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Review

# Protein Aggregation and Proteostasis Failure in Neurodegenerative Diseases: Mechanisms, Structural Insights, and Therapeutic Strategies

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## Abstract

Protein aggregation is a defining pathological hallmark of major neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and tauopathies. These disorders share a convergent failure of proteostasis—the integrated network that regulates protein synthesis, folding, trafficking, and clearance—rather than isolated, disease-specific anomalies. Neurons, as long-lived post-mitotic cells, are particularly vulnerable to proteostasis collapse, which promotes accumulation of misfolded proteins such as amyloid- $\beta$ ,  $\alpha$ -synuclein, tau, and TDP-43. Aggregates exhibit structural polymorphism, prion-like propagation, and strain-specific conformations that dictate regional vulnerability and clinical heterogeneity. Mechanistically, clearance pathways—including the ubiquitin–proteasome system, autophagy–lysosomal axis, endosomal–lysosomal trafficking, and molecular chaperones—operate in a highly coordinated manner; their dysfunction accelerates aggregation and neurotoxicity. Emerging evidence implicates liquid–liquid phase separation as a precursor to amyloid formation, while post-translational modifications such as phosphorylation, ubiquitination, and truncation modulate aggregation kinetics and pathogenicity. Aging further exacerbates proteostasis failure through impaired autophagic flux, lysosomal dysfunction, and glymphatic clearance decline, amplifying aggregate burden and prion-like dissemination. Despite extensive development of aggregation inhibitors, immunotherapies, and proteostasis enhancers, clinical translation remains limited due to poor blood–brain barrier penetration, off-target effects, and incomplete understanding of intermediate toxic species. Future strategies should integrate structural biology, seed amplification assays, and systems-level approaches to target early aggregation events, reinforce clearance pathways, and modulate neuroinflammation. This review synthesizes mechanistic insights, structural data, and therapeutic advances, emphasizing the complexity of protein aggregation as a dynamic, multifactorial process in neurodegeneration.

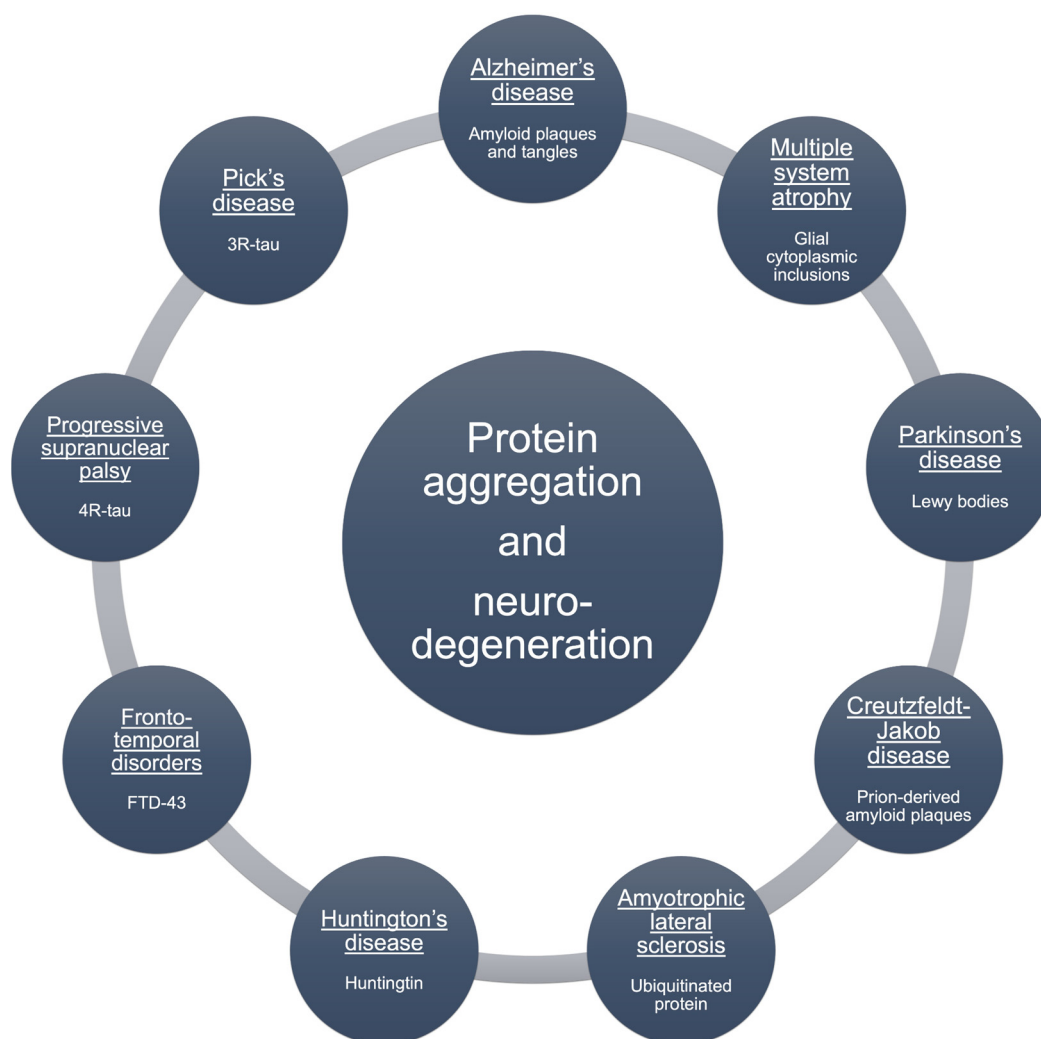
**Keywords:** protein aggregation; proteostasis; prion-like propagation; autophagy; mixed proteinopathies; neurodegeneration;  $\alpha$ -synuclein; tauopathies; amyloid- $\beta$ ; TDP-43

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## 1. Introduction

Neurodegenerative diseases are defined by the characteristic neuropathological findings, particularly the presence of specific misfolded protein aggregates in distinct areas of the central nervous system (CNS). These aggregates vary by disease: Lewy bodies (LBs) in Parkinson's disease (PD), amyloid- $\beta$  (A $\beta$ ) plaques and neurofibrillary tangles in Alzheimer's disease (AD), mutant huntingtin inclusions in Huntington's disease (HD), glial cytoplasmic inclusions in multiple system atrophy (MSA), prion-derived A $\beta$  plaques in prion diseases, and ubiquitinated protein aggregates in amyotrophic lateral sclerosis (ALS) (Figure 1). Despite differences in protein identity and anatomical distribution—nuclear and cytoplasmic inclusions in HD, cytoplasmic aggregates in PD and ALS, and

extracellular plaques in AD—the convergence on protein aggregation suggests a shared mechanism of proteostasis failure rather than isolated, disease-specific anomalies [1].



**Figure 1.** Protein aggregates in neurodegenerative diseases.

Although the term proteinopathy is often used interchangeably with abnormal protein aggregation, a more precise definition considers proteinopathy as a broader impairment of proteostasis. Proteostasis refers to the dynamic maintenance of a functional proteome through coordinated regulation of protein synthesis, folding, trafficking, and clearance [2]. Disruption of this network can lead to misfolded protein accumulation, overwhelming cellular quality-control systems and triggering neurodegeneration. Importantly, proteostasis failure and protein aggregation are not exclusive to classical neurodegenerative disorders; they also occur in certain non-neurodegenerative movement disorders, underscoring the relevance of protein homeostasis across a wider spectrum of neurological conditions.

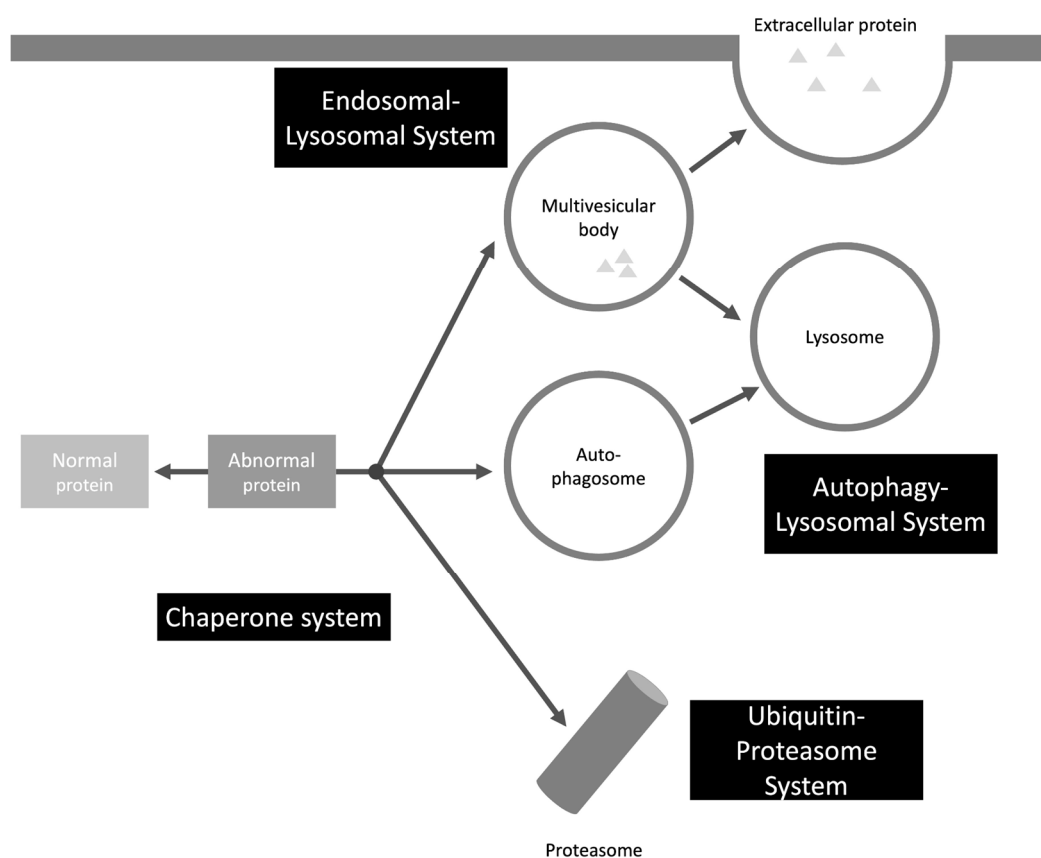
Liquid-liquid phase separation (LLPS) has emerged as a critical intermediate in the transition from soluble proteins to pathogenic aggregates in neurodegenerative diseases. LLPS enables intrinsically disordered proteins such as  $\alpha$ -synuclein ( $\alpha$ Syn), tau, and TDP-43 to form dynamic condensates that concentrate aggregation-prone species within membranellar compartments. These condensates exhibit liquid-like properties initially but can mature into gel-like or solid amyloid states under stress conditions, post-translational modifications (PTMs), or altered ionic environments. LLPS is modulated by protein isoforms, charge distribution, and cofactors such as RNA and lipids, which influence condensate stability and aggregation kinetics. Recent studies demonstrate that  $\alpha$ Syn condensates recruit synaptic vesicles and SNARE proteins, linking phase separation to synaptic

dysfunction [3], while tau LLPS accelerates nucleation of paired helical filaments in the presence of polyanions [4]. Importantly, LLPS provides a mechanistic framework for understanding how cellular stress and crowding bias aggregation pathways, suggesting that targeting condensate formation or maturation could represent a novel therapeutic strategy [5,6].

