

REVIEW

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Misfolded protein oligomers: mechanisms of formation, cytotoxic effects, and pharmacological approaches against protein misfolding diseases

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Abstract

The conversion of native peptides and proteins into amyloid aggregates is a hallmark of over 50 human disorders, including Alzheimer's and Parkinson's diseases. Increasing evidence implicates misfolded protein oligomers produced during the amyloid formation process as the primary cytotoxic agents in many of these devastating conditions. In this review, we analyze the processes by which oligomers are formed, their structures, physicochemical properties, population dynamics, and the mechanisms of their cytotoxicity. We then focus on drug discovery strategies that target the formation of oligomers and their ability to disrupt cell physiology and trigger degenerative processes.

Keywords Aggregation kinetics, Secondary nucleation, Protofibrils, Lecanemab, Cellular interactions, Amyloid toxicity, Drug discovery, Diagnostics, Therapeutics, Fibril fragmentation, Biophysics

Background

It has been known for many years that numerous normally soluble proteins can misfold and self-assemble into amyloid fibrils [1–5]. The architecture of these aberrant aggregates is highly organized, with a characteristic cross- β core formed by β -strands arranged perpendicular to the main axis of the fibril, creating an extensive network of hydrogen bonds that confer high stability to the amyloid state [1–4, 6]. Amyloid fibrils are associated with a variety of human diseases involving either

the central nervous system (neuropathic conditions), a multiplicity of tissues and organs other than the brain (non-neuropathic systemic amyloidoses), or a specific organ (non-neuropathic localized amyloidoses) [1]. Individual amyloid-associated diseases (Table 1) are generally characterized by the loss of native function for specific peptides or proteins, and these biomolecules can form aberrant and destructive aggregates [1, 7]. Collectively, these conditions affect dozens of millions of people worldwide [1]. To cite a few examples, the deposition in the brain of the tau protein into intracellular neurofibrillary tangles [8, 9] and the amyloid- β peptide (A β) into extracellular plaques [10, 11] is associated with Alzheimer's disease (AD). Parkinson's disease (PD) is characterized by the aggregation of α -synuclein into Lewy Bodies of dopaminergic neurons [12, 13], and type II diabetes (T2D) by the self-assembly of islet amyloid polypeptide (IAPP, also known as amylin) in the islets of Langerhans in the pancreas [14, 15]. Consequently, the characterization of the various species that are formed within each

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Table 1 Non-exhaustive list of protein misfolding diseases with their associated peptides or proteins, and in vitro and ex vivo structures of corresponding amyloid fibrils. Putative mechanisms of aggregation and toxicity are also indicated

Disease	Proteins	Length	Prospective most toxic species	Dominant aggregation mechanism	In vitro amyloid structure	Ex vivo amyloid structure
Alzheimer's disease (AD)	Amyloid- β (A β) Tau (3R + 4R)	40 or 42 352–441	A β : Oligomers [3, 16, 17] Tau: Oligomers [18–20]	Secondary nucleation [21, 22] Secondary nucleation [23]	A β_{40} [24] A β_{42} [25] Tau [26]	A β_{40} [27, 28] A β_{42} [5] Tau [29, 30]
Parkinson's disease (PD)	α -synuclein	140	Oligomers [31–33]	Condition-dependent [34], including lipid-induced aggregation [35]	[36–39]	[40–42]
Dementia with Lewy Bodies (DLB)	α -synuclein	140	Pre-synaptic aggregates [43] & oligomers [44, 45]	Not yet known, secondary pathways [46]	-	[40, 47]
PD dementia (PDD)	α -synuclein	140	Not yet known, possibly oligomers [48]	-	-	[40]
Multiple system atrophy (MSA)	α -synuclein	140	Oligomers [49, 50]	-	-	[42, 51]
Huntington's disease (HD)	Huntingtin	variable	Not yet known, possibly oligomers [52–54]	Possibly stochastic nucleation [55]	[56]	-
Chronic traumatic encephalopathy (CTE)	Tau (3R + 4R)	352–441	Not yet known	Not yet known, possibly secondary pathways [57]	[26]	[58]
Pick's disease (PiD)	Tau (3R)	352–410	Not yet known	Not yet known, possibly secondary pathways [59]	-	[60]
Corticobasal degeneration (CBD)	Tau (4R)	383–441	Not yet known	Not yet known, possibly secondary pathway [61]	-	[62]
Progressive supranuclear palsy (PSP)	Tau (4R)	383–441	Not yet known	-	-	[4]
Argyrophilic grain disease (AGD)	Tau (4R)	383–441	Not yet known	Not yet known, possibly secondary pathways [63]	-	[4]
Globular glial tauopathy (GGT)	Tau (4R)	383–441	Not yet known	Not yet known, possibly secondary pathways [64]	-	[4]
Spongiform encephalopathies	Prion protein (PrP)	208	PrP ^{Sc} [65], possibly oligomers [66, 67]	Fragmentation [68]	[69–72]	Mouse PrP [73, 74] RML prion [75] Scrapie [70] Bovine serum encephalopathy [76]
Type II diabetes	Amylin (IAPP)	37	Oligomers [77, 78]	Secondary nucleation [79]	[80, 81]	[82]
Cataracts	Crystallins	175	Aggregates of damaged proteins [83, 84]	-	-	-
AA amyloidosis	Serum amyloid A	104	Fibrils [85] and oligomers [86]	Secondary pathways [87]	[85, 88]	[85, 88]
Transthyretin amyloidosis	ATTRwt Val30Met ATTR	127	Various species [89–91]	-	-	[92, 93]
Ig-related amyloidosis (AL)	Immunoglobulin light chain	Proteolytic fragments	Fibrils [94] and oligomers [95]	Not yet known, seed-competent [96]	-	[97, 98]

aggregation reaction, and the study of the mechanisms by which they contribute to cellular dysfunction and death, would help reveal the molecular origins of protein misfolding diseases and provide insights into possible therapeutic and diagnostic methods to combat these conditions.

The fibrillar species of amyloidogenic proteins were initially thought to be the most toxic aggregate forms in many protein misfolding diseases. However, increasing evidence has shown that smaller, intermediate and metastable soluble aggregates, known as misfolded protein oligomers, are in many cases more toxic than their mature fibrillar counterparts [18, 19, 77, 99–104]. In other protein misfolding diseases, amyloid fibrils are cytotoxic either by sequestering functional proteins (loss-of-function) or by directly damaging the cells and tissues where they form (toxic gain-of-function) [1, 100, 105, 106]. Moreover, once formed, amyloid fibrils can, in certain amyloid systems, establish a positive feedback loop that further promotes the proliferation of more oligomers in a multiplicative manner through surface-catalyzed secondary nucleation [21, 107]. Furthermore, amyloid fibrils can act as reservoirs of oligomers that can detach from the fibril ends [108]. Mature fibrillar aggregates also remain main pathological biomarkers and histological hallmarks [109–112], while oligomers may be a crucial target for effective drug screening programs [16, 101, 113, 114].

Because of the elusive nature of misfolded protein oligomers, they can vary in their characteristics, including differences in their size and hydrophobicity, as well as their degree of metastability [1, 115, 116]. The detection of oligomers *ex vivo* for diagnostic purposes thus presents challenges, particularly due to their heterogeneous structure, transient nature, and low concentrations [115]. A β oligomers have been detected in AD brains using conformation-sensitive antibodies specific for well-defined oligomers with low reactivity towards monomers, fibrils and other oligomer types [115, 117–122]. A β oligomers have also been detected in peripheral fluids, including the cerebrospinal fluid (CSF), where they are present in the circa attomolar to picomolar concentration range [115, 123, 124], and tau oligomers have been found in the CSF at approximately femtomolar concentrations [125].

Despite these challenges, progress has been made to reinforce our understanding of misfolded protein oligomers on several fronts, as we will describe in this review article. We consider here these oligomers with respect to their biophysical characterization and population dynamics, quantification techniques, the processes by which they form, and the consequences of their dysregulated

presence. We then conclude by summarizing recent developments in drug discovery against oligomers.

The amyloid state of proteins

Many proteins in their physiological states are frequently expressed at levels close to their solubility limits [126, 127]. These proteins are thermodynamically metastable in their native states and over time tend to convert into aggregates [128] (Fig. 1). The amyloid state is characterized by the presence of fibrillar aggregates consisting of a number of β -sheet structures running along the fibril axis [129, 130]; a fibrillar morphology with characteristic cross- β structure and signature tinctorial properties, including binding of the dyes thioflavin-T and Congo red, are commonly accepted as key hallmarks of the amyloid state [1]. It is accessible independently of the sequence, structure and function of the precursor native proteins and is now recognized to often be the most stable state of a protein, even more stable than the native state, at the high protein concentrations present in the cellular environment [131, 132]. Breakthrough developments in solid-state nuclear magnetic resonance (ssNMR) spectroscopy and then cryogenic electron microscopy (cryo-EM), particularly the increased sensitivity of instruments [133] and the advancement of analytical software [134], have facilitated the determination of high-resolution structures (approaching the 2 Å limit) of brain-derived amyloid fibrils of disease-associated proteins and peptides. Filamentous structures have been solved for many amyloidogenic biomolecules, including for tau [135], TDP-43 [136], α -synuclein [40], and amyloid- β [5], although those of TDP-43 do not exhibit cross- β structure with the 10–11 Å spacing and binding to amyloid diagnostic dyes [136]. Remarkably, it has been shown that the tau protein can self-assemble into a range of different amyloid structures, known as polymorphs, in a pathology-dependent manner, including in AD, Pick's disease (PiD), chronic traumatic encephalopathy (CTE), corticobasal degeneration (CBD), and progressive supranuclear palsy (PSP) [4, 135] (Table 1), and the same is true for amyloid fibrils formed from other proteins [137]. Polymorphism has also been observed for A β fibrils in AD, with different polymorphs in different patients [5, 138], and in familial and sporadic forms [139].

We also note that in some cases amyloid species can be functional, and be formed both intra- and extracellularly in diverse organisms ranging from bacteria to mammals [142–144]. For example, functional amyloids serve roles in curli formation in *E. coli*, in the control of nitrogen catabolism in yeast, and as scaffolds that promote melanin synthesis in human melanocytes [142, 143]. Unlike pathological amyloid

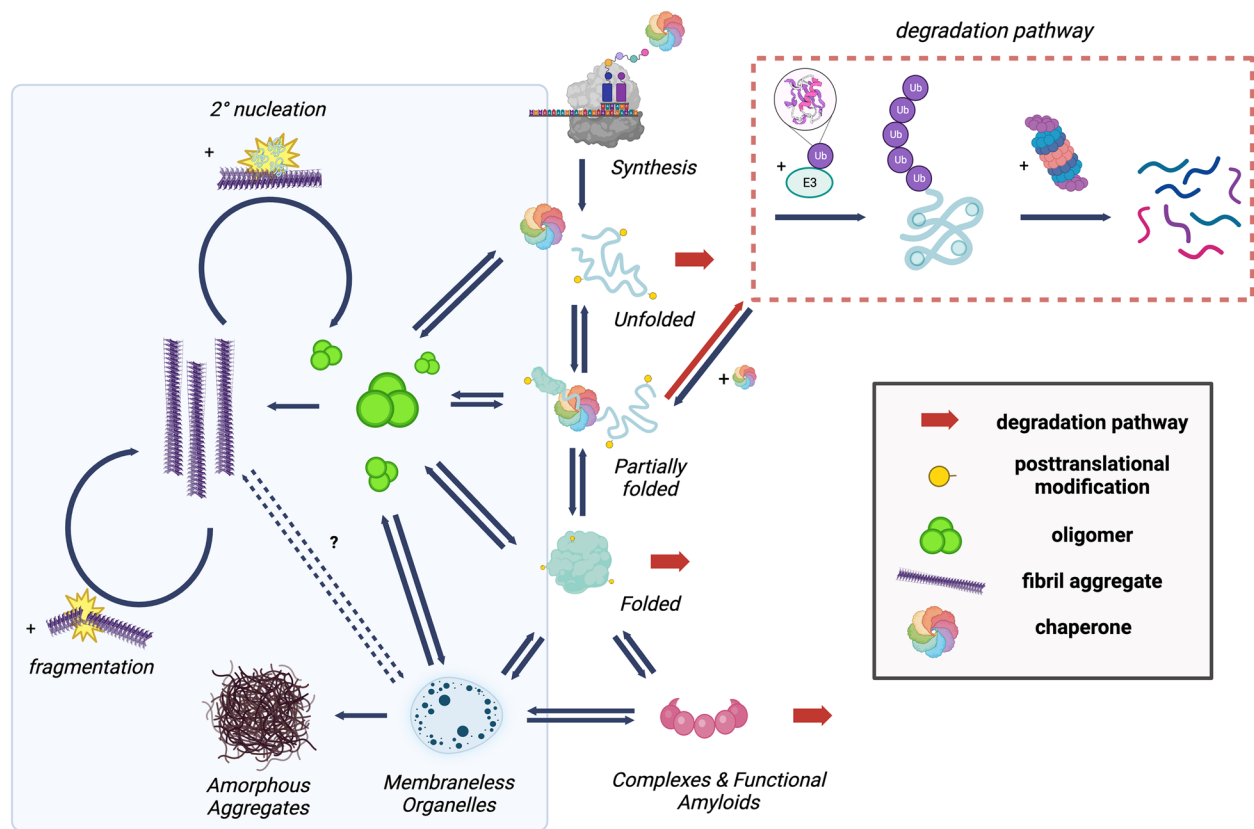


Fig. 1 Proteins interconvert between different conformational states in the cell. After its biosynthesis by the ribosome, a protein may fold into its native state and be trafficked to its correct cellular location, assemble into a functional complex, condense into membraneless organelles, or misfold and aggregate. These processes are regulated by the proteostasis network (PN) [1, 140]. Accessing the amyloid state is a process that typically involves the conversion of monomeric proteins into oligomers and ultimately, highly ordered, rigid cross- β sheet fibrillar structures [1]. In addition to being associated with disease, amyloid fibrils can also be functional, and for this reason they have applications in material sciences, biomedical engineering, and drug discovery [1, 2, 141]. Created with biorender.com

fibrils, functional amyloids form under controlled conditions and seem to have evolved to avoid secondary pathways, possibly in order to eliminate autocatalytic processes that would be difficult to regulate [145]. From a materials chemistry perspective, functional synthetic amyloids have been leveraged for a variety of biotechnological applications, such as silk micrococoon for antibody drug delivery [146], and amyloid-coated purification systems to reduce heavy metal concentrations in contaminated water sources [147]. Although predominantly studied for their pathogenicity, fibrillar species from protein misfolding diseases have also demonstrated a capacity to catalyze chemical reactions. For example, it has been shown recently that various amyloid fibrils can also catalyze chemical reactions, including the hydrolysis of para-nitrophenyl acetate and dephosphorylation of para-nitrophenyl-orthophosphate induced by α -synuclein fibrils [148]

and the degradation of specific neurotransmitters induced by $A\beta$ fibrils [149].

Structure and mechanism of oligomer formation

The conversion of proteins from their soluble native state to amyloid fibrils is a complex process that involves a number of intermediate states. We will refer to the intermediates that are multimeric but small enough to remain soluble as misfolded protein oligomers. A great assortment of oligomers has been described for the widely studied $A\beta_{40}/A\beta_{42}$ system and a wide range of names have been given them, such as spherical and chain-like protofibrils, paranuclei, pentamers, globulomers, amylo-spheroids, SDS-stable dimers/trimers, $A\beta$ -derived diffusible ligands (ADDLs), prefibrillar and fibrillar oligomers, and spherical amyloid intermediates. [101, 118, 120, 150–159]. Different oligomeric species have also been described for α -synuclein, including type A, type A*,

type B and type B* oligomers, amongst many other forms [33, 108, 160–163].

Comparison between structural characteristics of the various oligomers indicates that the β -sheet content generally increases with molecular weight, suggesting that an increase in oligomer size stabilizes their β -sheet structure. Such structure has generally been shown to involve both anti-parallel [157, 161, 164] and parallel but out-of-register strands [165], unlike fibrils where β -strands are generally parallel and in-register. When various oligomers of A β or α -synuclein appear sequentially with time during an aggregation process, the first species are unstructured, and the species containing β -sheet structure appear later [154, 159, 160, 165, 166]. The aggregation of globular proteins recapitulates many of these characteristics if the process takes place under conditions promoting their unfolding [167, 168]. However, when it is initiated under native conditions, it often leads to early aggregates where the individual monomers populate native-like states, which later convert into β -sheet containing protofibrils/fibrils [169, 170].

The largest oligomers with the highest β -sheet content, such as the fibrillar oligomers, annular protofibrils, chain-like protofibrils and amylospheroids for A β and type B or B* oligomers for α -synuclein, represent off-pathway species that need to reassemble at least partially before forming amyloid fibrils [164, 171, 172]. These species are large and have antiparallel or parallel but out-of-register β -sheet arrangements that needs to be substantially reorganized to form the parallel in-register cross- β structure of the fibrils. Their off-pathway nature is also shown by dedicated kinetic tests [164, 171, 173]. On-pathway oligomers are more difficult to detect and isolate because they generally convert into other oligomers or fibrils. Indeed, one important class of oligomers include nuclei of fibril formation, which can be identified kinetically, as described in the next section.

Kinetic mechanisms of amyloid fibril formation to reveal oligomeric nuclei

Macroscopic measurements

Quantitative kinetic analysis of amyloid fibril formation makes it possible to gain insight into the mechanism of misfolded protein oligomer generation that are on-pathway to fibril formation, as well as their population dynamics [174]. Chemical kinetics enables the establishment of models to describe the conversion of monomeric proteins into fibrillar products by breaking the process down into a series of elementary steps governed by rate laws [175]. Fluorescent dyes are commonly used to monitor fibril formation, as they exhibit a substantial increase in quantum yield upon their interaction with β -sheet rich structures (Fig. 2) [176, 177]. Consequently, the

binding of these dyes to amyloid fibrils induces a large fluorescence emission increase that, over time, manifests as a classic sigmoidal curve in *in vitro* aggregation assays. Macroscopically, this sigmoidal curve can be thought of as having three major phases: (1) a lag phase where aggregation is already under way, but the amount of fibrillar structures is too low to be detectable, (2) a growth phase dominated by secondary processes (i.e. microscopic steps that depend on the presence of fibrils), and (3) a plateau phase that begins when the concentration of the monomers remaining in solution becomes rate limiting [178]. However, because of their ability to recognize molecular grooves of a fibrillar surface, the dyes are not ideally suited to quantify the heterogeneous species formed in the early stages of the aggregation process, including oligomers and protofibrils. Biophysical methods can instead be used, such as dynamic light scattering (DLS) or microfluidic free-flow electrophoresis [115], amongst others discussed later in this review.

To connect these macroscopic observables with the microscopic processes that contribute to the overall reaction, one can use the formalism of chemical kinetics (Table 2). In this approach, the elementary steps underlying the aggregation process are described by a system of differential equations, known as a master equation. This equation defines the time dependence of the populations of the intermediate species produced during the reaction, which cannot be readily measured, from the knowledge of the time dependence of the populations of the reactants and products, which can instead be measured [180]. The steps accounted for within the master equation approach for amyloid systems can be classified into two groups: those that affect the aggregate mass (growth), such as fibril elongation and monomer dissociation, and those that contribute to the total number of aggregates, such as primary nucleation, secondary nucleation, and fibril fragmentation.

In practice, a series of aggregation assays are conducted with different initial conditions to probe specific microscopic mechanisms [175, 181]. Specifically, the addition of low concentrations of fibrillar seeds at the start of the aggregation reaction can bypass primary nucleation, while the addition of high concentrations of seeds bypasses both primary and secondary nucleation, thus assessing elongation. The combination of unseeded and seeded aggregation assays can offer sufficient constraints to enable the determination of the microscopic rate constants by a global fit of the kinetic data [180, 182–184]. The web platform Amylofit, which is freely available (<https://amylofit.com/>), has been developed to facilitate this type of analysis [181], and it can solve molecular mechanisms and kinetic parameters with and without additives like small molecules. The differential

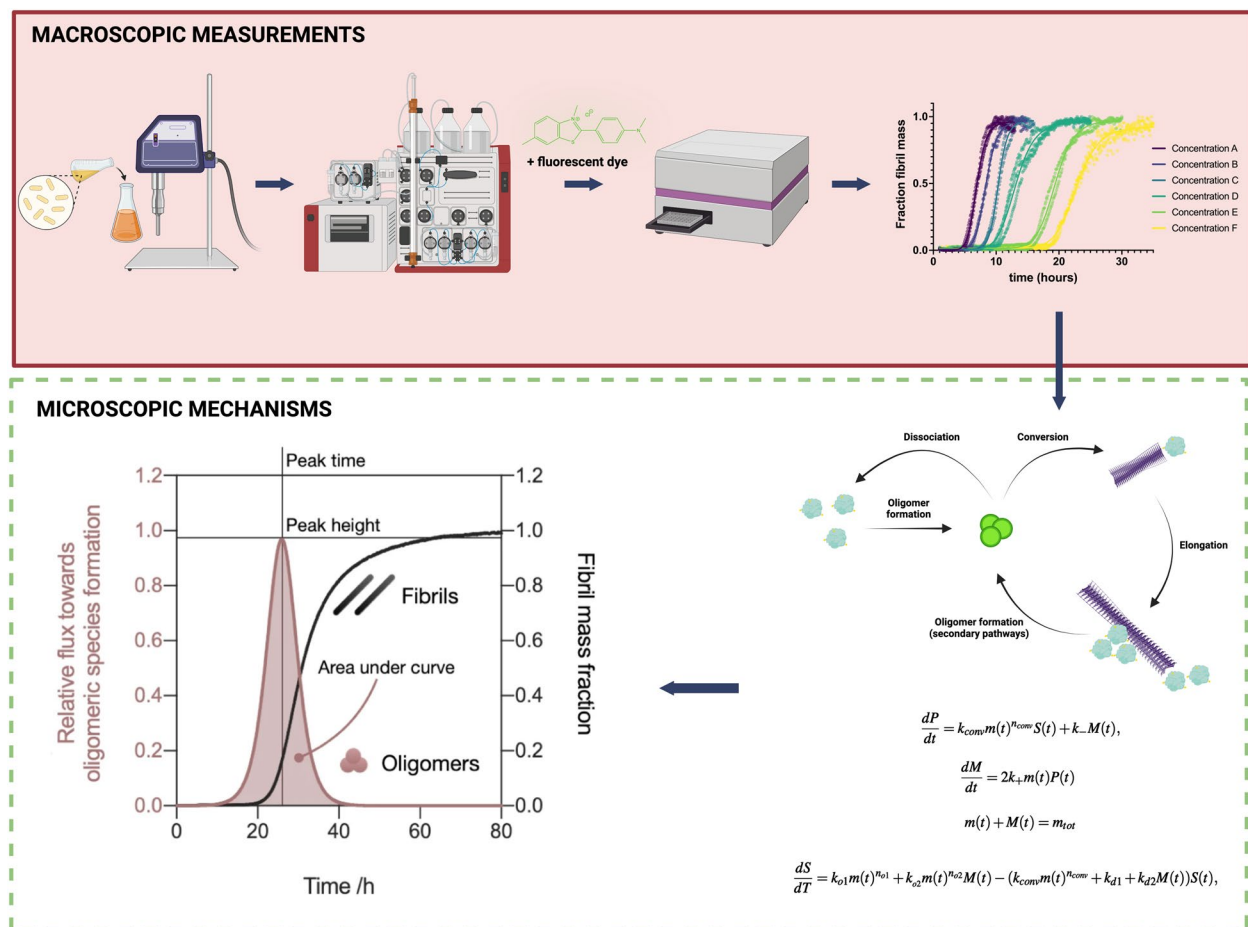


Fig. 2 From macroscopic measurements to microscopic mechanisms of protein aggregation. In a typical in vitro aggregation experiment, recombinant proteins are purified using a number of procedures, including fast protein liquid chromatography. Samples containing purified proteins are aliquoted with an amyloid-binding fluorophore in a low-bind multiwell plate. A plate reader tracks the time-dependent evolution of the overall fibril mass, and kinetic traces can be subsequently analyzed using chemical kinetics to resolve the mechanism of aggregation, as well as the effects of additive species, such as aggregation inhibitors. The reactive flux towards oligomeric species can be calculated using this approach [179]. Relative flux graphic reprinted from Staats et.al [179]. Created with biorender.com

equations for key elementary steps in the amyloid pathway are summarized in Table 2, and the microscopic steps relevant to oligomer formation (Fig. 3) are analyzed in the following sections. Examples of individual kinetic traces from several different protein systems analyzed with this analytical procedure and single-molecule biophysical experiments are also shown in Fig. 4.

Fibril elongation

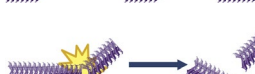
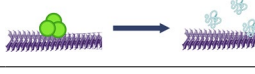
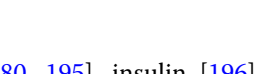
Elongation is a microscopic process in which a monomer is added to a fibril end. The rate at which this process takes place, specified by the rate constant k_{+} , is highly dependent upon conditions such as temperature, pH, and ionic strength [187]. Generally, fibril elongation sees the adsorption of monomer to the ends of a growing fibril, followed by a rapid conversion step. However, at sufficiently

high concentrations of monomers, fibril elongation can be saturated, suggesting a two-step process [188].

