Received: 10 February 2023 | Revised: 17 May 2023 | Accepted: 24 May 2023

DOI: 10.1002/pro.4686

ARTICLE

THE PROTEIN SOCIETY WILEY

Big versus small: The impact of aggregate size in disease

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Funding information
National Institutes of Health, Grant/Award Numbers: R35 GM134864, 1RF1 AG071675-01, 1R01 AT012053; Passan Foundation; National Science Foundation, Grant/Award Number: 2210963

Review Editor: Nir Ben-Tal

Abstract

Protein aggregation results in an array of different size soluble oligomers and larger insoluble fibrils. Insoluble fibrils were originally thought to cause neuronal cell deaths in neurodegenerative diseases due to their prevalence in tissue samples and disease models. Despite recent studies demonstrating the toxicity associated with soluble oligomers, many therapeutic strategies still focus on fibrils or consider all types of aggregates as one group. Oligomers and fibrils require different modeling and therapeutic strategies, targeting the toxic species is crucial for successful study and therapeutic development. Here, we review the role of different-size aggregates in disease, and how factors contributing to aggregation (mutations, metals, post-translational modifications, and lipid interactions) may promote oligomers opposed to fibrils. We review two different computational modeling strategies (molecular dynamics and kinetic modeling) and how they are used to model both oligomers and fibrils. Finally, we outline the current therapeutic strategies targeting aggregating proteins and their strengths and weaknesses for targeting oligomers versus fibrils. Altogether, we aim to highlight the importance of distinguishing the difference between oligomers and fibrils and determining which species is toxic when modeling and creating therapeutics for protein aggregation in disease.

KEYWORDS

aggregation, fibril, kinetic modeling, molecular dynamics simulation, neurodegeneration, oligomer, post-translational modification

1 | INTRODUCTION

Protein aggregates are any protein species of a higher molecular weight than the expected native species. Unlike external pathogens, protein aggregation diseases are caused by native proteins which have crucial functions within cells. Through mutations, external factors such as toxins or metals, post-translational modifications

(PTMs), crowding, or a combination of effects, these native proteins aggregate into a range of soluble and insoluble species. We will refer to smaller soluble aggregates as oligomers and larger insoluble aggregates as fibrils since the latter species typically form β-sheet rich strands (Sipe & Cohen, 2000).

In the context of disease, fibrils were originally thought to be the toxic species since they were readily identified in patient samples. However, it has since been shown that fibril concentration does not correlate with disease severity (Kuemmerle et al., 1999; Tiwari & Kepp, 2016). Beginning in the 2000s, soluble oligomers began to be investigated as the potential toxic species in many protein aggregation diseases (A. K. R. Dasari et al., 2019; Ferreira et al., 2007; Haass & Selkoe, 2007; Lasagna-Reeves et al., 2013; Martinelli et al., 2019; Proctor et al., 2016; C. Wells et al., 2021), whereas fibrils were shown to either have a protective effect or no effect (Congdon & Duff, 2008; Hnath & Dokholyan, 2022; Zhu et al., 2018). Yet, the aggregation field still debates the toxicity of oligomers versus fibrils, with many therapeutic and biomarker strategies still targeting the fibrils (Alam et al., 2019; Cascella et al., 2022; Stefani, 2010; Taneja et al., 2015). Fibrils are visualized in tissue samples using immunostaining, β-sheet binding dyes such as Congo red and thioflavin T, or just by the naked eye in some cases (Nilsson, 2004; Sipe & Cohen, 2000). Oligomers are difficult to identify in patient samples since they typically cannot be visualized using β-sheet binding dyes and some oligomer species are structurally independent from the native protein structure and fibril structure, hence most antibodies cannot detect them (Doig et al., 2017; Hnath & Dokholyan, 2022). While the fibril secondary structure is typically composed of β-sheets, α-helices have been identified as a prominent structure component in many disease-associated oligomers, and many disordered proteins adopt helical-rich structures when in contact with lipids or in specific environments (Figure 1; D. Ghosh et al., 2015; Hnath & Dokholyan, 2022; Pannuzzo et al., 2013). Most protein aggregation diseases involve a toxic gain of function from aggregates developing aberrant interactions that are not present in the normal functional state of the proteins (Hoffner & Djian, 2002; Rajagopalan & Andersen, 2001; Redler &

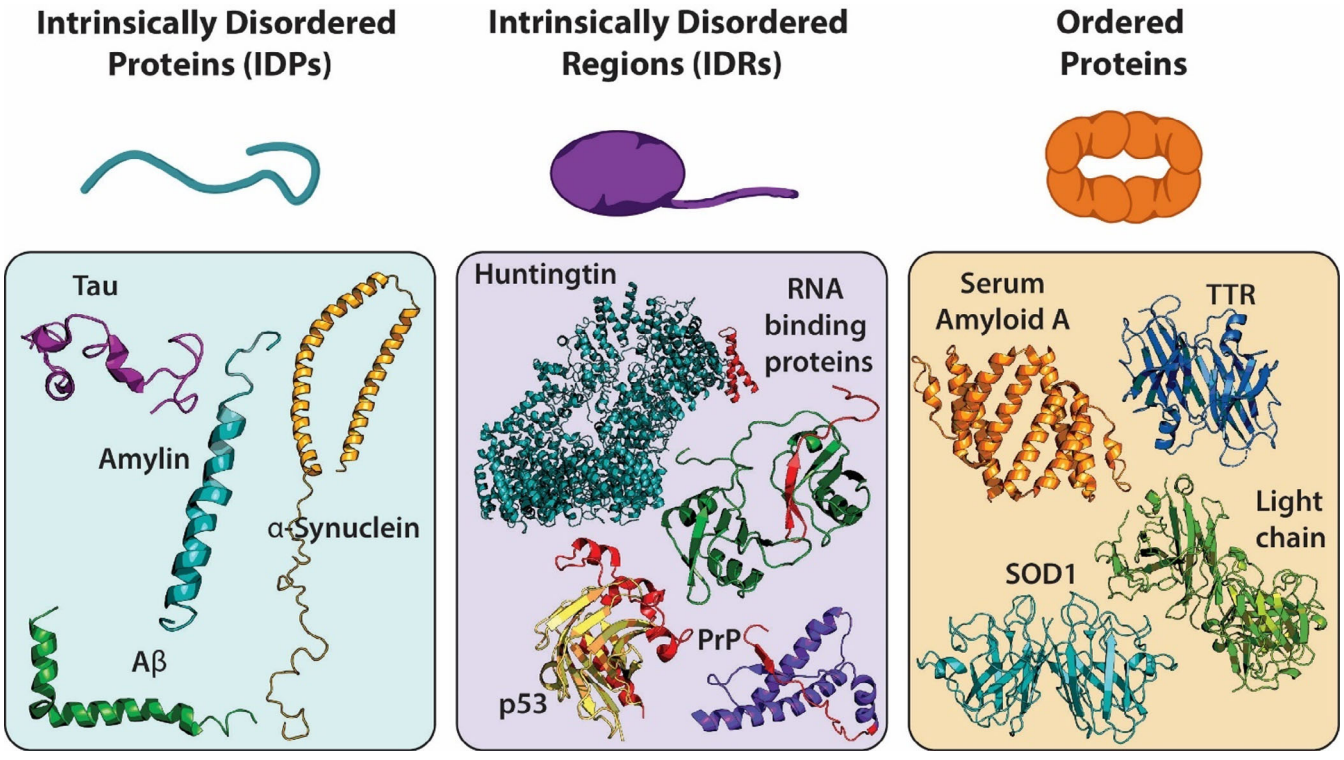

FIGURE 1 Different types of aggregating proteins. Intrinsically disordered proteins have unstable tertiary or secondary structures such as Tau (PDB 2MZ7), Amylin (PDB 2KB8), Aβ (PDB 1IYT), and α-synuclein (PDB 1XQ8). Proteins with intrinsically disordered regions have an unstable region that is prone to aggregation, additive mutations typically occur in this region (red region of proteins). Some examples of IDRs are Huntingtin (PDB 6RMH), p53 (PDB 1T4W), PrP (PDB 1QLX), and RNA binding proteins such as FUS and TDP-43 (PDB 4BS2). Ordered proteins have stable secondary structures which must be destabilized for aggregation to occur such as hexameric Serum Amyloid A (PDB 4IP9, depicts a dimeric piece of the whole structure), tetrameric TTR (PDB 3TFB), dimeric SOD1 (PDB 1SPD), and dimeric antibody light chain (PDB 1YUH).