Building on these emerging concepts, this review integrates mechanistic, structural, and translational perspectives to dissect the complexity of protein aggregation in neurodegenerative diseases. We first examine the architecture and dynamics of proteostasis networks—including the ubiquitin–proteasome system (UPS), autophagy–lysosomal pathways, endosomal trafficking, and molecular chaperones—that collectively safeguard neuronal protein homeostasis. Next, we explore how their failure converges with aggregation-prone proteins such as  $A\beta$ ,  $\alpha$ Syn, tau, and TDP-43, emphasizing structural polymorphism, prion-like propagation, and modulatory factors such as PTMs, lipid interactions, and aging. Finally, we synthesize current and emerging therapeutic strategies, ranging from aggregation inhibitors and immunotherapies to network-level interventions targeting epichaperomes and proteostasis enhancers. By framing aggregation as a dynamic, multifactorial process rather than a static endpoint, this review underscores the need for integrated approaches that combine structural biology, systems-level analysis, and precision therapeutics to effectively mitigate neurodegeneration.

## 2. Protein Clearance Mechanisms – Intraneuronal

Neurons are particularly vulnerable to proteostasis disruption because they are long-lived, post-mitotic cells that cannot dilute misfolded proteins through cell division. This intrinsic limitation means that even minor perturbations in protein homeostasis can have profound consequences for neuronal function and survival. To counteract this risk, neurons rely on an intricate proteostasis network comprising molecular chaperones, the UPS, and autophagy-lysosomal pathways, which collectively regulate protein folding, trafficking, and clearance (Figure 2) [7].



**Figure 2.** Intraneuronal Protein Clearance Mechanisms. Schematic representation of major pathways involved in protein quality control within neurons, including the UPS, molecular chaperones, the autophagy–lysosomal pathway, and the endosomal–lysosomal system.

### 2.1. UPS

UPS is the primary pathway for selective intracellular protein degradation, ensuring proteostasis by removing misfolded, damaged, or short-lived proteins. Ubiquitin acts as a molecular tag, covalently attached to substrate proteins through an enzymatic cascade involving E1 activating enzymes, E2 conjugating enzymes, and E3 ubiquitin ligases. Substrates can be modified with a single ubiquitin or polyubiquitin chains, which dictate their fate—typically proteasomal degradation for K48-linked chains or signaling for other linkages [8,9]. The 26S proteasome recognizes ubiquitinated substrates and catalyzes their degradation into peptides, maintaining neuronal homeostasis. Given neurons' post-mitotic nature and high metabolic demand, UPS dysfunction leads to accumulation of toxic aggregates, a hallmark of neurodegenerative diseases such as PD, AD, and HD [10].

UPS activity is tightly regulated by ubiquitin ligases and deubiquitinating enzymes (DUBs), which control the addition and removal of ubiquitin chains. Genetic mutations affecting these enzymes have been implicated in movement disorders. For example, pathogenic variants in ataxin-3 (ATXN3), a DUB, cause spinocerebellar ataxia type 3 (SCA3) [11], while mutations in E3 ligases such as STUB1/CHIP lead to autosomal recessive cerebellar ataxia (SCAR16) and spinocerebellar ataxia type 48 (SCA48) [12,13]. Similarly, RNF216 mutations underlie Gordon Holmes syndrome [14], and AMFR variants cause hereditary spastic paraplegia (SPG89) [15,16].

Beyond genetic causes, UPS impairment is exacerbated by aging and cellular stress, reducing proteasomal efficiency and promoting aggregation of proteins. Recent studies emphasize that UPS dysfunction often coexists with autophagy deficits, forming a dual failure of proteostasis in neurodegeneration [17]. Therapeutic strategies targeting UPS components—such as small-molecule activators of proteasome function, modulators of E3 ligases, and DUB inhibitors—are under investigation to restore protein clearance and mitigate disease progression [12].

### 2.2. Endosomal-Lysosomal System

The endosomal–lysosomal system is a critical arm of the cellular proteostasis network, responsible for degrading membrane proteins, extracellular cargo, and aggregate-prone species that escape proteasomal clearance. Proteins destined for disposal are sorted into endosomes and subsequently trafficked to lysosomes for degradation. This process relies on the coordinated action of Rab GTPases, which regulate vesicle budding, trafficking, and fusion events, and the endosomal sorting complexes required for transport (ESCRT) machinery, which mediates cargo sequestration into intraluminal vesicles (ILVs) within multivesicular bodies (MVBs) [18,19]. These ILVs are typically degraded upon lysosomal fusion; however, they can also be secreted as exosomes, contributing to intercellular communication and, in pathological contexts, the spread of misfolded proteins such as tau and  $\alpha$ Syn [20].

Genetic disruptions in endosomal–lysosomal components underscore their importance in movement disorders. Mutations in ESCRT-I subunit UBAP1 cause hereditary spastic paraplegia type 80 (SPG80), characterized by axonal degeneration linked to defective endosomal cargo sorting [21]. Similarly, variants in Rab GTPases, such as RAB32, have been associated with atypical parkinsonism, implicating impaired vesicular trafficking and mitochondrial–endolysosomal crosstalk in disease pathogenesis [22].

Beyond genetic causes, emerging evidence suggests that endosomal–lysosomal dysfunction synergizes with autophagy impairment and proteasomal overload in neurodegenerative conditions. Defective ESCRT-mediated membrane repair and lysosomal degradation promote accumulation of toxic aggregates and exacerbate prion-like propagation of pathogenic proteins via exosomes [23]. Therapeutic strategies aimed at enhancing lysosomal biogenesis, modulating Rab GTPase activity, or

targeting ESCRT components are being explored to restore vesicular homeostasis and mitigate disease progression [24].

### 2.3. Autophagy-Lysosomal System

The autophagy–lysosomal pathway (ALP) is a major degradative system that maintains neuronal homeostasis by eliminating misfolded proteins, aggregates, and damaged organelles. During macroautophagy, cytoplasmic cargo is sequestered into double-membraned autophagosomes, which then fuse with lysosomes for degradation by acidic hydrolases [25]. This process is highly dependent on vesicular trafficking regulators such as Rab GTPases and the HOPS complex, which mediate autophagosome–lysosome fusion. Genetic evidence underscores the importance of these components: mutations in VPS16 and VPS41 [26], encoding HOPS subunits, cause early-onset dystonia and spastic ataxia (SCAR29, DYT30), highlighting the link between autophagy failure and movement disorders [27].

Beyond canonical PI3P-dependent autophagy, recent studies have identified a noncanonical pathway mediated by PI5P, which can substitute for PI3P in recruiting autophagy machinery [28]. PI5P synthesis by PIKfyve and its regulation by PI5P4 kinases modulate autophagosome biogenesis independently of VPS34. Inhibition of PI5P4Ks enhances clearance of aggregate-prone proteins, including mutant huntingtin, positioning these kinases as promising therapeutic targets for neurodegenerative diseases [29].

Autophagy dysfunction is increasingly recognized as a convergent mechanism in AD and PD, where impaired autophagosome formation or lysosomal fusion promotes accumulation of tau and  $\alpha$ Syn aggregates [30]. Factors such as excess  $\alpha$ Syn, nitric oxide signaling, and mutations in trafficking proteins like VPS35 exacerbate these defects [31], while GWAS implicates loci such as CALM [32], which regulate SNARE-mediated vesicle fusion, in tau pathology. Therapeutic strategies aimed at enhancing lysosomal function, modulating PI5P signaling, or restoring HOPS-mediated fusion hold promise for mitigating neurodegeneration by reinforcing the autophagy–lysosomal axis [33].

### 2.4. Chaperone System

Molecular chaperones, primarily heat shock proteins (HSPs), are essential components of the proteostasis network, ensuring proper protein folding and preventing aggregation under physiological and stress conditions. HSP families such as HSP70 and HSP90 bind nascent or misfolded polypeptides, facilitating correct folding or refolding, and directing terminally misfolded proteins toward degradation pathways like the UPS or autophagy [34,35]. These chaperones act in concert with co-chaperones and ATP-dependent cycles to maintain neuronal protein homeostasis, which is particularly critical in post-mitotic neurons vulnerable to proteotoxic stress. Dysregulation of chaperone activity contributes to the accumulation of pathogenic aggregates driving neurodegenerative processes [36].

The expression of HSPs is transcriptionally regulated by heat shock factor 1 (HSF1), a master stress-response regulator that activates heat shock genes during proteotoxic stress. HSF1 dysfunction has been implicated in movement disorders; notably, recent genetic studies identified intronic VNTR expansions in HSF1 that reduce its expression and confer risk for essential tremor, linking impaired chaperone induction to disease pathogenesis [37]. Beyond genetic factors, aging and chronic stress diminish HSF1 activity, weakening the cellular defense against misfolded proteins. Pharmacological strategies aimed at modulating HSF1 or enhancing HSP expression—such as small molecules that disrupt HSP90–HSF1 interaction to activate HSF1—are emerging as promising therapeutic approaches for neurodegenerative and movement disorders [38].