Primary nucleation

In this process, individual monomers self-associate into small, disordered oligomers. The majority of the oligomers produced in this way dissociate back into monomers [174], though in some cases they can persist long enough to convert into ordered oligomers, which are effectively fibril fragments and can act as seed for fibril growth [174]. The presence of surfaces, for example other proteins or lipid membranes, can, in certain instances, serve as a catalyst that induces heterogeneous primary nucleation [189, 190]. It is difficult to observe individual primary nuclei in a macroscopic sample due to their low concentration, transient nature, and a lack of molecular probes that bind them with specificity. The use of

Table 2 Time evolution of the fibril number concentration (P), fibril mass concentration (M) and oligomer mass concentration (S) for the microscopic processes involved in amyloid formation [181]. $m(t)$, time-dependent monomer concentration; $M(t)$, time-dependent fibril mass concentration; $P(t)$, time-dependent fibril number concentration; $S(t)$, time-dependent oligomer mass concentration; k_n , primary nucleation rate constant; k_2 , secondary nucleation rate constant; k_+ , elongation rate constant; k_- , fragmentation rate constant; k_d , oligomer dissociation rate constant; k_{o1} , primary oligomer association rate constant; k_{o2} , secondary oligomer association rate constant; k_{conv} , oligomer conversion rate constant; k_{d2} , fibril-mediated oligomer dissociation rate constant; n_c , reaction order of primary nucleation; n_2 , reaction order of secondary nucleation; n_{conv} , reaction order of oligomer conversion; n_{o1} , reaction order of primary oligomer association; n_{o2} , reaction order of secondary oligomer association; K_E , Michaelis–Menten constant for saturating elongation (monomer concentration at which the rate of elongation is half the maximal velocity, V_{max}); K_M (Michaelis–Menten constant for saturating secondary nucleation (monomer concentration at which the rate of secondary nucleation is half the maximal velocity, V_{max}))

Molecular process	Time evolution model	Illustration of the model
Primary nucleation	$\frac{dP}{dt} = k_n m(t)^{n_c}$	
Heterogeneous primary nucleation	<i>varies</i>	
Elongation	$\frac{dM}{dt} = 2k_+ m(t)P(t)$	
Saturating elongation	$\frac{dM}{dt} = \frac{2k_+ m(t)P(t)}{1 + \frac{m(t)}{K_E}}$	
Fragmentation	$\frac{dP}{dt} = k_- M(t)$	
Secondary nucleation	$\frac{dP}{dt} = k_2 m(t)^{n_2} M(t)$	
Multistep secondary nucleation	$\frac{dP}{dt} = \frac{k_2 m(t)^{n_2} M(t)}{\left(1 + \frac{m(t)}{K_M}\right)^{n_2}}$	
Primary association of oligomers	$\frac{dS}{dt} = k_{o1} m(t)^{n_{o1}}$	
Primary dissociation of oligomers	$\frac{dS}{dt} = k_d S(t)$	
Oligomer conversion	$\frac{dS}{dt} = k_{conv} m(t)^{n_{conv}} S(t)$	
Secondary association of oligomers	$\frac{dS}{dt} = k_{o2} m(t)^{n_{o2}} M(t)$	
Fibril-mediated oligomer dissociation	$\frac{dS}{dt} = k_{d2} S(t)M(t)$	

microfluidic techniques has, however, enabled their visualization, thus providing novel insight into the role of system size on primary nucleation events and the propagative nature of the amyloid cascade [191].

Secondary nucleation

The process by which new aggregates form by a monomer adsorbing onto and nucleating at the surface of an existing fibril is known as secondary nucleation [192]. It has been observed crystalline systems [193] and a range of protein deposition reactions, including for sickle-cell

hemoglobin [194], IAPP [80, 195], insulin [196], A β_{42} [21], α -synuclein [35, 197], and tau [23], with increasing evidence implicating its key role in disease and pathology. Secondary nucleation in protein aggregation has been directly visualized using direct stochastic optical reconstruction microscopy (dSTORM) [198] and total internal reflection fluorescence (TIRF) microscopy [199]. Furthermore, the formation of oligomers is greatest when both monomers and fibrils are involved in an aggregation reaction, compared to any other molecular process in the amyloid network. Thus, secondary nucleation, markedly

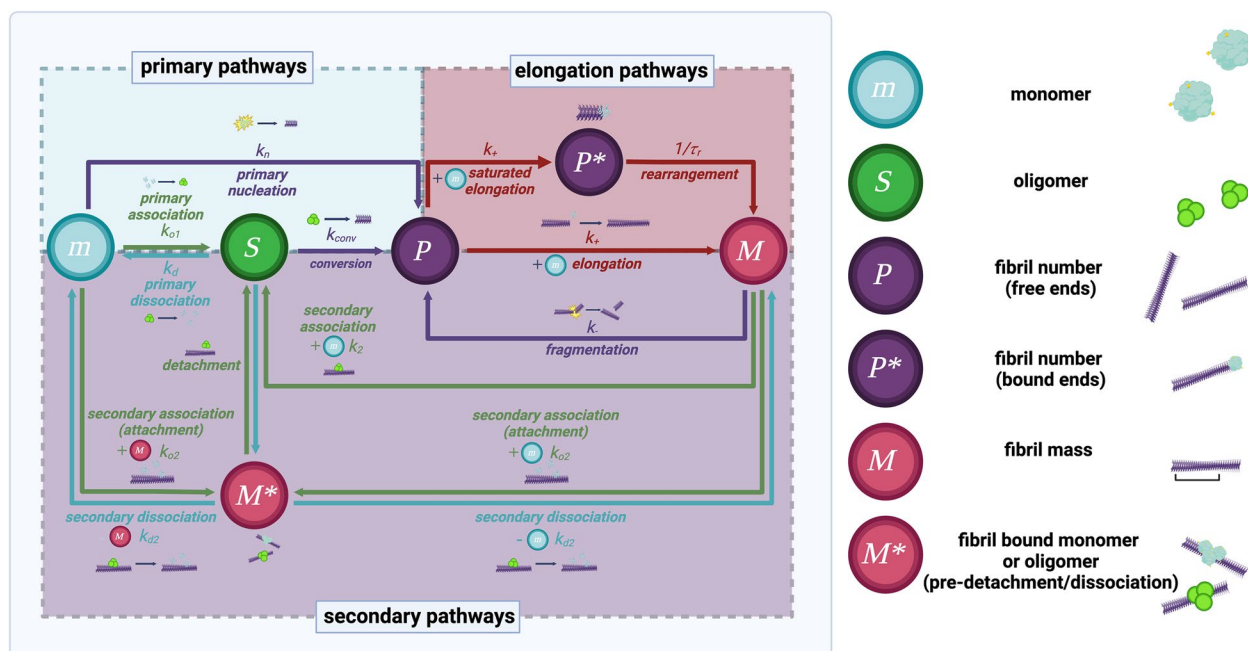


Fig. 3 Petri net representation of the reaction network that models an aggregation reaction. The monomer mass concentration (m), fibril mass concentration (M), fibril number concentration (P) and oligomer mass concentration (S), as well as the rate constants of their interconversions (see Table 2 for definitions), are indicated. M^* represents monomer bound to fibril prior to its conversion or detachment, and P^* represents a multistep elongation process including association and rearrangement. These processes fall into three categories: growth processes (elongation pathways), primary pathways, and secondary pathways (i.e. those that require the presence of fibrils). Note that no fibrillar mass is lost due to secondary nucleation or fragmentation, unless non-fibrillar oligomers are capable of detaching from fibril ends. Pathways shown in red and purple increase the relative fibrillar mass and number, respectively. In green are the pathways considered to be 'pro-oligomer,' meaning they lead to a net increase in the oligomer population. Conversely, pathways shown in blue represent microscopic processes that lead to oligomer dissociation. Adapted from Meisl et al. [185], to include the reactive flux towards and away from oligomers. Created with biorender.com

more so than other microscopic steps, can be often implicated as a major source of toxic misfolded protein oligomers (Fig. 3) [200].

Fragmentation

Concurrent with or independent of secondary nucleation depending on solution conditions, fragmentation is another secondary pathway which may be particularly relevant, such as for the aggregation of insulin [180], β -lactoglobulin [201, 202], and the prion glycoprotein [203]. In a process dominated by fibril fragmentation, a fibril breaks into shorter fibril fragments, leading to a proliferation of fibril ends and therefore an exponential increase in fibril mass in the presence of fibril elongation. Fibril fragmentation may result from thermal fluctuations and mechanical stress [204], or regulatory processes, such as by molecular chaperones [205]. In the real-time quaking-induced conversion (RT-QuIC) assay, monomeric substrate can be seeded by pathogenic fibrils present in a diluted brain homogenate sample alongside shaking to induce fragmentation, including for 3R [59, 206] and 4R [61] tauopathies, and synucleinopathies [207]. Like secondary nucleation, fragmentation can

enhance the overall cytotoxicity within the amyloid cascade [208]. Exacerbated cytotoxicity may stem from the ability for oligomers to detach from fibril ends [108], the exponential growth of fibril ends for monomer adsorption, or enhanced cellular uptake of, on average, shorter fibrils [209–211].

Oligomer dynamics

Recent advances in experimental methods to detect and quantify oligomers [115] (see below) have facilitated the development of kinetic models that explicitly include the formation and disruption of oligomers, including ones that are both on- and off-pathway (Table 2). This inclusion of oligomer dynamics in the rate equations for protein aggregation, and their subsequent fitting to data using amyloid-binding dyes that monitor for fibril formation, now enables the detailed description of the kinetics of oligomer populations formed during aggregation reactions for multiple protein systems [174, 186, 212].

As done with amyloid assembly kinetics, the evolution of oligomeric species can be broken down into a series of microscopic elementary steps: monomer association into oligomers, oligomer dissociation into monomers,

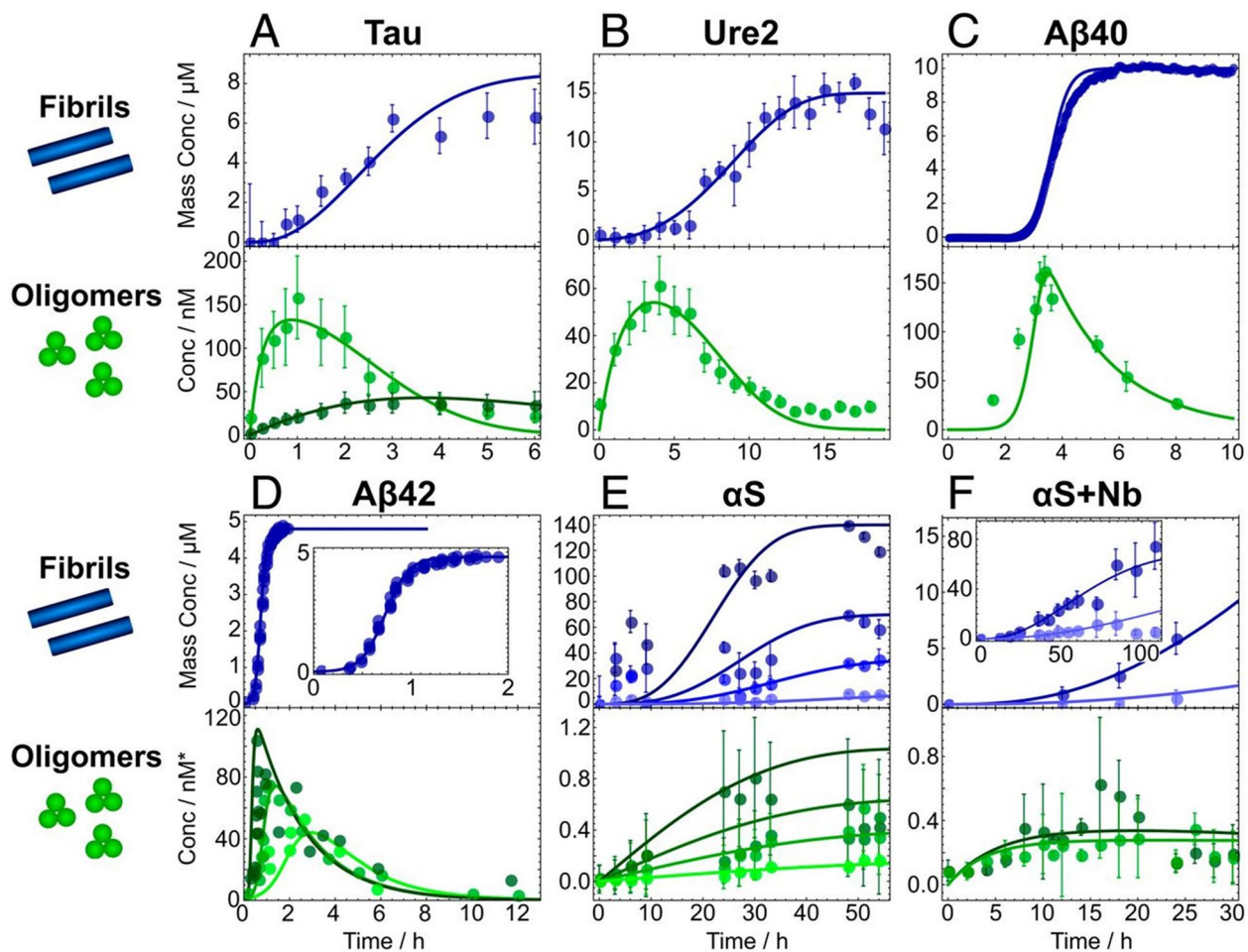


Fig. 4 Global simultaneous fits for fibril mass concentration using amyloid-binding dyes and soluble oligomer concentration. Fibril (top panels) and oligomer (bottom panels) concentrations were determined using amyloid-binding dyes and single molecule biophysical techniques, respectively. Fitting parameters are summarized herein and described in detail in ref. [186]. This study shows that it is possible to monitor oligomer dynamics from macroscopic amyloid-dye binding experiments. Reprinted from Dear et. al [186]

oligomer conversion into oligomers competent for fibril elongation (fibrillar oligomers), and elongation of fibrillar oligomers to fibrils (Fig. 3, Table 2) [186]. Non-fibrillar (i.e. non-converted) oligomers can form via primary nucleation, which is a fibril-independent pathway, or via fibril-dependent secondary mechanisms (Table 2). Upon formation, oligomers can be depleted by either their conversion into elongation-competent fibrillar species, or their dissociation back into monomers (Table 2). Because of the reversibility of secondary nucleation, it is also possible that oligomers dissociate back to monomers upon interaction with the fibril surface. This is included in Fig. 3 and Table 2 as secondary dissociation or fibril-mediated oligomer dissociation. Each of these steps contributes to the oligomer population dynamics for a given amyloid system, which can be summarized by four main parameters that differ considerably from one amyloid

system to another. These parameters are: persistence, productivity, abundance, and peak half-time.

The first of these, oligomer persistence, measures the decay, whether through dissociation or conversion, of the oligomer population upon reaching the peak concentration. It is governed by the average lifetime of the oligomers. Revisiting available *in vitro* kinetics data [21, 22, 213, 214] with a mechanistic model accounting for oligomeric reactions revealed that the intermediate species have different lifetimes ranging from a few minutes (PrP) to hundreds of hours (α -synuclein) under their corresponding conditions of analysis.

Another parameter to describe the dynamics of oligomers, the kinetic productivity, measures the tendency of oligomers to convert into fibrillar nuclei instead of dissociating. Like persistence, the productivity of oligomers varies substantially between the various amyloid systems studied so far. From tau to α -synuclein, oligomer

Table 3 Analytical expressions for various descriptors of oligomer population dynamics [114]. ρ_{o1} and ρ_{o2} represent the rates of oligomer formation via primary and secondary nucleation, respectively, ρ_+ represents the rate for fibril elongation, ρ_c represents the rate of oligomer conversion, and ρ_e represents the combined rates of oligomer conversion and dissociation

Descriptor	Non-equilibrium oligomerisation	Quasi-equilibrium oligomerisation
Secondary nucleation-dominated		
Peak time	$\tau_{peak} = \frac{1}{(\rho_{o2}\rho_c\rho_+)^{\frac{1}{3}}}$	$\tau_{peak} = \frac{1}{(\rho_{o2}\rho_+)^{\frac{1}{2}}} \sqrt{\frac{\rho_e}{\rho_c}}$
Persistence	$\tau_{decay} \simeq \frac{1}{\rho_c + \rho_d}$	$\tau_{decay} \simeq \frac{1}{(\rho_{o2}\rho_+)^{\frac{1}{2}}} \sqrt{\frac{\rho_e}{\rho_c}}$
Productivity	$\rho = \frac{\rho_c}{\rho_e}$	$\rho = \frac{\rho_c}{\rho_e}$
Abundance	$\frac{c_{peak}}{m(0)} = \frac{\rho_{o2}}{\rho_e}$	$\frac{c_{peak}}{m(0)} = \frac{\rho_{o2}}{\rho_e}$
Primary nucleation-dominated		
Peak time	$\tau_{peak} \simeq \frac{1}{(\rho_{o1}\rho_+)^{\frac{1}{2}}} \sqrt{\frac{\rho_e}{\rho_c}}, \text{ or } \frac{1}{\sqrt{\rho_+k_-}} \text{ (if fragmentation dominated)}$	
Persistence	$\tau_{decay} \simeq \frac{1}{\rho_c + \rho_d}$	$\tau_{decay} \simeq \frac{1}{(\rho_{o1}\rho_+)^{\frac{1}{2}}} \sqrt{\frac{\rho_e}{\rho_c}}$
Productivity	$\rho = \frac{\rho_c}{\rho_e}$	$\rho = \frac{\rho_c}{\rho_e}$
Abundance	$\frac{c_{peak}}{m(0)} = \frac{\rho_{o1}}{\rho_e}$	$\frac{c_{peak}}{m(0)} = \frac{\rho_{o1}}{\rho_e}$

productivity can vary by over four orders of magnitude, reaching values up to approximately 23% [114]. A higher rate of productivity effectively translates to a reduction in the concentration of oligomers at any given time.

Abundance, the maximum concentration of oligomers that can be estimated theoretically, is determined by the maximal rate of oligomer formation relative to the maximal rate of depletion. These rates are determined by the rates of primary nucleation and/or secondary nucleation, as applicable [114, 186]. This parameter is generally less variable between systems compared to the differences in productivity and half time, with predicted values as low as 0.3% for Ure2 and as high as 8% for α -synuclein and A β 42 [186].

Finally, the peak time refers to the timepoint in the experiment when oligomer concentration is maximal and is set by the characteristic rate of aggregation and initial monomer concentration, and it varies by approximately one order of magnitude for different proteins.

For systems dominated by secondary nucleation, these four experimentally observable parameters, peak time, productivity, persistence, and abundance, can be mathematically represented (Table 3). Consequently, these metrics facilitate the investigation of the various effects of potential therapeutics or additive agents on targeting one or more microscopic steps (Fig. 5).

Amyloid aggregation within protein condensates

The process by which proteins form a liquid-like condensed phase is also becoming increasingly recognized as relevant in both physiology [132, 215–217] and pathology

[218, 219]. This phenomenon, which is known as protein phase separation (PPS), may take place when protein–protein interactions become more favorable than protein–solvent interactions [217]. Driving forces for this process include electrostatic interactions (cation–anion, dipole–dipole cation– π , sp²– π , π – π), polar interactions mediated by hydrogen bonds, and hydrophobic interactions, amongst others [220–223]. The environmental conditions influence greatly protein phase separation, where factors like protein concentration, RNA interaction partners, co-solutes, temperature, pH, salt type and concentration, and crowding agents can drastically change the way in which proteins interact with each other. Although most proteins appear capable of protein phase separation [132], we are only beginning to understand the sequence-based determinants of the propensities of different proteins to do so [223–228].

The presence of liquid-like condensates opens the possibility of an alternative pathway to amyloid aggregation. This “condensation pathway” is distinct from the direct formation of amyloid aggregates from the native state occurring through oligomeric species, which is known as the “deposition pathway”. Formation of solid deposits either directly through the deposition pathway or from liquid droplets through the condensation pathway have been observed even within the same cell and protein system undergoing self-assembly [229–232].

The role of oligomers is not yet clear, however, when amyloid aggregation takes place within condensates. TDP-43 oligomers were found to form in both the deposition and condensation pathways, and to form before

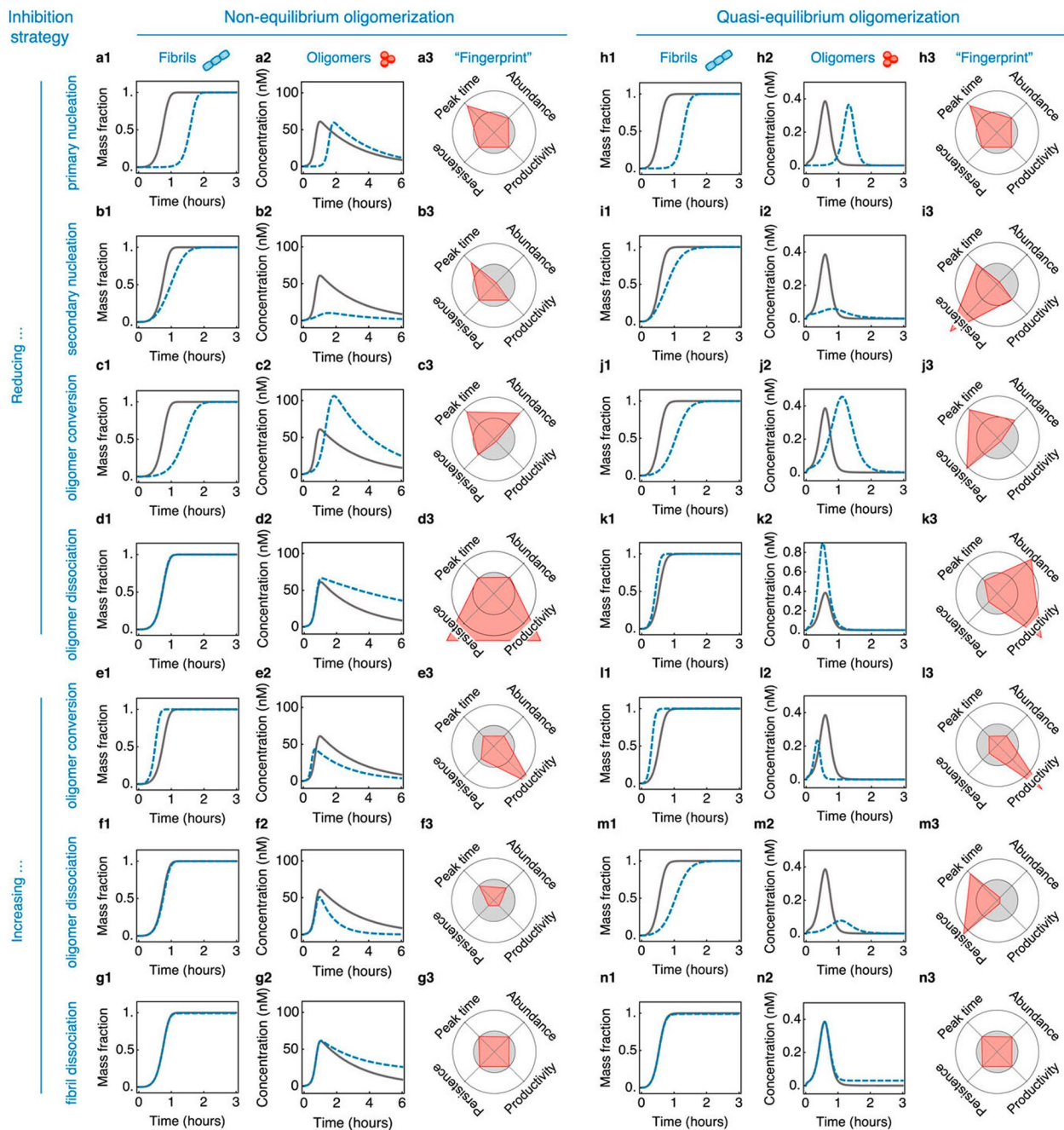


Fig. 5 Simulated effects on the time evolution of fibril and oligomer populations upon addition of compounds that inhibit specific microscopic steps in the aggregation reaction of a given protein. Reprinted from Michaels et. al [114], with the permission of AIP Publishing

solid aggregates emerge within a condensed gel-like phase [233]. It has also been reported that α -synuclein oligomers formed immediately following phase separation of the monomeric protein into a hydrogel, where monomers, oligomers and fibrils co-exist and where the hydrogels entrap, rather than release, oligomeric and fibrillar α -synuclein in a highly cytotoxic state [234]. Similarly, it was found for tau that liquid-liquid phase

separation precedes gel formation and then aggregation in vitro [235], and induces a pathogenic conformation and oligomerization [235, 236]. Another RNA-binding protein, TIA1, further potentiates tau phase separation, facilitating the oligomerization and subsequent cytotoxicity of the microtubule-associated protein [237]. The investigation of a coarse-grained peptide also found the

formation of both metastable and stable oligomers in a dense phase [238].

Oligomer detection methods

Although oligomers are aberrant assemblies that can interact with a wide range of cellular components [1, 100], their concentration remains low during the amyloid aggregation process. For example, at the half-point of an aggregation reaction in vitro where half the monomers have converted into fibrils, the concentration of oligomers can be two or three orders of magnitude lower than the monomer concentration [21]. It is therefore often necessary to isolate oligomers at higher concentrations or stabilize them to facilitate their investigation in vitro [161, 239–241]. It is important to note that on-pathway oligomers are the focus of the kinetic models described above, whereas stabilization methods typically redirect the aggregation reaction towards the formation of off-pathway oligomers at experimentally amenable concentrations.