Dokholyan, 2012; Sharma et al., 2016) as opposed to a toxic loss of function in which aggregates sequester the proteins and prevent them from performing crucial jobs (still observed in a few exceptions; Broeck et al., 2014; Choi & Dokholyan, 2021).

In this review, we describe how different size protein aggregates (oligomers vs. fibrils) are toxic or protective in many diseases, and explain how factors that promote aggregation (mutations, metals, PTMs, or lipid interactions) may differentially promote oligomers or fibrils. Additionally, we will outline computational modeling and therapeutic strategies targeting different aggregate sizes. Distinguishing whether oligomers or fibrils are being targeted is crucial when modeling aggregation in disease and designing therapeutics.

2 | PROTEIN AGGREGATION IN DISEASE

There are three groups of proteins associated with disease progression: intrinsically disordered proteins (IDPs), proteins with intrinsically disordered regions (IDRs), and ordered proteins which aggregate after the native form is destabilized (Figure 1). IDPs are defined as a group of proteins with unstable tertiary or secondary structures under physiological circumstances and are represented by a heterogeneous conformational ensemble (Wright & Jane Dyson, 2015). The disorder may alternatively be limited to specific protein regions, which are called IDRs (R. Lee et al., 2014). The amino acid compositions of IDPs/IDRs are significantly different from those of structured globular proteins or regions in terms of hydrophobicity, charge, sequence complexity, and other properties (Uversky et al., 2008). Although IDPs/IDRs are highly flexible, some of them transition from the disordered to an ordered state when interacting with partners (Uversky, 2013). Misfolded IDPs/IDRs may aggregate and form toxic species that cause neurodegenerative diseases, diabetes, cardiovascular disease, and cancer (Martinelli et al., 2019). In this section, we highlight some IDPs, proteins with IDRs, and misfolded ordered proteins and their aggregation associated with diseases.

α-Synuclein, an IDP with 140 amino acids commonly studied in association with Parkinson disease (PD), is encoded by the SNCA gene and widely exists in the presynaptic terminals of neurons (Iwai et al., 1995). α-Synuclein interacts with synaptic vesicle membranes and plays an important role in synaptic vesicle homeostasis and neurotransmitter release (Fortin, 2004). Monomeric α-synuclein has flexible structures and is characterized by a heterogenous conformational ensemble (J. Chen, Zaer, et al., 2021). Bartels et al. reported that native α-synuclein isolated from various mammalian cell lines, mouse brain tissue, and living human cells exists primarily as a helically folded tetramer, while monomeric, dimeric, and trimeric forms were detected in small amounts in certain cell types (Bartels et al., 2011). On the contrary, using in-cell NMR, Binolfi et al. discovered that native α-synuclein is predominantly monomeric and disordered inside intact *Escherichia coli* cells (Binolfi et al., 2012). Due to these controversial findings, the most abundant physiologic species of α-synuclein in vivo are undetermined and need to be investigated thoroughly in the future. α-Synuclein aggregates including toxic oligomers and fibrils act as precursors in Lewy body formation, a hallmark in synucleinopathies (e.g., PD, multiple system atrophy [MSA], and Lewy body dementia [LBD]; Alam et al., 2019). Although the specific toxic α-synuclein species is still undetermined and debated, increasing research suggests that soluble α-synuclein oligomers are cytotoxic, whereas larger Lewy inclusions might be the consequence of a protective response (Martinelli et al., 2019).

Prion protein (PrP) is a glycoprotein abundantly present on the outer surface of neurons (Kovač & Šerbec, 2022). Accumulation of toxic prion aggregates in the brain contributes to prion disease, also named as transmissible spongiform encephalopathies, occurring in several species including humans (C. Chen & Dong, 2016), felines (Colby & Prusiner, 2011), cervids (N. A. Rivera et al., 2019), cattle (G. A. H. Wells et al., 1991), goats and sheep (Acín et al., 2021). PrP consists of an intrinsically disordered N-terminal region and a structured C-terminal portion comprised of three α-helices and two β-sheets (Kovač & Šerbec, 2022). The protein is localized on the cell membrane through a glycosylphosphatidylinositol anchor at the C-terminus (Kovač & Šerbec, 2022). The normal form of PrP has been implicated in several important biological processes including myelin maintenance, mitochondrial function, metal ion homeostasis, circadian rhythm, intercellular signaling, and neuroprotection (Castle & Gill, 2017; Fevrier et al., 2004; Zamponi & Stys, 2009). This conformation of prion protein converts into a β-sheet-rich structure, which stimulates autocatalytic transformation and protein aggregation (Yamaguchi & Kuwata, 2018). Although prion diseases have been associated with abnormal protein aggregation, the molecular mechanism underlying the conversion of prion protein from normal to pathological forms and the development of prion disease are still poorly understood.

Proteins with prion-like amino acid composition or prion-like domains induce aggregation, cause neurotoxicity, spread between cells (Porta et al., 2018), and are associated with many diseases, showing similar features as

those of PrP (Feiler et al., 2015; Furukawa et al., 2011; Laferrière et al., 2019; Nonaka et al., 2013). For instance, RNA-binding proteins containing prion-like domains, including fused in sarcoma (FUS), Ewing sarcoma breakpoint region 1 (EWSR1), TAR DNA-binding protein 43 (TDP-43), TATA-binding protein associated factor 15 (TAF15), and heterogeneous nuclear ribonucleoproteins A1 and A2 (hnRNPA1 and hnRNPA2), are known to generate pathological inclusions that are related to neurodegenerative diseases, such as frontotemporal disorders (FTD), multisystem proteinopathy (MSP), and amyotrophic lateral sclerosis (ALS; Harrison & Shorter, 2017). These proteins share a similar modular architecture consisting of a disordered prion-like domain and a structured RNA-recognition motif or other RNA-binding domains (Sprunger & Jackrel, 2021). Prion-like domains assist with the function of RNA-binding proteins, while also increasing protein misfolding and aggregation, which contributes to neurodegenerative diseases (Harrison & Shorter, 2017).

Human p53 is a G1-S checkpoint transcription factor playing essential roles in cell proliferation, apoptosis, angiogenesis, senescence, and DNA repair. Pathological aggregation of p53 has been discovered in tumors and is proposed to cause functional alterations that finally influence tumor progression (Oliveira et al., 2020). P53 is composed of 393 residues that can be divided into several domains: an intrinsically disordered N-terminal transactivation domain, a proline-rich domain, a structured DNA-binding domain, and a structurally disordered C-terminal (Cho et al., 1994; Clore et al., 1995; S. Fields & Jang, 1990; Haupt et al., 1997; Honda et al., 1997; Joerger & Fersht, 2008; W. Lee et al., 1994). The disordered regions mediate interactions with partner proteins and facilitate p53 function. Under physiological conditions, p53 oligomerize through the oligomerization domain at the C-terminal. Monomer and dimer of p53 distribute in the cytoplasm, while its tetramer localizes in the nucleus and is responsible for transcriptional activation (Stommel, 1999). The most toxic aggregates of p53 are small prefibrillar aggregates and soluble oligomers (Lasagna-Reeves et al., 2013). In contrast, large p53 amyloid aggregation is non-toxic and serves biological roles for tumor cells (Ham et al., 2019; Xu et al., 2011). Mechanisms of p53 aggregation are summarized by J. Li et al. (2022).