Recent advances highlight the therapeutic potential of targeting chaperone systems directly. Approaches include HSP90 inhibitors, which reduce tau and A $\beta$  toxicity in AD models, and HSP70 activators, which promote disaggregation and clearance of misfolded proteins [39]. Novel concepts such as epichaperomes—stable, disease-specific chaperone assemblies—offer network-level intervention strategies to restore proteostasis in complex disorders [40]. Unlike transient chaperone

complexes, epichaperomes act as scaffolding hubs that rewire protein–protein interaction networks, amplifying synaptic dysfunction and proteotoxic stress. Their persistence under chronic stress creates a maladaptive connectivity state that cannot be corrected by conventional chaperone modulators. Recent studies highlight small molecules such as PU-H71 and PU-AD (icapamespib) that selectively dismantle epichaperomes, restoring dynamic chaperone function and network integrity without broadly inhibiting heat shock proteins [40,41]. Chemical biology approaches have advanced clickable probes (PU-TCO) for single-cell mapping of epichaperomes in AD and PD, enabling precision diagnostics and therapeutic stratification [42]. Systems-level interactome analyses confirm that epichaperome disruption reverses synaptic and cognitive deficits in preclinical models, positioning these assemblies as actionable targets for network-centric interventions [43]. This strategy contrasts with single-protein aggregation inhibitors by addressing upstream proteostasis failure and restoring connectivity across signaling and trafficking pathways.

### 2.5. Additional Selective Clearance Pathways

Beyond canonical UPS and macroautophagy, selective clearance of protein aggregates leverages ESCRT-driven microautophagy, proteaphagy of proteasomes, receptor-guided aggrephagy (NBR1, OPTN, TOLLIP), and LLPS chaperone control (HSP70/RNA), together forming an integrated network that funnels diverse substrates to lysosomes or stabilizes pre-aggregate condensates.

Autophagy-linked FYVE domain-containing protein (ALFY, encoded by WDFY3) is a large (400 kDa), evolutionarily conserved scaffold essential for selective macroautophagy (aggrephagy). ALFY mediates clearance of ubiquitinated aggregates by bridging cargo and autophagic machinery. Its FYVE domain binds PI3P on autophagic membranes, WD40 repeats interact with Atg5 and GABARAP, and its PH-BEACH domain binds p62/SQSTM1, enabling recruitment of autophagosomes to ubiquitinated inclusions [44,45]. Under basal conditions, ALFY localizes to the nucleus but translocates to the cytoplasm upon stress or aggregation, colocalizing with pathogenic proteins such as mutant huntingtin, TDP-43, and SOD1 [46,47]. Loss of ALFY impairs selective autophagic degradation of detergent-insoluble aggregates, accelerating neurodegenerative phenotypes in models of HD [44,48]. Importantly, ALFY is dispensable for starvation-induced autophagy, underscoring its specificity for aggregate clearance [49].

Proteomic analysis of insoluble aggregates identified CRAM-1, a worm protein with a ubiquitin-binding domain, as a key modulator. Knockdown of CRAM-1 reduced aggregate burden by up to 86%, despite its minor representation in aggregate mass, indicating a leveraged effect [50]. Mechanistically, CRAM-1 binds ubiquitin and oligoubiquitin with high affinity, interfering with p62/sequestosome-mediated delivery of misfolded proteins to proteasomes and autophagosomes. Functional assays confirmed that CRAM-1 knockdown rescues motility and chemotaxis in A $\beta$ -expressing worms, but only when proteasome activity is intact, implicating CRAM-1 as a negative regulator of proteasomal clearance [51].

CRAM-1 knockdown extended worm lifespan by 11%, consistent with prior findings for its mammalian orthologs (SURF1/2, HYPC). However, worms lacking CRAM-1 exhibited reduced early fecundity, suggesting evolutionary retention of aggregation-promoting proteins due to reproductive advantage despite late-life proteotoxicity [50]. This supports the antagonistic pleiotropy hypothesis, where genes beneficial for early fitness persist despite deleterious aging effects.

## 3. Protein Clearance Mechanisms - Extraneuronal

### 3.1. Glymphatic System

The glymphatic system facilitates bulk clearance of interstitial solutes and proteins through a perivascular network. Cerebrospinal fluid (CSF) enters the brain parenchyma from periarterial spaces, propelled by arterial pulsatility and regulated by aquaporin-4 (AQP4) channels concentrated on astrocytic endfeet [52]. This arrangement enables efficient CSF–interstitial fluid exchange and drives convective flow through the neuropil [53]. After solute exchange, CSF exits along perivenous

spaces toward the subarachnoid space and cervical lymphatics, forming a continuous clearance route for extracellular metabolites and proteins.

Glymphatic activity is state-dependent: tracer studies demonstrate markedly increased clearance during non-REM sleep and under anesthesia, while wakefulness significantly reduces flow [54]. Human imaging studies using intrathecal gadolinium-enhanced MRI confirmed this pathway *in vivo*, validating its role in solute transport [55]. Initially, A $\beta$  was identified as a glymphatic substrate, linking this system to AD pathophysiology [52]. More recent evidence shows  $\alpha$ Syn clearance via glymphatic flow, implicating it in PD and related synucleinopathies [56].

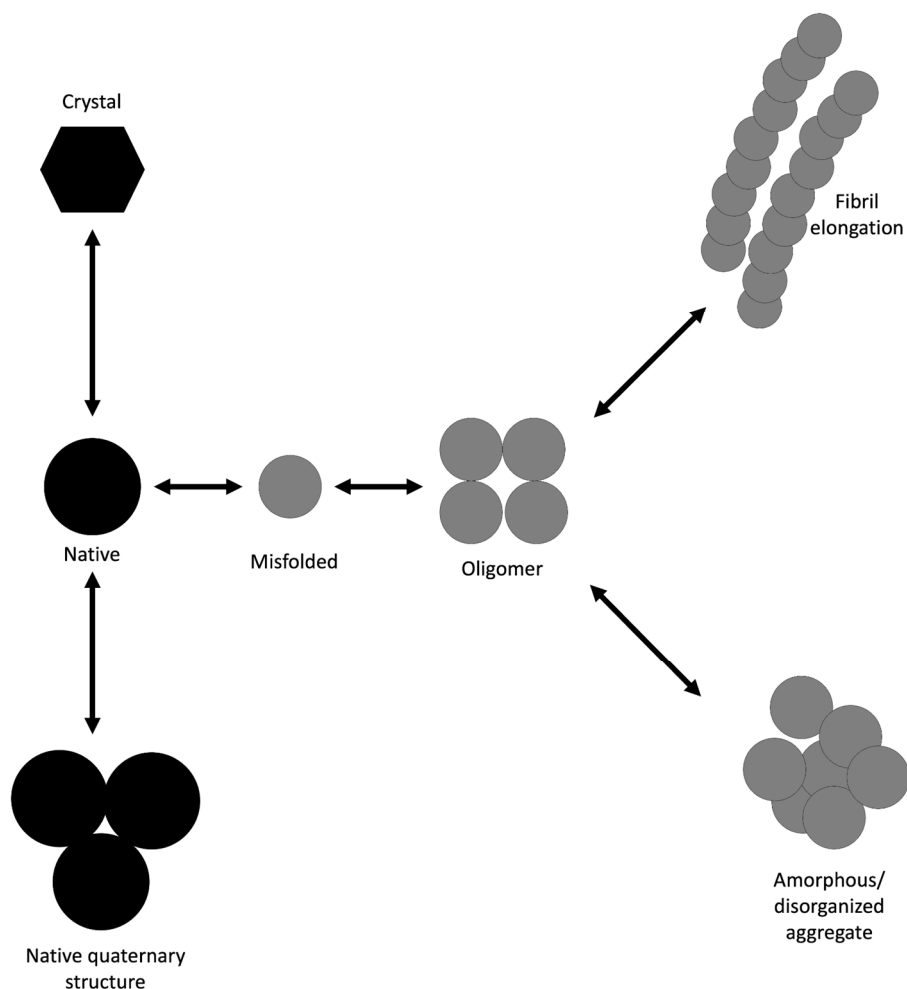
### 3.2. Glial Cells

Glial cells are essential for maintaining extracellular proteostasis through phagocytosis of protein aggregates and cellular debris. Microglia, the brain's resident immune cells, exhibit the highest phagocytic capacity among glial populations. They internalize  $\alpha$ Syn aggregates via receptor-mediated endocytosis involving Toll-like receptors (TLR2, TLR4) and Fc $\gamma$  receptors, followed by degradation through lysosomal pathways. *In vivo* studies using human  $\alpha$ Syn transgenic mice (h $\alpha$ Syn-Tg) confirmed microglial uptake and clearance of fibrillar  $\alpha$ Syn, which is accompanied by activation of inflammatory signaling cascades [57,58]. Microglia also phagocytose tau and A $\beta$  aggregates, with efficiency influenced by complement receptor pathways (CR3) and triggering receptor expressed on myeloid cells 2 (TREM2) [59].

Astrocytes contribute to extracellular clearance by engulfing  $\alpha$ Syn and tau aggregates through endocytosis and macropinocytosis, followed by lysosomal degradation. Recent studies show astrocytes accumulate  $\alpha$ Syn inclusions in PD models, suggesting a secondary clearance role when microglial capacity is exceeded [60]. Astrocytic uptake is mediated by LRP1 and other scavenger receptors, and astrocytes can form glial scars that sequester aggregates, limiting their spread. Oligodendrocytes and oligodendrocyte precursor cells (OPCs) also exhibit phagocytic activity, particularly relevant in MSA, where they internalize  $\alpha$ Syn and contribute to glial cytoplasmic inclusion formation [61].

## 4. Protein Aggregation

Neurons bearing protein aggregates exhibit widespread cellular damage (Figure 3). Aggregated species such as  $\alpha$ Syn and tau disrupt nuclear integrity, causing DNA double-strand breaks and transcriptional stress, while RNA metabolism is impaired through sequestration of RNA-binding proteins [62]. These aggregates activate innate immune pathways, including TLR signaling and inflammasome components, amplifying neuroinflammation and accelerating degeneration [63]. Cytoskeletal collapse is another hallmark: aggregates destabilize microtubules and actin networks, impairing axonal transport and synaptic vesicle trafficking, which compromises neuronal connectivity and cortical circuit function [64].



**Figure 3.** Schematic representation of protein aggregation pathways. Proteins in their native state can undergo misfolding, forming oligomers that serve as intermediates for different aggregation routes. These include fibril elongation, leading to ordered A $\beta$  fibrils, or the formation of amorphous, disorganized aggregates. Native proteins may also assemble into quaternary structures or crystalline forms under specific conditions.

Mitochondrial impairment emerges as a critical consequence of aggregation rather than a primary trigger. Experimental models using optogenetic control of  $\alpha$ Syn aggregation demonstrate that mitochondrial depolarization, ATP depletion, and fragmentation occur after aggregate formation, mediated by cardiolipin externalization and mitophagy activation [65,66]. These changes coincide with reduced oxidative phosphorylation and metabolic collapse, ultimately engaging apoptotic and necroptotic pathways. Importantly, overexpression of  $\alpha$ Syn without aggregation does not reproduce these mitochondrial defects, reinforcing the causative role of aggregate formation in bioenergetic failure [65].