Many techniques are available for the detection of oligomers [115]. Immunoassays are commonly used and are based on conformation- or sequence-specific antibodies that capture and trap oligomers in solution, ideally without appreciably detecting monomers or fibrils [118, 242]. Examples include the A11 polyclonal antibody to detect prefibrillar spherical oligomers by A β and other systems [118], the OC polyclonal antibody against A β fibrillar oligomers [120], the polyclonal M94 antiserum against A β ADDLs [243], ASyO2 to bind 600 kDa α -syn oligomers [244], mAB-O that binds 25–150 kDa A β ₄₂ oligomers [244], 71A1 to bind 670+ kDa A β oligomers [245], and another that binds A β oligomers markedly more so than its monomers or fibrils [246]. Importantly, antibodies of this type have been used to detect oligomers in AD brains that were absent in aged-matched healthy individuals [118, 120, 243].

A variety of antibody-based assays are used for detecting oligomers in various samples [115, 123, 247]. However, generating antibodies with high specificity for oligomers remains challenging, as many antibodies initially reported to be oligomer-specific often do not differentiate well between oligomers and fibrils [248]. Despite this, developments have been made with biosensors [115], which allow for a label-free capture and detection of oligomers through antibodies. Biolayer interferometry (BLI) uses oligomer-specific antibodies attached to glass fibre tips for their functionalization [249]. Using surface plasmon resonance (SPR), interactions between immobilized conformation-specific antibodies and oligomers are observable through changes in reflected light. SPR can be end-coupled to mass-spectrometry for further characterization (i.e., mass, stoichiometry, topology, charge,

etc.) and quantification of oligomers [250–254]. Other oligomer detection techniques include dye-derived fluorescence spectroscopy and microscopy, electron microscopy, atomic force microscopy (AFM), DLS, filter-trap assays, radiolabeling, mass photometry, and numerous others [115].

Characterization methods of oligomer structure

The physicochemical properties of oligomers are important mediators of their cytotoxicity. A variety of experimental techniques have been developed to monitor these properties, some of which are discussed here. Hydrophobicity is readily quantified in certain experimental settings using 8-anilino-1-naphthalene-sulfonate (ANS) fluorescence (or its derivative bis-ANS), as its intensity increases and its wavelength of maximum fluorescence undergoes a blue shift upon binding to hydrophobic regions that are solvent exposed in a protein [255]. Oligomers induce these changes more than fibrils, whereas monomers typically exhibit minimal changes in ANS fluorescence [241].

Oligomer size can be quantified through a wide array of methods with varying levels of sensitivity, including microfluidic diffusional sizing [246, 256], single-molecule TIRF and dSTORM super-resolution imaging [246], static and dynamic light scattering (SLS and DLS, respectively) [257–259], size-exclusion chromatography [91], several types of polyacrylamide gel electrophoresis [260], photo-induced cross-linking of unmodified protein (PICUP) [261], AFM [262], and cryo-EM [161].

The secondary structure of oligomers has also been widely investigated, for example using circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopy [241]. Based on these studies, our current understanding is that secondary structure is not clearly linked to oligomer toxicity [263], unlike size and hydrophobicity. Analogous to the case for ANS binding, CD and FTIR spectra differ significantly for oligomers in comparison to monomers or fibrils. While ANS binding tends to be higher for oligomers, the secondary structure of oligomers assessed by CD or FTIR tends to be intermediate between monomeric and fibrillar preparations.

Site-specific structural information has been gained using a variety of experimental approaches. Protein engineering to substitute a given residue to cysteine has been used to label the same residue with a probe that is either fluorescent or paramagnetic and reporting on a specific structural type of information, such as solvent exposure, degree of packing, and distance from another residue via fluorescence resonance energy transfer (FRET), using either fluorescence or electron paramagnetic resonance (EPR) [240, 264–266]. Proline scanning mutagenesis has also been used to scan the involvement of any residue in

oligomer structure [267]. Various applications of solution and solid-state nuclear magnetic resonance (NMR) spectroscopy have been utilized to map out residues with β -sheet structure within the oligomers [33, 154, 165, 266]. Further advancement awaits technological progress in applications of cryo-EM, which is not as advanced in providing oligomer structures as it is on fibril structures.

Oligomers in protein misfolding diseases

Despite a quest that has already lasted over two decades, it has been difficult to obtain direct evidence that misfolded protein oligomers are cytotoxic species in protein misfolding diseases. While there is a wealth of information about the cytotoxicity of oligomers in vitro on cultured cells, primary neurons and brain slices using a variety of peptide and protein systems and a variety of biological observables, it has proven much more challenging to establish direct links between oligomer formation, cellular dysfunction and disease phenotype in vivo [104]. Perhaps one of the strongest elements of support for the oligomer toxicity hypothesis comes from recent clinical trials on AD patients, and consequent Food and Drug Administration (FDA) accelerated and then traditional approval to treat AD, of lecanemab, an antibody raised against high molecular weight A β oligomers, also known as soluble protofibrils, which has shown that targeting these species slows down cognitive decline in AD human cases [268], and reduces brain and CSF A β oligomers in a mouse model [269]. The mechanism of action of lecanemab is consistent with that of aducanumab [270, 271], another, but more controversial, antibody approved by the FDA for AD treatment, which has been shown to target mature forms of A β and reduce oligomer formation by inhibiting secondary nucleation in vitro [272]. In particular, lecanemab, aducanumab, and gantenerumab have been characterized to bind differentially to various A β species, where lecanemab demonstrated a 10-fold stronger binding affinity for protofibrils over fibrils, and aducanumab and gantenerumab showed preferential binding to fibrils instead of protofibrils [273]. It was also shown that all three antibodies had a low affinity for monomers, but lecanemab and aducanumab showed very weak monomer binding [273].

Well before these achievements, the hypothesis that small oligomers, as opposed to fibrils, are the main pathogenic species in AD had been supported by many lines of circumstantial evidence [1, 100, 102, 103]. These include: (1) the higher cytotoxicity and synaptotoxicity of oligomers to cultured cells, primary neurons, and iPSC-derived human neurons [50, 100, 274], (2) impairment of social memory, reduced brain volume, increased caspase-3-positive cells, microglial and

pro-inflammatory responses, following the injection of human A β in rat brains [275], (3) evidence that severity of AD and synaptic loss does not correlate with the extent of amyloid plaque formation, but with the biochemically detected amount of soluble A β (including soluble oligomers) [276], (4) observations that in some transgenic APP mouse models, biochemical and electrophysiological evidence of synaptic alteration and cognitive impairment precedes amyloid plaque formation, but occurs after A β levels start to rise steadily [277], and (5) the E693G mutation associated with familial early AD promotes protofibril rather than fibril formation [278].

One of the reasons for the lack of direct evidence of a causal role of oligomers in disease is the difficulties in isolating oligomers from post-mortem brain tissue due to their small size, low stabilities, low concentrations, transient nature, and extensive structural heterogeneity. Structures for toxic oligomers have been determined from in vitro preparations and were cylindrical in shape, including for example α B-crystallin [279] and α -synuclein [161]. While there are over 250 amyloid fibril structures resolved by ssNMR or cryo-EM in the Amyloid Atlas [137], a key challenge remains analyzing the structural motifs of the oligomers present in human pathology. It is clear that different fibril polymorphs are associated with different diseases and phenotypes, but it remains to be determined if this phenomenon holds true also for oligomeric aggregates. Moreover, supernatants of high-speed ultracentrifugation preparations from aqueous AD brain extracts have been recently reported to contain fibrils with the same structure as those from plaques, and these fibrils bound lecanemab resulting in their diminished synaptotoxicity [280].

Nevertheless, conformation-sensitive antibodies have allowed the detection of well-defined oligomers in AD and PD patients relative to aged-matched controls [43–45, 121, 243, 281–286] and increased levels of oligomers have been observed in the CSF of AD [284, 287] and PD cases [45, 288, 289], although these observations do not stand per se as a conclusive proof that oligomers are the causative agents of protein misfolding diseases. Recently, soluble protein aggregates were isolated from eight brain regions for AD patients at Braak stage III [290]. Soluble aggregates approximately 2 nm in diameter and less than 100 nm in length from all these regions were neuroinflammatory and permeabilized liposomes to varying extents, suggesting that this early stage of disease is characterized by a global pathology occurring to differing extents in various regions but simultaneously in the entire brain. Another study using gentle extraction methods rather than conventional brain tissue

homogenization found that only a critical minority of A β consisted of diffusible oligomers, which were responsible for inducing toxicity [291].

Comparing the CSF of individuals with AD, mild cognitive impairment (MCI), and healthy controls, MCI cases demonstrated a greater extent of small aggregates that could induce membrane permeabilization, while AD individuals exhibited larger aggregates that robustly triggered pro-inflammatory responses in glial cells [292]. These results in part suggest that the number and size distributions of aggregates evolves over time during the progression of AD, the latter of which could be quantified in CSF samples [292].

Oligomer toxicity in animal models

Oligomers have been investigated in transgenic animal models, including A β oligomers [293–295] and α -synuclein oligomers [296, 297]. Many studies have linked these small aggregates in particular to the onset and development of cellular dysfunction and neurodegeneration [31, 43, 100, 298, 299]. APP transgenic mice with the E693 delta mutation exhibit extensive A β oligomerization without fibril formation alongside impairments to synaptic plasticity and memory, abnormal tau phosphorylation, microglial and astrocyte activation, and neuronal loss at varying time points from 8–24 months [294]. Injection of oligomers from various sources in mice or rat brains resulted in severe impairments. For example, toxic misfolded HypF-N oligomers injected into rodent brains caused loss of cholinergic neurons, spatial memory impairments, synaptic colocalization in primary neurons, and attenuated long-term potentiation (LTP) in hippocampal brain slices [274, 300]. Tau oligomers injected into the wild-type mouse brain also triggered synaptic and mitochondrial dysfunction [18]. Natural A β oligomers formed within specific intracellular vesicles and subsequently secreted extracellularly were also found to be effective at inhibiting LTP in rats in vivo upon cerebral microinjection [301]. Oligomeric A β also markedly potentiated intracellular Ca²⁺ ion influx upon the exogenous treatment of healthy mice brains with soluble A β oligomers [302]. In addition, A β oligomers triggered tau pathology, caused synaptic loss and axon transport dysfunction, insulin resistance, cholinergic impairment, choline acetyltransferase inhibition, neuroinflammation, and epigenetic changes [284, 303]. Injection of toxic α -synuclein type B* oligomers into the mouse striatum induced a small but significant loss of dopaminergic neurons in the *substantia nigra pars compacta*, although a higher effect was found when injecting small short fibrils of the same protein as a result of the ability of fibrils to spread and amplify α -synuclein aggregation [304].

Oligomer interactions with cellular targets

Misfolded protein oligomers have been shown to bind generically to biological membranes resulting in a toxic gain of function, to specific membrane receptors resulting in a loss of native function, and to cytosolic proteins and nucleic acids, wherein these mechanisms can occur simultaneously [1]. It is unlikely that a single molecular interaction, mechanism of action or cellular cascade is responsible for causing pathology in protein misfolding diseases [1]. Rather, the toxicity of protein aggregates, including misfolded protein oligomers, is likely a consequence of their misfolded structure and extensive heterogeneity, which enables them to induce a wide range of dysfunctional cellular interactions in a litany of cellular compartments, including lipid bilayers, discrete receptors, soluble proteins, RNAs, and metabolites and culminating in cell death [1]. In the last sections of review, we consider the role that oligomeric species play in the events associated with amyloid-associated cytotoxicity, with a focus on how oligomers interact with cells, the cellular consequences of these interactions, and a subsequent discussion of therapeutic efforts aimed at these approaches.

Membrane disruption caused by protein misfolded oligomers leads to neurotoxicity characterized by calcium imbalance, mitochondrial dysfunction, and intracellular reactive oxygen species (ROS) production [240, 305, 306]. With respect to how oligomers induce membrane perturbation, which can also be accomplished by fibrils albeit often to a lower extent [106, 108, 307], a clear relationship exists between size, hydrophobicity and toxicity of misfolded protein oligomers, where oligomers that are small and have a greater extent of hydrophobic amino acids being solved exposed are the most cytotoxic (Fig. 6). Small oligomers have greater diffusional mobility and therefore reach the cell membrane more frequently [308, 309], while oligomers with enhanced hydrophobicity are able to embed and readily insert into the interior of lipid bilayers [33, 160, 240, 264, 310, 311], therein perturbing the membrane and inducing toxicity. High molecular weight oligomers isolated from AD brains were found to be only mildly neurotoxic, whereas their dissociation into lower molecular weight oligomers in mildly alkaline buffer markedly increased their toxicities [312].

Molecular chaperones have been shown to alleviate oligomer toxicity by increasing their size [308]. A size-toxicity relationship has been established for A β aggregates, where low molecular weight oligomers are markedly more cytotoxic than larger oligomers and fibrillar aggregates display the least toxicity [100]. By isolating A β ₄₂ soluble aggregates of different sizes using gradient ultracentrifugation, smaller soluble aggregates were found to

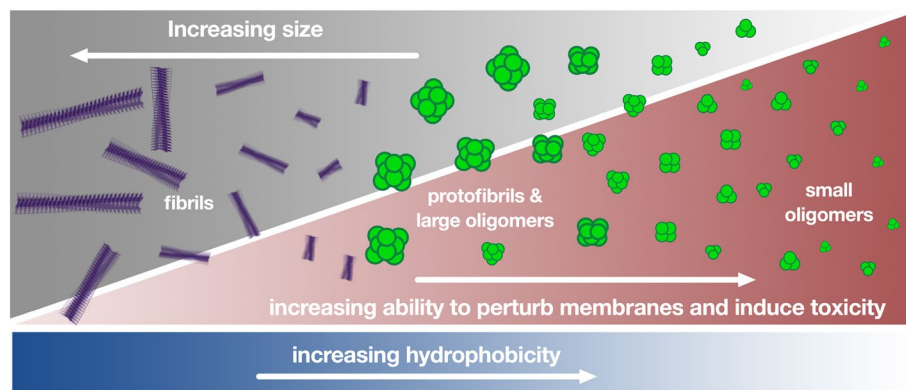


Fig. 6 Physicochemical parameters that influence the toxicity of protein misfolded oligomers. The toxicity typically scales with increasing hydrophobicity and decreasing size. Created with biorender.com

more overtly induce membrane permeabilization, suggesting an inverse relationship between size and toxicity, while larger soluble aggregates more potently induced an inflammatory response in microglia cells [313]. Recently, small soluble aggregates of α -synuclein less than 100–200 nm were identified as the toxic species in PD through the comparison of in vitro oligomers to soluble aggregates in post-mortem PD brains [32].

The importance of the hydrophobicity-toxicity connection is exemplified from the observation that oligomers of similar size and dissimilar hydrophobicity can be experimentally stabilized, where only the oligomers of greater solvent exposed hydrophobicity are capable of inducing significant levels of cellular toxicity and dysfunction. This phenomenon has been observed for pairs of toxic and nontoxic oligomers [116] for HypF-N [240, 264], sup35NM [311], α -synuclein [33, 160], and A β [310].

In addition to the properties of oligomers that can mitigate their binding to cell membranes, the composition of the cell membranes themselves plays an important role in oligomer binding and in the induction of toxicity [314]. In fact, cell membranes enriched in the monosialotetrahexosylganglioside GM1, which is abundant in lipid rafts alongside cholesterol and sphingomyelin, exhibit heightened A β_{42} and HypF-N oligomer binding [306]. A key finding of that study is that the quantified toxicity was directly proportional to the extent of oligomer binding [306].

Beside interacting with biological membranes, misfolded protein oligomers have been reported to interact with, or modulate the activity of, a variety of cellular components and receptors, including the cellular form of the prion protein (PrP^C) [315], $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ -nAChR) [316, 317], low-density lipoprotein receptor-related protein-1 (LRP1) [315, 318], and many others. In particular, A β oligomers have been described to interact with over 20 types of receptors

[319] and also extracellular and intracellular synaptic proteins, including Na/K-ATPase, synGap, and Shank3 [320]. Calcium dyshomeostasis has been associated with AD via overactivation of glutamatergic receptors, and A β oligomers have been found to activate to a small extent AMPA receptors and to a large extent NMDA receptors resulting in the rapid influx of calcium ions into the cytoplasm [321]. The misfolded oligomers interact indirectly with these receptors, and the activation was caused by oligomer-induced changes in membrane tension that were sensed by mechanosensitive NMDA and AMPA receptors [321]. Specific receptors for A β oligomers were shown to recognize features of both toxic oligomers and fibril ends. In particular, PrP^C, Fc γ receptor IIb (Fc γ RIIb), and leukocyte immunoglobulin-like receptor B2 (LilrB2) were characterized to bind the ends of fibrils, neurotoxic oligomers, and protofibrils, therein inhibiting fibril growth [322].

While A β oligomers can bind membrane bilayers and proteins and trigger aberrant intracellular cascades, evidence also suggests that A β oligomer formation in the cell, or internalization into the cell, is also important. A β aggregation induced by cell uptake contributes to cell death and culminates in the release of amyloid aggregates outside the cell [323]. A β can enter the cell by pore formation, endocytosis, and via specific receptors [324–326]. Moreover, oligomers can initiate aberrant protein–protein interactions. The hyperphosphorylation of tau and its aggregation, for example, can potentiate A β oligomer-induced dysfunction in AMPA receptor signaling [327]. A β oligomers have also been shown to promote the internalization of fibrillar tau seeds resulting in increased intracellular tau aggregation [328]. Different conformations of neurodegeneration-linked proteins can also impact one another. For example, α -synuclein monomers inhibit A β_{42} secondary nucleation, whereas

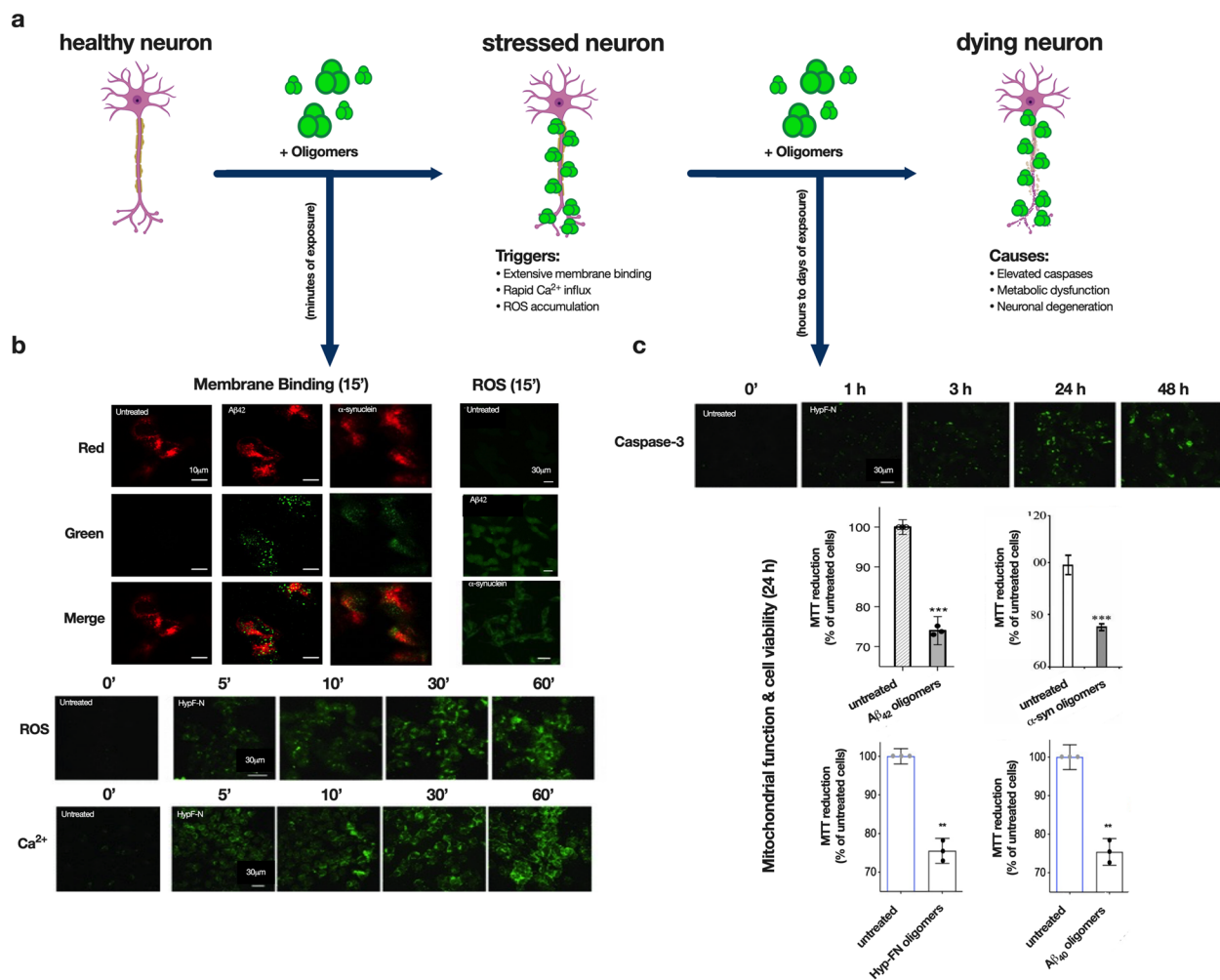


Fig. 7 Oligomers induce cytotoxic effects that can be monitored over time. **a** In healthy cells, over short durations (minutes up to one hour), toxic oligomers exhibit extensive membrane binding, induce rapid influx of Ca^{2+} ions, and then reactive oxygen species (ROS) accumulation [321]. Longer incubations (hours to days) induce elevated caspase-3 levels, metabolic dysfunction, and ultimately death of the cell [331]. Created with biorender.com. **b** Examples of observable impacts of $\text{A}\beta_{42}$ and α -synuclein oligomer treatment for short durations to SH-SY5Y human neuroblastoma cells [257, 332]. Membrane binding: oligomers (green channel) and membranes (red channel) [257]. ROS production (green). Intracellular calcium ions (green). **c** Examples of observable impacts of HypF-N oligomer treatment for longer durations (hours to days) to SH-SY5Y cells, including caspase-3 production (green) [300] and metabolic defects as assessed using the MTT assay for $\text{A}\beta_{40}$, $\text{A}\beta_{42}$, α -synuclein, and HypF-N oligomers [257, 258, 332]. Panels were adapted from Limbocker et. al [257], Zampagni et.al [300], Perni et. al [332], and Limbocker et. al [258]

fibrillar α -synuclein stimulates $\text{A}\beta_{42}$ heterogeneous nucleation [329]. Pathological α -synuclein accumulation was recently shown to disrupt the decapping module of P-bodies, therein disrupting mRNA stability in iPSC-derived neuronal models of PD [330]. Figure 7 summarizes a subset of the deleterious interactions and effects that results from the interaction between cells and misfolded protein oligomers.