Amylin, also known as islet amyloid polypeptide, is an IDP with 37 amino acids. It is co-secreted with insulin from the same pancreatic cells (Cooper et al., 1987; Mosselman et al., 1988; Westermark et al., 1987). Amylin aggregates are the major component of the amyloid deposits in pancreatic islets, a hallmark feature of type 2 diabetes (Hieronymus & Griffin, 2015). Amylin aggregation follows a sigmoidal formation curve (first-order kinetics) with lag, log, and plateau phases (Rhoades et al., 2000). The lag phase initiates by the interaction of one structurally disordered monomer to another, and further addition of more monomers to produce oligomers (Abedini et al., 2016). These oligomers function as a seed to form higher-order oligomers and fibrils. The formation of fibrils facilitates the production of new fibrils, and the elongation of fibrils is accelerated in this log phase (Padrick & Miranker, 2002; Patil et al., 2011). Eventually, protein aggregation enters the plateau phase wherein proto-fibrils and amyloid fibrils rich in β-sheet structure are formed (Rambaran & Serpell, 2008). At first, amylin fibrils were thought to be the main cause of cytotoxicity. However, it was later found that small oligomers were the most toxic species among the various intermediates. Amylin misfolding and aggregation are associated with a gradual loss of pancreatic cell function and mass in diabetic patients (Bhowmick et al., 2022). It causes cytotoxicity by inducing endoplasmic reticulum stress (C. Huang et al., 2007), oxidative stress (Zraika et al., 2009), mitochondrial malfunction (X.-L. Li, Chen, et al., 2011), inflammatory cytokine release (Westwell-Roper et al., 2011), and autophagy pathway disruption (J. F. Rivera et al., 2011).

Alzheimer disease (AD) is the most common neurodegenerative disorder, responsible for 60%–70% of dementia cases, which involves the aggregation of two major kinds of IDPs, extracellular amyloid-β (Aβ) aggregates and intracellular neurofibrillary Tau tangles. Both types of aggregates form highly ordered cross-β structures (Bulic et al., 2009). The major component of extracellular aggregates is the Aβ peptide, a cleavage product of the amyloid precursor protein. β-sheet-rich structures of Aβ tend to associate with other monomers to aggregate into oligomers and fibrils. Early studies suggested that Aβ fibrils are the toxic agents that cause neuronal cell death and other hallmarks of AD. Further studies have shown that Aβ oligomers rather than Aβ fibrils are toxic to neuronal cells (G. Chen et al., 2017; D. Y. Zhang et al., 2022). Bernstein et al. determined that the aggregation prone form of Aβ, Aβ42, has a different oligomer-size distribution than the more commonly found form, Aβ40 (Bernstein et al., 2009). The crystal structure of amyloid-like fibrils revealed that the formation of highly ordered parallel or antiparallel β-sheets and the steric zipper interface between β-sheets are two essential elements of amyloid fibril formation (Nelson et al., 2005). The major component of the intracellular neurofibrillary is Tau, which plays important roles in regulating microtubules, neurite outgrowth, and axonal transport. Tau adopts β-sheet structures resulting in a highly ordered morphology of paired helical filaments.

Huntington disease is an incurable late-onset and progressive neurodegenerative disorder caused by a mutation in the IT-15 gene, which encodes a 350 kDa protein of unknown function, huntingtin (Ross & Poirier, 2004). One of the hallmarks of Huntington disease is the aggregation of mutant huntingtin protein (Bonfanti et al., 2019; Khare et al., 2005; Lakhani et al., 2010). The mutant huntingtin protein which contains an IDR of expanded polyglutamine from CAG trinucleotide expansion in the gene is prone to misfolding and aggregating. The amyloid of mutant huntingtin protein, β-sheet structures tightly held together by hydrogen bonds, is considered a main cause of Huntington disease pathogenesis, causing dysfunction and death of neurons (S. Kim & Kim, 2014). Oligomeric Htt residue in the cytoplasm translocates to the nucleus in neurons as well as in the cytosol in non-neurons. The huntingtin oligomers affect motor function and cognition (C. Wells et al., 2021). There are over 900 protein interactions involving oligomeric Htt with a major role of interfering with nuclear transport (Woerner et al., 2016). Oligomers may play a pivotal and toxic role in Huntington disease and several studies have shown that fibril formation prevents cell death, similarly to other neurodegenerative diseases (Arrasate et al., 2004; Leitman et al., 2013).

Unlike IDPs, the three main proteins associated with systemic amyloid diseases (amyloidosis; Eisele et al., 2015); transthyretin (TTR), antibody light chain, and serum amyloid A, all have ordered structures that are necessary for the proteins' primary functions. Transthyretin is a tetrameric protein responsible for the transport of retinol and the thyroid hormone thyroxine to the liver (Saelices et al., 2015), antibody light chain is a two-part small polypeptide subunit of an antibody (Blancas-Mejia et al., 2018), and serum amyloid A is a hexameric apolipoprotein responsible for the transport of lipids and the recruitment of immune cells (J. Lu et al., 2014). Dissociation of each protein's primary conformation is a necessary first step for aggregation. The location of protein aggregates in the body dictates how the disease presents, with aggregate presentation typically leading to the affected organ failing (Eisele et al., 2015). Soluble oligomers of transthyretin have been shown to be more toxic than fibrils (A. K. R. Dasari et al., 2019), but the toxicity of different size aggregates of antibody light chain and serum amyloid A are still undetermined (Allen et al., 2012; Blancas-Mejia et al., 2018).

Superoxide dismutase-1 (SOD1) is a 32-kDa homodimeric antioxidant enzyme, binding copper and zinc ions (Ding et al., 2012; Khare et al., 2003, 2004, 2005). SOD1 was the first genetic factor associated with ALS, a fatal neurodegenerative disease characterized by the loss of motor neurons, leading to paralysis and eventual death (Redler & Dokholyan, 2012). The main known function of SOD1 is as a dismutase that removes dangerous superoxide radicals by metabolizing them into molecular oxygen and hydrogen peroxide (Nguyen et al., 2021). SOD1 dimer dissociation and loss of metals are necessary precursors for aggregation to occur (Khare et al., 2004; Wilcox et al., 2009). SOD1 apo-monomers were shown to aggregate into large insoluble fibrils or competing toxic trimers (Hnath & Dokholyan, 2022; Redler et al., 2011, 2014). Toxic trimeric SOD1 is structurally different from other larger aggregates, presenting a new therapeutic target for ALS (Hnath & Dokholyan, 2022; Proctor et al., 2016; Zhu et al., 2018).

Protein aggregation diseases are complex and not fully understood; many lack a known cure (Croce & Yamamoto, 2021). Cell mechanisms for maintaining proteostasis exist, but misfolded proteins take on aberrant functions or loss of function that manifests clinically (Hegde et al., 2023). In many aggregation-related diseases smaller soluble oligomers are beginning to be identified as the toxic species while larger insoluble fibrils may be protective. IDPs proteins with IDRs are affected by processes promoting aggregation (mutations, metals, or PTMs) in different ways than ordered proteins which misfold prior to aggregation.