Protein aggregates propagate through prion-like mechanisms, spreading along neuronal networks and amplifying pathology in connected regions [67]. Computational models integrating connectome architecture and prion-like kinetics replicate observed progression patterns, identifying hubs that accelerate dissemination [68]. Despite widespread exposure, not all neurons succumb to aggregate toxicity, suggesting cell-intrinsic resilience mechanisms, such as enhanced lysosomal degradation or tissue-specific anti-aggregation pathways like SAPA [69]. These protective programs highlight heterogeneity in neuronal vulnerability and represent key determinants of disease trajectory.

Although A $\beta$ ,  $\alpha$ Syn, tau, and TDP-43 share common principles such as  $\beta$ -sheet-driven assembly and prion-like propagation, their aggregation pathways exhibit distinct molecular signatures. A $\beta$

forms extracellular cross- $\beta$  fibrils with high polymorphism and secondary nucleation dominance [70], whereas  $\alpha$ Syn aggregates in the cytoplasm via NAC-driven fibrillization strongly influenced by lipid interactions [71]. Tau aggregation requires polyanionic cofactors and displays isoform-dependent filament folds (3R vs 4R) [72], while TDP-43 undergoes phase separation before solidification, producing non-classical amyloid-like structures modulated by diverse PTMs [73]. These differences in architecture, nucleation dynamics, cellular localization, and regulatory mechanisms are summarized in Table 1.

**Table 1.** Comparative Structural and Mechanistic Features of A $\beta$ ,  $\alpha$ Syn, Tau, and TDP-43 Aggregation.

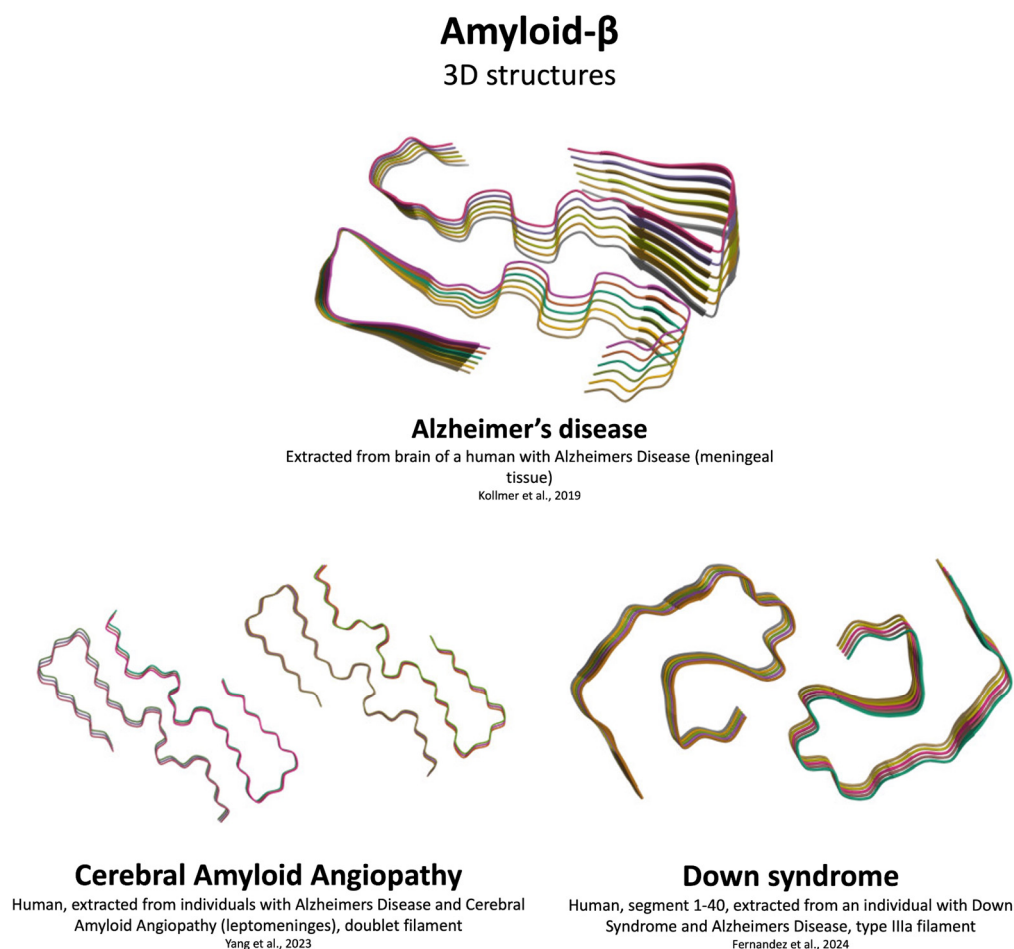
Feature	A $\beta$	$\alpha$ Syn	Tau	TDP-43
Primary aggregation domain	Full-length (A $\beta$ 40/42), hydrophobic core (residues 17–21, 30–42)	NAC region (61–95) critical for fibrillization	Microtubule-binding repeats (R2, R3; VQIINK, VQIVYK motifs)	Low-complexity domain (LCD; residues 282–360)
Architecture	Canonical cross- $\beta$ fibrils; cryo-EM shows S-shaped, $\nu$ -shaped polymorphs	Cross- $\beta$ fibrils; multiple polymorphs (MSA vs PD)	Paired helical filaments (PHFs) and straight filaments; disease-specific folds	Amyloid-like but non-classical cross- $\beta$ ; short $\beta$ -strands, spiral folds
Nucleation mechanism	Primary nucleation + secondary nucleation dominates oligomer formation	Primary nucleation; secondary nucleation accelerates oligomer growth	Heparin-induced nucleation in vitro; secondary nucleation less defined	LLPS precedes solidification; oligomeric seeds nucleate fibrils
Polymorphism	High; A $\beta$ 40 vs A $\beta$ 42 differ; multiple brain-derived folds	High; strain-specific folds linked to disease phenotype	High; AD vs PiD vs CBD folds differ by isoform composition (3R vs 4R)	Present; LCD truncations yield distinct fibril cores
Cellular localization	Extracellular plaques (parenchyma, vasculature)	Cytoplasmic inclusions (LBs, neurites)	Cytoplasmic neurofibrillary tangles; axonal threads	Cytoplasmic inclusions; nuclear clearance
PTMs influencing aggregation	N-terminal truncation, oxidation	Phosphorylation, nitration	Hyperphosphorylation, acetylation	Phosphorylation, ubiquitination, SUMOylation,

				citrullination
Propagation	Prion-like seeding; extracellular spread	Prion-like; vesicle-mediated transmission	Prion-like; templated misfolding	Prion-like; LLPS-driven seeding and spread
Toxic species	Soluble oligomers (membrane pore formation)	Oligomers; disrupt vesicle trafficking	Oligomers; impair microtubule stability	Oligomers; RNA sequestration, splicing defects

Abbreviations: A $\beta$ , amyloid-beta; AD, Alzheimer's disease; CAA, cerebral amyloid angiopathy; EM, electron microscopy; FTLN, frontotemporal lobar degeneration; LCD, low-complexity domain; LLPS, liquid-liquid phase separation; MSA, multiple system atrophy; PHF, paired helical filament; PTM, post-translational modification; SSNMR, solid-state nuclear magnetic resonance.

#### 4.1. A $\beta$ Aggregation

Amyloid fibrils display a cross- $\beta$  arrangement in which  $\beta$ -strands are perpendicular to the fibril axis and  $\beta$ -sheets stack parallel, yielding 4.8 Å inter-strand and 10 Å inter-sheet spacings reproducibly resolved by X-ray and cryo-EM across sequences [74]. Representative cryo-EM structures of A $\beta$  filaments associated with AD, cerebral amyloid angiopathy, and Down syndrome are shown in Figure 4 [75–78]. In human AD brain, A $\beta$ 42 filaments adopt two S-shaped protofilament folds (Type I/II) with Type I enriched in sporadic AD and Type II in familial AD, and these *in vivo* folds differ from *in vitro* assemblies; AppNL-F knock-in mice recapitulate Type II deposits [79]. Brain-seeded samples expand this repertoire beyond S-shapes to v- and v-shaped cores with opposite helical handedness and intersubunit salt bridges (e.g., K16–A42; partial K28–A42) that define distinct packing topologies and solvent accessibilities [80]. A $\beta$ 40 filaments from leptomeningeal vessels (CAA) show anti-parallel packing of H14–G37 within protofilament pairs and ordered cores spanning D1–G38, establishing architecture that is proteinopathy-specific and distinct from parenchymal A $\beta$ 42 [76].



**Figure 4.** Structural diversity of human A $\beta$  filaments in different pathological conditions. The panel shows representative 3D structures of A $\beta$  extracted from human brain tissue in AD, cerebral amyloid angiopathy, and Down syndrome with AD. These cryo-EM structures reveal distinct filament conformations and polymorphs associated with disease-specific pathology. Data from Kollmer et al. [75], Yang et al. [76], and Fernandez et al. [77]. Image created using RCSB PDB Mol 3D Viewer by Sehnal et al. [78].

A $\beta$  aggregation is governed by primary nucleation  $\rightarrow$  elongation  $\rightarrow$  secondary nucleation, with surface-catalyzed secondary nucleation on existing fibrils emerging as the dominant microscopic step that amplifies oligomer production under physiological conditions [81]. Denaturant-specific effects dissect these steps: urea reduces overall aggregation by decreasing fibril surface coverage and suppressing secondary/primary nucleation more than elongation, whereas low GuHCl accelerates aggregation via electrostatic screening before higher concentrations restore denaturation-dominated slowing [82]. pH provides step selectivity; titration near the histidine pKa (7.0) enhances primary nucleation while secondary nucleation and elongation remain pH-independent, linking acidic endo-lysosomes to accelerated nucleus formation without changing elongation [83]. These observations align with the backbone-driven nature of cross- $\beta$  assembly (hydrogen-bond ladders), with side-chain and solvent constraints selecting polymorph-specific interfaces and tissues [84].