Cellular consequences of oligomer interactions

Toxic misfolded protein oligomers demonstrate a preferential ability to penetrate the lipid bilayers of cell

membranes (Fig. 8) [116]. These interactions trigger numerous events, including calcium influx into the cell, intracellular production of ROS, lipid peroxidation, cell membrane leaking and the escape of intracellular molecules, caspase-3 activation, and mitochondrial damage [116, 240, 300, 305, 333, 334]. Among the earliest events that take place after misfolded protein oligomer binding to the cell are the influx of calcium ions from the extracellular medium that is mediated by NMDA receptors. Oligomeric $\text{A}\beta$, for example, is known to markedly potentiate intracellular Ca^{2+} influx in cell culture [306, 321, 335, 336], which then triggers ROS

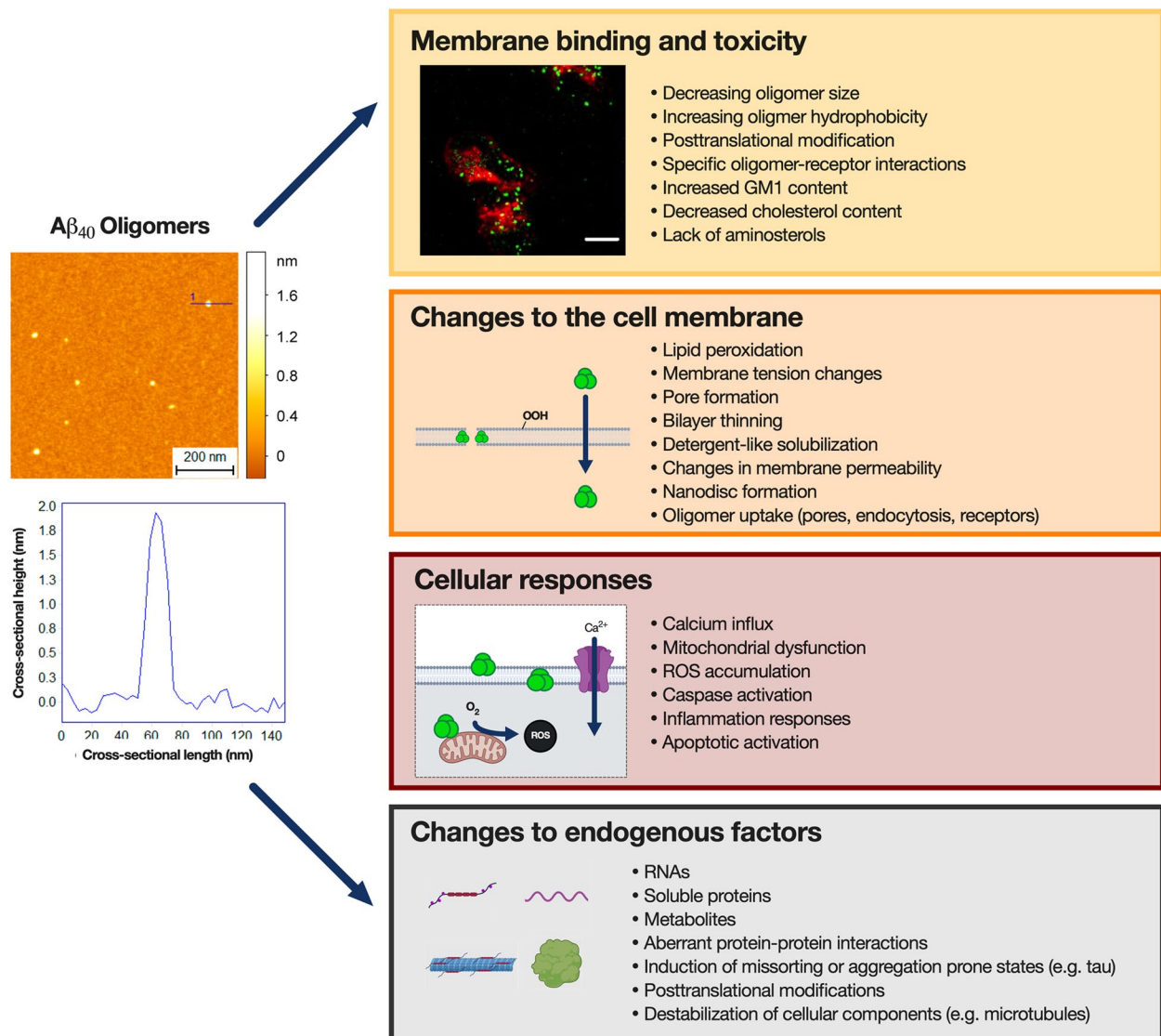


Fig. 8 Consequences of the exposure of cells to misfolded protein oligomers. AFM cross-sectional profile of A β_{40} oligomers stabilized by Zn $^{2+}$. Misfolded protein oligomers of this type typically are 2–6 nm in height, and they induce membrane binding and toxicity accompanied with deleterious changes to the properties of cell membranes, cellular responses, and changes to endogenous factors. The AFM map and cross-sectional profile were adapted from Limbocker et. al [337]. The membrane binding panel was adapted from Limbocker et. al [257]. Created with biorender.com

accumulation and following events [335]. Changes in Ca $^{2+}$ levels precede synaptic damage in vivo [302].

Another hallmark of neurodegenerative diseases arising from misfolded proteins is the presence of elevated levels of ROS [338, 339]. Misfolded protein oligomers are able to induce extensive ROS generation leading to a cascade of intracellular consequences, such as ROS-mediated activation of apoptosis signal-regulating kinase 1 (ASK1) associated with the toxicity of A β in AD [340]. High levels of ROS can also stimulate lipid peroxidation of cell membranes, which

in turn can impact protein aggregation and lead to the loss of organelle function [341]. In cardiomyopathy, the deposition of transthyretin (TTR) increased the production of ROS, correlating with left ventricular systolic dysfunction, though likely indirectly from endoplasmic reticulum stress and calcium dyshomeostasis by non-fibrillar TTR species [89, 342–344].

Mitochondrial dysfunction is also associated with the presence of misfolded protein oligomers [345]. Oligomers or protofibrils have been shown to perturb mitochondrial membranes and induce significantly the influx of Ca $^{2+}$

[240, 321], interrupt normal metabolic processes during oxidative phosphorylation [345], and trigger apoptotic pathways [346, 347]. For example, IAPP oligomers associated with type II diabetes are toxic by increasing Ca^{2+} influx [336] and disrupting the mitochondrial membrane in pancreatic β -cells [78].

In addition, A β oligomers can also interact with proteins or lipids at synapses and inhibit LTP, which is a correlate of synaptic plasticity [291]. They can also induce an inflammatory response in microglial cells upregulating, among other factors, the major histocompatibility complex class II, inducible nitric oxide synthase, and CD40 in the hippocampus of AD transgenic mice [348] and release pro-inflammatory cytokines [349]. Several key dysfunctional responses caused by oligomers to cell membranes, whole cells and endogenous factors are summarized in Fig. 8.

Therapeutic approaches targeting misfolded protein oligomers

Numerous strategies have been studied *in vitro* to attenuate the toxicity of misfolded protein oligomers, including reducing their concentration and lifetime, increasing their size, neutralizing their hydrophobic surface, targeting their toxic interactions with molecular targets, such as specific receptors or cell membranes, or enhancing their clearance [101, 309, 350]. As covered in the previous sections, misfolded oligomers can induce cytotoxicity in many ways, which is in large part a result of their intrinsic heterogeneity and ability to interact with a wide variety of cellular components. While it is critical to understand the diverse means by which these oligomers can damage many parts of the cell, it is unlikely that blocking specific oligomer-target interactions will be sufficient to arrest the toxicity of amyloid pathologies. The clinical relevance of many of the strategies discussed here will become clearer over the next few decades by building upon the momentum of the recent successes obtained with monoclonal antibodies for the treatment of AD.

Reduction of oligomer formation

The protein homeostasis system is capable of inhibiting specific microscopic steps of an aggregation process using molecular chaperones, such as α B-crystallin that inhibits α -synuclein and A β elongation or Hsp70 that can inhibit tau primary nucleation as well as sequester oligomeric and mature tau into inert and seeding-incompetent species [351–354]. This strategy has been extensively investigated as a therapeutic approach for protein misfolding diseases with small molecules [355, 356]. A challenge, however, is that there is no direct relationship between the reduction in the number of amyloid aggregates and the reduction in the number of oligomers [114]. For example, a fibril

elongation inhibitor delays the aggregation process, but contributes to the accumulation of oligomers [114]. It has been estimated that the inhibition of fibril elongation by one order of magnitude can generate a five-fold increase in the concentration of oligomers [212].

The inhibition of primary nucleation may offer opportunities for therapy, as it delays formation of oligomeric species (Fig. 5). In fact, several candidates have been identified for primary nucleation inhibition [332, 357–362]. Of great promise, targeting secondary nucleation would be particularly beneficial therapeutically as it causes the autocatalytic proliferation of aggregates and is primarily responsible for oligomer production (Fig. 5) [21, 114, 363]. This approach has been realized by several molecular agents, including a group of small molecules [364] and antibodies [365] that target secondary nucleation in A β_{42} aggregation to differing extents. A similar approach was utilized to evaluate a library of flavones against α -synuclein aggregation, with focus on drugs that most inhibit oligomer formation [179]. Moreover, specific aminosterols can inhibit fibril amplification secondary processes in α -synuclein aggregation [362, 366]. Molecular chaperones can also target secondary nucleation in A β_{42} aggregation [354, 360]. Structure-kinetic activity relationship (SKAR) rules have also been leveraged to convert an inactive rhodamine molecule into a derivative that could inhibit secondary nucleation in A β_{42} aggregation [113].

A kinetic analysis of four anti-A β antibodies in different stages of clinical trials at the time of that publication found that aducanumab (granted accelerated approval by the FDA [367]) targets secondary nucleation in the A β_{42} aggregation process and therefore the reactive flux of oligomers, whereas bapineuzumab, solanezumab, and gantenerumab impacted other microscopic processes including elongation or primary nucleation (Fig. 9) [272]. Of interest, the monoclonal antibody ACU193 is suggested to be an A β oligomer-selective immunotherapeutic and is in clinical trials [368].

Neutralization of oligomers through binding

Compounds that bind directly misfolded oligomers have also been identified and their mechanism has been investigated. While binding oligomers can have the effect of stabilizing them, it can also neutralize their hydrophobic surfaces, inhibit their action as nuclei, or remodel the aggregation pathway towards the formation of less toxic species. Phage display was used to identify a soluble inhibitor specific to A β_{42} oligomers and able to inhibit their ability to act as nuclei [369]. Similarly, a rational design strategy was used to obtain oligomer-specific antibodies that undermine secondary nucleation [246]. It is also possible to drive off-pathway aggregation processes with small molecules, as observed for the polyphenol

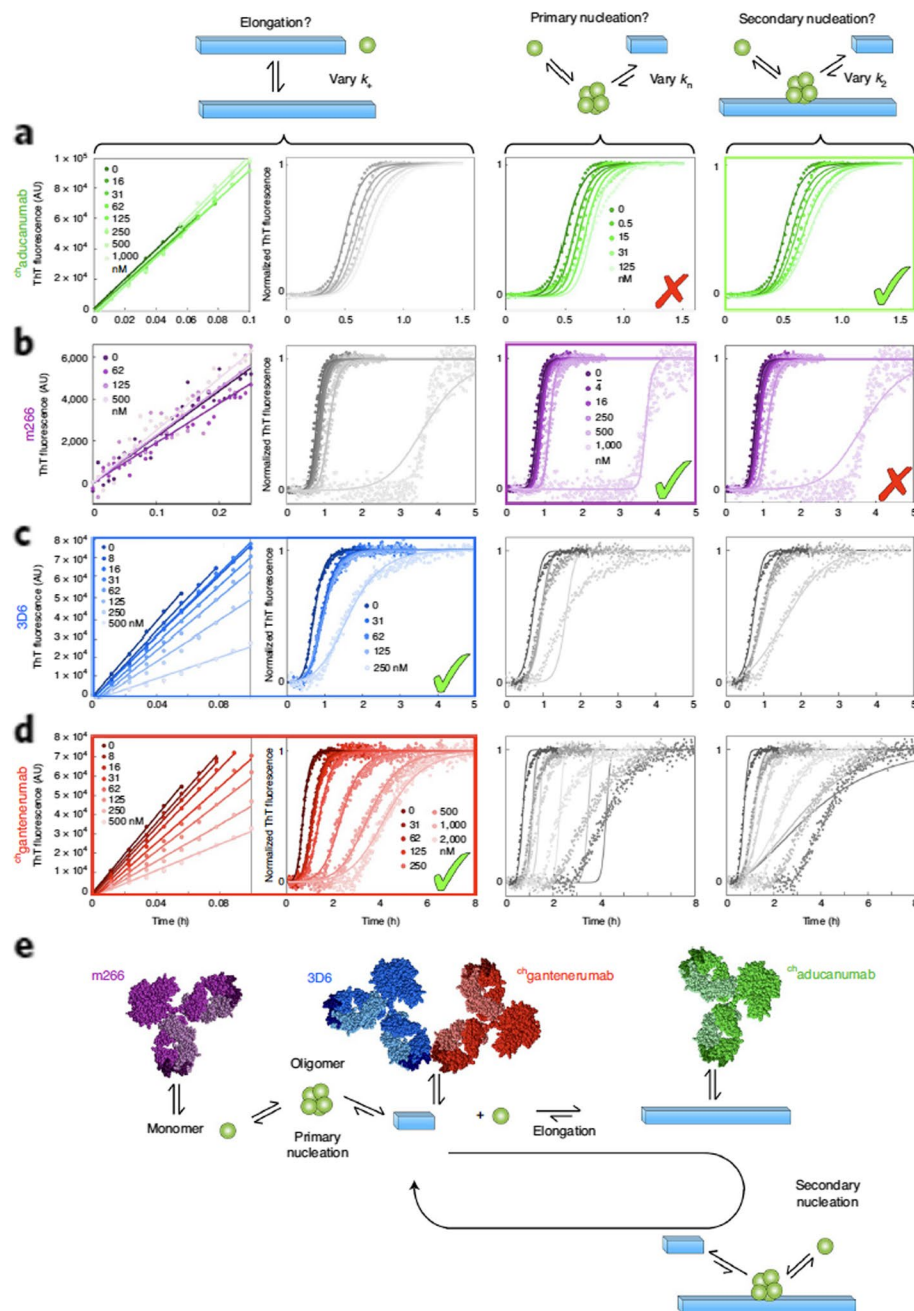


Fig. 9 Clinical-stage antibodies against AD target different microscopic steps in $A\beta_{42}$ aggregation. From Linse et. al [272]

(-)-epigallocatechin gallate (EGCG) that can form oligomers of α -synuclein and $A\beta$ that are non-toxic [370] or resveratrol that can remodel $A\beta_{42}$ into nontoxic, high molecular weight species [371].

Stabilization of the native state

Another approach to prevent aggregation is the stabilization of the native state of proteins. This approach

has been implemented in the case of protein misfolding diseases caused by amyloid aggregation of transthyretin, through the small molecule tafamidis [372, 373], which was approved by the European Medicines Agency (EMA) in 2011 for the treatment of stage I ATTRv-polyneuropathy, after successful completion of a phase III clinical trial [374]. Then, in 2019 and 2020, the drug was approved by the FDA and EMA, respectively, for

the treatment of ATTRv- and ATTRwt-cardiomyopathy at stages I and II, after a successful phase III clinical trial was noted [375]. Tafamidis was the first approved drug to slow down the progression of an amyloid disease. A similar approach is also being explored for light chain amyloidosis (AL) by using small molecules that bind at the native monomer–monomer interface of native dimeric immunoglobulin light chains [376]. Native state stabilization has been shown to be effective more generally for three other protein misfolding diseases that are not associated with amyloid formation, through the use of drugs is approved in all three cases by both FDA and EMA [377]. This approach, however, is challenging for disordered proteins, such as A β , tau and α -synuclein, since these proteins do not exhibit stable binding pockets for small molecules [378, 379]. Binding mechanisms in which the contribution to the free energy of binding comes from entropy have been explored [380]. The small molecule 10074-G5 was recently found to increase the conformational entropy of monomeric A β alongside decreasing its hydrophobic exposure, resulting in the stabilization of the monomeric state and the prevention of aggregation [378].

Oligomer clearance

Other promising therapies are being explored to potentiate oligomer degradation through a variety of mechanisms, including activating the unfolded protein response, stimulating autophagy, aiding extracellular clearance, rebalancing the proteostasis network (PN) by targeting specific heat shock factors, and exploiting molecular chaperones working as disaggregases [381]. However, approaches based on the exploitation of the PN are beyond the scope of the present review. A β oligomer clearance by passive immunization is of course a very promising strategy, as shown by the recent accelerated and then regular approval by the FDA of lecanemab, designed to target mainly soluble A β protofibrils among other species [16, 268]. Major efforts have also been devoted to reducing the amount of aggregation-prone A β monomers by targeting the proteolytic cleavage of APP, its precursor protein, by secretases [382, 383]. This in theory would reduce the number of oligomers formed, but this strategy has demonstrated limited success in clinical trials.

Reduction of oligomer interactions

In addition to inhibiting the formation of oligomers, promoting their clearance, changing their structure, and attenuating the aberrant interactions of the oligomers with their biological targets, another promising approach is the mitigation of specific oligomer-membrane protein interactions. This can be accomplished through targeting

the action of oligomers on specific receptors, as exemplified by studies on microglial A β -induced P2X7R-dependent stimulation of inflammation and toxicity, which can be eliminated by the dihydropyridine nimodipine [384], and molecules that inhibited A β -LilrB2 interactions on the cell surface with reduced cytotoxicity [385]. Whether preventing the interaction of misfolded protein oligomers with cell membranes generally [366] or via specific receptor proteins in the membrane [385], these approaches aim at reducing the interaction of A β with the cell membrane, inhibit cell membrane destabilization and uptake of A β into the cytosol of the cell, which are known to lessen the toxicity of soluble A β species [386].

Several molecular chaperones have demonstrated capacity to modulate the size or hydrophobicity of misfolded protein oligomers. Sub-stoichiometric concentrations of α B-crystallin, heat shock protein 70, clusterin, haptoglobin, and α 2-macroglobulin reduced the toxicity of A β ₄₂, IAPP, and HypF-N oligomers by promoting their assembly into markedly larger species with reduced diffusional mobility and reactive surfaces of the oligomers [308]. On the other hand, super-stoichiometric concentrations of the same chaperones can bind to the hydrophobic surfaces of the oligomers and neutralize them in the absence of their further clustering [387]. Heat shock protein B1 and transthyretin have also been characterized to sequester A β ₄₂ oligomers into inert species [388–391]. Clusterin has been shown to bind to hydrophobic portions of A β ₄₂ oligomers, therein slowing down its aggregation by inhibiting primary and secondary nucleation [392]. Of note, small molecules and protein engineering have been used to stimulate or attenuate the activity of specific molecular chaperones as therapeutic means [381].

It has also been reported that cell membranes were protected from the deleterious effects of misfolded protein oligomers using compounds that do not bind to oligomers or impact their structures, but rather integrate directly into the cell membrane [366]. Key aminosterols such as squalamine and trodusquemine strongly prevent and displace the binding of oligomers of α -synuclein, A β ₄₀, A β ₄₂, and HypF-N to cell membranes, resulting in the attenuation of their toxicity to cultured cells [257, 258, 332, 362, 393], and in transgenic *C. elegans* models of PD [332, 362] and AD [257] diseases. These molecules additionally have effects on the kinetics of amyloid formation and therefore also impact the rate of oligomer formation [257, 332, 362]. Similarly, one report found that EGCG caused a partial reduction in the binding of α -synuclein oligomers to vesicles and cells without impacting the secondary structure or size of the isolated oligomers [394]. Modulating the lipid composition of the neuronal membrane with endogenous factors such as

GM1 and cholesterol can also mitigate oligomer binding and their associated toxicity [306, 314]. Collectively, these results highlight the importance of physicochemical properties for both misfolded protein oligomers and plasma membranes in mediating the binding and ultimate toxicity of oligomeric aggregates.

It is also possible to target the deleterious immunological effects of misfolded protein oligomer toxicity.

Elevated markers of brain inflammation such as interleukin (IL)-1 β , tumor necrosis factor (TNF), and IL-6 have been found in the CSF, brains, and serum of patients with various neurodegenerative diseases [395, 396]. Recent work demonstrated that IL-1 β regulates the dysfunction induced by A β oligomers to mitochondrial proteins [397], and blocking TNF receptor-1 genetically or pharmacologically was beneficial in APP/PS1 transgenic mice

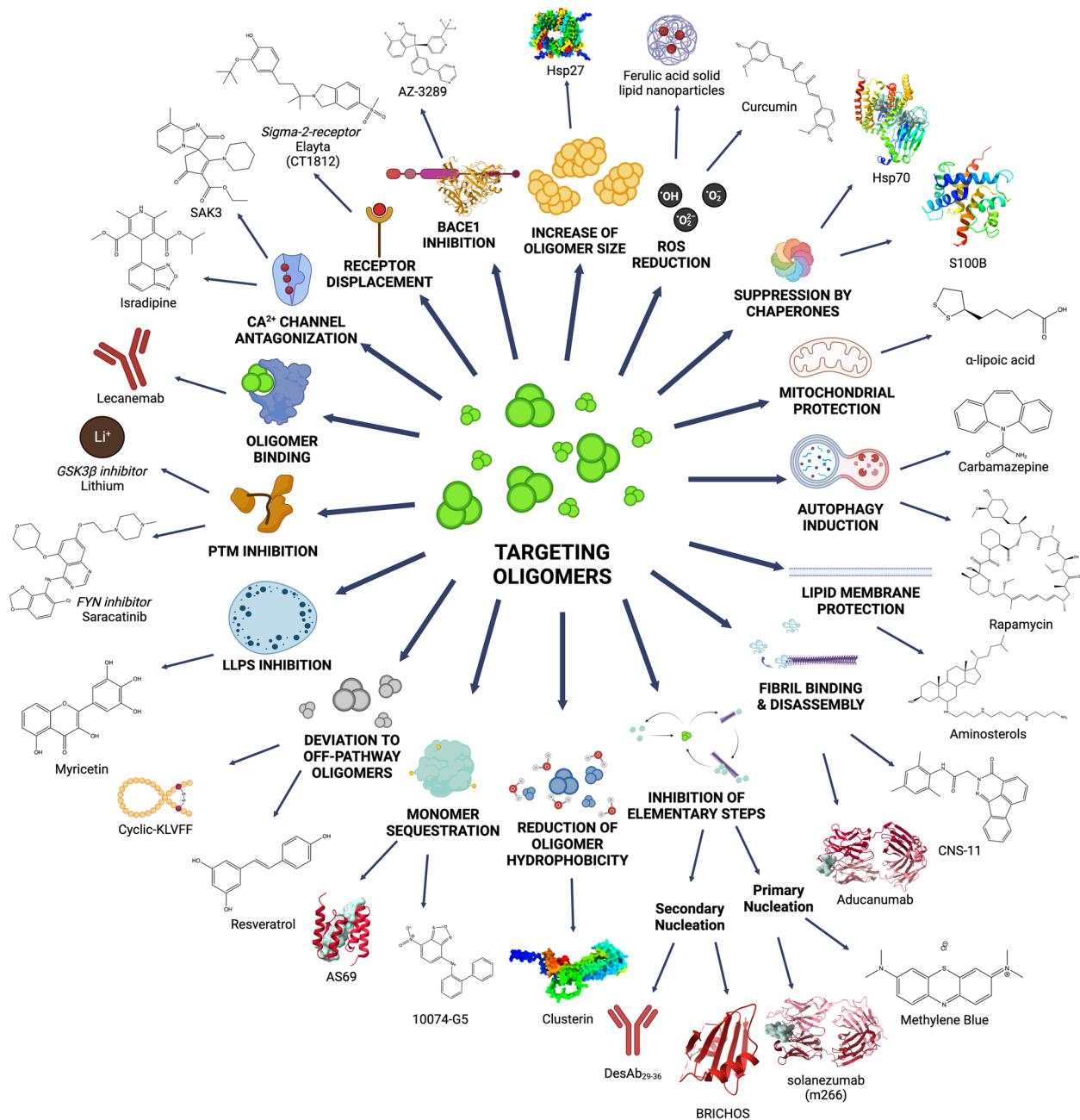


Fig. 10 Overview of approaches under development that could be potentially able to suppress the formation, reduce the lifetime, or decrease the toxicity of misfolded protein oligomers. Biological or chemical structures of prototypical drugs are shown for each class of therapeutic. Created with biorender.com

[398]. The molecule nimodipine has also been shown to reduce IL-1 β levels caused by intra-hippocampal inoculation with A β [384].

An overview of a subset of the different classes of therapeutics being developed to target oligomers is summarized in Fig. 10, including seminal molecules corresponding to each approach [101, 179, 272, 371, 378, 388, 392, 399–430].

Conclusions

In order to provide a molecular-level understanding of current therapeutic strategies that are being explored for the treatment of protein misfolding diseases, and to inspire new ones, we have described the mechanisms by which misfolded protein oligomers form, interact with cells and induce cytotoxicity, as well as approaches investigated to mitigate their toxicity. Support to therapeutic strategies targeting misfolded protein oligomers, such as those discussed in this review, has come from the recent accelerated approval of the antibodies lecanemab and aducanumab by the FDA for the treatment of AD. These advances, however, should not confuse the fact that AD is a multifactorial disorder characterized not just by A β and tau aggregation, but also by excitotoxicity, synaptic loss, inflammation, cholinergic dysfunction, oxidative stress, glucose hypometabolism, alterations of the gut microbiome, the immune pathway, the endocrine pathway, and bacteria-derived metabolites [431]. Other neurodegenerative diseases such as PD are also multifactorial. On the misfolded protein oligomer side, further progress will require major developments in two areas. The first is the establishment of quantitative methods for the detection of oligomers in vivo and for investigating their mechanisms of formation. The second is the development of toxicity assays that recapitulate pathological mechanism relevant in disease. With continued research, we anticipate that more effective strategies to both achieve early diagnosis and develop compounds to target oligomeric species will lead to the generation of effective disease-modifying therapeutics for a wide variety of protein misfolding diseases.

Abbreviations

3R	Three-repeat Tau
4R	Four-repeat Tau
A β	Amyloid- β peptide
AD	Alzheimer's Disease
ADDLs	Amyloid-derived diffusible ligands
AFM	Atomic force microscopy
AMPA	α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANS	8-Anilinonaphthalene-1-sulfonate
ASK1	Apoptosis signal-regulating kinase 1
ATTR	Amyloid transthyretin amyloidosis
BLI	Biolayer interferometry

CBD	Corticobasal degeneration
CD	Circular dichroism
Cryo-EM	Cryogenic electron microscopy
CSF	Cerebrospinal fluid
CTE	Chronic traumatic encephalopathy
DLS	Dynamic light scattering
EGCG	(-)-Epigallocatechin gallate
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
Fc γ RIIb	Fc γ receptor IIb
FDA	U.S. Food and Drug Administration
FRET	Förster resonance energy transfer
FTIR	Fourier transform infrared spectroscopy
HD	Huntington's disease
IAPP	Islet Amyloid Polypeptide (amylin)
IL	Interleukin
LilrB2	Leukocyte immunoglobulin-like receptor B2
LRP1	Low-density lipoprotein receptor-related protein-1
MCI	Mild cognitive impairment
MSA	Multiple system atrophy
NMDA	N-methyl-D-aspartate
PD	Parkinson's Disease
PICUP	Photo-induced cross-linking of unmodified protein
PiD	Pick's disease
PrP	Prion protein
PSP	Progressive supranuclear palsy
ROS	Reactive oxygen species
RT-QuIC	Real-time quaking-induced conversion
SKAR	Structure-kinetic activity relationship
SPR	Surface plasmon resonance
ssNMR	Solid-state nuclear magnetic resonance
T2D	Type II Diabetes
TDP-43	TAR DNA-binding protein 43
TNF	Tumor necrosis factor
TTR	Transthyretin

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Availability of data and materials

New data were not generated for this review.