3 | PROCESSES PROMOTING AGGREGATION

3.1 | Mutations

Most mutations that promote aggregation do so by either increasing the length or hydrophobicity of the prion-like region. Length-increasing mutations can occur in three main ways; repeats in a specific amino acid (Huntington disease and Ataxias), repeats of a specific domain (Tau and c9orf72), or increasing the length of a tail region (Aβ42). Huntington disease is caused by an expanded CAG trinucleotide repeat in the Huntingtin gene. This trinucleotide repeat region is typically repeated 10–26 times in healthy individuals but is repeated over 40 times in individuals with Huntington disease (Barton et al., 2007; Bates et al., 2015; Budworth & McMurray, 2013; Lakhani et al., 2010). Similarly, spinocerebellar ataxias also occur due to CAG trinucleotide repeats in the long polyglutamine track of ataxin proteins (Fan et al., 2014). Insertion mutations in prion protein cause additional copies of an octapeptide repeat domain at the N-terminus of the protein; there are typically five copies of the octapeptide repeat, but mutations insert up to eight additional domains (Kovács et al., 2002). Half of the known tau mutations have their primary effect at the

RNA level. Some of these mutations increase the splicing of exon 10, overproducing four-repeat tau which is more prone to aggregation (Goedert, 2005; Panda et al., 2003). A hexanucleotide repeat expansion in chromosome 9 open reading frame 72 (c9orf72), found in 40%–50% of familial ALS patients and 5%–10% of sporadic ALS patients, produces a glycine-arginine repeat protein that promotes the aggregation of TDP-43 (Cook et al., 2014; Gitler & Tsuiji, 2016; Umoh et al., 2016). Finally, the aggregation-prone form of amyloid beta (Aβ42) has a longer tail and is formed due to mutations in the amyloid precursor protein (Finder & Glockshuber, 2007). An increased number of inserted repeats in a protein directly correlates with decreased solubility and rate of aggregation (Adegbuyiro et al., 2017; Yu et al., 2007). Ordway et al. demonstrated that even inserting a glutamine repeat in a protein not associated with disease (hypoxanthine phosphoribosyl transferase) could induce neurological symptoms, shorten the lifespan, and trigger cerebral inclusions in mice (Brais et al., 1998; Ordway et al., 1997; Perutz, 1999). Expanding the length of a protein through insertion mutations promotes aggregation, increasing the formation of oligomers and fibrils.

In addition to insertion mutations, point mutations also promote aggregation by increasing the hydrophobicity of prion regions leading to both mislocalization and aggregation. TDP-43 (TARDBP), FUS, and other RNA binding proteins naturally contain low complexity domains contributing to liquid–liquid phase separation and the formation of aggregated RNA granules. RNA granules need to be dynamic for normal cell function; the granules must be stable enough to facilitate RNA transport and other cellular processes, but flexible enough to dissociate when necessary. ALS-related mutations in TARDBP and FUS promote highly stable aggregates which affect the necessary dynamics of RNA granules (Maziuk et al., 2017). Some ALS mutations to TARDBP have even been observed to disrupt RNA granules, forming smaller oligomers instead (Conicella et al., 2016; Fang et al., 2014; B. S. Johnson et al., 2009). TDP-43 and FUS are both nuclear-localized proteins, but point mutations to the C-terminus region affect their nuclear localization signal causing them to enter the cytoplasm (H. Deng et al., 2014; Prasad et al., 2019). Further, point mutations in α-synuclein (Srinivasan et al., 2021), TARDBP (Prasad et al., 2019), and FUS (H. Deng et al., 2014) all increase the hydrophobicity of the N-terminus, promoting aggregation. α-Synuclein point mutations, which result in the most severe dopaminergic loss in the substantia nigra, promote oligomer formation and toxic interactions with membranes (Winner et al., 2011). While mutations in aggregating proteins are associated with diseases, there are other factors that promote aggregation without the need for mutations.

3.2 | Metals

Protein-metal interactions either promote or prevent aggregation. The copper and zinc ions in functional SOD1 are necessary for the superoxide function of the protein, loss of the metals destabilize the dimer interface leading to aggregation (Ding & Dokholyan, 2008; Khare et al., 2004; Khare & Dokholyan, 2006). Similarly, copper and zinc binding to the octapeptide repeat domain of prion protein partially fold the domains preventing aggregation (Inanami et al., 2005; Jackson et al., 2001; Leclerc et al., 2006). Alternatively, metal binding to the prion domains of α-synuclein (Hillmer et al., 2010; Rasia et al., 2005; Uversky et al., 2001; Yamin et al., 2003), Aβ (Bolognin et al., 2011; Sarell et al., 2010; Tõugu et al., 2011), tau (Yamamoto et al., 2004; Yang et al., 2010), and ataxin (Ricchelli et al., 2007; Stawoska et al., 2009) increase the hydrophobicity of the proteins and promote aggregation. The binding of different metals affects whether soluble oligomers or large insoluble fibrils are formed, the soluble forms are typically more toxic while the large insoluble fibrils are less toxic (Breydo & Uversky, 2011; Drago et al., 2008; T. D. Kim et al., 2000; B. Liu et al., 2011; Miyake et al., 2011). The differing effects of different metals on what type of aggregates are formed (oligomers versus fibrils), along with the high concentrations of zinc and copper at synapses may be a contributing factor to why many aggregating proteins contribute to neurodegeneration (E. P. Huang, 1997; L. Wang et al., 2020).

3.3 | Post-translational modifications

PTMs are a common cause of protein aggregation, specifically in the context of traumatic injuries and age-related neurodegenerative disorders. PTMs are broadly defined as any covalent attachment of a functional group or targeted cleavage that occurs to a protein following its synthesis. While over 600 PTMs have been described (Bradley, 2022), only a subset have been experimentally confirmed to play a role in protein aggregation. In broad terms, PTMs influence protein aggregation similarly to mutations and metals, primarily by modifying protein folding or altering solubility. Shaffert and Carter have recently reviewed the influence of PTMs on aggregation and their role in neurodegenerative diseases (Schaffert & Carter, 2020). Here we provide a brief overview of the

most well-characterized PTMs and their role in protein aggregation.

Protein phosphorylation is defined as the covalent addition of a phosphate group to a receiving protein, a process typically catalyzed by a kinase. Physiologically, phosphorylation is utilized by cells to transfer and store energy, activate or deactivate a protein through a conformational change, and transmit information through signaling pathways. Aberrant phosphorylation occurs when kinase function becomes dysregulated, frequently due to a genetic error, trauma, or other causes of cellular stress (Gendron & Petrucelli, 2009; Giasson & Mushynski, 1996; Perluigi et al., 2016). Proteins that have been shown to aggregate upon phosphorylation include Aβ (Jamasbi et al., 2017; Kumar & Walter, 2011), tau (Buée et al., 2000; G. V. W. Johnson & Stoothoff, 2004; Lippens et al., 2007), α-synuclein (Fujiwara et al., 2002; Samuel et al., 2016), TDP-43 (Carlomagno et al., 2014; Hasegawa et al., 2008), and PrP^c (Giannopoulos et al., 2009). However, the phosphorylation of these proteins does not always result in aggregate formation. Numerous groups have demonstrated that the phosphorylation of certain residues in Aβ (Kumar et al., 2016), tau (W. Hu et al., 2016), and TDP-43 (Brady et al., 2011; H.-Y. Li, Yeh, et al., 2011) do not promote aggregation. Phosphorylation of different residues could also contribute to whether oligomers or fibrils are formed (Kumar et al., 2016).

While phosphorylation is perhaps the most well-studied PTM that leads to pathologic protein aggregation, other pathways have been implicated in the formation of aggregates. Oxidation has been shown to promote aggregate formation by altering kinase function (T. Zhang et al., 2015), as well as disrupting the structural conformation of proteins and damaging genetic elements that disrupt protein structure after translation (Butterfield & Kanski, 2001). Specifically, the oxidation of α-synuclein (El-Agnaf & Brent Irvine, 2000) and Aβ (Oda et al., 1995) are well characterized. Nitration has also been demonstrated to increase aggregate formation in multiple neurodegenerative disorders (Hyun et al., 2004; Ischiropoulos & Beckman, 2003; Reynolds et al., 2007; Uversky et al., 2005).