A $\beta$  oligomers are the principal cytotoxins, producing ion-conducting pores that dysregulate Ca<sup>2+</sup> homeostasis and trigger apoptotic/inflammatory signaling in neurons [85]. Single-channel electrophysiology in model bilayers shows A $\beta$ 1-42 pores < 5 Å in diameter with short-lived openings and conductances comparable to gramicidin, supporting an A $\beta$  channelopathy [86]. High-resolution oligomer structures in membrane mimetics reveal tetramers/octamers with hydrophilic edge residues forming lipid-stabilized “edge-conductivity” pores that allow water permeation and leakage [87].

Lipid composition is decisive: outer-leaflet phosphatidylserine ( $\geq 10$  mol %) accelerates A $\beta$ 1-42 oligomerization and pore formation within 30 min, whereas neutral DOPC resists adsorption/leakage [88]. Free POPC in extracellular microdomains co-aggregates with A $\beta$ 42, increasing compactness/sphericity of octamer/decamer oligomers via hydrophobic tail interactions and exposed hydrophobic patches that may nucleate further oligomerization [89].

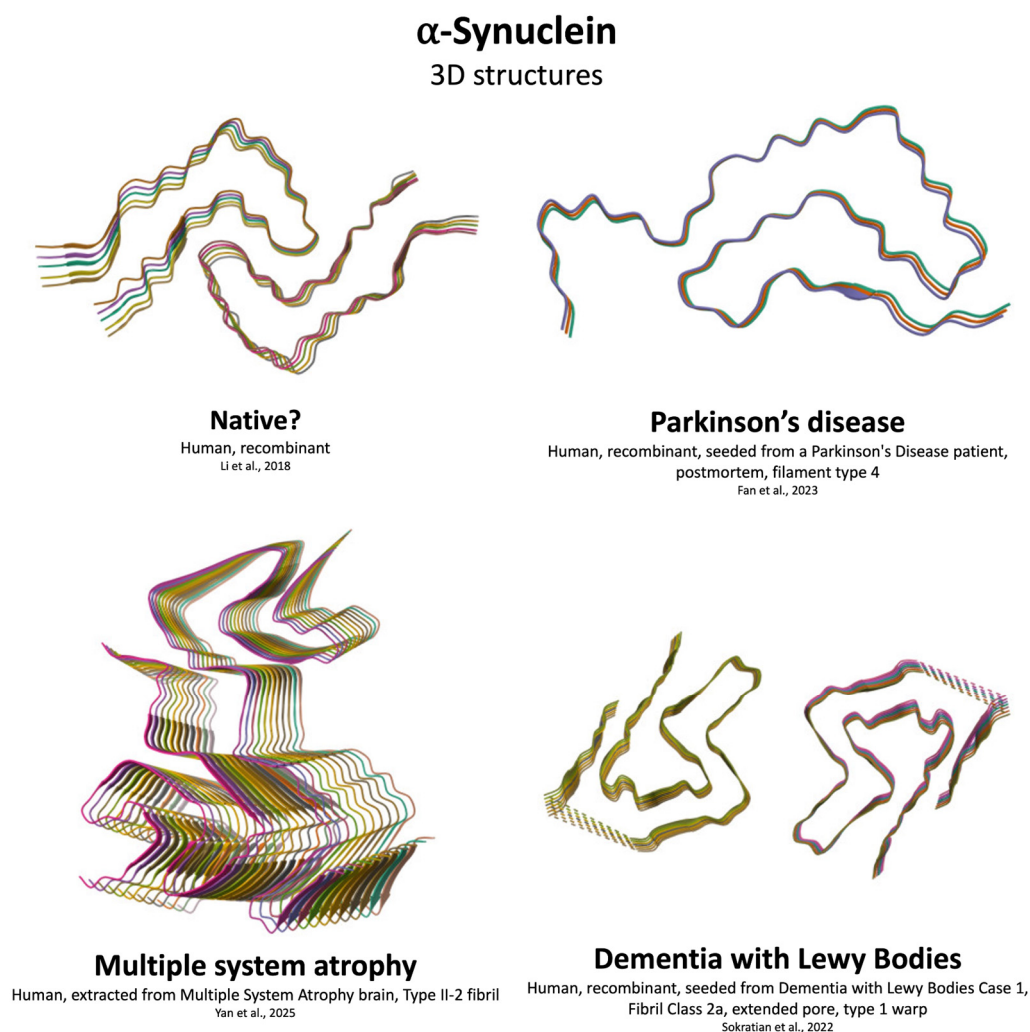
A $\beta$ -tau and A $\beta$ - $\alpha$ Syn interactions reshape aggregation pathways. A $\beta$  oligomers increase tau oligomer binding and internalization at human synapses via membrane/endocytic proteins, mechanistically linking A $\beta$  burden to tau spread [90]. Tau microtubule-binding repeats form hetero-adducts with A $\beta$ , altering A $\beta$  aggregation kinetics and toxicity in intra- and extracellular milieus [91]. For  $\alpha$ Syn, secondary nucleation on fibril surfaces is the dominant source of oligomers under neutral pH/ionic strength [92], mirroring A $\beta$ 's surface catalysis [93]. Direct A $\beta$ - $\alpha$ -syn co-assembly shows A $\beta$ 42 heterogeneous nucleation of  $\alpha$ -syn condensation, whereas A $\beta$ 40 is sequestered into  $\alpha$ -syn condensates that accelerate the liquid-to-solid transition to amyloid, accounting for mixed deposits in AD/Lewy body spectra [94].

Not all cross- $\beta$  assemblies are pathological. The *Drosophila* Orb2 (CPEB) functional amyloid forms three-fold symmetric filaments with a hydrophilic glutamine-packed core that switches Orb2 from translation repressor to activator, providing a stable yet regulatable substrate for memory persistence [95]. This hydrophilic-core architecture contrasts with the hydrophobic cores typical of pathogenic fibrils, illustrating how sequence-context and packing encode biological function versus toxicity [95].

#### 4.2. $\alpha$ Syn Aggregation

$\alpha$ Syn is a 140-amino-acid, intrinsically disordered presynaptic protein whose misfolding and self-assembly into oligomers and fibrils underlie the hallmark Lewy body and Lewy neurite pathology of PD and related synucleinopathies [96]. Over the last five years, converging structural, biophysical, and cellular studies have refined our understanding of how  $\alpha$ -syn transitions from dynamic monomers to conformationally diverse assemblies, how membranes/lipids and post-translational modifications (PTMs) modulate this process, and how strain polymorphism and prion-like mechanisms shape propagation and disease heterogeneity [97,98].

High-resolution cryo-EM has mapped multiple  $\alpha$ -syn fibril polymorphs, highlighting distinct protofilament interfaces, buried cores, and asymmetric architectures (Figure 5) [78,99–102]. Notably, brain-derived fibrils from MSA exhibit protofilaments with extensive interfacial contacts and asymmetric pairing, distinct from recombinant fibrils [103]. Familial mutations reconfigure fibril structure; for example, A53T alters the protofilament interface and increases neuronal seeding/toxicity [104]. Methodological advances now routinely achieve  $\leq 2.5$  Å maps and robust workflows for helical reconstruction, extending resolution of residues 36–97 in the fibril core and newly resolving N-terminal density islands [103,105].



**Figure 5.** Structural diversity of human  $\alpha$ Syn in different states and disease conditions. The panel shows representative 3D structures of  $\alpha$ Syn in its recombinant form and aggregated states associated with neurodegenerative disorders. Structures were obtained from cryo-EM studies and illustrate distinct fibril conformations and polymorphs linked to disease-specific pathology. Data from Li et al. [100], Sokratian et al. [102], Fan et al. [99], and Yan et al. [101]. Image created using RCSB PDB Mol 3D Viewer by Sehnaal et al. [78].

Beyond classical nucleation–elongation, recent work shows  $\alpha$ -syn undergoes LLPS to form protein condensates that can mature via liquid-to-solid phase transitions into amyloid [5,106]. Alternative splice isoforms modulate LLPS and aggregation kinetics: even minor fractions of  $\alpha$ -syn-112 significantly accelerate phase separation and co-aggregation of full-length  $\alpha$ -syn-140 [107]. SNARE interactions provide a cellular handle on LLPS. VAMP2 drives  $\alpha$ -syn phase separation via electrostatic contacts between its juxtamembrane domain and the  $\alpha$ -syn C-terminus, with condensates that sequester vesicles and recruit complexes, linking LLPS to synaptic organization [3]. Importantly, solution conditions decouple LLPS and amyloid formation. Salt promotes LLPS monotonically, but aggregation shows non-monotonic salt dependence, reflecting different intra- vs. intermolecular interaction landscapes [6]. These observations place condensate biology at the gateway of  $\alpha$ -syn aggregation and suggest that cellular context (protein isoforms, binding partners, ionic milieu) chooses between metastable condensates and irreversible fibrils [106,107].

$\alpha$ -syn's amphipathic N-terminus binds negatively charged phospholipids, and lipids are now recognized as active co-reactants in aggregation, not merely passive surfaces [108,109]. Kinetic and scattering studies show that binding to anionic vesicles remodels membranes, producing small lipid–protein particles that subsequently co-assemble into fibrils with lipid incorporation in the fibril material [109,110]. At organelle contact sites,  $\alpha$ -syn localizes to mitochondria-associated ER

membranes (MAMs), where it perturbs phosphatidylserine metabolism; lipidomics of human PD brain reveals region-specific phosphatidylserine alterations aligned with vulnerability [111]. These findings support a reciprocal coupling: lipid composition and curvature bias  $\alpha$ -syn assembly pathways, while aggregates feedback to alter membrane structure and lipid homeostasis [108,111].

PTMs on soluble  $\alpha$ -syn gate amplification of pathological seeds. Systematic mapping of human  $\alpha$ -syn uncovered new phosphorylation and acetylation sites whose presence modulates seeded transmission in site- and conformation-specific ways [112]. Non-enzymatic PTMs linked to metabolism (e.g., glycation by methylglyoxal) enhance aggregation, increase pSer129-positive deposits, and activate microglia, providing molecular traction for the PD–diabetes comorbidity [113,114]. Conversely, O-GlcNAcylation has context-dependent effects on monomer dynamics and early oligomer formation: glycosylation at T72 slows aggregation whereas S87 may favor small oligomer formation without accelerating late fibrillation, underscoring residue-specific outcomes [115]. Collectively, PTMs serve as rheostats that redirect  $\alpha$ -syn across condensed, oligomeric, and fibrillar states [112,115].