Declarations

Ethics approval and consent to participate

This study did not directly involve human participants, human data or human tissue and therefore did not require ethical approval or consent for participation.

Consent for publication

Not applicable.

Competing interests

M.V. is a co-founder of Wren Therapeutics Limited, which is pursuing inhibitors of protein misfolding and aggregation. The remaining authors declare no competing interests. The views expressed herein are those of the authors and do not reflect the position of the United States Military Academy, the Department of the Army, or the Department of Defense.

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References

- Chiti F, Dobson CM. Protein misfolding, amyloid formation, and human disease: a summary of progress over the last decade. *Annu Rev Biochem.* 2017;86:27–68.
- Knowles TPJ, Vendruscolo M, Dobson CM. The amyloid state and its association with protein misfolding diseases. *Nat Rev Mol Cell Biol.* 2014;15:384–96.
- Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med.* 2016;8:595–608.
- Shi Y, Zhang W, Yang Y, Murzin AG, Falcon B, Kotecha A, et al. Structure-based classification of tauopathies. *Nature.* 2021;598:359–63.
- Yang Y, Arseni D, Zhang W, Huang M, Lövestam S, Schweighauser M, et al. Cryo-EM structures of amyloid- β 42 filaments from human brains. *Science.* 2022;375:167–72.
- Buxbaum JN, Dispenzieri A, Eisenberg DS, Fändrich M, Merlini G, Saraiva MJM, et al. Amyloid nomenclature 2022: update, novel proteins, and recommendations by the International Society of Amyloidosis (ISA) Nomenclature Committee. *Amyloid.* 2022;29:213–9.
- Merlini G, Bellotti V. Molecular mechanisms of amyloidosis. *N Engl J Med.* 2003;349:583–96.
- Wood JG, Mirra SS, Pollock NJ, Binder LI. Neurofibrillary tangles of Alzheimer disease share antigenic determinants with the axonal microtubule-associated protein tau. *Proc Natl Acad Sci.* 1986;83:4040–3.
- Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *J Biol Chem.* 1986;261:6084–9.
- Hardy JA, Higgins GA. Alzheimer's Disease: The amyloid cascade hypothesis. *Science.* 1992;256:184–5.
- Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc Natl Acad Sci.* 1985;82:4245–9.
- Spillantini MG, Schmidt ML, Lee VM-Y, Trojanowski JQ, Jakes R, Goedert M. α -Synuclein in Lewy bodies. *Nature.* 1997;388:839–40.
- Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M. α -Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proc Natl Acad Sci U S A.* 1998;95:6469–73.
- Palato LM, Pilcher S, Oakes A, Lamba A, Torres J, Ledesma Monjaraz LI, et al. Amyloidogenicity of naturally occurring fulllength animal IAPP variants. *J Pept Sci.* 2019;25:e3199.
- Westermarck P, Wernstedt C, Wilander E, Sletten K. A novel peptide in the calcitonin gene related peptide family as an amyloid fibril protein in the endocrine pancreas. *Biochem Biophys Res Commun.* 1986;140:827–31.
- van Dyck CH, Swanson CJ, Aisen P, Bateman RJ, Chen C, Gee M, et al. Lecanemab in early Alzheimer's disease. *N Engl J Med.* 2023;388:9–21.
- Sengupta U, Nilson AN, Kaye R. The role of amyloid- β oligomers in toxicity, propagation, and immunotherapy. *eBioMedicine.* 2016;6:42–9.
- Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Clos AL, Jackson GR, Kaye R. Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. *Mol Neurodegener.* 2011;6:39.
- Du F, Yu Q, Kanaan NM, Yan SS. Mitochondrial oxidative stress contributes to the pathological aggregation and accumulation of tau oligomers in Alzheimer's disease. *Hum Mol Genet.* 2022;31:2498–507.
- Guerrero-Muñoz MJ, Gerson J, Castillo-Carranza DL. Tau oligomers: The toxic player at synapses in Alzheimer's disease. *Front Cell Neurosci.* 2015;9:1–10. Available from: <https://www.frontiersin.org/articles/10.3389/fncel.2015.00464>.
- Cohen SIA, Linse S, Luheshi LM, Hellstrand E, White DA, Rajah L, et al. Proliferation of amyloid- β 42 aggregates occurs through a secondary nucleation mechanism. *Proc Natl Acad Sci USA.* 2013;110:9758–63.
- Meisl G, Yang X, Hellstrand E, Frohm B, Kirkegaard JB, Cohen SIA, et al. Differences in nucleation behavior underlie the contrasting aggregation kinetics of the A β 40 and A β 42 peptides. *Proc Natl Acad Sci USA.* 2014;111:9384–9.
- Rodriguez Camargo DC, Sileikis E, Chia S, Axell E, Bernfur K, Cataldi RL, et al. Proliferation of Tau 304–380 fragment aggregates through autocatalytic secondary nucleation. *ACS Chem Neurosci.* 2021;12:4406–15.
- Sachse C, Fändrich M, Grigorieff N. Paired β -sheet structure of an A β (1–40) amyloid fibril revealed by electron microscopy. *Proc Natl Acad Sci USA.* 2008;105:7462–6.
- Gremer L, Schölzel D, Schenk C, Reinartz E, Labahn J, Ravelli RBG, et al. Fibril structure of amyloid- β (1–42) by cryo-electron microscopy. *Science.* 2017;358:116–9.
- Lövestam S, Koh FA, van Knippenberg B, Kotecha A, Murzin AG, Goedert M, et al. Assembly of recombinant tau into filaments identical to those of Alzheimer's disease and chronic traumatic encephalopathy. *eLife.* 2022;11:e76494.
- Ghosh U, Thurber KR, Yau W-M, Tycko R. Molecular structure of a prevalent amyloid- β fibril polymorph from Alzheimer's disease brain tissue. *Proc Natl Acad Sci USA.* 2021;118:e2023089118.
- Kollmer M, Close W, Funk L, Rasmussen J, Bsoul A, Schierhorn A, et al. Cryo-EM structure and polymorphism of A β amyloid fibrils purified from Alzheimer's brain tissue. *Nat Commun.* 2019;10:4760.
- Fitzpatrick AWP, Falcon B, He S, Murzin AG, Murshudov G, Garringer HJ, et al. Cryo-EM structures of tau filaments from Alzheimer's disease. *Nature.* 2017;547:185–90.
- Falcon B, Zhang W, Schweighauser M, Murzin AG, Vidal R, Garringer HJ, et al. Tau filaments from multiple cases of sporadic and inherited Alzheimer's disease adopt a common fold. *Acta Neuropathol (Berl).* 2018;136:699–708.
- Winner B, Jappelli R, Maji SK, Desplats PA, Boyer L, Aigner S, et al. In vivo demonstration that alpha-synuclein oligomers are toxic. *Proc Natl Acad Sci USA.* 2011;108:4194–9.
- Emin D, Zhang YP, Lobanova E, Miller A, Li X, Xia Z, et al. Small soluble α -synuclein aggregates are the toxic species in Parkinson's disease. *Nat Commun.* 2022;13:5512.
- Fusco G, Chen SW, Williamson PTF, Cascella R, Perni M, Jarvis JA, et al. Structural basis of membrane disruption and cellular toxicity by α -synuclein oligomers. *Science.* 2017;358:1440–3.
- Horne RI, Metrick MAI, Man W, Rinauro DJ, Brotzakis ZF, Chia S, et al. Secondary processes dominate the quiescent, spontaneous aggregation of α -synuclein at physiological pH with sodium salts. *ACS Chem Neurosci.* 2023;14:3125–31.
- Buell AK, Galvagnion C, Gaspar R, Sparr E, Vendruscolo M, Knowles TPJ, et al. Solution conditions determine the relative importance of nucleation and growth processes in α -synuclein aggregation. *Proc Natl Acad Sci USA.* 2014;111:7671–6.
- Guerrero-Ferreira R, Taylor NM, Mona D, Ringler P, Lauer ME, Riek R, et al. Cryo-EM structure of alpha-synuclein fibrils. *eLife.* 2018;7:e36402.
- Li Y, Zhao C, Luo F, Liu Z, Gui X, Luo Z, et al. Amyloid fibril structure of α -synuclein determined by cryo-electron microscopy. *Cell Res.* 2018;28:897–903.
- Boyer DR, Li B, Sun C, Fan W, Sawaya MR, Jiang L, et al. Structures of fibrils formed by α -synuclein hereditary disease mutant H50Q reveal new polymorphs. *Nat Struct Mol Biol.* 2019;26:1044–52.
- Tuttle MD, Comellas G, Nieuwkoop AJ, Covell DJ, Berthold DA, Kloepper KD, et al. Solid-state NMR structure of a pathogenic fibril of full-length human α -synuclein. *Nat Struct Mol Biol.* 2016;23:409–15.
- Yang Y, Shi Y, Schweighauser M, Zhang X, Kotecha A, Murzin AG, et al. Structures of α -synuclein filaments from human brains with Lewy pathology. *Nature.* 2022;610:791–5.
- Burger D, Fenyl A, Bousset L, Stahlberg H, Melki R. Cryo-EM structure of alpha-synuclein fibrils amplified by PMCA from PD and MSA patient brains. *bioRxiv.* 2021;2021.07.08.451588. Available from: <https://www.biorxiv.org/content/10.1101/2021.07.08.451588v1>.
- Strohäker T, Jung BC, Liou S-H, Fernandez CO, Riedel D, Becker S, et al. Structural heterogeneity of α -synuclein fibrils amplified from patient brain extracts. *Nat Commun.* 2019;10:5535.
- Kramer ML, Schulz-Schaeffer WJ. Presynaptic alpha-synuclein aggregates, not Lewy bodies, cause neurodegeneration in dementia with Lewy bodies. *J Neurosci.* 2007;27:1405–10.
- Paleologou KE, Kragh CL, Mann DMA, Salem SA, Al-Shami R, Allsop D, et al. Detection of elevated levels of soluble alpha-synuclein oligomers

- in post-mortem brain extracts from patients with dementia with Lewy bodies. *Brain J Neurol.* 2009;132:1093–101.
45. Ingelsson M. Alpha-synuclein oligomers—Neurotoxic molecules in parkinson's disease and other lewy body disorders. *Front Neurosci.* 2016;10:1–10. Available from: <https://www.frontiersin.org/article/10.3389/fnins.2016.00408>.
 46. Bongianini M, Ladogana A, Capaldi S, Klotz S, Baiardi S, Cagnin A, et al. α -Synuclein RT-QuIC assay in cerebrospinal fluid of patients with dementia with Lewy bodies. *Ann Clin Transl Neurol.* 2019;6:2120–6.
 47. Dhavale DD, Barclay AM, Borcik CG, Basore K, Gordon IR, Liu J, et al. Structure of alpha-synuclein fibrils derived from human Lewy body dementia tissue. *bioRxiv.* 2023;2023.01.09.523303. Available from: <https://www.biorxiv.org/content/10.1101/2023.01.09.523303v1>.
 48. Hansson O, Hall S, Ohrfelt A, Zetterberg H, Blennow K, Minthon L, et al. Levels of cerebrospinal fluid α -synuclein oligomers are increased in Parkinson's disease with dementia and dementia with Lewy bodies compared to Alzheimer's disease. *Alzheimers Res Ther.* 2014;6:25.
 49. Pountney DL, Lowe R, Quilty M, Vickers JC, Voelcker NH, Gai WP. Annular α -synuclein species from purified multiple system atrophy inclusions. *J Neurochem.* 2004;90:502–12.
 50. Cascella R, Bigi A, Cremades N, Cecchi C. Effects of oligomer toxicity, fibril toxicity and fibril spreading in synucleinopathies. *Cell Mol Life Sci.* 2022;79:174.
 51. Schweighauser M, Shi Y, Tarutani A, Kametani F, Murzin AG, Ghetti B, et al. Structures of α -synuclein filaments from multiple system atrophy. *Nature.* 2020;585:464–9.
 52. Tabrizi SJ, Flower MD, Ross CA, Wild EJ. Huntington disease: new insights into molecular pathogenesis and therapeutic opportunities. *Nat Rev Neurol.* 2020;16:529–46.
 53. Wetzel R. Exploding the repeat length paradigm while exploring amyloid toxicity in Huntington's disease. *Acc Chem Res.* 2020;53:2347–57.
 54. Gropp MHM, Klaips CL, Hartl FU. Formation of toxic oligomers of polyQ-expanded Huntingtin by prion-mediated cross-seeding. *Mol Cell.* 2022;82:4290–4306.e11.
 55. Sinnige T, Meisl G, Michaels TCT, Vendruscolo M, Knowles TPJ, Morimoto RI. Kinetic analysis reveals that independent nucleation events determine the progression of polyglutamine aggregation in *C. elegans*. *Proc Natl Acad Sci USA.* 2021;118:e2021888118.
 56. Nazarov S, Chiki A, Boudeffa D, Lashuel HA. Structural basis of Huntingtin fibril polymorphism revealed by cryogenic electron microscopy of exon 1 HTT fibrils. *J Am Chem Soc.* 2022;144:10723–35.
 57. Katsumoto A, Takeuchi H, Tanaka F. Tau pathology in chronic traumatic encephalopathy and Alzheimer's disease: similarities and differences. *Front Neurol.* 2019;10:1–9. Available from: <https://www.frontiersin.org/articles/10.3389/fneur.2019.00980>.
 58. Falcon B, Zivanov J, Zhang W, Murzin AG, Garringer HJ, Vidal R, et al. Novel tau filament fold in chronic traumatic encephalopathy encloses hydrophobic molecules. *Nature.* 2019;568:420–3.
 59. Metrick MA, Ferreira N do C, Saijo E, Kraus A, Newell K, Zanusso G, et al. A single ultrasensitive assay for detection and discrimination of tau aggregates of Alzheimer and Pick diseases. *Acta Neuropathol Commun.* 2020;8:22.
 60. Falcon B, Zhang W, Murzin AG, Murshudov G, Garringer HJ, Vidal R, et al. Structures of filaments from Pick's disease reveal a novel tau protein fold. *Nature.* 2018;561:137.
 61. Saijo E, Metrick MA, Koga S, Parchi P, Litvan I, Spina S, et al. 4-Repeat tau seeds and templating subtypes as brain and CSF biomarkers of fronto-temporal lobar degeneration. *Acta Neuropathol (Berl).* 2020;139:63–77.
 62. Zhang W, Tarutani A, Newell KL, Murzin AG, Matsubara T, Falcon B, et al. Novel tau filament fold in corticobasal degeneration. *Nature.* 2020;580:283–7.
 63. Woerman AL, Aoyagi A, Patel S, Kazmi SA, Lobach I, Grinberg LT, et al. Tau prions from Alzheimer's disease and chronic traumatic encephalopathy patients propagate in cultured cells. *Proc Natl Acad Sci USA.* 2016;113:E8187–96.
 64. Chung DC, Carlomagno Y, Cook CN, Jansen-West K, Daugherty L, Lewis-Tuffin LJ, et al. Tau exhibits unique seeding properties in globular glial tauopathy. *Acta Neuropathol Commun.* 2019;7:36.
 65. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. *Science.* 1982;216:136–44.
 66. Caughey B, Baron GS, Chesebro B, Jeffrey M. Getting a grip on prions: oligomers, amyloids and pathological membrane interactions. *Annu Rev Biochem.* 2009;78:177–204.
 67. Sigurdson CJ, Bartz JC, Glatzel M. Cellular and molecular mechanisms of prion disease. *Annu Rev Pathol.* 2019;14:497–516.
 68. Meisl G, Kurt T, Condado-Morales I, Bett C, Sorce S, Nuvolone M, et al. Scaling analysis reveals the mechanism and rates of prion replication in vivo. *Nat Struct Mol Biol.* 2021;28:365–72.
 69. Wang L-Q, Zhao K, Yuan H-Y, Li X-N, Dang H-B, Ma Y, et al. Genetic prion disease-related mutation E196K displays a novel amyloid fibril structure revealed by cryo-EM. *Sci Adv.* 2021;7:eabg9676.
 70. Kraus A, Hoyt F, Schwartz CL, Hansen B, Artikis E, Hughson AG, et al. High-resolution structure and strain comparison of infectious mammalian prions. *Mol Cell.* 2021;81:4540–4551.e6.
 71. Wang L-Q, Zhao K, Yuan H-Y, Wang Q, Guan Z, Tao J, et al. Cryo-EM structure of an amyloid fibril formed by full-length human prion protein. *Nat Struct Mol Biol.* 2020;27:598–602.
 72. Glynn C, Sawaya MR, Ge P, Gallagher-Jones M, Short CW, Bowman R, et al. Cryo-EM structure of a human prion fibril with a hydrophobic, protease-resistant core. *Nat Struct Mol Biol.* 2020;27:417–23.
 73. Manka SW, Wenborn A, Betts J, et al. A structural basis for prion strain diversity. *Nat Chem Biol.* 2023;19:607–13. <https://doi.org/10.1038/s41589-022-01229-7>.
 74. Manka SW, Zhang W, Wenborn A, Betts J, Joiner S, Saibil HR, et al. 2.7 Å cryo-EM structure of ex vivo RML prion fibrils. *Nat Commun.* 2022;13:4004.
 75. Hoyt F, Standke HG, Artikis E, Schwartz CL, Hansen B, Li K, et al. Cryo-EM structure of anchorless RML prion reveals variations in shared motifs between distinct strains. *Nat Commun.* 2022;13:4005.
 76. Kamali-Jamil R, Vázquez-Fernández E, Tancowny B, Rathod V, Amidian S, Wang X, et al. The ultrastructure of infectious L-type bovine spongiform encephalopathy prions constrains molecular models. *PLOS Pathog.* 2021;17:e1009628.
 77. Bram Y, Frydman-Marom A, Yanai I, Gilead S, Shaltiel-Karyo R, Amdursky N, et al. Apoptosis induced by islet amyloid polypeptide soluble oligomers is neutralized by diabetes-associated specific antibodies. *Sci Rep.* 2014;4:4267.
 78. Gurlo T, Ryazantsev S, Huang C, Yeh MW, Reber HA, Hines OJ, et al. Evidence for proteotoxicity in β cells in Type 2 Diabetes: toxic islet amyloid polypeptide oligomers form intracellularly in the secretory pathway. *Am J Pathol.* 2010;176:861–9.
 79. Rodriguez Camargo DC, Chia S, Menzies J, Mannini B, Meisl G, Lundqvist M, et al. Surface-Catalyzed Secondary Nucleation Dominates the Generation of Toxic IAPP Aggregates. *Front Mol Biosci.* 2021;8:1–11. Available from: <https://www.frontiersin.org/article/10.3389/fmols.2021.757425>.
 80. Gallardo R, Iadanza MG, Xu Y, Heath GR, Foster R, Radford SE, et al. Fibril structures of diabetes-related amylin variants reveal a basis for surface-templated assembly. *Nat Struct Mol Biol.* 2020;27:1048–56.
 81. Cao Q, Boyer DR, Sawaya MR, Ge P, Eisenberg DS. Cryo-EM structure and inhibitor design of human IAPP (amylin) fibrils. *Nat Struct Mol Biol.* 2020;27:653–9.
 82. Cao Q, Boyer DR, Sawaya MR, Abskharon R, Saelices L, Nguyen BA, et al. Cryo-EM structures of hIAPP fibrils seeded by patient-extracted fibrils reveal new polymorphs and conserved fibril cores. *Nat Struct Mol Biol.* 2021;28:724–30.
 83. Meehan S, Berry Y, Luisi B, Dobson CM, Carver JA, MacPhee CE. Amyloid fibril formation by lens crystallin proteins and its implications for cataract formation. *J Biol Chem.* 2004;279:3413–9.
 84. Hayashi J, Carver JA. The multifaceted nature of α B-crystallin. *Cell Stress Chaperones.* 2020;25:639–54.
 85. Bansal A, Schmidt M, Rennegarbe M, Haupt C, Liberta F, Stecher S, et al. AA amyloid fibrils from diseased tissue are structurally different from in vitro formed SAA fibrils. *Nat Commun.* 2021;12:1013.
 86. Jayaraman S, Gantz DL, Haupt C, Gursky O. Serum amyloid A forms stable oligomers that disrupt vesicles at lysosomal pH and contribute to the pathogenesis of reactive amyloidosis. *Proc Natl Acad Sci USA.* 2017;114:E6507–15.
 87. Westermark GT, Westermark P. Serum amyloid A and protein AA: Molecular mechanisms of a transmissible amyloidosis. *FEBS Lett.* 2009;583:2685–90.