There is emerging evidence that methylation (Balmik & Chinnathambi, 2021) and ubiquitination promote aggregation in specific contexts (Berke & Paulson, 2003; Dantuma & Bott, 2014); however, these PTMs may affect other proteins in unique ways. Similarly, multiple groups have described how the addition of long-chain functional groups result in aggregate formation. Acetylation of tau (S. I. A. Cohen et al., 2013; Cook et al., 2014) and TDP-43 (T. J. Cohen et al., 2015), glycation of tau (K. Liu et al., 2016), glycosylation of tau (F. Liu et al., 2002), and SUMOylation of tau (X. Chen, Zhang, et al., 2021; Luo et al., 2014) all result in protein aggregation. Glutathionylation of SOD1 destabilizes the dimer leading to aggregation (Redler et al., 2011). While decades of research have dissected individual modifications and their role in the overall aggregate formation, research has only recently begun to investigate how these modifications affect oligomers versus fibrils (Barrett & Timothy Greenamyre, 2015; Ercan-Herbst et al., 2019; Kumar et al., 2016).

3.4 | Lipid interactions

Protein–lipid interactions promote aggregation and contribute to cell death in many aggregation-related diseases. One of the key factors contributing to Aβ toxicity in AD is the interaction between the cell membrane and Aβ. There are several ways in which the lipid membrane interacts with Aβ (Arispe et al., 1993). For instance, membranes can act as a surface for Aβ to adsorb onto, leading to increased concentration and proximity which affects aggregation (Yanagisawa et al., 2011). Certain lipid components, such as cholesterol and sphingolipids, can promote the formation of Aβ oligomers by altering the physical properties of the lipid membrane, modifying the conformation of Aβ and forming ion channels. Moreover, the membrane can also serve as a nucleation site for Aβ aggregation, promoting the formation of toxic oligomers and fibrils (Vetrivel & Thinakaran, 2010). Aβ peptides can insert themselves into the lipid bilayer and disrupt the normal structure and function of the membrane, leading to increased membrane permeability and cellular dysfunction. In addition, the membrane can facilitate the interaction between Aβ and other proteins on the membrane, such as Apolipoprotein E. The interaction between the membrane and Aβ can also activate intracellular signaling pathways that affect Aβ toxicity (Reed-Geaghan et al., 2009).

α-Synuclein and lipid interactions play essential roles in both maintaining normal physiological functions as well as contributing to the pathogenesis of several neurodegenerative diseases, termed as synucleinopathies (Runwal & Edwards, 2021). α-Synuclein monomers and oligomers bind to lipid membranes through electrostatic interactions between the negatively charged lipid headgroups and the positively charged lysine-rich N-terminus of α-synuclein (Galvagnion, 2017; Grey et al., 2011; Rooijen et al., 2008). Both monomeric and oligomeric forms of α-synuclein have a high binding affinity to loosely packed membranes and small unilamellar vesicles with high surface curvature (Middleton & Rhoades, 2010; Ouberai et al., 2013). Among all different species of

α-synuclein, the oligomeric forms cause neurotoxicity and are likely responsible for the onset and development of disease (Lashuel et al., 2013). The cytotoxic effects of α-synuclein oligomers are attributed to the interactions with lipid membranes, deteriorating membrane integrity and impairing cellular homeostasis (Alam et al., 2019; Fusco et al., 2017; Iyer & Claessens, 2019; Killinger et al., 2019). Several scenarios have been proposed to explain the cellular toxicity caused by α-synuclein oligomer–membrane interactions; including bilayer thinning, detergent-like solubilization, and pore and nanodisc formation, which have been summarized by Musteikytė et al. (2021). On the other hand, several studies found that lipid membranes could trigger α-synuclein aggregation and oligomer formation in vivo (Bae et al., 2013; Galvagnion, 2017; Näsström et al., 2011). Assayag et al. reported that alterations in the level of polyunsaturated fatty acids on lipid membrane induced cytotoxic α-synuclein oligomer formation in the cytoplasm, which preceded Lewy-like inclusions in dopaminergic cells (Assayag et al., 2007).

Aβ and α-synuclein lipid interactions are the most widely studied, but there is also evidence that Tau oligomers and misfolded SOD1 can interact with, and may disrupt, mitochondrial membranes (Abbasabadi et al., 2013; Britti et al., 2020; Camilleri et al., 2020; H.-X. Deng et al., 2006; Lin & Flint Beal, 2006). Mitochondrial dysfunction and energy deficiency are established components of many neurodegenerative diseases (Cozzolino & Carrì, 2012; Johri & Flint Beal, 2012; X. Wang et al., 2014). Overall, lipid interactions contribute to protein aggregation and maybe a central component of cell death in some diseases (Gonzalez-Garcia et al., 2021).

4 | MODELING PROTEIN AGGREGATION

4.1 | Molecular dynamics modeling of protein aggregation

Protein aggregation is involved in many neurodegenerative diseases, and research on the mechanism of protein aggregation will aid the development of therapeutic interventions that modulate protein aggregation. Although known aggregated proteins have different sequences, many of them adopt β-sheet-rich structures in coincidence for aggregation. One of the hypotheses is that hydrogen-bond interactions unrelated to amino acid side chains play a key role in the process of protein aggregation. The validation of this hypothesis and the exploration of other mechanisms of protein aggregation rely on wet-lab experiments. However, many proteins that aggregate are disordered in nature and only form specific three-dimensional structures transiently, which makes studying them experimentally very hard. An alternative approach is to resort to computational molecular dynamics (MD) simulation. Here we review computational studies of the aggregation of Aβ, α-synuclein, and SOD1 with all-atom MD simulations as well as coarse-grained molecular dynamics. More research on the aggregation of other proteins can be found in the reviews of Kulkarni et al. (2022) and Thirumalai (2003).

Extensive modeling of Aβ monomers, intermediates, and fibrils has been performed by multiple groups. Sanches et al. utilized the energy landscape visualization method (ELViM) to analyze coarse-grained simulations of normal Aβ-40 and Aβ-42 monomers, along with six single-point mutations associated with early-onset disease. They found that the aggregation rate is correlated with the β-strand content of the monomers, and most of the contacts necessary to seed the aggregation have already been formed in Aβ42 monomers and the monomers of less soluble mutants, while the monomers of Aβ40 need to unfold some helical structure before aggregating (Sanches et al., 2022). Baumketner et al. performed both ion mobility mass spectrometry and theoretical modeling to study the conformational states of the monomeric Aβ42 peptide. Their simulations revealed that Aβ42 in aqueous solution adopts both extended chains as well as collapsed-coil structures. Aβ42 did not display a unique fold like a typical protein, but rather a mixture of rapidly interconverting conformations. They analyzed the secondary structure and found that Aβ42 peptide conformations are dominated by loops and turns but show some helical structure in the C-terminal hydrophobic tail (Baumketner, 2006). Klimov and Thirumalai conducted a series of MD simulations for Aβ and found that Aβ16–22 peptides form antiparallel β-sheet structures, and α-helical intermediates are transiently populated. They proposed that Aβ forms by fibrils maximizing the number of salt bridges and hydrophobic interactions, and the oligomers must have a high α-helical content (Klimov & Thirumalai, 2003). Studying monomers and fibril intermediates gives some information on the structure of oligomeric intermediates, but further simulations expand on the structure of specific-size oligomers.

