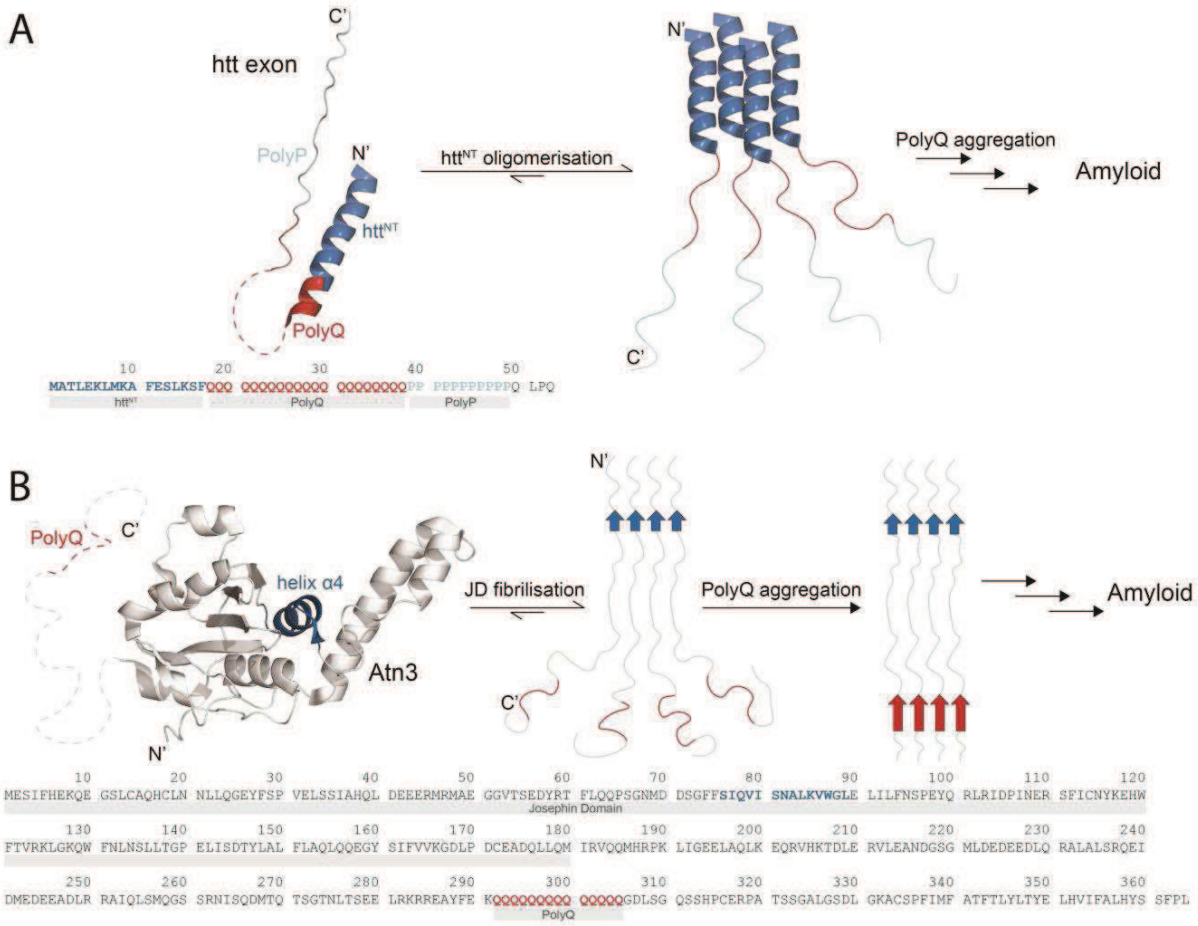


Figure 4

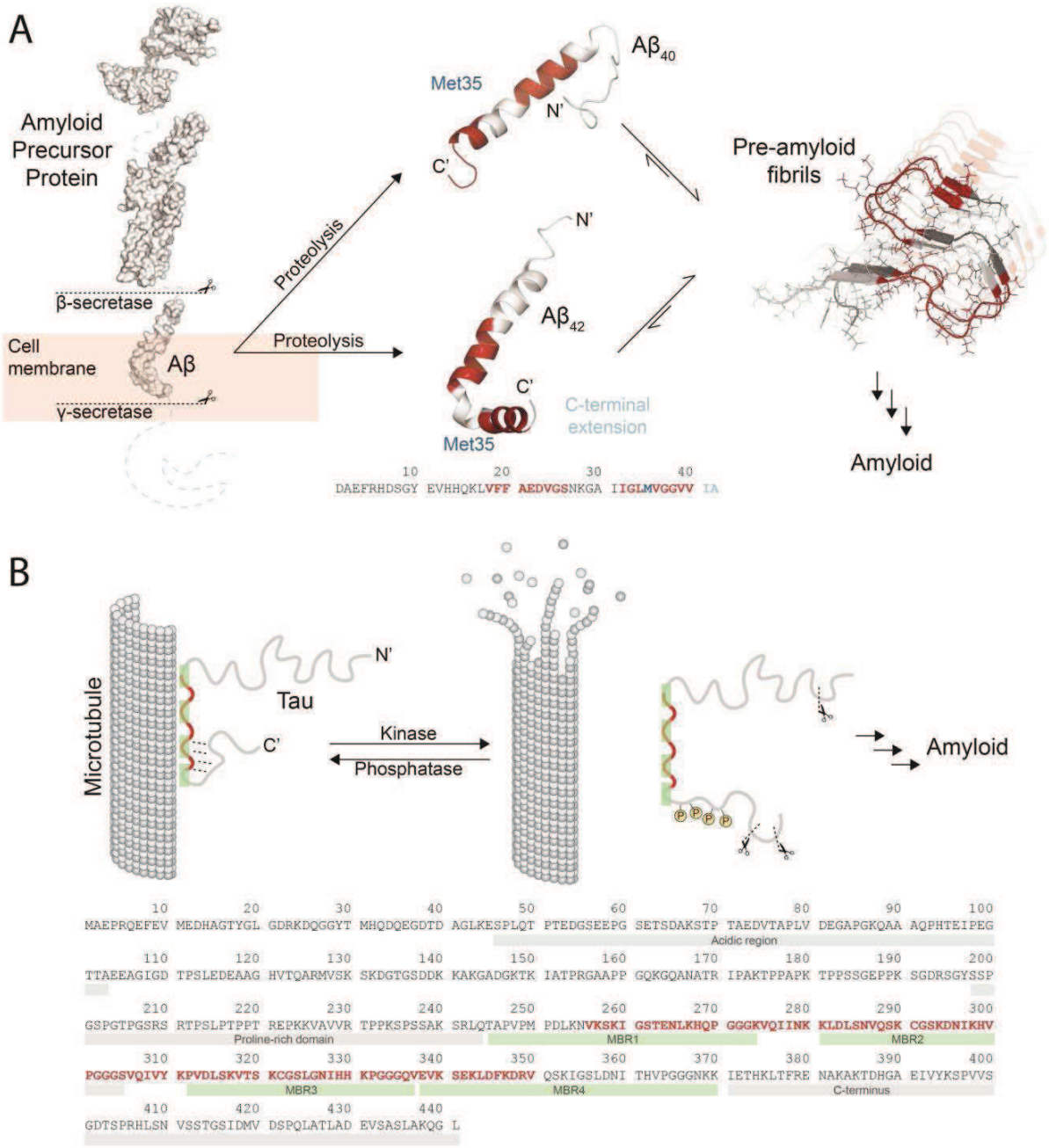
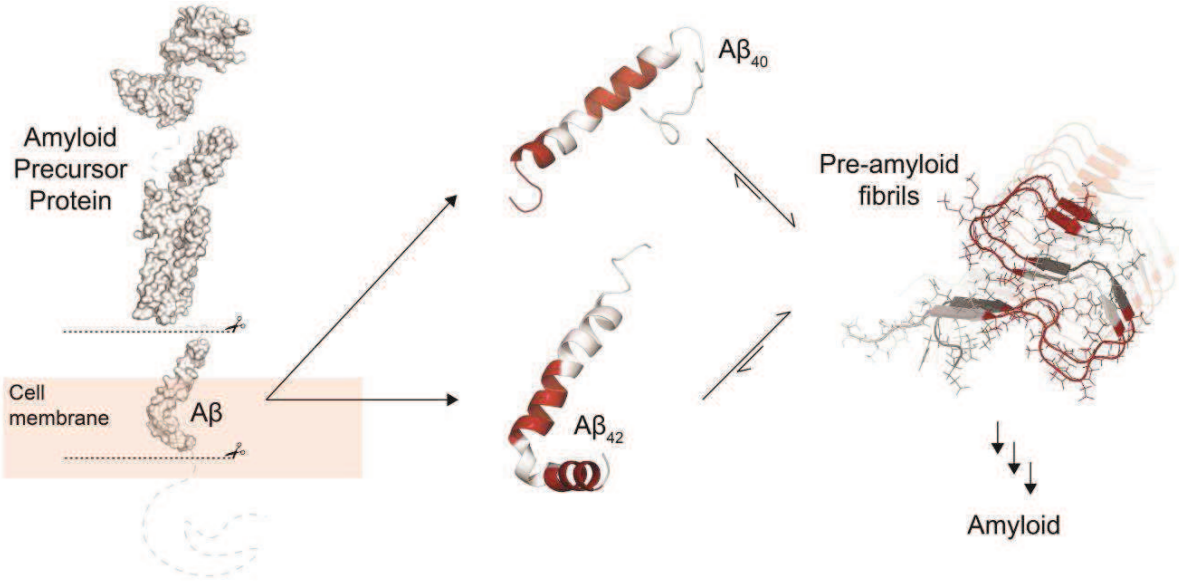


Figure 5



Graphical absract

Highlights:

- Protein aggregation to amyloid forms the molecular basis of a range of diseases
- Early events in protein misfolding define the aggregation pathway
- Recent detailed atomic and temporal insights have explained early misfolding events
- Distal regions beyond the classically amyloidogenic domains define aggregation
- Therapeutic targeting of distal regions is likely to aid in disease treatment