88. Heerde T, Rennegarbe M, Biedermann A, Savran D, Pfeiffer PB, Hitzengerber M, et al. Cryo-EM demonstrates the in vitro proliferation of an ex vivo amyloid fibril morphology by seeding. *Nat Commun*. 2022;13:85.
89. Teixeira PF, Cerca F, Santos SD, Saraiva MJ. Endoplasmic reticulum stress associated with extracellular aggregates: evidence from transthyretin deposition in familial amyloid polyneuropathy. *J Biol Chem*. 2006;281:21998–2003.
90. Andersson K, Olofsson A, Nielsen EH, Svehaug S-E, Lundgren E. Only amyloidogenic intermediates of transthyretin induce apoptosis. *Biochem Biophys Res Commun*. 2002;294:309–14.
91. Reixach N, Deechongkit S, Jiang X, Kelly JW, Buxbaum JN. Tissue damage in the amyloidosis: Transthyretin monomers and nonnative oligomers are the major cytotoxic species in tissue culture. *Proc Natl Acad Sci USA*. 2004;101:2817–22.
92. Steinebrei M, Gottwald J, Baur J, Röcken C, Hegenbart U, Schönland S, et al. Cryo-EM structure of an ATTRwt amyloid fibril from systemic non-hereditary transthyretin amyloidosis. *Nat Commun*. 2022;13:6398.
93. Schmidt M, Wiese S, Adak V, Engler J, Agarwal S, Fritz G, et al. Cryo-EM structure of a transthyretin-derived amyloid fibril from a patient with hereditary ATTR amyloidosis. *Nat Commun*. 2019;10:5008.
94. Merlini G, Dispenzieri A, Sanchowala V, Schönland SO, Palladini G, Hawkins PN, et al. Systemic immunoglobulin light chain amyloidosis. *Nat Rev Dis Primer*. 2018;4:1–19.
95. Imperlini E, Gneccchi M, Rognoni P, Sabidò E, Ciuffreda MC, Palladini G, et al. Proteotoxicity in cardiac amyloidosis: amyloidogenic light chains affect the levels of intracellular proteins in human heart cells. *Sci Rep*. 2017;7:15661.
96. Blancas-Mejía LM, Ramirez-Alvarado M. Recruitment of light chains by homologous and heterologous fibrils shows distinctive kinetic and conformational specificity. *Biochemistry*. 2016;55:2967–78.
97. Radamaker L, Baur J, Huhn S, Haupt C, Hegenbart U, Schönland S, et al. Cryo-EM reveals structural breaks in a patient-derived amyloid fibril from systemic AL amyloidosis. *Nat Commun*. 2021;12:875.
98. Swuec P, Lavatelli F, Tasaki M, Papissoni C, Rognoni P, Maritan M, et al. Cryo-EM structure of cardiac amyloid fibrils from an immunoglobulin light chain AL amyloidosis patient. *Nat Commun*. 2019;10:1269.
99. Dasari AKR, Yi S, Coats MF, Wi S, Lim KH. Toxic Misfolded transthyretin oligomers with different molecular conformations formed through distinct oligomerization pathways. *Biochemistry*. 2022;61:2358–65.
100. Bemporad F, Chiti F. Protein misfolded oligomers: experimental approaches, mechanism of formation, and structure-toxicity relationships. *Chem Biol*. 2012;19:315–27.
101. Kreiser RP, Wright AK, Block NR, Hollows JE, Nguyen LT, LeFort K, et al. Therapeutic strategies to reduce the toxicity of misfolded protein oligomers. *Int J Mol Sci*. 2020;21:8651.
102. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β -peptide. *Nat Rev Mol Cell Biol*. 2007;8:101–12.
103. Benilova I, Karran E, De Strooper B. The toxic A β oligomer and Alzheimer's disease: an emperor in need of clothes. *Nat Neurosci*. 2012;15:349–57.
104. Hampel H, Hardy J, Blennow K, Chen C, Perry G, Kim SH, et al. The amyloid- β pathway in Alzheimer's disease. *Mol Psychiatry*. 2021;26:5481–503.
105. Marin-Argany M, Lin Y, Misra P, Williams A, Wall JS, Howell KG, et al. Cell damage in light chain amyloidosis. *J Biol Chem*. 2016;291:19813–25.
106. Pieri L, Madiona K, Bousset L, Melki R. Fibrillar α -synuclein and huntingtin exon 1 assemblies are toxic to the cells. *Biophys J*. 2012;102:2894–905.
107. Scheidt T, Łapińska U, Kumita JR, Whiten DR, Klenerman D, Wilson MR, et al. Secondary nucleation and elongation occur at different sites on Alzheimer's amyloid- β aggregates. *Sci Adv*. 2019;5:eaau3112.
108. Cascella R, Chen SW, Bigi A, Camino JD, Xu CK, Dobson CM, et al. The release of toxic oligomers from α -synuclein fibrils induces dysfunction in neuronal cells. *Nat Commun*. 2021;12:1814.
109. Josephs KA, Ahlskog JE, Parisi JE, Boeve BF, Crum BA, Giannini C, et al. Rapidly progressive neurodegenerative dementias. *Arch Neurol*. 2009;66:201–7.
110. Rösler TW, Tayanian Marvian A, Brendel M, Nykänen N-P, Höllerhage M, Schwarz SC, et al. Four-repeat tauopathies. *Prog Neurobiol*. 2019;180: 101644.
111. Irwin DJ, Lee VM-Y, Trojanowski JQ. Parkinson's disease dementia: convergence of α -synuclein, tau and amyloid- β pathologies. *Nat Rev Neurosci*. 2013;14:626–36.
112. Dugger BN, Dickson DW. Pathology of neurodegenerative diseases. *Cold Spring Harb Perspect Biol*. 2017;9: a028035.
113. Chia S, Habchi J, Michaels TCT, Cohen SIA, Linse S, Dobson CM, et al. SAR by kinetics for drug discovery in protein misfolding diseases. *Proc Natl Acad Sci USA*. 2018;115:10245–50.
114. Michaels TCT, Dear AJ, Cohen SIA, Vendruscolo M, Knowles TPJ. Kinetic profiling of therapeutic strategies for inhibiting the formation of amyloid oligomers. *J Chem Phys*. 2022;156: 164904.
115. Kulenkampff K, Wolf Perez A-M, Sormanni P, Habchi J, Vendruscolo M. Quantifying misfolded protein oligomers as drug targets and biomarkers in Alzheimer and Parkinson diseases. *Nat Rev Chem*. 2021;5(4):277–94. <https://doi.org/10.1038/s41570-021-00254-9>.
116. Limbocker R, Cremades N, Cascella R, Tessier PM, Vendruscolo M, Chiti F. Characterization of pairs of toxic and nontoxic misfolded protein oligomers elucidates the structural determinants of oligomer toxicity in protein misfolding diseases. *Acc Chem Res*. 2023;56:1395–405.
117. McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, et al. Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann Neurol*. 1999;46:860–6.
118. Kaye R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, et al. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science*. 2003;300:486–9.
119. Lacor PN, Buniel MC, Furlow PW, Clemente AS, Velasco PT, Wood M, et al. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *J Neurosci*. 2007;27:796–807.
120. Kaye R, Head E, Sarsoza F, Saing T, Cotman CW, Necula M, et al. Fibril specific, conformation dependent antibodies recognize a generic epitope common to amyloid fibrils and fibrillar oligomers that is absent in prefibrillar oligomers. *Mol Neurodegener*. 2007;2:18.
121. Noguchi A, Matsumura S, Dezawa M, Tada M, Yanazawa M, Ito A, et al. Isolation and characterization of patient-derived, toxic, high mass amyloid beta-protein (Abeta) assembly from Alzheimer disease brains. *J Biol Chem*. 2009;284:32895–905.
122. Hillen H, Barghorn S, Striebing A, Labkovsky B, Müller R, Nimmrich V, et al. Generation and therapeutic efficacy of highly oligomer-specific β -amyloid antibodies. *J Neurosci*. 2010;30:10369–79.
123. Hölttä M, Hansson O, Andreasson U, Hertz J, Minthon L, Nägga K, et al. Evaluating amyloid- β oligomers in cerebrospinal fluid as a biomarker for Alzheimer's disease. *PLoS ONE*. 2013;8: e66381.
124. Savage MJ, Kalinina J, Wolfe A, Tugusheva K, Korn R, Cash-Mason T, et al. A sensitive A β oligomer assay discriminates Alzheimer's and aged control cerebrospinal fluid. *J Neurosci*. 2014;34:2884–97.
125. Sengupta U, Portelius E, Hansson O, Farmer K, Castillo-Carranza D, Woltjer R, et al. Tau oligomers in cerebrospinal fluid in Alzheimer's disease. *Ann Clin Transl Neurol*. 2017;4:226–35.
126. Vecchi G, Sormanni P, Mannini B, Vandelli A, Tartaglia GG, Dobson CM, et al. Proteome-wide observation of the phenomenon of life on the edge of solubility. *Proc Natl Acad Sci USA*. 2020;117:1015–20.
127. Tartaglia GG, Pechmann S, Dobson CM, Vendruscolo M. Life on the edge: a link between gene expression levels and aggregation rates of human proteins. *Trends Biochem Sci*. 2007;32:204–6.
128. Baldwin AJ, Knowles TPJ, Tartaglia GG, Fitzpatrick AW, Devlin GL, Shamas SL, et al. Metastability of native proteins and the phenomenon of amyloid formation. *J Am Chem Soc*. 2011;133:14160–3.
129. Eisenberg D, Jucker M. The amyloid state of proteins in human diseases. *Cell*. 2012;148:1188–203.
130. Sunde M, Serpell LC, Bartlam M, Fraser PE, Pepys MB, Blake CCF. Common core structure of amyloid fibrils by synchrotron X-ray diffraction. *J Mol Biol*. 1997;273:729–39.
131. Dobson CM. Protein misfolding, evolution and disease. *Trends Biochem Sci*. 1999;24:329–32.
132. Fuxreiter M, Vendruscolo M. Generic nature of the condensed states of proteins. *Nat Cell Biol*. 2021;23:587–94.

133. Callaway E. Revolutionary cryo-EM is taking over structural biology. *Nature*. 2020;578:201.
134. Scheres SHW. RELION: Implementation of a Bayesian approach to cryo-EM structure determination. *J Struct Biol*. 2012;180:519–30.
135. Scheres SH, Zhang W, Falcon B, Goedert M. Cryo-EM structures of tau filaments. *Curr Opin Struct Biol*. 2020;64:17–25.
136. Arseni D, Hasegawa M, Murzin AG, Kametani F, Arai M, Yoshida M, et al. Structure of pathological TDP-43 filaments from ALS with FTLD. *Nature*. 2022;601:139–43.
137. Sawaya MR, Hughes MP, Rodriguez JA, Riek R, Eisenberg DS. The expanding amyloid family: Structure, stability, function, and pathogenesis. *Cell*. 2021;184:4857–73.
138. Lu J-X, Qiang W, Yau W-M, Schwieters CD, Meredith SC, Tycko R. Molecular structure of β -amyloid fibrils in Alzheimer's disease brain tissue. *Cell*. 2013;154:1257–68.
139. Qiang W, Yau W-M, Lu J-X, Collinge J, Tycko R. Structural variation in amyloid- β fibrils from Alzheimer's disease clinical subtypes. *Nature*. 2017;541:217–21.
140. Hartl FU, Bracher A, Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. *Nature*. 2011;475:324–32.
141. Knowles TP, Fitzpatrick AW, Meehan S, Mott HR, Vendruscolo M, Dobson CM, et al. Role of intermolecular forces in defining material properties of protein nanofibrils. *Science*. 2007;318:1900–3.
142. Fowler DM, Koulou AV, Balch WE, Kelly JW. Functional amyloid – from bacteria to humans. *Trends Biochem Sci*. 2007;32:217–24.
143. Otzen D, Riek R. Functional Amyloids. *Cold Spring Harb Perspect Biol*. 2019;11: a033860.
144. Hervas R, Rau MJ, Park Y, Zhang W, Murzin AG, Fitzpatrick JAJ, et al. Cryo-EM structure of a neuronal functional amyloid implicated in memory persistence in *Drosophila*. *Science*. 2020;367:1230–4.
145. Meisl G, Xu CK, Taylor JD, Michaels TCT, Levin A, Otzen D, et al. Uncovering the universality of self-replication in protein aggregation and its link to disease. *Sci Adv*. 2022;8:eabn6831.
146. Shimanovich U, Ruggeri FS, Genst ED, Adamcik J, Barros TP, Porter D, et al. Silk micrococoon for protein stabilisation and molecular encapsulation. *Nat Commun*. 2017;8:15902.
147. Bolisetti S, Mezzenga R. Amyloid-carbon hybrid membranes for universal water purification. *Nat Nanotechnol*. 2016;11:365–71.
148. Horvath I, Wittung-Stafshede P. Amyloid fibers of α -synuclein catalyze chemical reactions. *ACS Chem Neurosci*. 2023;14:603–8.
149. Arad E, Baruch Leshem A, Rapaport H, Jelinek R. β -Amyloid fibrils catalyze neurotransmitter degradation. *Chem Catal*. 2021;1:908–22.
150. Walsh DM, Lomakin A, Benedek GB, Condron MM, Teplow DB. Amyloid beta-protein fibrillogenesis. Detection of a protofibrillar intermediate. *J Biol Chem*. 1997;272:22364–72.
151. Harper JD, Wong SS, Lieber CM, Lansbury PT. Observation of metastable A β amyloid protofibrils by atomic force microscopy. *Chem Biol*. 1997;4:119–25.
152. Podlisny MB, Ostaszewski BL, Squazzo SL, Koo EH, Rydel RE, Teplow DB, et al. Aggregation of secreted amyloid beta-protein into sodium dodecyl sulfate-stable oligomers in cell culture. *J Biol Chem*. 1995;270:9564–70.
153. Ahmed M, Davis J, Aucoin D, Sato T, Ahuja S, Aimoto S, et al. Structural conversion of neurotoxic amyloid- β 1-42 oligomers to fibrils. *Nat Struct Mol Biol*. 2010;17:561–7.
154. Chimon S, Shaibat MA, Jones CR, Calero DC, Aizezi B, Ishii Y. Evidence of fibril-like β -sheet structures in a neurotoxic amyloid intermediate of Alzheimer's β -amyloid. *Nat Struct Mol Biol*. 2007;14:1157–64.
155. Hoshi M, Sato M, Matsumoto S, Noguchi A, Yasutake K, Yoshida N, et al. Spherical aggregates of β -amyloid (amylospheroid) show high neurotoxicity and activate tau protein kinase I/glycogen synthase kinase-3 β . *Proc Natl Acad Sci USA*. 2003;100:6370–5.
156. Barghorn S, Nimmrich V, Striebing A, Krantz C, Keller P, Janson B, et al. Globular amyloid beta-peptide oligomer - a homogenous and stable neuropathological protein in Alzheimer's disease. *J Neurochem*. 2005;95:834–47.
157. Kaye R, Pensalfini A, Margol L, Sokolov Y, Sarsoza F, Head E, et al. Annular protofibrils are a structurally and functionally distinct type of amyloid oligomer. *J Biol Chem*. 2009;284:4230–7.
158. Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, et al. Diffusible, nonfibrillar ligands derived from A β 1–42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci USA*. 1998;95:6448–53.
159. Bitan G, Kirkitadze MD, Lomakin A, Vollers SS, Benedek GB, Teplow DB. Amyloid β -protein (A β) assembly: A β 40 and A β 42 oligomerize through distinct pathways. *Proc Natl Acad Sci USA*. 2003;100:330–5.
160. Cremades N, Cohen SIA, Deas E, Abramov AY, Chen AY, Orte A, et al. Direct observation of the interconversion of normal and toxic forms of α -synuclein. *Cell*. 2012;149:1048–59.
161. Chen SW, Drakulic S, Deas E, Oubrai M, Aprile FA, Arranz R, et al. Structural characterization of toxic oligomers that are kinetically trapped during α -synuclein fibril formation. *Proc Natl Acad Sci USA*. 2015;112:E1994–2003.
162. Gallea JI, Celej MS. Structural insights into amyloid oligomers of the Parkinson disease-related protein α -synuclein. *J Biol Chem*. 2014;289:26733–42.
163. Lorenzen N, Nielsen SB, Buell AK, Kaspersen JD, Arosio P, Vad BS, et al. The role of stable α -Synuclein oligomers in the molecular events underlying amyloid formation. *J Am Chem Soc*. 2014;136:3859–68.
164. Stroud JC, Liu C, Teng PK, Eisenberg D. Toxic fibrillar oligomers of amyloid- β have cross- β structure. *Proc Natl Acad Sci USA*. 2012;109:7717–22.
165. Parthasarathy S, Inoue M, Xiao Y, Matsumura Y, Nabeshima Y, Hoshi M, et al. Structural insight into an alzheimer's brain-derived spherical assembly of amyloid β by solid-state NMR. *J Am Chem Soc*. 2015;137:6480–3.
166. O'Nuallain B, Freir DB, Nicoll AJ, Risse E, Ferguson N, Herron CE, et al. Amyloid β -protein dimers rapidly form stable synaptotoxic protofibrils. *J Neurosci*. 2010;30:14411–9.
167. Carulla N, Zhou M, Arimon M, Gairi M, Giral E, Robinson CV, et al. Experimental characterization of disordered and ordered aggregates populated during the process of amyloid fibril formation. *Proc Natl Acad Sci USA*. 2009;106:7828–33.
168. Modler AJ, Gast K, Lutsch G, Damaschun G. Assembly of amyloid protofibrils via critical oligomers—a novel pathway of amyloid formation. *J Mol Biol*. 2003;325:135–48.
169. Bouchard M, Zurdo J, Nettleton EJ, Dobson CM, Robinson CV. Formation of insulin amyloid fibrils followed by FTIR simultaneously with CD and electron microscopy. *Protein Sci Publ Protein Soc*. 2000;9:1960–7.
170. Plakoutsi G, Bemporad F, Calamai M, Taddei N, Dobson CM, Chiti F. Evidence for a mechanism of amyloid formation involving molecular reorganisation within native-like precursor aggregates. *J Mol Biol*. 2005;351:910–22.
171. Matsumura S, Shinoda K, Yamada M, Yokojima S, Inoue M, Ohnishi T, et al. Two distinct amyloid β -protein (A β) assembly pathways leading to oligomers and fibrils identified by combined fluorescence correlation spectroscopy, morphology, and toxicity analyses. *J Biol Chem*. 2011;286:11555–62.
172. Fu Z, Aucoin D, Davis J, Van Nostrand WE, Smith SO. Mechanism of nucleated conformational conversion of A β 42. *Biochemistry*. 2015;54:4197–207.
173. Lasagna-Reeves CA, Glabe CG, Kaye R. Amyloid- β annular protofibrils evade fibrillar fate in Alzheimer disease brain. *J Biol Chem*. 2011;286:22122–30.
174. Michaels TCT, Šarić A, Curk S, Bernfur K, Arosio P, Meisl G, et al. Dynamics of oligomer populations formed during the aggregation of Alzheimer's A β 42 peptide. *Nat Chem*. 2020;12:445–51.
175. Cohen SIA, Vendruscolo M, Dobson CM, Knowles TPJ. From macroscopic measurements to microscopic mechanisms of protein aggregation. *J Mol Biol*. 2012;421:160–71.
176. Lee VM-Y. Amyloid binding ligands as Alzheimer's disease therapies. *Neurobiol Aging*. 2002;23:1039–42.
177. Biancalana M, Koide S. Molecular mechanism of thioflavin-t binding to amyloid fibrils. *Biochim Biophys Acta*. 2010;1804:1405–12.
178. Ferrone F. Analysis of protein aggregation kinetics. *Methods Enzymol*. 1999;309:256–74.
179. Staats R, Michaels TCT, Flagmeier P, Chia S, Horne RI, Habchi J, et al. Screening of small molecules using the inhibition of oligomer formation in α -synuclein aggregation as a selection parameter. *Commun Chem*. 2020;3:1–9.
180. Knowles TPJ, Waudby CA, Devlin GL, Cohen SIA, Aguzzi A, Vendruscolo M, et al. An Analytical solution to the kinetics of breakable filament assembly. *Science*. 2009;326:1533–7.

181. Meisl G, Kirkegaard JB, Arosio P, Michaels TCT, Vendruscolo M, Dobson CM, et al. Molecular mechanisms of protein aggregation from global fitting of kinetic models. *Nat Protoc.* 2016;11:252–72.
182. Cohen SIA, Vendruscolo M, Welland ME, Dobson CM, Terentjev EM, Knowles TPJ. Nucleated polymerization with secondary pathways. I. Time evolution of the principal moments. *J Chem Phys.* 2011;135:065105.
183. Cohen SIA, Vendruscolo M, Dobson CM, Knowles TPJ. Nucleated polymerization with secondary pathways II. Determination of self-consistent solutions to growth processes described by non-linear master equations. *J Chem Phys.* 2011;135:065106.
184. Cohen SIA, Vendruscolo M, Dobson CM, Knowles TPJ. Nucleated polymerization with secondary pathways III. Equilibrium behavior and oligomer populations. *J Chem Phys.* 2011;135:065107.
185. Meisl G, Rajah L, I. Cohen SA, Pfammatter M, Šarić A, Hellstrand E, et al. Scaling behaviour and rate-determining steps in filamentous self-assembly. *Chem Sci.* 2017;8:7087–97.
186. Dear AJ, Michaels TCT, Meisl G, Klenerman D, Wu S, Perrett S, et al. Kinetic diversity of amyloid oligomers. *Proc Natl Acad Sci USA.* 2020;117:12087–94.
187. Buell AK. The growth of amyloid fibrils: rates and mechanisms. *Biochem J.* 2019;476:2677–703.
188. Young LJ, Schierle GSK, Kaminski CF. Imaging A β (1–42) fibril elongation reveals strongly polarised growth and growth incompetent states. *Phys Chem Chem Phys.* 2017;19:27987–96.
189. Habchi J, Chia S, Galvagnion C, Michaels TCT, Bellaiche MMJ, Ruggeri FS, et al. Cholesterol catalyses A β 42 aggregation through a heterogeneous nucleation pathway in the presence of lipid membranes. *Nat Chem.* 2018;10:673–83.
190. Galvagnion C, Buell AK, Meisl G, Michaels TCT, Vendruscolo M, Knowles TPJ, et al. Lipid vesicles trigger α -synuclein aggregation by stimulating primary nucleation. *Nat Chem Biol.* 2015;11:229–34.
191. Knowles TPJ, White DA, Abate AR, Agresti JJ, Cohen SIA, Sperling RA, et al. Observation of spatial propagation of amyloid assembly from single nuclei. *Proc Natl Acad Sci USA.* 2011;108:14746–51.
192. Törnquist M, T. Michaels TC, Sanagavarapu K, Yang X, Meisl G, A. Cohen SI, et al. Secondary nucleation in amyloid formation. *Chem Commun.* 2018;54:8667–84.
193. Botsaris GD. Secondary nucleation — a review. In: Mullin JW. (eds) *Industrial crystallization*. Boston: Springer; 1976. https://doi.org/10.1007/978-1-4615-7258-9_1.
194. Ferrone FA, Hofrichter J, Eaton WA. Kinetics of sickle hemoglobin polymerization: II. A double nucleation mechanism. *J Mol Biol.* 1985;183:611–31.
195. Ruschak AM, Miranker AD. Fiber-dependent amyloid formation as catalysis of an existing reaction pathway. *Proc Natl Acad Sci USA.* 2007;104:12341–6.
196. Foderà V, Librizzi F, Groenning M, van de Weert M, Leone M. Secondary nucleation and accessible surface in insulin amyloid fibril formation. *J Phys Chem B.* 2008;112:3853–8.
197. Gaspar R, Meisl G, Buell AK, Young L, Kaminski CF, Knowles TPJ, et al. Secondary nucleation of monomers on fibril surface dominates α -synuclein aggregation and provides autocatalytic amyloid amplification. *Q Rev Biophys.* 2017;50:1–12. Available from: <https://www.cambridge.org/core/journals/quarterly-reviews-of-biophysics/article/secondary-nucleation-of-monomers-on-fibril-surface-dominates-synuclein-aggregation-and-provides-autocatalytic-amyloid-amplification/36C9559FB74EE301AC7440C31A2E6151>.
198. Thacker D, Barghouth M, Bless M, Zhang E, Linse S. Direct observation of secondary nucleation along the fibril surface of the amyloid β 42 peptide. *Proc Natl Acad Sci USA.* 2023;120: e2220664120.
199. Zimmermann MR, Bera SC, Meisl G, Dasadikari S, Ghosh S, Linse S, et al. Mechanism of secondary nucleation at the single fibril level from direct observations of A β 42 aggregation. *J Am Chem Soc.* 2021;143:16621–9.
200. Jan A, Adolfsson O, Allaman I, Buccarello A-L, Magistretti PJ, Pfeifer A, et al. Abeta42 neurotoxicity is mediated by ongoing nucleated polymerization process rather than by discrete Abeta42 species. *J Biol Chem.* 2011;286:8585–96.
201. Hill EK, Krebs B, Goodall DG, Howlett GJ, Dunstan DE. Shear flow induces amyloid fibril formation. *Biomacromol.* 2006;7:10–3.
202. Xue W-F, Homans SW, Radford SE. Systematic analysis of nucleation-dependent polymerization reveals new insights into the mechanism of amyloid self-assembly. *Proc Natl Acad Sci USA.* 2008;105:8926–31.
203. Bett C, Joshi-Barr S, Lucero M, Trejo M, Liberski P, Kelly JW, et al. Biochemical properties of highly neuroinvasive prion strains. *PLOS Pathog.* 2012;8: e1002522.
204. Kundel F, Hong L, Falcon B, McEwan WA, Michaels TCT, Meisl G, et al. Measurement of tau filament fragmentation provides insights into prion-like spreading. *ACS Chem Neurosci.* 2018;9:1276–82.
205. Shorter J, Lindquist S. Hsp104 catalyzes formation and elimination of self-replicating sup35 prion conformers. *Science.* 2004;304:1793–7.
206. Kraus A, Saijo E, Metrick MA, Newell K, Sigurdson CJ, Zanusso G, et al. Seeding selectivity and ultrasensitive detection of tau aggregate conformers of Alzheimer disease. *Acta Neuropathol (Berl).* 2019;137:585–98.
207. Saijo E, Groveman BR, Kraus A, Metrick M, Orr CD, Hughson AG, et al. Ultrasensitive RT-QuIC seed amplification assays for disease-associated tau, α -synuclein, and prion aggregates. *Protein Misfolding Dis Methods Protoc.* 2019:19–37. https://doi.org/10.1007/978-1-4939-8820-4_2.
208. Xue W-F, Hellewell AL, Gosal WS, Homans SW, Hewitt EW, Radford SE. Fibril fragmentation enhances amyloid cytotoxicity. *J Biol Chem.* 2009;284:34272–82.
209. Koffie RM, Meyer-Luehmann M, Hashimoto T, Adams KW, Mielke ML, Garcia-Alloza M, et al. Oligomeric amyloid beta associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. *Proc Natl Acad Sci USA.* 2009;106:4012–7.
210. Zhang X, Wesén E, Kumar R, Bernson D, Gallud A, Paul A, et al. Correlation between cellular uptake and cytotoxicity of fragmented α -synuclein amyloid fibrils suggests intracellular basis for toxicity. *ACS Chem Neurosci.* 2020;11:233–41.
211. Bett C, Lawrence J, Kurt TD, Orru C, Aguilar-Calvo P, Kincaid AE, et al. Enhanced neuroinvasion by smaller, soluble prions. *Acta Neuropathol Commun.* 2017;5:32.
212. Michaels TCT, Lazell HW, Arosio P, Knowles TPJ. Dynamics of protein aggregation and oligomer formation governed by secondary nucleation. *J Chem Phys.* 2015;143: 054901.
213. Yang J, Dear AJ, Michaels TCT, Dobson CM, Knowles TPJ, Wu S, et al. Direct observation of oligomerization by single molecule fluorescence reveals a multistep aggregation mechanism for the yeast prion protein Ure2. *J Am Chem Soc.* 2018;140:2493–503.
214. Iljina M, Garcia GA, Horrocks MH, Tosatto L, Choi ML, Ganzinger KA, et al. Kinetic model of the aggregation of alpha-synuclein provides insights into prion-like spreading. *Proc Natl Acad Sci USA.* 2016;113:E1206–15.
215. Babinchak WM, Surewicz WK. Liquid–liquid phase separation and its mechanistic role in pathological protein aggregation. *J Mol Biol.* 2020;432:1910–25.
216. Alberti S, Gladfelter A, Mittag T. Considerations and challenges in studying liquid–liquid phase separation and biomolecular condensates. *Cell.* 2019;176:419–34.
217. Banani SF, Lee HO, Hyman AA, Rosen MK. Biomolecular condensates: organizers of cellular biochemistry. *Nat Rev Mol Cell Biol.* 2017;18:285–98.
218. Mitrea DM, Mittasch M, Gomes BF, Klein IA, Murcko MA. Modulating biomolecular condensates: a novel approach to drug discovery. *Nat Rev Drug Discov.* 2022;21:841–62.
219. Vendruscolo M, Fuxreiter M. Protein condensation diseases: therapeutic opportunities. *Nat Commun.* 2022;13:5550.
220. Farahi N, Lazar T, Wodak SJ, Tompa P, Pancsa R. Integration of data from liquid–liquid phase separation databases highlights concentration and dosage sensitivity of LLPs drivers. *Int J Mol Sci.* 2021;22:3017.
221. Krainer G, Welsh TJ, Joseph JA, Espinosa JR, Wittmann S, de Csiláry E, et al. Reentrant liquid condensate phase of proteins is stabilized by hydrophobic and non-ionic interactions. *Nat Commun.* 2021;12:1085.
222. Martin EW, Mittag T. Relationship of sequence and phase separation in protein low-complexity regions. *Biochemistry.* 2018;57:2478–87.
223. Vendruscolo M, Fuxreiter M. Towards sequence-based principles for protein phase separation predictions. *Curr Opin Chem Biol.* 2023;75: 102317.
224. Alberti S, Halfmann R, King O, Kapila A, Lindquist S. A systematic survey identifies prions and illuminates sequence features of prionogenic proteins. *Cell.* 2009;137:146–58.