Urbanc et al. studied the formation of Aβ dimer by discrete molecular dynamics (DMD) (Urbanc et al., 2004). They first studied the formation of the Aβ dimer through coarse-grained simulations and then studied the thermodynamic properties through all-atom simulation. They found that the free energy of the dimer is generally higher than that of the monomer, and there is no significant difference in the free energy of the Aβ42

dimer and Aβ40 dimer. Barz et al. utilized transition networks derived from all-atom molecular dynamics to determine that the oligomer size distribution is different between Aβ42 and Aβ40 due to higher solvent exposure of hydrophobic residues in Aβ42. Additionally, dimers and tetramers of Aβ42 and Aβ40 were structurally different which could contribute to their differences in aggregation (Barz et al., 2018). Ma and Nussinov simulated the stabilities of oligomeric AGAAAAGA and AAAAAAAA (A8) with MD simulations and found these two peptides are stable in aggregates of 6–8 monomers, providing insight into the mechanism of seed growth with a multilayer β-sheet model (Ma & Nussinov, 2009). They further studied the oligomers of Aβ fragments 16–22, 16–35, and 10–35. Their simulations indicated that the antiparallel β-sheet orientation is the most stable for the Aβ16-22, in agreement with a solid state NMR-based model (Ma & Nussinov, 2002). In addition, recent crystal structures of Aβ16-22 suggest that the region 16–22 can form multiple polymorphs, with variations in their molecular structure, hydrogen-bonding network, and conformational dynamics (Colvin et al., 2016; S. Dasari & Mallik, 2020; J.-X. Lu et al., 2013; Qiang et al., 2017; Tycko, 2015; Wälti et al., 2016). The structural diversity of these polymorphs may contribute to the heterogeneity of Alzheimer disease and its clinical subtypes. Zhang et al. have performed MD simulations to computationally investigate the interactions between Aβ and GM1, a member of the ganglion series of gangliosides. They performed 100 ns MD simulations for GM1 membranes and five Aβ42 monomers and found that GM1 tightly binds Aβ42. They found that the fifth residue (arginine, R5) of Aβ is within 1–2 Å from GM1, suggesting that R5 stably binds GM1. They further computationally substituted R5 to glycine, and they found that the average distance between G5 and GM1 increased to >10 Å and was within a binding distance (<2 Å) for as little as 2% of the time, indicating that the R5G mutation disrupted the tight binding interaction between the fifth residue and GM1. Thus, the fifth residue in Aβ42 plays a critical role in the direct binding between AC and GM1 (D. Y. Zhang et al., 2022). The recently-solved cryo-EM structures of Aβ have provided valuable insights into the molecular architecture of amyloid fibrils and their role in the pathogenesis of Alzheimer disease (Fitzpatrick et al., 2017; Gremer et al., 2017; Q. Li et al., 2021; R. Zhang et al., 2009). These structures can enable researchers to perform detailed molecular dynamics simulations to better understand the dynamics and stability of the fibrils. It is important to model different size Aβ oligomers to be able to determine the toxicity of different oligomers. More MD research on Aβ can be found in the review by Nasica-Labouze et al. (2015).

Chen et al. used inter-dye distance distributions from bulk time-resolved Förster resonance energy transfer as constraints in DMD simulations to map the conformational space of α-synuclein monomers (J. Chen, Zaer, et al., 2021). They found that some conformations of α-synuclein are surprisingly stable, exhibiting conformational transitions in milliseconds. Their comprehensive analysis of conformational ensembles revealed fundamental structural properties and underlying conformations that facilitate their various functions in membrane interactions or oligomer and fibril formation. By integrating cross-linking mass spectrometry with DMD simulations, the same group utilized cross-linking data as constraints to simulate the dimer structures of α-synuclein. They identified one compact and stable dimer structure where tyrosine 39 is sufficiently close to forming a dityrosine covalent bond, which may be involved in α-synuclein amyloid fibril formation (Zamel et al., 2019).

To computationally propose potential SOD1 trimer structures, DMD was used to simulate several nanoseconds of SOD1 trimer trajectory. To obtain constraints for certain interatomic distances and to narrow the structural search space, Proctor et al. applied limited proteolysis to SOD1 trimers and used mass spectrometry to identify likely solvent-exposed sites on the surface of the trimer. A custom bias potential was designed iteratively so that the experimentally deduced solvent-exposed sites were solvent-exposed in the DMD simulations (Proctor et al., 2016).

DMD has proven to be a useful tool for the investigation of protein aggregation. DMD applies discrete step function potentials to define interatomic interactions rather than continuous potentials widely adopted in traditional molecular dynamics simulations (Brodie et al., 2019; Dokholyan et al., 1998; Proctor et al., 2011; Shirvanyants et al., 2012). This innovative application greatly reduces calculations and therefore enhances sampling and allows DMD to achieve protein folding, especially for smaller oligomers, in a practical time scale (Ding et al., 2008; Dokholyan et al., 1998). For intrinsically disordered proteins, to the best of our knowledge no experimental technique characterizes their conformational ensembles at the atomic level. Therefore, experimentally guided DMD simulations provide the best solution. DMD simulations are combined with various experimental technologies, such as FRET, Mass Spectrometry, which have been demonstrated by previous studies (J. Chen, Zaer, et al., 2021; Proctor et al., 2016; Zamel et al., 2019). Protein structures predicted by DMD simulations that are guided and validated by experimental data are more accurate than those predicted solely by computational methods (J. Chen, Zaer, et al., 2021;

Seffernick & Lindert, 2020) making DMD a crucial tool to observe complex conformational changes in the monomeric, oligomeric, and aggregated forms of Aβ, α-synuclein, and SOD1.

4.2 | Kinetic models of protein aggregation

Without detailed knowledge of a protein's structure in the aggregated state, it is still possible to propose systems of ordinary differential equations (ODEs) that model the formation of aggregates of various size (Hall et al., 2005, 2015; Hirota et al., 2019). Toy models of protein aggregation proposed by Knowles, Dobson, Vendruscolo, Hall, Hirota, and others use physical reasoning to propose reasonable systems of ODEs and predict the relative abundances of the various sizes of amyloid as a function of initial conditions, particularly the size of the initial nucleation site (Hirota et al., 2019; Hall et al., 2005, 2015; Knowles & Buehler, 2011; Knowles et al., 2007; 2009). Such modeling often uses simplifying assumptions, such as irreversible misfolding of the protein upon aggregation, and roughly spherical shapes for the protein; however, the results are flexible enough to fit a wide variety of time-dependent concentration signals.

An extensive accounting of protein aggregation in humans and other organisms is found in the review by Chiti and Dobson (2006). Important factors in aggregation include increased hydrophobicity of amino acid side chains and neutral total protein charge, as aggregates are energetically favored in proteins with these properties. Investigation into Aβ42 aggregates has shown that the major factor for formation and growth is the presence of existing fibrils (S. I. A. Cohen et al., 2013), which is termed a secondary nucleation process. Since Aβ monomer is known to be an intrinsically disordered protein (Uversky et al., 2008), the assumptions made in ODE modeling of aggregation are thought to hold, since there is no preferred native monomer structure that would bias the aggregation pathways. Additionally, metal-binding may play an important role in aggregate nucleation and growth, since Aβ plaques found in Alzheimer disease are enriched in copper and zinc (Faller et al., 2014).

Proteins can exist in different structural states, including ordered and intrinsically disordered forms. The mechanisms of protein aggregation for these two forms are distinct from each other. In the case of ordered proteins, they need to undergo a destabilization of their unique structures to form amyloidogenic intermediates. This process is typically referred to as denaturation and misfolding, which can eventually lead to the formation of amyloid fibrils. On the other hand, IDPs require at least partial folding to form amyloidogenic or aggregation-prone species (Uversky & Fink, 2004). It has been shown that IDPs can aggregate in the absence of a well-defined tertiary structure and that the nature of the disordered regions can influence the kinetics and thermodynamics of the aggregation process (Avni et al., 2019; Uversky, 2014; W. Wang et al., 2010). Therefore, understanding the differences in the mechanisms of protein aggregation for ordered and intrinsically disordered proteins is important for developing effective strategies to prevent or treat protein misfolding diseases.