A consensus continues to crystallize that soluble oligomers are the primary cytotoxic species, with polymorphic oligomers showing distinct membrane disruption, mitochondrial impairment, and proteostasis stress [116,117]. Structural work distinguishes off-pathway, highly toxic oligomers—stable, often  $\alpha$ -helical-rich assemblies—from on-pathway species that mature into less dynamic fibrils [116,118]. In vivo, neuronal fibrils penetrating the nucleus have been observed in MSA, where early nuclear envelope damage and loss of lamin integrity tie aggregation state to rapid neurodegeneration [119]. These data support a damage continuum in which oligomer–membrane interactions, organelle stress, and nuclear invasion are mechanistically linked [116,119].

Recent studies refine the prion-like propagation framework. Soluble oligomers/protofibrils display seeding capacity that depends on assembly size/shape and exposed epitopes [120]. Strain propagation occurs independently of cellular prion protein (PrP<sup>c</sup>), pointing to alternate receptors/pathways for uptake and neuroinvasion [121]. In vivo imaging and computational modeling reveal network-like atrophy after PFF inoculation that depends on epicenter (striatal vs. hippocampal) and host genotype, emphasizing connectivity and regional vulnerability over a simplistic domino spread [120,122]. Complementarily, stochastic misfolding under identical conditions yields multiple strains in vitro and in vivo, offering a parsimonious explanation for clinical heterogeneity across synucleinopathies [123].

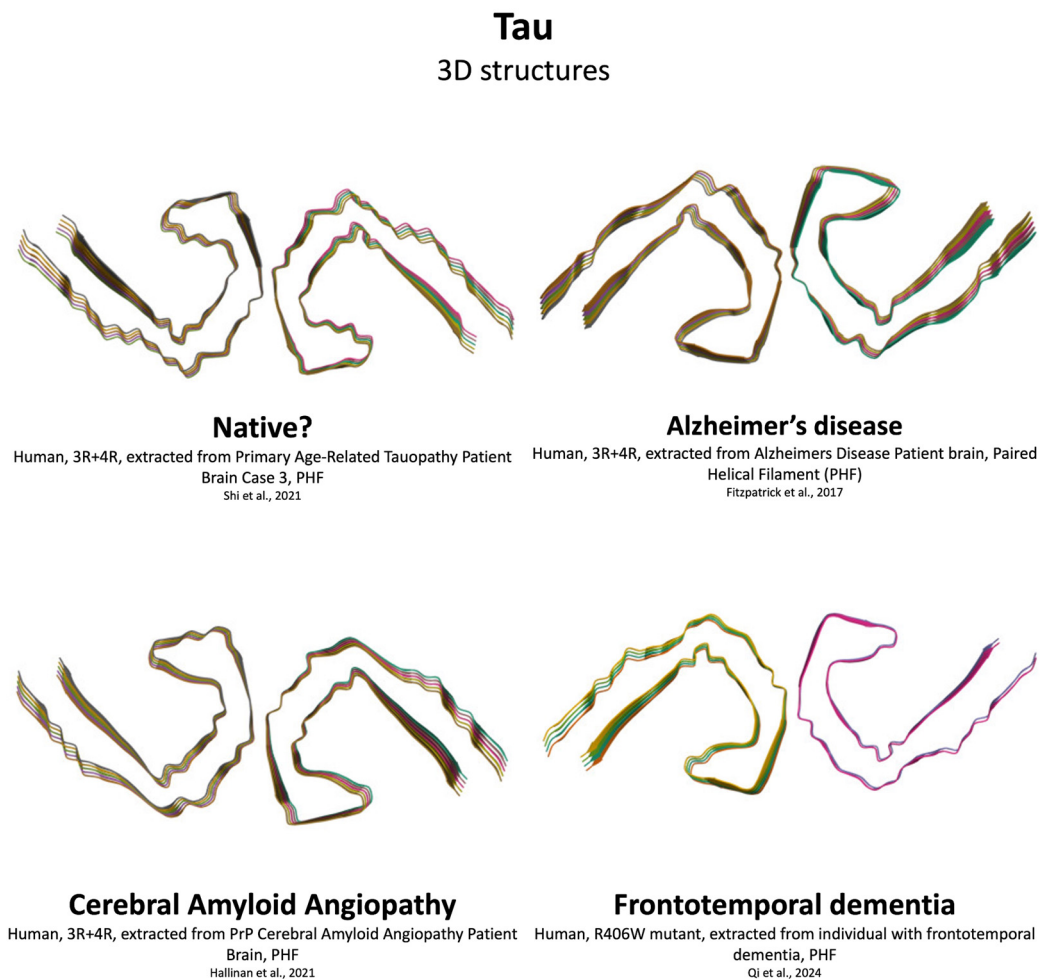
Mounting clinical, neuropathological, and experimental data implicate the gut–brain axis in initiation and modulation of  $\alpha$ -syn pathology. Reviews and models support ENS-to-brainstem propagation via the vagus pathway in a “gut-first” PD subtype, consistent with early gastrointestinal prodromes [124–126]. Microbiome dysbiosis and metabolites shape aggregation and inflammation: short-chain fatty acids aggravate  $\alpha$ -syn deposition and NLRP3-mediated neuroinflammation via GPR43, linking peripheral metabolism to central pathology [127,128]. These lines of evidence situate aggregation biology within a systems framework, where peripheral immune–metabolic states and barrier integrity bias  $\alpha$ -syn assembly and spread [124,127].

### 4.3. Tau Aggregation

Tau aggregation is strongly driven by its primary structure, particularly the microtubule-binding repeats (R1–R4) located between residues 244–369. Within these repeats, two hexapeptide motifs—VQIINK (R2, residues 275–280) and VQIVYK (R3, residues 306–311)—are critical nucleation sites for  $\beta$ -sheet formation and filament assembly [129]. These motifs exhibit high  $\beta$ -propensity and form steric zippers in crystallographic studies, providing the structural basis for paired helical filament (PHF) formation [130]. Mutations within these motifs, such as  $\Delta$ K280 or P301L, enhance aggregation by stabilizing  $\beta$ -structure, whereas proline insertions disrupt fibrillization [131]. Additionally, cysteine residues at positions 291 and 322 can form intermolecular disulfide bonds, further promoting filament elongation [132].

Tau is intrinsically disordered in its monomeric state, exhibiting minimal  $\alpha$ -helix or  $\beta$ -sheet content [133]. However, aggregation involves a coil-to- $\beta$ -sheet transition, particularly within R2 and R3, which nucleates filament growth [134,135]. Cryo-EM studies reveal that  $\beta$ -sheet formation in these repeats underpins the amyloid-like cross- $\beta$  architecture of PHFs and straight filaments [136]. Tertiary structural rearrangements, such as the opening of the “paper-clip” conformation—where N- and C-terminal domains interact—are prerequisites for aggregation [137]. Phosphorylation at Thr231 within the proline-rich region induces cis-trans isomerization via Pin1 [138], destabilizing local  $\alpha$ -helices and favoring aggregation-prone conformations [139].

Aggregated tau exhibits remarkable structural polymorphism, with disease-specific filament folds revealed by cryo-EM (Figure 6) [78,136,140–142]. AD filaments incorporate residues 273–380, forming paired helical and straight filaments, whereas Pick’s disease filaments (3R isoforms) span residues 254–378 and adopt distinct conformations [143]. Corticobasal degeneration filaments (4R isoforms) display unique folds involving residues 274–380. Heparin-induced filaments *in vitro* recapitulate some, but not all, disease-specific architectures, emphasizing the influence of cofactors and isoform composition on structural outcomes [144]. These polymorphic assemblies likely represent distinct “strains” of tau, analogous to prions, and may underlie phenotypic diversity across tauopathies [145].



**Figure 6.** Structural diversity of human tau protein in different pathological conditions. The panel shows representative 3D structures of tau filaments extracted from human brain tissue. These cryo-EM structures illustrate distinct filament conformations and polymorphs as-associated with disease-specific pathology. Data from Shi et al. [140], Fitzpatrick et al. [136], Hallinan et al. [141], and Qi et al.[142]. Image created using RCSB PDB Mol 3D Viewer by Sehnal et al. [78].

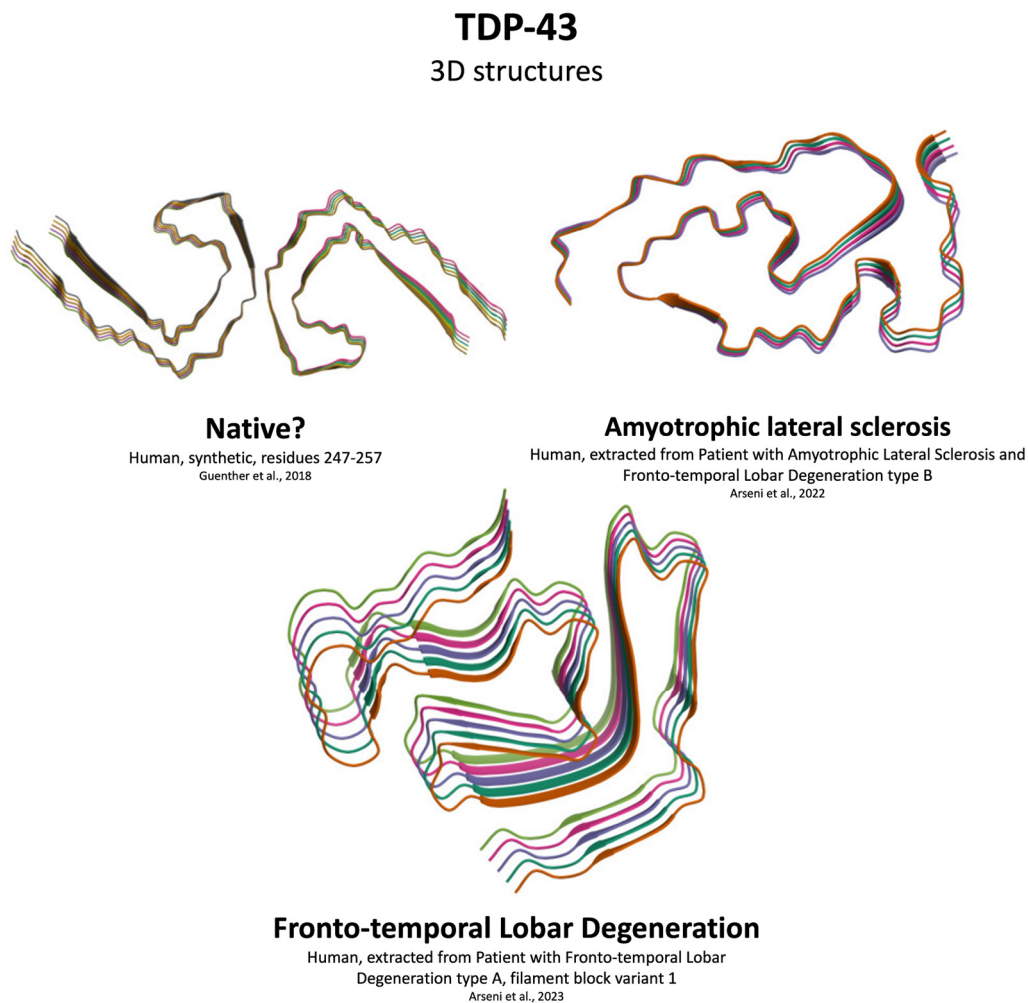
Tau aggregation follows a nucleation-elongation mechanism, where oligomeric intermediates precede fibril formation [146]. In vitro, polyanions such as heparin or RNA accelerate nucleation by neutralizing electrostatic repulsion between positively charged repeat domains [147]. Seeding experiments demonstrate that short fibrils are the most potent species for templating aggregation, consistent with prion-like propagation observed in transgenic mouse models [145]. Hexapeptide deletion mutants lacking VQIINK or VQIVYK fail to seed aggregation, confirming their essential role in templated assembly [148]. Moreover, aggregation is concentration-dependent and modulated by PTMs, including phosphorylation and acetylation, which alter charge distribution and steric accessibility of nucleating motifs [149].