225. Franzmann TM, Alberti S. Prion-like low-complexity sequences: Key regulators of protein solubility and phase behavior. *J Biol Chem*. 2019;294:7128–36.
226. Vendruscolo M, Fuxreiter M. Sequence determinants of the aggregation of proteins within condensates generated by liquid-liquid phase separation. *J Mol Biol*. 2022;434: 167201.
227. Marsh JA, Forman-Kay JD. Sequence determinants of compaction in intrinsically disordered proteins. *Biophys J*. 2010;98:2383–90.
228. Das RK, Pappu RV. Conformations of intrinsically disordered proteins are influenced by linear sequence distributions of oppositely charged residues. *Proc Natl Acad Sci USA*. 2013;110:13392–7.
229. Cascella R, Bigi A, Riffert DG, Gagliani MC, Ermini E, Moretti M, et al. A quantitative biology approach correlates neuronal toxicity with the largest inclusions of TDP-43. *Sci Adv*. 2022;8:eabm6376.
230. Chen Y, Cohen TJ. Aggregation of the nucleic acid-binding protein TDP-43 occurs via distinct routes that are coordinated with stress granule formation. *J Biol Chem*. 2019;294:3696–706.
231. Hans F, Glasebach H, Kahle PJ. Multiple distinct pathways lead to hyperubiquitinated insoluble TDP-43 protein independent of its translocation into stress granules. *J Biol Chem*. 2020;295:673–89.
232. Hardenberg M, Horvath A, Ambrus V, Fuxreiter M, Vendruscolo M. Widespread occurrence of the droplet state of proteins in the human proteome. *Proc Natl Acad Sci USA*. 2020;117:33254–62.
233. French RL, Grese ZR, Aligredy H, Dhavale DD, Reeb AN, Kedia N, et al. Detection of TAR DNA-binding protein 43 (TDP-43) oligomers as initial intermediate species during aggregate formation. *J Biol Chem*. 2019;294:6696–709.
234. Kumar R, Das S, Mohite GM, Rout SK, Halder S, Jha NN, et al. Cytotoxic oligomers and fibrils trapped in a gel-like state of α -synuclein assemblies. *Angew Chem Int Ed*. 2018;57:5262–6.
235. Wegmann S, Eftekhazadeh B, Tepper K, Zoltowska KM, Bennett RE, Dujardin S, et al. Tau protein liquid-liquid phase separation can initiate tau aggregation. *EMBO J*. 2018;37: e98049.
236. Kanaan NM, Hamel C, Grabinski T, Combs B. Liquid-liquid phase separation induces pathogenic tau conformations in vitro. *Nat Commun*. 2020;11:2809.
237. Ash PEA, Lei S, Shattuck J, Boudeau S, Carlomagno Y, Medalla M, et al. TIA1 potentiates tau phase separation and promotes generation of toxic oligomeric tau. *Proc Natl Acad Sci USA*. 2021;118: e2014188118.
238. Xing Y, Nandakumar A, Kallinen A, Sun Y, Davis TP, Ke PC, et al. Amyloid Aggregation under the Lens of Liquid-Liquid Phase Separation. *J Phys Chem Lett*. 2021;12:368–78.
239. Walsh DM, Hartley DM, Kusumoto Y, Fezoui Y, Condron MM, Lomakin A, et al. Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. *J Biol Chem*. 1999;274:25945–52.
240. Campioni S, Mannini B, Zampagni M, Pensalfini A, Parrini C, Evangelisti E, et al. A causative link between the structure of aberrant protein oligomers and their toxicity. *Nat Chem Biol*. 2010;6:140–7.
241. Mannini B, Habchi J, Chia S, Ruggeri FS, Perni M, Knowles TPJ, et al. Stabilization and characterization of cytotoxic A β 40 oligomers isolated from an aggregation reaction in the presence of zinc ions. *ACS Chem Neurosci*. 2018;9:2959–71.
242. Lassen LB, Gregersen E, Isager AK, Betzer C, Kofoed RH, Jensen PH. ELISA method to detect α -synuclein oligomers in cell and animal models. *PLoS ONE*. 2018;13: e0196056.
243. Gong Y, Chang L, Viola KL, Lacor PN, Lambert MP, Finch CE, et al. Alzheimer's disease-affected brain: Presence of oligomeric A β ligands (ADDLs) suggests a molecular basis for reversible memory loss. *Proc Natl Acad Sci USA*. 2003;100:10417–22.
244. Brännström K, Lindhagen-Persson M, Gharibyan AL, Iakovleva I, Vestling M, Sellin ME, et al. A generic method for design of oligomer-specific antibodies. *PLoS ONE*. 2014;9: e90857.
245. Liu L, Kwak H, Lawton TL, Jin S-X, Meunier AL, Dang Y, et al. An ultra-sensitive immunoassay detects and quantifies soluble A β oligomers in human plasma. *Alzheimers Dement*. 2022;18:1186–202.
246. Aprile FA, Sormanni P, Podpolny M, Chhangur S, Needham L-M, Ruggeri FS, et al. Rational design of a conformation-specific antibody for the quantification of A β oligomers. *Proc Natl Acad Sci USA*. 2020;117(24):13509–18.
247. Majbour NK, Vaikath NN, van Dijk KD, Ardah MT, Varghese S, Vestergaard LB, et al. Oligomeric and phosphorylated alpha-synuclein as potential CSF biomarkers for Parkinson's disease. *Mol Neurodegener*. 2016;11:7.
248. Kumar ST, Jagannath S, Francois C, Vanderstichele H, Stoops E, Lashuel HA. How specific are the conformation-specific α -synuclein antibodies? Characterization and validation of 16 α -synuclein conformation-specific antibodies using well-characterized preparations of α -synuclein monomers, fibrils and oligomers with distinct structures and morphology. *Neurobiol Dis*. 2020;146: 105086.
249. Chatterjee T, Knappik A, Sandford E, Tewari M, Choi SW, Strong WB, et al. Direct kinetic fingerprinting and digital counting of single protein molecules. *Proc Natl Acad Sci USA*. 2020;117:22815–22.
250. Kool J, Jonker N, Irth H, Niessen WMA. Studying protein-protein affinity and immobilized ligand-protein affinity interactions using MS-based methods. *Anal Bioanal Chem*. 2011;401:1109.
251. Borch J, Jørgensen TJ, Roepstorff P. Mass spectrometric analysis of protein interactions. *Curr Opin Chem Biol*. 2005;9:509–16.
252. Buijs J, Franklin GC. SPR-MS in functional proteomics. *Brief Funct Genomic Proteomic*. 2005;4:39–47.
253. Zhang G, Ueberheide BM, Waldemarson S, Myung S, Molloy K, Eriksson J, et al. Protein quantitation using mass spectrometry. *Methods Mol Biol*. 2010;673:211–22.
254. Heck AJR. Native mass spectrometry: a bridge between interactomics and structural biology. *Nat Methods*. 2008;5:927–33.
255. Mannini B, Mulvihill E, Sgromo C, Cascella R, Khodarahmi R, Ramazzotti M, et al. Toxicity of protein oligomers is rationalized by a function combining size and surface hydrophobicity. *ACS Chem Biol*. 2014;9:2309–17.
256. Horrocks MH, Tosatto L, Dear AJ, Garcia GA, Iljina M, Cremades N, et al. Fast flow microfluidics and single-molecule fluorescence for the rapid characterization of α -synuclein oligomers. *Anal Chem*. 2015;87:8818–26.
257. Limboccker R, Chia S, Ruggeri FS, Perni M, Cascella R, Heller GT, et al. Trodusquemine enhances A β 42 aggregation but suppresses its toxicity by displacing oligomers from cell membranes. *Nat Commun*. 2019;10:225.
258. Limboccker R, Mannini B, Ruggeri FS, Cascella R, Xu CK, Perni M, et al. Trodusquemine displaces protein misfolded oligomers from cell membranes and abrogates their cytotoxicity through a generic mechanism. *Commun Biol*. 2020;3:1–10.
259. Modler AJ, Fabian H, Sokolowski F, Lutsch G, Gast K, Damaschun G. Polymerization of proteins into amyloid protofibrils shares common critical oligomeric states but differs in the mechanisms of their formation. *Amyloid*. 2004;11:215–31.
260. Upadhaya AR, Lungin I, Yamaguchi H, Fändrich M, Thal DR. High-molecular weight A β oligomers and protofibrils are the predominant A β species in the native soluble protein fraction of the AD brain. *J Cell Mol Med*. 2012;16:287–95.
261. Bitan G, Lomakin A, Teplow DB. Amyloid beta-protein oligomerization: prenucleation interactions revealed by photo-induced cross-linking of unmodified proteins. *J Biol Chem*. 2001;276:35176–84.
262. Ruggeri FS, Šneideris T, Vendruscolo M, Knowles TPJ. Atomic force microscopy for single molecule characterisation of protein aggregation. *Arch Biochem Biophys*. 2019;664:134–48.
263. Vivoli Vega M, Cascella R, Chen SW, Fusco G, De Simone A, Dobson CM, et al. The toxicity of misfolded protein oligomers is independent of their secondary structure. *ACS Chem Biol*. 2019;14:1593–600.
264. Capitini C, Patel JR, Natalello A, D'Andrea C, Relini A, Jarvis JA, et al. Structural differences between toxic and nontoxic HypF-N oligomers. *Chem Commun*. 2018;54:8637–40.
265. Wu JW, Breydo L, Isas JM, Lee J, Kuznetsov YG, Langen R, et al. Fibrillar oligomers nucleate the oligomerization of monomeric amyloid beta but do not seed fibril formation. *J Biol Chem*. 2010;285:6071–9.
266. Gu L, Liu C, Guo Z. Structural insights into A β 42 oligomers using site-directed spin labeling. *J Biol Chem*. 2013;288:18673–83.
267. Williams AD, Segal M, Chen M, Kheterpal I, Geva M, Bertheliev V, et al. Structural properties of A β protofibrils stabilized by a small molecule. *Proc Natl Acad Sci USA*. 2005;102:7115–20.
268. Swanson CJ, Zhang Y, Dhadda S, Wang J, Kaplow J, Lai RYK, et al. A randomized, double-blind, phase 2b proof-of-concept clinical trial in early Alzheimer's disease with lecanemab, an anti-A β protofibril antibody. *Alzheimers Res Ther*. 2021;13:80.

269. Tucker S, Möller C, Tegerstedt K, Lord A, Laudon H, Sjö Dahl J, et al. The murine version of BAN2401 (mAb158) selectively reduces amyloid- β protofibrils in brain and cerebrospinal fluid of tg-ArcSwe mice. *J Alzheimers Dis*. 2015;43:575–88.
270. Cummings J, Aisen P, Lemere C, Atri A, Sabbagh M, Salloway S. Aducanumab produced a clinically meaningful benefit in association with amyloid lowering. *Alzheimers Res Ther*. 2021;13:98.
271. Cummings J, Aisen P, Apostolova LG, Atri A, Salloway S, Weiner M. Aducanumab: appropriate use recommendations. *J Prev Alzheimers Dis*. 2021;8:398–410.
272. Linse S, Scheidt T, Bernfur K, et al. Kinetic fingerprints differentiate the mechanisms of action of anti-A β antibodies. *Nat Struct Mol Biol*. 2020;27:1125–33. <https://doi.org/10.1038/s41594-020-0505-6>.
273. Söderberg L, Johannesson M, Nygren P, Laudon H, Eriksson F, Osswald G, et al. Lecanemab, Aducanumab, and Gantenerumab — binding profiles to different forms of amyloid-beta might explain efficacy and side effects in clinical trials for Alzheimer's Disease. *Neurotherapeutics*. 2023;20:195–206.
274. Tatini F, Pugliese AM, Traini C, Niccoli S, Maraula G, Ed Dami T, et al. Amyloid- β oligomer synaptotoxicity is mimicked by oligomers of the model protein HypF-N. *Neurobiol Aging*. 2013;34:2100–9.
275. Baerends E, Soud K, Folke J, Pedersen A-K, Henmar S, Konrad L, et al. Modeling the early stages of Alzheimer's disease by administering intracerebroventricular injections of human native A β oligomers to rats. *Acta Neuropathol Commun*. 2022;10:113.
276. Lue LF, Kuo YM, Roher AE, Brachova L, Shen Y, Sue L, et al. Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am J Pathol*. 1999;155:853–62.
277. Kitazawa M, Medeiros R, LaFerla FM. Transgenic mouse models of alzheimer disease: developing a better model as a tool for therapeutic interventions. *Curr Pharm Des*. 2012;18:1131–47.
278. Nilsberth C, Westlind-Danielsson A, Eckman CB, Condrin MM, Axelman K, Forsell C, et al. The "Arctic" APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation. *Nat Neurosci*. 2001;4:887–93.
279. Laganowsky A, Liu C, Sawaya MR, Whitelegge JP, Park J, Zhao M, et al. Atomic view of a toxic amyloid small oligomer. *Science*. 2012;335:1228–31.
280. Stern AM, Yang Y, Meunier AL, Liu W, Cai Y, Ericsson M, et al. Abundant A β fibrils in ultracentrifugal supernatants of aqueous extracts from Alzheimer's disease brains. *bioRxiv*:2022.10.18.512754. Available from: <https://doi.org/10.1016/j.neuron.2023.04.007>.
281. Tomic JL, Pensalfini A, Head E, Glabe CG. Soluble fibrillar oligomer levels are elevated in Alzheimer's disease brain and correlate with cognitive dysfunction. *Neurobiol Dis*. 2009;35:352–8.
282. Esparza TJ, Zhao H, Cirrito JR, Cairns NJ, Bateman RJ, Holtzman DM, et al. Amyloid- β oligomerization in Alzheimer dementia versus high-pathology controls. *Ann Neurol*. 2013;73:104–19.
283. McDonald JM, Savva GM, Brayne C, Welzel AT, Forster G, Shankar GM, et al. The presence of sodium dodecyl sulphate-stable Abeta dimers is strongly associated with Alzheimer-type dementia. *Brain J Neurol*. 2010;133:1328–41.
284. Cline EN, Bicca MA, Viola KL, Klein WL. The amyloid- β oligomer hypothesis: beginning of the third decade. *J Alzheimers Dis*. 2018;64:S567–610.
285. Pham E, Crews L, Ubhi K, Hansen L, Adame A, Cartier A, et al. Progressive accumulation of amyloid-beta oligomers in Alzheimer's disease and in amyloid precursor protein transgenic mice is accompanied by selective alterations in synaptic scaffold proteins. *FEBS J*. 2010;277:3051–67.
286. Stern AM, Yang Y, Jin S, Yamashita K, Meunier AL, Liu W, Cai Y, Ericsson M, Liu L, Goedert M, Scheres SHW, Selkoe DJ. Abundant A β fibrils in ultracentrifugal supernatants of aqueous extracts from Alzheimer's disease brains. *Neuron*. 2023;111(13):2012–2020.e4. <https://doi.org/10.1016/j.neuron.2023.04.007>.
287. Georganopoulou DG, Chang L, Nam J-M, Thaxton CS, Mufson EJ, Klein WL, et al. Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease. *Proc Natl Acad Sci USA*. 2005;102:2273–6.
288. Park MJ, Cheon S-M, Bae H-R, Kim S-H, Kim JW. Elevated levels of α -synuclein oligomer in the cerebrospinal fluid of drug-naïve patients with Parkinson's disease. *J Clin Neurol*. 2011;7:215–22.
289. Tokuda T, Qureshi MM, Ardah MT, Varghese S, Shehab S a. S, Kasai T, et al. Detection of elevated levels of α -synuclein oligomers in CSF from patients with Parkinson disease. *Neurology*. 2010;75:1766–72.
290. Sideris DI, Danial JSH, Emin D, Ruggeri FS, Xia Z, Zhang YP, et al. Soluble amyloid beta-containing aggregates are present throughout the brain at early stages of Alzheimer's disease. *Brain Commun*. 2021;3:fcab147.
291. Hong W, Wang Z, Liu W, O'Malley TT, Jin M, Willem M, et al. Diffusible, highly bioactive oligomers represent a critical minority of soluble A β in Alzheimer's disease brain. *Acta Neuropathol (Berl)*. 2018;136:19–40.
292. De S, Whiten DR, Ruggeri FS, Hughes C, Rodrigues M, Sideris DI, et al. Soluble aggregates present in cerebrospinal fluid change in size and mechanism of toxicity during Alzheimer's disease progression. *Acta Neuropathol Commun*. 2019;7:120.
293. Ochiishi T, Kaku M, Kiyosue K, Doi M, Urabe T, Hattori N, et al. New Alzheimer's disease model mouse specialized for analyzing the function and toxicity of intraneuronal Amyloid β oligomers. *Sci Rep*. 2019;9:17368.
294. Tomiyama T, Matsuyama S, Iso H, Umeda T, Takuma H, Ohnishi K, et al. A mouse model of amyloid β oligomers: their contribution to synaptic alteration, abnormal tau phosphorylation, glial activation, and neuronal loss in vivo. *J Neurosci*. 2010;30:4845–56.
295. Kass B, Schemmert S, Zafiu C, Pils M, Bannach O, Kutzsche J, et al. A β oligomer concentration in mouse and human brain and its drug-induced reduction ex vivo. *Cell Rep Med*. 2022;3: 100630.
296. Kiechle M, von Einem B, Höfs L, Voehringer P, Grodzanov V, Markx D, et al. In vivo protein complementation demonstrates presynaptic α -synuclein oligomerization and age-dependent accumulation of 8–16-mer oligomer species. *Cell Rep*. 2019;29:2862–2874.e9.
297. Tsika E, Moysidou M, Guo J, Cushman M, Gannon P, Sandaltzopoulos R, et al. Distinct region-specific α -synuclein oligomers in A53T transgenic mice: implications for neurodegeneration. *J Neurosci*. 2010;30:3409–18.
298. Bucciantini M, Giannoni E, Chiti F, Baroni F, Formigli L, Zurdo J, et al. Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature*. 2002;416:507–11.
299. Cleary JP, Walsh DM, Hofmeister JJ, Shankar GM, Kuskowski MA, Selkoe DJ, et al. Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. *Nat Neurosci*. 2005;8:79–84.
300. Zampagni M, Cascella R, Casamenti F, Grossi C, Evangelisti E, Wright D, et al. A comparison of the biochemical modifications caused by toxic and non-toxic protein oligomers in cells. *J Cell Mol Med*. 2011;15:2106–16.
301. Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, et al. Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. *Nature*. 2002;416:535–9.
302. Arbel-Ornath M, Hudry E, Boivin JR, Hashimoto T, Takeda S, Kuchibhotla KV, et al. Soluble oligomeric amyloid- β induces calcium dyshomeostasis that precedes synapse loss in the living mouse brain. *Mol Neurodegener*. 2017;12:27.
303. Cline EN, Das A, Bicca MA, Mohammad SN, Schachner LF, Kamel JM, et al. A novel crosslinking protocol stabilizes amyloid β oligomers capable of inducing Alzheimer's-associated pathologies. *J Neurochem*. 2019;148:822–36.
304. Froula JM, Castellana-Cruz M, Anabtawi NM, Camino JD, Chen SW, Thrasher DR, et al. Defining α -synuclein species responsible for Parkinson's disease phenotypes in mice. *J Biol Chem*. 2019;294:10392–406.
305. Cenini G, Lloret A, Cascella R. Oxidative stress in neurodegenerative diseases: from a mitochondrial point of view. *Oxid Med Cell Longev*. 2019;2019: e2105607.
306. Evangelisti E, Cascella R, Becatti M, Marrazza G, Dobson CM, Chiti F, et al. Binding affinity of amyloid oligomers to cellular membranes is a generic indicator of cellular dysfunction in protein misfolding diseases. *Sci Rep*. 2016;6:32721.
307. Monsellier E, Bousset L, Melki R. α -Synuclein and huntingtin exon 1 amyloid fibrils bind laterally to the cellular membrane. *Sci Rep*. 2016;6:19180.
308. Mannini B, Cascella R, Zampagni M, van Waarde-Verhagen M, Meehan S, Roodveldt C, et al. Molecular mechanisms used by chaperones to reduce the toxicity of aberrant protein oligomers. *Proc Natl Acad Sci USA*. 2012;109:12479–84.