Recently, it has been determined that the formation of superoxide dismutase-1 (SOD1) trimers implicated in the development of amyotrophic lateral sclerosis form off-pathway from the larger aggregates (Khare & Dokholyan, 2006; Proctor et al., 2016; Zhu et al., 2018). The theoretical justification for this result was that the model from Hall and Hirota predicts that off-pathway trimer pathway aggregation results in a strong correlation between the decrease in trimer with an increase in aggregate (Hirota et al., 2019), which was observed in SOD1 aggregation data ($r = -0.78$ to 0.98, $p < 0.001$). Despite the exact structures of the SOD1 trimer and larger aggregates being unknown at present, there is indirect evidence that SOD1 trimers form off-pathway (Hnath & Dokholyan, 2022).

Efforts to understand the formation of Aβ from both the computation (Auer et al., 2007; Blondelle et al., 1997) and experiments (Fandrich, 2002; X. Hu et al., 2009; Nelson et al., 2005; Serio et al., 2000) point to a nucleated growth mechanism, in which high concentrations of protein are needed for the initial formation of misfolded seeds, before a first-order kinetic process results in the exponential growth of Aβ fibrils (Ding et al., 2005). However, it is currently unknown how nucleation occurs, since the concentration needed for the misfolded seeds to form are in the mM range (Auer et al., 2007; Ilie & Caflisch, 2019), whereas Aβ is physiologically found at nM to μM concentrations, both in cerebrospinal fluid (Andreasen et al., 1999) and neuronal tissue (Gong et al., 2003; Lazarevic et al., 2017). Recently, it has been proposed that Aβ may interact with the plasma membrane to form seeds (D. Y. Zhang et al., 2022), with the GM1 ganglioside serving as a catalyst that binds and localizes Aβ monomer.

5 | THERAPEUTIC STRATEGIES TO TREAT PROTEIN AGGREGATION

Four main therapeutic strategies are used to target different stages of protein aggregation in disease; protein reduction, aggregation inhibition, breaking down

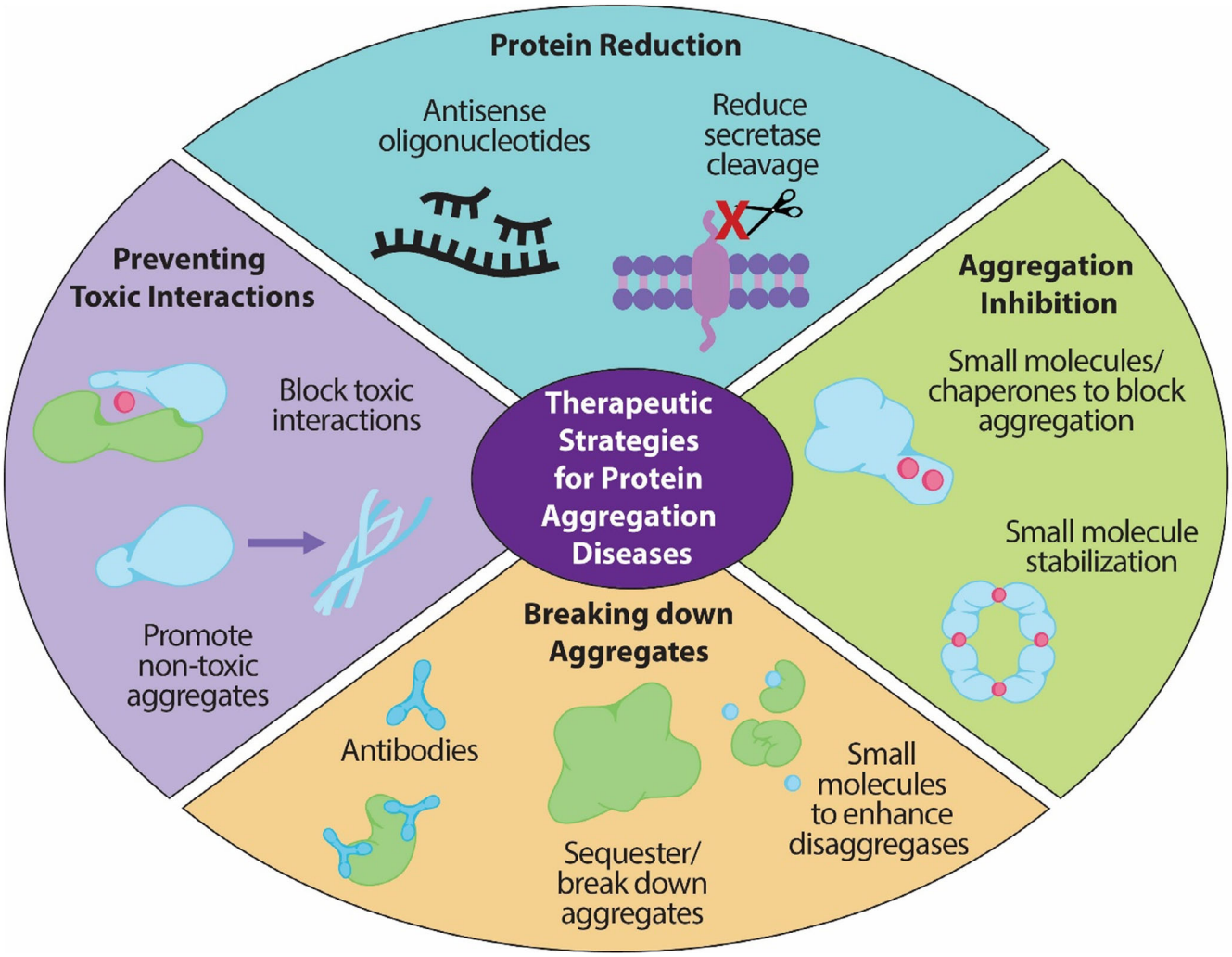

FIGURE 2 Therapeutic strategies for protein aggregation diseases. The four current strategies for targeting aggregating proteins are through (1) overall protein reduction, (2) inhibiting aggregation using small molecules or chaperones to block protein interactions or stabilizing the native form, (3) breaking down formed aggregates using antibodies or disaggregases, or (4) preventing toxic interactions by blocking the interaction or promoting non-toxic aggregates.

aggregates or preventing toxic interactions (Figure 2). For most of these strategies, it is necessary to know the native function, toxic aggregate size and structure, and mechanism of cell death of the aggregating proteins, of which parts remain unknown for most diseases associated with aggregating proteins. Very few therapeutics targeting protein aggregations have been successful in clinical trials (Doig et al., 2017; Lang, 2010; Petrov et al., 2017).

Crowding and increased protein concentration contribute to protein aggregation (Munishkina et al., 2004); for example, copy number gains of α-synuclein are regularly seen in Parkinson disease (Mokretar et al., 2018). To combat overexpression and prevent aggregation many different methods have been tested to reduce the level of aggregation-prone proteins. Antisense oligonucleotide therapy uses short strands of modified nucleotides to target RNA and reduce the protein expression (Kuijper et al., 2021). This therapy is currently being tested to reduce SOD1 (Meyer et al., 2023; Miller et al., 2022), tau (Mummery et al., 2021), huntingtin (Smith & Tabrizi, 2020), amylin (Novials et al., 1998), α-synuclein (Alarcón-Arís et al., 2018), FUS (Korobeynikov et al., 2022), and TTR (Teresa Coelho et al., 2013) with various degrees of success in clinical trials. To reduce the amount of Aβ, strategies focus on decreasing the activity of β and γ-secratases (A. K. Ghosh & Osswald, 2014). Protein reduction is only a feasible therapeutic strategy for protein aggregates with a toxic gain of function. Another issue with this strategy is that many aggregating proteins have crucial native roles in the body, decreasing the overall level of some proteins causes a different phenotype due to a toxic loss of function. Protein reduction strategies need to carefully reduce the expression level of aggregating proteins without losing the native function,

and the dosing may not transfer from person to person due to natural differences in protein expression levels (Southwell et al., 2012). Since this strategy is aimed at reducing all aggregation instead of just targeting fibrils it may be helpful against oligomers.