PTMs of misfolded proteins are increasingly recognized as critical determinants of aggregation dynamics, pathogenicity, and clinical outcomes in neurodegenerative diseases. In AD, phosphorylation of tau at threonine 217 (pT217) is strongly associated with progressive cognitive decline and serves as a predictive biomarker for conversion from mild cognitive impairment (MCI) to AD [150]. Furthermore, tau seeding activity and specific PTMs exhibit domain-specific correlations with cognitive impairment and can differentiate AD-related tau aggregation from primary age-related tauopathies, highlighting their diagnostic relevance [151].

Direct inhibition of tau aggregation has been achieved using small molecules that interfere with  $\beta$ -sheet stacking or hexapeptide interactions. Rhodanine derivatives exhibit submicromolar  $IC_{50}$  values for both aggregation inhibition and fibril disassembly, mediated by hydrogen bonding and hydrophobic interactions with repeat domains [152]. Phenylthiazolyl-hydrazides and benzothiazoles similarly disrupt filament elongation, with structure-activity relationships emphasizing planarity, aromaticity, and hydrogen bond donors as key pharmacophores [153]. Methylene blue (methylthioninium chloride), a phenothiazine derivative, demonstrated clinical efficacy in reducing cognitive decline in patients with AD, supporting the therapeutic potential of aggregation inhibitors [154]. However, challenges remain regarding blood-brain barrier permeability, off-target effects, and the risk of generating toxic oligomeric intermediates during disassembly. Rational optimization of these scaffolds requires integration of pharmacokinetic and pharmacodynamic parameters early in development.

#### 4.4. TDP-43 Aggregation

TDP-43 is a ubiquitously expressed RNA/DNA-binding protein whose pathological aggregation is a defining feature of ALS and frontotemporal lobar degeneration (FTLD), occurring in >97% of ALS and 45% of FTLD cases [155,156]. Cryo-EM studies of patient-derived inclusions reveal that TDP-43 filaments adopt an amyloid-like architecture comprising a single protofilament with a double-spiral fold spanning residues 282–360 in the low-complexity domain (LCD) [157], distinct from in vitro assemblies (Figure 7) [78,157–159]. This fold is stabilized by glycine-rich turns and polar residues, resulting in short  $\beta$ -strands and an absence of classical cross- $\beta$  stacking, producing chemically heterogeneous surfaces that may mediate ligand interactions [160,161]. Solid-state NMR further demonstrates structural polymorphism among LCD fragments (e.g., 300–414 vs. 314–414), where minor sequence truncations generate fibrils with comparable morphology but distinct local packing, highlighting the role of aggregation-prone segments in polymorphic assembly and neurotoxicity [160].



**Figure 7.** Structural diversity of human TDP-43 filaments in different conditions. The panel shows representative 3D structures of TDP-43 in a synthetic fragment and aggregated states associated with neurodegenerative disorders. These cryo-EM structures reveal distinct filament conformations and polymorphs linked to disease-specific pathology. Data from Guenther et al. [158], Arseni et al. [157], and Arseni et al. [159]. Image created using RCSB PDB Mol 3D Viewer by Sehnal et al. [78].

Pathological TDP-43 aggregation is initiated by nuclear clearance and cytoplasmic mislocalization, followed by LLPS and transition to solid fibrils [162]. Disease-associated mutations and stressors (oxidative, nitrosative) accelerate this process. S-nitrosylation of cysteine residues promotes disulfide-linked oligomerization and prion-like propagation in neurons and in vivo models [163]. Recent work shows that amyloidogenic oligomers derived from the LCD act as potent nucleators, inducing phase separation of full-length TDP-43 and enhancing phosphorylation of cytosolic condensates, particularly in ALS-linked mutants such as A315T [164]. These oligomers exhibit high toxicity and recruit stress granule components, while chaperones like HSP70 modulate their material state, delaying irreversible gelation [164]. PTMs critically regulate aggregation. Hyperphosphorylation at C-terminal serines, long considered a pathological hallmark, may paradoxically antagonize LLPS and aggregation under certain contexts [165,166]. Conversely, SUMO2/3 conjugation during oxidative stress stabilizes RNA-free TDP-43 in stress granules, preventing irreversible aggregation, whereas inhibition of SUMOylation accelerates fibrillization [167]. Novel PTMs such as citrullination have been identified as irreversible modifications that alter TDP-43 structure and promote pathology progression [168].

TDP-43 aggregates propagate via prion-like seeding, templating misfolding of native TDP-43 and spreading along corticospinal and frontotemporal networks, consistent with neuropathological

staging of ALS and FTLD [156,169]. Seeding experiments in neurons demonstrate that aggregate uptake triggers loss of nuclear TDP-43, cryptic exon activation (e.g., STMN2, UNC13A), and disruption of autoregulatory feedback, establishing a toxic feed-forward loop [155,170]. Aggregates also sequester RNA and splicing factors, impairing transcriptome integrity, while mitochondrial dysfunction emerges as a downstream effect. Mislocalized TDP-43 interacts with mitochondrial membranes, activating the mitochondrial unfolded protein response (UPR<sub>mt</sub>) and perturbing oxidative phosphorylation [171,172]. Heterotypic interactions further exacerbate pathology— $\alpha$ Syn co-assembly with TDP-43 LCD generates distinct fibril polymorphs with enhanced protease resistance and neurotoxicity, explaining mixed protein deposits in LATE and Lewy body dementias [173].

## 5. Protein-Based Self-Propagation Mechanism

The concept of templated conversion, first established in prion biology, describes how misfolded proteins act as seeds that recruit native monomers, inducing them to adopt the same pathogenic conformation. This mechanism drives fibril elongation through iterative cycles of monomer incorporation and structural corruption [174]. In Lewy body disorders, a similar process occurs with  $\alpha$ Syn. Soluble monomeric  $\alpha$ Syn undergoes conformational refolding into  $\beta$ -sheet-rich fibrils, forming the characteristic Lewy fold [175].

Recent studies confirm that  $\alpha$ Syn aggregates propagate via prion-like mechanisms, meaning they self-template and spread across interconnected neural circuits [176]. Misfolded  $\alpha$ Syn seeds can be internalized by neurons, escape endosomal compartments, and induce conformational conversion of endogenous  $\alpha$ Syn, amplifying pathology [177,178]. This process underlies the spatial and temporal progression of Lewy pathology described by Braak, which begins in the olfactory bulb and dorsal motor nucleus of the vagus and ascends to limbic and cortical regions [179].

Cryo-EM studies reveal that  $\alpha$ Syn fibrils exhibit polymorphic structures, with distinct protofilament interfaces and strain-specific conformations that dictate neurotoxicity and regional targeting [103,180]. These strains behave analogously to prions: they maintain biochemical identity upon serial propagation and produce disease-specific phenotypes in experimental models [121,181]. Strain adaptation during cross-species transmission has also been documented, suggesting selective pressures shape  $\alpha$ Syn prion variants [182].

$\alpha$ Syn seeds initiate aggregation through nucleation-dependent polymerization, recruiting monomers into growing fibrils. Advanced assays such as real-time quaking-induced conversion (RT-QuIC) and seed amplification assays (SAA) exploit this property to detect pathogenic  $\alpha$ Syn in cerebrospinal fluid and blood, demonstrating that amplified seeds retain strain-specific features [183–185].

Emerging evidence shows that  $\alpha$ Syn interacts with other amyloidogenic proteins, notably tau, enabling cross-seeding and synergistic aggregation. This interplay contributes to overlapping pathologies in PD, dementia with LBs, and MSA, challenging traditional disease classifications [72].

## 6. Impact of Aging on Protein Aggregation

Aging drives a global erosion of proteostasis in the nervous system, so neurons accumulate misfolded/aggregated proteins and become more vulnerable to prion-like spread within networks [186]. For  $\alpha$ Syn, aging acts as a rate-limiting cofactor that promotes both aggregation and long-range propagation from enteric/peripheral sites to brainstem and cortex according to recent syntheses of age-dependent  $\alpha$ -syn biology [187,188]. Also, Morley et al. demonstrated that huntingtin aggregation increases with aging in *in vivo* models using *C. elegans* [189]. Parallel analyses of protein degradation and cellular senescence show age-driven declines in proteome turnover and intracellular proteolysis, further linking aging to aggregate accumulation across tissues [190].

The autophagy–lysosome axis exhibits region- and cell-type specific aging phenotypes. Certain brain regions (e.g., dentate gyrus, prefrontal cortex) show reduced autolysosome abundance/flux

with age, while other tissues display compensatory or even increased autophagy—underscoring that “autophagy decline” is not uniform and must be assessed as flux rather than expression alone [191]. Integrated reviews detail how aging perturbs autophagosome formation, trafficking, fusion, and lysosomal pH/ion homeostasis, tipping neurons toward aggregate retention and organelle stress, with these failures emerging as primary drivers of late-age neurodegeneration [25]. At the organelle level, lysosome-repair capacity itself appears to fail with aging, fueling dys-proteostasis and sterile inflammation in aged and AD-derived neurons—providing a mechanistic bridge between normal aging and AD pathology [192]. Complementary syntheses position lysosomes as dynamic regulators of metabolic signaling in aging and neurodegeneration, tying lysosomal homeostasis to systemic hallmarks such as inflammation and mitochondrial dysfunction [193].