309. Mannini B, Chiti F. Chaperones as suppressors of protein misfolded oligomer toxicity. *Front Mol Neurosci*. 2017;10:1–8. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5380756/>.
310. Ladiwala ARA, Litt J, Kane RS, Aucoin DS, Smith SO, Ranjan S, et al. Conformational Differences between two amyloid β oligomers of similar size and dissimilar toxicity. *J Biol Chem*. 2012;287:24765–73.
311. Krishnan R, Goodman JL, Mukhopadhyay S, Pacheco CD, Lemke EA, Deniz AA, et al. Conserved features of intermediates in amyloid assembly determine their benign or toxic states. *Proc Natl Acad Sci USA*. 2012;109:11172–7.
312. Yang T, Li S, Xu H, Walsh DM, Selkoe DJ. Large soluble oligomers of amyloid β -protein from Alzheimer brain are far less neuroactive than the smaller oligomers to which they dissociate. *J Neurosci*. 2017;37:152–63.
313. De S, Wirthensohn DC, Flagmeier P, Hughes C, Aprile FA, Ruggeri FS, et al. Different soluble aggregates of A β 42 can give rise to cellular toxicity through different mechanisms. *Nat Commun*. 2019;10:1541.
314. Evangelisti E, Cecchi C, Cascella R, Sgromo C, Becatti M, Dobson CM, et al. Membrane lipid composition and its physicochemical properties define cell vulnerability to aberrant protein oligomers. *J Cell Sci*. 2012;125(10):2416–2427.
315. Rushworth JV, Griffiths HH, Watt NT, Hooper NM. Prion protein-mediated toxicity of amyloid- β oligomers requires lipid rafts and the transmembrane LRP1. *J Biol Chem*. 2013;288:8935–51.
316. Wang HY, Lee DH, D'Andrea MR, Peterson PA, Shank RP, Reitz AB. beta-Amyloid(1–42) binds to alpha7 nicotinic acetylcholine receptor with high affinity. Implications for Alzheimer's disease pathology. *J Biol Chem*. 2000;275:5626–32.
317. Mroczko B, Groblewska M, Litman-Zawadzka A, Kornhuber J, Lewczuk P. Cellular receptors of amyloid β oligomers (A β Os) in Alzheimer's disease. *Int J Mol Sci*. 2018;19:1884.
318. Benoit ME, Hernandez MX, Dinh ML, Benavente F, Vasquez O, Tenner AJ. C1q-induced LRP1B and GPR6 proteins expressed early in Alzheimer disease mouse models, are essential for the C1q-mediated protection against amyloid- β neurotoxicity. *J Biol Chem*. 2013;288:654–65.
319. Huang Y, Liu R. The toxicity and polymorphism of β -Amyloid oligomers. *Int J Mol Sci*. 2020;21:4477.
320. Ding Y, Zhao J, Zhang X, Wang S, Viola KL, Chow FE, et al. Amyloid beta oligomers target to extracellular and intracellular neuronal synaptic proteins in Alzheimer's disease. *Front Neurol*. 2019;10:1–16. Available from: <https://www.frontiersin.org/article/10.3389/fneur.2019.01140>.
321. Fani G, Mannini B, Vecchi G, Cascella R, Cecchi C, Dobson CM, et al. A β oligomers dysregulate calcium homeostasis by mechanosensitive activation of AMPA and NMDA receptors. *ACS Chem Neurosci*. 2021;12:766–81.
322. Amin L, Harris DA. A β receptors specifically recognize molecular features displayed by fibril ends and neurotoxic oligomers. *Nat Commun*. 2021;12:3451.
323. Friedrich RP, Tepper K, Röncke R, Soom M, Westermann M, Reymann K, et al. Mechanism of amyloid plaque formation suggests an intracellular basis of Abeta pathogenicity. *Proc Natl Acad Sci USA*. 2010;107:1942–7.
324. Serra-Batiste M, Ninot-Pedrosa M, Bayoumi M, Gairi M, Maglia G, Carulla N. A β 42 assembles into specific β -barrel pore-forming oligomers in membrane-mimicking environments. *Proc Natl Acad Sci USA*. 2016;113:10866–71.
325. Wesén E, Jeffries GDM, Matson Dzebo M, Esbjörner EK. Endocytic uptake of monomeric amyloid- β peptides is clathrin- and dynamin-independent and results in selective accumulation of A β (1–42) compared to A β (1–40). *Sci Rep*. 2017;7:2021.
326. Jarosz-Griffiths HH, Noble E, Rushworth JV, Hooper NM. Amyloid- β receptors: the good, the bad, and the prion protein. *J Biol Chem*. 2016;291:3174–83.
327. Miller EC, Teravskis PJ, Dummer BW, Zhao X, Hugarir RL, Liao D. Tau phosphorylation and tau mislocalization mediate soluble A β oligomer-induced AMPA glutamate receptor signaling deficits. *Eur J Neurosci*. 2014;39:1214–24.
328. Shin WS, Di J, Cao Q, Li B, Seidler PM, Murray KA, et al. Amyloid β -protein oligomers promote the uptake of tau fibril seeds potentiating intracellular tau aggregation. *Alzheimers Res Ther*. 2019;11:86.
329. Chia S, Flagmeier P, Habchi J, Lattanzi V, Linse S, Dobson CM, et al. Monomeric and fibrillar α -synuclein exert opposite effects on the catalytic cycle that promotes the proliferation of A β 42 aggregates. *Proc Natl Acad Sci USA*. 2017;114(30):8005–10.
330. Hallaceli E, Kayatekin C, Nazeen S, Wang XH, Sheinkopf Z, Sathyakumar S, et al. The Parkinson's disease protein alpha-synuclein is a modulator of processing bodies and mRNA stability. *Cell*. 2022;185:2035–2056.e33.
331. Bucciantini M, Rigacci S, Berti A, Pieri L, Cecchi C, Nosi D, et al. Patterns of cell death triggered in two different cell lines by HypF-N prefibrillar aggregates. *FASEB J*. 2005;19:437–9.
332. Perni M, Galvagnion C, Maltsev A, Meisl G, Müller MBD, Challa PK, et al. A natural product inhibits the initiation of α -synuclein aggregation and suppresses its toxicity. *Proc Natl Acad Sci USA*. 2017;114:E1009–17.
333. Errico S, Ramshini H, Capitini C, Canale C, Spaziano M, Barbut D, et al. Quantitative measurement of the affinity of toxic and nontoxic misfolded protein oligomers for lipid bilayers and of its modulation by lipid composition and trodusquemine. *ACS Chem Neurosci*. 2021;12:3189–202.
334. Nguyen PT, Zottig X, Sebastiao M, Arnold AA, Marcotte I, Bourgault S. Identification of transmissible proteotoxic oligomer-like fibrils that expand conformational diversity of amyloid assemblies. *Commun Biol*. 2021;4:1–14.
335. Fani G, La Torre CE, Cascella R, Cecchi C, Vendruscolo M, Chiti F. Misfolded protein oligomers induce an increase of intracellular Ca²⁺ causing an escalation of reactive oxidative species. *Cell Mol Life Sci*. 2022;79:500.
336. Demuro A, Mina E, Kayed R, Milton SC, Parker I, Glabe CG. Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. *J Biol Chem*. 2005;280:17294–300.
337. Limbocker R, Mannini B, Cataldi R, Chhangur S, Wright AK, Kreiser RP, et al. Rationally designed antibodies as research tools to study the structure–toxicity relationship of amyloid- β oligomers. *Int J Mol Sci*. 2020;21:4542.
338. Kim GH, Kim JE, Rhie SJ, Yoon S. The role of oxidative stress in neurodegenerative diseases. *Exp Neurobiol*. 2015;24:325–40.
339. Patten DA, Germain M, Kelly MA, Slack RS. Reactive oxygen species: stuck in the middle of neurodegeneration. *J Alzheimers Dis*. 2010;20(Suppl 2):S357–367.
340. Kadowaki H, Nishitoh H, Urano F, Sadamitsu C, Matsuzawa A, Takeda K, et al. Amyloid β induces neuronal cell death through ROS-mediated ASK1 activation. *Cell Death Differ*. 2005;12:19–24.
341. Iuchi K, Takai T, Hisatomi H. Cell death via lipid peroxidation and protein aggregation diseases. *Biology*. 2021;10:399.
342. Hou X, Parkington HC, Coleman HA, Mechler A, Martin LL, Aguilar M-I, et al. Transthyretin oligomers induce calcium influx via voltage-gated calcium channels. *J Neurochem*. 2007;100:446–57.
343. Sartiani L, Bucciantini M, Spinelli V, Leri M, Natalello A, Nosi D, et al. Biochemical and electrophysiological modification of amyloid transthyretin on cardiomyocytes. *Biophys J*. 2016;111:2024–38.
344. Nakano T, Onoue K, Terada C, Terasaki S, Ishihara S, Hashimoto Y, et al. Transthyretin amyloid cardiomyopathy: impact of transthyretin amyloid deposition in myocardium on cardiac morphology and function. *J Pers Med*. 2022;12:792.
345. Gonzalez-Garcia M, Fusco G, De Simone A. Membrane interactions and toxicity by misfolded protein oligomers. *Front Cell Dev Biol*. 2021;9:1–12. Available from: <https://www.frontiersin.org/article/10.3389/fcell.2021.642623>.
346. Guglielmotto M, Monteleone D, Piras A, Valsecchi V, Tropiano M, Ariano S, et al. A β 1–42 monomers or oligomers have different effects on autophagy and apoptosis. *Autophagy*. 2014;10:1827–43.
347. Sölvander S, Nikitidou E, Brolin R, Söderberg L, Sehlin D, Lannfelt L, et al. Accumulation of amyloid- β by astrocytes result in enlarged endosomes and microvesicle-induced apoptosis of neurons. *Mol Neurodegener*. 2016;11:38.
348. Ferretti MT, Bruno MA, Ducatenzeiler A, Klein WL, Cuello AC. Intracellular A β -oligomers and early inflammation in a model of Alzheimer's disease. *Neurobiol Aging*. 2012;33:1329–42.
349. Richter M, Vidovic N, Biber K, Dolga A, Culmsee C, Dodel R. The neuroprotective role of microglial cells against amyloid beta-mediated

- toxicity in organotypic hippocampal slice cultures. *Brain Pathol Zurich Switz*. 2020;30:589–602.
350. Karran E, De Strooper B. The amyloid hypothesis in Alzheimer disease: new insights from new therapeutics. *Nat Rev Drug Discov*. 2022;21:306–18.
 351. Waudby CA, Knowles TPJ, Devlin GL, Skepper JN, Ercroft H, Carver JA, et al. The interaction of α -crystallin with mature α -synuclein amyloid fibrils inhibits their elongation. *Biophys J*. 2010;98:843–51.
 352. Shammas SL, Waudby CA, Wang S, Buell AK, Knowles TPJ, Ercroft H, et al. Binding of the molecular chaperone α B-Crystallin to A β amyloid fibrils inhibits fibril elongation. *Biophys J*. 2011;101:1681–9.
 353. Kundel F, De S, Flagmeier P, Horrocks MH, Kjaergaard M, Shammas SL, et al. Hsp70 inhibits the nucleation and elongation of tau and sequesters tau aggregates with high affinity. *ACS Chem Biol*. 2018;13:636–46.
 354. Cohen SA, Arosio P, Presto J, Kurundenkandy FR, Biverstål H, Dolfe L, et al. A molecular chaperone breaks the catalytic cycle that generates toxic A β oligomers. *Nat Struct Mol Biol*. 2015;22:207–13.
 355. Kiuchi Y, Isobe Y, Fukushima K. Entactin-induced inhibition of human amyloid β -protein fibril formation in vitro. *Neurosci Lett*. 2001;305:119–22.
 356. Dandanell Agerschou E, Borgmann V, M. Wördehoff M, Hoyer W. Inhibitor and substrate cooperate to inhibit amyloid fibril elongation of α -synuclein. *Chem Sci*. 2020;11:11331–7.
 357. Doytchinova I, Atanasova M, Salamanova E, Ivanov S, Dimitrov I. Curcumin inhibits the primary nucleation of amyloid-beta peptide: a molecular dynamics study. *Biomolecules*. 2020;10:1323.
 358. Du W-J, Guo J-J, Gao M-T, Hu S-Q, Dong X-Y, Han Y-F, et al. Brazilin inhibits amyloid β -protein fibrillogenesis, remodels amyloid fibrils and reduces amyloid cytotoxicity. *Sci Rep*. 2015;5:7992.
 359. Nagaraj M, Najarzadeh Z, Pansieri J, Biverstål H, Musteikyte G, Smirnovas V, et al. Chaperones mainly suppress primary nucleation during formation of functional amyloid required for bacterial biofilm formation. *Chem Sci*. 2022;13:536–53.
 360. Ghadami SA, Chia S, Ruggeri FS, Meisl G, Bemporad F, Habchi J, et al. Transthyretin inhibits primary and secondary nucleations of amyloid- β peptide aggregation and reduces the toxicity of its oligomers. *Biomacromolecules*. 2020;21:1112–25.
 361. Meade RM, Morris KJ, Watt KJC, Williams RJ, Mason JM. The library derived 4554w peptide inhibits primary nucleation of α -synuclein. *J Mol Biol*. 2020;432: 166706.
 362. Perni M, Flagmeier P, Limbocker R, Cascella R, Aprile FA, Galvagnion C, et al. Multistep inhibition of α -synuclein aggregation and toxicity in vitro and in vivo by trodusquemine. *ACS Chem Biol*. 2018;13:2308–19.
 363. Horne RI, Andrzejewska E, Alam P, Brotzakis ZF, Srivastava A, Aubert A, et al. Discovery of potent inhibitors of α -synuclein aggregation using structure-based iterative learning. *bioRxiv*. 2023:2021.11.10.468009. Available from: <https://www.biorxiv.org/content/10.1101/2021.11.10.468009v3>.
 364. Habchi J, Chia S, Limbocker R, Mannini B, Ahn M, Perni M, et al. Systematic development of small molecules to inhibit specific microscopic steps of A β 42 aggregation in Alzheimer's disease. *Proc Natl Acad Sci USA*. 2017;114:E200–8.
 365. Aprile FA, Sormanni P, Perni M, Arosio P, Linse S, Knowles TPJ, et al. Selective targeting of primary and secondary nucleation pathways in A β 42 aggregation using a rational antibody scanning method. *Sci Adv*. 2017;3: e1700488.
 366. Limbocker R, Errico S, Barbut D, Knowles TPJ, Vendruscolo M, Chiti F, et al. Squalamine and trodusquemine: two natural products for neurodegenerative diseases, from physical chemistry to the clinic. *Nat Prod Rep*. 2022;39:742–53.
 367. FDA's Decision to Approve New Treatment for Alzheimer's Disease. FDA; 2021. Available from: <https://www.fda.gov/drugs/news-events-human-drugs/fdas-decision-approve-new-treatment-alzheimers-disease>. Accessed 2 Mar 2023.
 368. Krafft GA, Jerecic J, Siemsen E, Cline EN. ACU193: An immunotherapeutic poised to test the amyloid β oligomer hypothesis of Alzheimer's disease. *Front Neurosci*. 2022;16:1–16. Available from: <https://www.frontiersin.org/article/10.3389/fnins.2022.848215>.
 369. Linse S, Sormanni P, O'Connell DJ. An aggregation inhibitor specific to oligomeric intermediates of A β 42 derived from phage display libraries of stable, small proteins. *Proc Natl Acad Sci USA*. 2022;119: e2121966119.
 370. Ehrnhoefer DE, Bieschke J, Boeddrich A, Herbst M, Masino L, Lurz R, et al. ECGG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers. *Nat Struct Mol Biol*. 2008;15:558–66.
 371. Ladiwala ARA, Lin JC, Bale SS, Marcelino-Cruz AM, Bhattacharya M, Dordick JS, et al. Resveratrol selectively remodels soluble oligomers and fibrils of amyloid α into off-pathway conformers. *J Biol Chem*. 2010;285:24228–37.
 372. Connelly S, Choi S, Johnson SM, Kelly JW, Wilson IA. Structure-based design of kinetic stabilizers that ameliorate the transthyretin amyloidosis. *Curr Opin Struct Biol*. 2010;20:54–62.
 373. Bulawa CE, Connelly S, Devit M, Wang L, Weigel C, Fleming JA, et al. Tafamidis, a potent and selective transthyretin kinetic stabilizer that inhibits the amyloid cascade. *Proc Natl Acad Sci USA*. 2012;109:9629–34.
 374. Coelho T, Maia LF, Martins da Silva A, Waddington Cruz M, Planté-Bordeneuve V, Lozeron P, et al. Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. *Neurology*. 2012;79:785–92.
 375. Maurer MS, Schwartz JH, Gundapaneni B, Elliott PM, Merlini G, Waddington-Cruz M, et al. Tafamidis treatment for patients with transthyretin amyloid cardiomyopathy. *N Engl J Med*. 2018;379:1007–16.
 376. Yan NL, Santos-Martins D, Nair R, Chu A, Wilson IA, Johnson KA, et al. Discovery of potent coumarin-based kinetic stabilizers of amyloidogenic immunoglobulin light chains using structure-based design. *J Med Chem*. 2021;64:6273–99.
 377. Chiti F, Kelly JW. Small molecule protein binding to correct cellular folding or stabilize the native state against misfolding and aggregation. *Curr Opin Struct Biol*. 2022;72:267–78.
 378. Heller GT, Aprile FA, Michaels TCT, Limbocker R, Perni M, Ruggeri FS, et al. Small-molecule sequestration of amyloid- β as a drug discovery strategy for Alzheimer's disease. *Sci Adv*. 2021;6:eabb5924.
 379. Löhr T, Kohlhoff K, Heller GT, Camilloni C, Vendruscolo M. A small molecule stabilizes the disordered native state of the Alzheimer's A β Peptide. *ACS Chem Neurosci*. 2022;13:1738–45.
 380. Heller GT, Sormanni P, Vendruscolo M. Targeting disordered proteins with small molecules using entropy. *Trends Biochem Sci*. 2015;40:491–6.
 381. Sweeney P, Park H, Baumann M, Dunlop J, Frydman J, Kopito R, et al. Protein misfolding in neurodegenerative diseases: implications and strategies. *Transl Neurodegener*. 2017;6:6.
 382. Selkoe D. β -secretase inhibitors for Alzheimer's disease: heading in the wrong direction? *Lancet Neurol*. 2019;18:624–6.
 383. Cole SL, Vassar R. The Alzheimer's disease β -secretase enzyme, BACE1. *Mol Neurodegener*. 2007;2:22.
 384. Chiozzi P, Sarti AC, Sanz JM, Giuliani AL, Adinolfi E, Vultaggio-Poma V, et al. Amyloid β -dependent mitochondrial toxicity in mouse microglia requires P2X7 receptor expression and is prevented by nimodipine. *Sci Rep*. 2019;9:6475.
 385. Cao Q, Shin WS, Chan H, Vuong CK, Dubois B, Li B, et al. Inhibiting amyloid- β cytotoxicity through its interaction with the cell surface receptor LILRB2 by structure-based design. *Nat Chem*. 2018;10:1213–21.
 386. Foley AR, Roseman GP, Chan K, Smart A, Finn TS, Yang K, et al. Evidence for aggregation-independent, PrPC-mediated A β cellular internalization. *Proc Natl Acad Sci USA*. 2020;117:28625–31.
 387. Cappelli S, Penco A, Mannini B, Cascella R, Wilson MR, Ercroft H, et al. Effect of molecular chaperones on aberrant protein oligomers in vitro: super-versus sub-stoichiometric chaperone concentrations. *Biol Chem*. 2016;397:401–15.
 388. Ojha J, Masilamani G, Dunlap D, Udoff RA, Cashikar AG. Sequestration of toxic oligomers by HspB1 as a cytoprotective mechanism. *Mol Cell Biol*. 2011;31:3146–57.
 389. Garai K, Posey AE, Li X, Buxbaum JN, Pappu RV. Inhibition of amyloid beta fibril formation by monomeric human transthyretin. *Protein Sci*. 2018;27:1252–61.
 390. Cascella R, Conti S, Tatini F, Evangelisti E, Scartabelli T, Casamenti F, et al. Extracellular chaperones prevent A β 42-induced toxicity in rat brains. *Biochim Biophys Acta*. 2013;1832:1217–26.
 391. Cascella R, Conti S, Mannini B, Li X, Buxbaum JN, Tiribilli B, et al. Transthyretin suppresses the toxicity of oligomers formed by misfolded proteins in vitro. *Biochim Biophys Acta BBA - Mol Basis Dis*. 2013;1832:2302–14.

392. Beeg M, Stravalaci M, Romeo M, Carrá AD, Cagnotto A, Rossi A, et al. Clusterin binds to $\alpha\beta 1-42$ oligomers with high affinity and interferes with peptide aggregation by inhibiting primary and secondary nucleation. *J Biol Chem*. 2016;291:6958–66.
393. Limbocker R, Staats R, Chia S, Ruggeri FS, Mannini B, Xu CK, et al. Squallamine and its derivatives modulate the aggregation of amyloid- β and α -synuclein and suppress the toxicity of their oligomers. *Front Neurosci*. 2021;15:1–17. Available from: <https://www.frontiersin.org/articles/10.3389/fnins.2021.680026/abstract>.
394. Andreasen M, Lorenzen N, Otzen D. Interactions between misfolded protein oligomers and membranes: a central topic in neurodegenerative diseases? *Biochim Biophys Acta*. 2015;1848:1897–907.
395. Mrak RE, Griffin WST. Interleukin-1, neuroinflammation, and Alzheimer's disease. *Neurobiol Aging*. 2001;22:903–8.
396. Kempuraj D, Thangavel R, Selvakumar GP, Zaheer S, Ahmed ME, Raikwar SP, et al. Brain and peripheral atypical inflammatory mediators potentiate neuroinflammation and neurodegeneration. *Front Cell Neurosci*. 2017;11:216.
397. Batista AF, Rody T, Forný-Germano L, Cerdeiro S, Bellio M, Ferreira ST, et al. Interleukin-1 β mediates alterations in mitochondrial fusion/fission proteins and memory impairment induced by amyloid- β oligomers. *J Neuroinflammation*. 2021;18:54.
398. Steeland S, Gorié N, Vandendriessche C, Balusu S, Brkic M, Van Cauwenbergh C, et al. Counteracting the effects of TNF receptor-1 has therapeutic potential in Alzheimer's disease. *EMBO Mol Med*. 2018;10:e8300.
399. Yan R. Stepping closer to treating Alzheimer's disease patients with BACE1 inhibitor drugs. *Transl Neurodegener*. 2016;5:13.
400. Xu B, Mo X, Chen J, Yu H, Liu Y. Myricetin inhibits α -synuclein amyloid aggregation by delaying the liquid-to-solid phase transition. *ChemBioChem*. 2022;23:e202200216.
401. Willander H, Presto J, Askarieh G, Biverstål H, Frohm B, Knight SD, et al. BRICHOS domains efficiently delay fibrillation of amyloid β -peptide. *J Biol Chem*. 2012;287:31608–17.
402. Wang MS, Boddapati S, Emadi S, Sierks MR. Curcumin reduces α -synuclein induced cytotoxicity in Parkinson's disease cell model. *BMC Neurosci*. 2010;11:57.
403. Smith SP, Shaw GS. A novel calcium-sensitive switch revealed by the structure of human S100B in the calcium-bound form. *Structure*. 1998;6:211–22.
404. Seidler PM, Murray KA, Boyer DR, Ge P, Sawaya MR, Hu CJ, et al. Structure-based discovery of small molecules that disaggregate Alzheimer's disease tissue derived tau fibrils in vitro. *Nat Commun*. 2022;13:5451.
405. Schofield DJ, Irving L, Calo L, Bogstedt A, Rees G, Nuccitelli A, et al. Pre-clinical development of a high affinity α -synuclein antibody, MEDI1341, that can enter the brain, sequester extracellular α -synuclein and attenuate α -synuclein spreading in vivo. *Neurobiol Dis*. 2019;132: 104582.
406. Sarkar S, C. Rubinsztein D. Small molecule enhancers of autophagy for neurodegenerative diseases. *Mol Biosyst*. 2008;4:895–901.
407. Picone P, Bondi ML, Picone P, Bondi ML, Montana G, Bruno A, et al. Ferulic acid inhibits oxidative stress and cell death induced by Ab oligomers: Improved delivery by solid lipid nanoparticles. *Free Radic Res*. 2009;43:1133–45.
408. Nygaard HB, Wagner AF, Bowen GS, Good SP, MacAvoy MG, Strittmatter KA, et al. A phase Ib multiple ascending dose study of the safety, tolerability, and central nervous system availability of AZD0530 (saracatinib) in Alzheimer's disease. *Alzheimers Res Ther*. 2015;7:35.
409. Musteikyte G, Ziaunys M, Smirnovas V. Methylene blue inhibits nucleation and elongation of SOD1 amyloid fibrils. *PeerJ*. 2020;8: e9719.
410. Moreira GG, Cantrelle F-X, Quezada A, Carvalho FS, Cristóvão JS, Sen-gupta U, et al. Dynamic interactions and Ca^{2+} -binding modulate the holdase-type chaperone activity of S100B preventing tau aggregation and seeding. *Nat Commun*. 2021;12:6292.
411. Mirecka EA, Shaykhalishahi H, Gauhar A, Akgül Ş, Lecher J, Willbold D, et al. Sequestration of a β -Hairpin for Control of α -Synuclein Aggregation. *Angew Chem Int Ed*. 2014;53:4227–30.
412. Kreiser RP, Wright AK, Sasser LR, Rinauro DJ, Gabriel JM, Hsu CM, et al. A brain-permeable aminosterol regulates cell membranes to mitigate the toxicity of diverse pore-forming agents. *ACS Chem Neurosci*. 2022;13:1219–31.
413. King MK, Pardo M, Cheng Y, Downey K, Jope RS, Beurel E. Glycogen synthase kinase-3 inhibitors: Rescuers of cognitive impairments. *Pharmacol Ther*. 2014;141:1–12.
414. Fukunaga K, Izumi H, Yabuki Y, Shinoda Y, Shioda N, Han F. Alzheimer's disease therapeutic candidate SAK3 is an enhancer of T-type calcium channels. *J Pharmacol Sci*. 2019;139:51–8.
415. Esteras N, Kundel F, Amodeo GF, Pavlov EV, Klenerman D, Abramov AY. Insoluble tau aggregates induce neuronal death through modification of membrane ion conductance, activation of voltage-gated calcium channels and NADPH oxidase. *FEBS J*. 2021;288:127–41.
416. Dieter F, Esselun C, Eckert GP. Redox active α -lipoic acid differentially improves mitochondrial dysfunction in a cellular model of alzheimer and its control cells. *Int J Mol Sci*. 2022;23:9186.
417. Dedmon MM, Christodoulou J, Wilson MR, Dobson CM. Heat shock protein 70 inhibits α -synuclein fibril formation via preferential binding to prefibrillar species. *J Biol Chem*. 2005;280:14733–40.
418. Dai B, Zhong T, Chen Z-X, Chen W, Zhang N, Liu X-L, et al. Myricetin slows liquid–liquid phase separation of Tau and activates ATG5-dependent autophagy to suppress Tau toxicity. *J Biol Chem*. 2021;297:1–17. Available from: [https://www.jbc.org/article/S0021-9258\(21\)01025-5/abstract](https://www.jbc.org/article/S0021-9258(21)01025-5/abstract).
419. Crespi GAN, Hermans SJ, Parker MW, Miles LA. Molecular basis for mid-region amyloid- β capture by leading Alzheimer's disease immunotherapies. *Sci Rep*. 2015;5:9649.
420. Cenini G, Voos W. Mitochondria as potential targets in alzheimer disease therapy: an update. *Front Pharmacol*. 2019;10:1–20. Available from: <https://www.frontiersin.org/articles/10.3389/fphar.2019.00902>.
421. Cai X, Zhang K, Xie X, Zhu X, Feng J, Jin Z, et al. Self-assembly hollow manganese Prussian white nanocapsules attenuate Tau-related neuropathology and cognitive decline. *Biomaterials*. 2020;231: 119678.
422. Beal MF. Mitochondria take center stage in aging and neurodegeneration. *Ann Neurol*. 2005;58:495–505.
423. Atwal JK, Chen Y, Chiu C, Mortensen DL, Meilandt WJ, Liu Y, et al. A therapeutic antibody targeting bace1 inhibits amyloid- β production in vivo. *Sci Transl Med*. 2011;3:84ra43–84ra43.
424. Arndt JW, Qian F, Smith BA, Quan C, Kilambi KP, Bush MW, et al. Structural and kinetic basis for the selectivity of aducanumab for aggregated forms of amyloid- β . *Sci Rep*. 2018;8:6412.
425. Arai T, Sasaki D, Araya T, Sato T, Sohma Y, Kanai M. A cyclic KLVFF-derived peptide aggregation inhibitor induces the formation of less-toxic off-pathway amyloid- β oligomers. *ChemBioChem*. 2014;15:2577–83.
426. Anekonda TS, Quinn JF. Calcium channel blocking as a therapeutic strategy for Alzheimer's disease: The case for isradipine. *Biochim Biophys Acta BBA - Mol Basis Dis*. 2011;1812:1584–90.
427. Agerschou ED, Flagmeier P, Saridaki T, Galvagnion C, Komnig D, Heid L, et al. An engineered monomer binding-protein for α -synuclein efficiently inhibits the proliferation of amyloid fibrils. *eLife*. 2019;8:e46112.
428. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, et al. Highly accurate protein structure prediction with AlphaFold. *Nature*. 2021;596:583–9.
429. Collier MP, Alderson TR, de Villiers CP, Nicholls D, Gastall HY, Allison TM, et al. HspB1 phosphorylation regulates its intramolecular dynamics and mechanosensitive molecular chaperone interaction with filament C. *Sci Adv*. 2019;5:eaav8421.
430. Vitek GE, Decourt B, Sabbagh MN. Lecanemab (BAN2401): an anti- β -amyloid monoclonal antibody for the treatment of Alzheimer disease. *Expert Opin Investig Drugs*. 2023;32:89–94.
431. Du X, Wang X, Geng M. Alzheimer's disease hypothesis and related therapies. *Transl Neurodegener*. 2018;7:2.

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