Another strategy for preventing or slowing aggregation is using small molecules or chaperones to inhibit aggregation. For ordered proteins such as SOD1 and TTR, small molecule therapeutics focus on stabilizing the dimer or tetramer interfaces to prevent the native proteins from dissociating (Amporndanai et al., 2020; Capper et al., 2018; T. Coelho et al., 2012; Cotrina et al., 2020; Tojo et al., 2006). For IDPs like amylin (López et al., 2016) and α-synuclein (C. R. Fields et al., 2019), small molecule therapeutics target the prion-like region to prevent aggregation. The same strategy of blocking the aggregating region is accomplished using chaperone proteins such as heat shock protein (Klucken et al., 2004; Webster et al., 2019) or other engineered chaperones (Ferretti et al., 2022). While this strategy may be effective against both oligomers and fibrils it relies on treating patients early in disease progression before the aggregates have spread and done extensive damage, which is difficult since most of the above-mentioned diseases do not have early biomarkers to test for the disease before the symptoms have progressed. More extensive descriptions of small molecule aggregation inhibitors are reviewed by Malik and Wiedau (2020), Jokar et al. (2019), and Pena-Diaz et al. (2020).

Once aggregates have already formed, antibodies or disaggregases modulate them or direct them to proteostasis machinery for sequestration or degradation. Both active and passive immunotherapy techniques have been tested to treat protein aggregation-related diseases with mixed results. Vaccines of small surface-exposed regions of the toxic forms of protein aggregates (active immunotherapy) or monoclonal/ polyclonal antibody treatments which target specific regions of toxic aggregates (passive immunotherapy) have recently begun evaluation in clinical trials (Eisele et al., 2015; Vassilakopoulou et al., 2021). In the past year, two different monoclonal antibody treatments targeting Aβ oligomers were given emergency FDA approval to treat AD (Aducanumab and Lecanemab; Marsool et al., 2023; Perneczky et al., 2023; Rahman et al., 2023; Vitek et al., 2023). The key difference between new immunotherapy methods and past methods is immunotherapy is now being designed to target smaller soluble oligomers of proteins or specific misfolded regions found in larger aggregates, as opposed to the previous strategy of designing antibodies that only detect large insoluble aggregates. In-depth descriptions of failed and new immunotherapy methods to treat different diseases were reviewed by Reiss et al. (2021), Schwab et al. (2020), Gittings and Sattler (2020), and Villoslada et al. (2008). Disaggregases are a category of chaperone proteins that help break down or remove aggregated proteins (Mogk & Bukau, 2004); Skd3 (Caseinolytic peptidase B protein homolog; Rizo et al., 2019) and heat shock proteins (specifically Hsp70 [Gao et al., 2015; Thackray et al., 2022], Hsp104 [Shorter & Southworth, 2019], and Hsp110 [O'Driscoll et al., 2015]) are the two groups of disaggregases typically studied. Small molecule treatments are also being investigated to promote the cell's own degradation processes to break down aggregates. For example, rapamycin has been shown to increase the clearance of α-synuclein due to its inhibition of mTOR, inducing autophagy (Maiese et al., 2013; Sarkar et al., 2007). Rapamycin and many of these degradation-enhancing small molecules have many other off-target effects (such as acting on other essential pathways involved in immunosuppression; Abraham & Wiederrecht, 1996), for this strategy to be effective different small molecules with fewer off-target effects need to be tested. Small soluble oligomers are beginning to be identified as the toxic species in many protein aggregation diseases (A. K. R. Dasari et al., 2019; Lasagna-Reeves et al., 2013; Martinelli et al., 2019; Proctor et al., 2016; C. Wells et al., 2021), while larger aggregates are protective (Ham et al., 2019; Hnath & Dokholyan, 2022; Leitman et al., 2013; Xu et al., 2011; Zhu et al., 2018). Strategies that target and break down larger aggregates may be increasing toxicity by undoing the sequestration of smaller oligomers and increasing the abundance of the toxic species. Antibody strategies have therapeutic potential if they are highly specific for the toxic forms of aggregates, but the debate is ongoing to determine what the primary toxic species are for most protein aggregation diseases.

The last therapeutic strategy in development to treat protein aggregation-related diseases is in the initial phases of exploration. Most protein aggregation diseases involve a toxic gain of function (Hoffner & Djian, 2002; Rajagopalan & Andersen, 2001; Redler & Dokholyan, 2012; Sharma et al., 2016), meaning the toxic aggregates interact with another protein or membrane in a way they should not be. To combat this, researchers are inhibiting the toxic interaction either by blocking the binding site (Akhtar et al., 2021; Choi & Dokholyan, 2021; with specific antibodies or small molecules) or by using chaperones to promote non-toxic aggregate formation (promote fibrils). Since the structures and toxic interactions of protein aggregates are largely unknown this strategy is in the early stages.

Many protein aggregation diseases occur in the central nervous system (Alzheimer, Parkinson, and Huntington diseases), so these therapeutic strategies have the added challenge of crossing the blood–brain barrier to be effective. Additionally, these strategies cannot reverse

tissue damage, so early diagnosis is crucial for any of the strategies to have any effect. Other therapies are in development that focus on treating cellular damage after it occurs instead of preventing it by targeting the protein aggregation (Poppe et al., 2014; Srivastava et al., 2021). Preventing aggregate formation from the beginning, either through reducing the overall amount of protein or the addition of small molecules/ chaperones, has the potential to prevent both oligomer and fibril formation if biomarkers are discovered which allows patients to be treated earlier in disease progression. Breaking down aggregates is one of the most widely used current therapeutic strategies, despite the dangerous possibility of increasing toxic species by breaking down protective fibrils. Targeting toxic interactions has the potential to be an effective therapeutic once more work is done to determine the proteins or membranes being affected during the toxic gain of function. Many drug design strategies that attack aggregation have failed in the past due to administration too late in disease progression or targeting the wrong size aggregate. Therapeutic strategies become not just ineffective but dangerous by not considering the toxicity of oligomers versus fibrils in different diseases.

6 | CONCLUSION

Protein aggregates are still studied as one group, despite soluble oligomers and insoluble fibrils having drastically different toxicities and structures. Aggregation of IDPs, proteins with IDRs, or ordered proteins all contribute to a variety of diseases, the majority form into toxic oligomers that begin a gain of function while insoluble fibrils have protective effects. Modifications to aggregation-prone proteins by mutations, metal interactions/ loss of metals, PTMs, or lipid interactions contribute to the rate of aggregation and what size aggregates are formed. Due to the span of sizes and structural conformations of aggregates, different computational methods need to be utilized to study oligomers and fibrils. Finally, many different therapeutic strategies have been attempted to target aggregated proteins in disease, but more research needs to be done into the actual toxic interactions instead of just breaking apart aggregates and potentially increasing toxic oligomers. Overall, it is crucial to determine what size aggregate is toxic in each disease. Specifically targeting the toxic size can increase the effectiveness of protein aggregation modeling and therapeutic strategies to treat protein aggregation-related diseases.

ACKNOWLEDGMENTS

We acknowledge support from the National Institutes for Health (R35 GM134864, 1RF1 AG071675-01, 1R01 AT012053), National Science Foundation (2210963), and the Passan Foundation.

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How to cite this article: Hnath B, Chen J, Reynolds J, Choi E, Wang J, Zhang D, et al. Big versus small: The impact of aggregate size in disease. Protein Science. 2023;32(7):e4686. https://doi.org/10.1002/pro.4686