Beyond intracellular mechanisms, brain-wide clearance pathways deteriorate with age. Human diffusion-MRI demonstrates a mismatch in glymphatic influx/efflux in older adults—higher arterial-driven influx yet lower paravenous efflux—implicating age-related changes in aquaporin-4 polarization and vascular pulsatility in waste retention ( $A\beta$ , tau,  $\alpha$ -syn) [194]. Large MRI cohorts further show an age-related fall in the ALPS index (diffusion along perivascular spaces), corroborating glymphatic slowing across adulthood [195]. Multimodal MRI now links declining BBB water exchange to lower ALPS and higher white-matter free water—indicating coupled BBB–glymphatic failure in healthy aging [196]. In vivo, aging enhances  $\alpha$ -syn gut-to-brain propagation and aggregate maturation/resistance, with autonomic dysfunction reminiscent of clinical PD—positioning impaired clearance and aging-biased aggregate dynamics as convergent drivers of late-life synucleinopathy risk [197].

## 7. Strategies to Limit Protein Aggregation and Spread

Strategies to mitigate protein aggregation encompass diverse mechanisms, including RNA interference to reduce expression, stabilization of non-toxic conformers, inhibition of oligomerization and fibril elongation, and enhancement of proteostasis through autophagy or proteasome activation [198]. Immunotherapeutic approaches—both passive and active—aim to neutralize extracellular seeds and limit prion-like propagation, while emerging modalities such as PROTACs and AUTOTACs enable targeted intracellular degradation. Natural compounds and rationally designed peptides further expand the therapeutic landscape, although challenges such as blood–brain barrier penetration and off-target effects persist. These approaches, along with their mechanisms, representative agents, and translational limitations, are summarized in Table 2.

**Table 2.** Therapeutic Strategies Targeting Protein Aggregation.

Strategy	Mechanism of action	Representative agents	Current Status / Limitations
Passive Immunotherapy	Monoclonal antibodies bind aggregated $\alpha$ Syn to block propagation and promote clearance	Prasinezumab (C-terminal epitope), Cinpanemab (N-terminal epitope), MEDI1341/TAK-341, LuAF82422, Exidavemab	Phase II trials; limited efficacy, poor BBB penetration
Active Immunotherapy	Vaccines induce endogenous antibodies against aggregated $\alpha$ Syn	UB-312, PD01A, PD03A	Phase I–II; risk of neuroinflammation, variable

			immunogenicity
Small Molecule Aggregation Inhibitors	Block oligomerization or fibril elongation	Anle138b, NPT200-11, PBT434	Phase I–II; off-target effects, CNS penetration issues
Natural Compounds	Polyphenols disrupt hydrophobic interactions and reduce oxidative stress	EGCG, Curcumin, Squalamine/ENT-01	Preclinical/Phase II; poor bioavailability
Peptide-Based Inhibitors	Target NAC domain or fibril ends to prevent nucleation and elongation	K84s, TO2, TO5, macrocyclic peptides (BD1), poly-arginine peptides (R18D)	Preclinical; short half-life, BBB limitations
Proteolysis-Targeting Chimeras (PROTACs)	Recruit E3 ligases to ubiquitinate $\alpha$ Syn for proteasomal degradation	Arginine-based PROTACs	Preclinical; limited aggregate clearance, BBB penetration
Autophagy-Targeting Chimeras (AUTOTACs)	Redirect aggregates to autophagosomes for lysosomal degradation	ATC161	Phase I; BBB penetration remains a challenge
Autophagy Activators	Enhance autophagic flux to clear aggregates	Trehalose, Metformin, Rapamycin, Lithium	Phase I–II; systemic toxicity, non-selective degradation
Proteasome Activators	Stimulate proteasomal activity to degrade misfolded proteins	Roflumilast, modified tetramethylpyrazine	Preclinical; insufficient potency, poor CNS penetration

### 7.1. Immunization

The mechanisms of passive immunization are hypothesized to inhibit  $\alpha$ Syn aggregation; decrease  $\alpha$ Syn release; inhibit cell-to-cell transmission. Other possible mechanisms are related to the active immunization with immune generated antibodies [199].

Pagano et al. [200] and Lang et al. [201] evaluated two anti- $\alpha$ Syn monoclonal antibodies—prasinezumab and cinpanemab—in early PD through randomized, double-blind, placebo-controlled trials. Prasinezumab, which targets the C-terminal region of aggregated  $\alpha$ Syn (aa 118–122), achieved target engagement but did not significantly slow clinical progression over 52 weeks, although imaging biomarkers suggested biological activity [200]. Cinpanemab, directed against the N-terminal epitope (aa 1–10), similarly failed to demonstrate meaningful benefit in motor or non-motor outcomes compared to placebo [201].

Beyond these two agents, multiple antibodies are under investigation to disrupt  $\alpha$ Syn propagation in PD and MSA. MEDI1341/TAK-341 (AstraZeneca-Takeda) binds both monomeric and aggregated  $\alpha$ Syn at aa 102–130 [202] and is in Phase II for MSA [203], while LuAF82422 (Lundbeck) [204] and Exidavemab (BioArctic) [205] target aggregated species with distinct C-terminal epitopes [206]. Despite strong mechanistic rationale, major limitations persist, including poor brain penetration of large immunoglobulins and uncertain reduction of  $\alpha$ Syn in cerebrospinal fluid. Future strategies may involve smaller constructs such as single-chain antibodies or nanobodies, which offer improved blood-brain barrier permeability but shorter half-life [207].

Active immunization strategies targeting  $\alpha$ Syn aim to elicit endogenous antibody production to reduce pathogenic aggregates in synucleinopathies. UB-312 (Vaxxinity) is a synthetic peptide vaccine that induces antibodies against insoluble  $\alpha$ Syn at the C-terminal region, successfully completing Phase I by generating anti- $\alpha$ Syn antibodies and lowering aggregated species in cerebrospinal fluid (CSF) of PD patients, though without affecting monomeric forms [208]. Similarly, AFFiRiS-developed peptides PDO1A and PDO3A target the same C-terminal epitope, with PDO1A demonstrating a more robust and sustained antibody response and advancing to Phase II trials [209]. Despite promising immunogenicity, these approaches face challenges including off-target binding, neuroinflammation, and potential autoimmune reactions. Future directions may involve adeno-associated virus (AAV)-mediated delivery of optimized  $\alpha$ Syn sequences to enhance specificity and durability of immune responses [210].

Beyond kinetics, modifying oligomer physicochemical properties—size and hydrophobicity— affects toxicity. Antibodies that enlarge oligomers may reduce membrane penetration, though increased surface hydrophobicity can offset benefits, yielding negligible net toxicity change.

### 7.2. Natural Compounds

Natural polyphenolic compounds such as epigallocatechin gallate (EGCG), gallic acid, and fisetin exhibit antioxidant and anti-inflammatory properties and can interact with hydrophobic regions of  $\alpha$ Syn, thereby inhibiting oligomerization and aggregation in preclinical models [211]. Curcumin, which binds both monomeric and aggregated  $\alpha$ Syn, has been evaluated in several clinical trials but failed to demonstrate significant disease-modifying effects, likely due to poor bioavailability and low target specificity [212]. In contrast, squalamine and its synthetic derivative ENT-01 (Enterin) prevent  $\alpha$ Syn aggregation and have shown additional benefits in alleviating constipation in PD patients, with Phase II trials completed successfully [213]. Despite these promising mechanisms, major limitations—including low CNS penetration and rapid metabolism—continue to hinder clinical translation, emphasizing the need for improved formulations or delivery systems to enhance therapeutic efficacy.

### 7.3. Small Molecules

Small-molecule inhibitors targeting  $\alpha$ Syn aggregation represent an alternative to immunotherapy for disease modification in synucleinopathies. Anle138b (Emrusolmin), developed by MODAG-Teva, prevents  $\alpha$ Syn oligomerization and aggregation and also inhibits tau and prion protein aggregation, currently in Phase Ib for PD and Phase II for MSA [214]. PBT434 (ATH434), an iron chelator from Alterity, reduces iron-mediated aggregation of  $\alpha$ Syn and is in Phase II trials, leveraging the link between metal dysregulation and synuclein pathology [215]. NPT200-11 (UCB-0599/minzasolmin) binds  $\alpha$ Syn to prevent misfolding and aggregation, with Phase II completed for MSA [216].

Despite promising mechanisms, challenges remain. Off-target effects and limited CNS penetration pose significant safety and efficacy concerns. SynuClean-D, identified as a potential therapeutic for PD due to its ability to inhibit aggregation and degrade existing fibrils, did not progress to clinical trials, reflecting translational hurdles [217]. Future directions include optimizing pharmacokinetics and specificity to minimize adverse effects while enhancing target engagement.

#### 7.4. Peptides

Peptide-based inhibitors of  $\alpha$ Syn aggregation span rationally selected sequences, macrocyclic scaffolds, and cationic arginine-rich constructs, each acting at distinct steps of the A $\beta$  pathway. High-throughput yeast screening identified K84s and K102s, two short peptides that suppress  $\alpha$ Syn toxicity and oligomerization at sub-stoichiometric ratios [218]; K84s additionally reduced aggregation in human cells, and in silico analyses mapped favorable binding interfaces on  $\alpha$ Syn consistent with direct anti-aggregation mechanisms. Designed derivatives TO2 (monomer-binding) and TO5 (oligomer-binding) follow the same logic—targeting regions proximal to the NAC domain to lower fibril formation in vitro—paralleling other NAC/N-terminal-focused inhibitory motifs that decelerate nucleation and elongation in ThT assays [219].

Macrocyclic peptides provide kinetic blockade at fibril ends or induce off-pathway remodeling. A RaPID-selected macrocycle (BD1) binds  $\alpha$ Syn fibril termini to halt extension without altering fibril morphology, demonstrating a tractable “end-capping” mode of action; solubility optimization further enhanced inhibitory potency [220]. Complementarily, self-assembled cyclic d,l- $\alpha$ -peptides (e.g., CP-2) interact with the N-terminal/NAC regions to redirect  $\alpha$ Syn toward amorphous, non-toxic species and disaggregate preformed fibrils in vitro and in cells overexpressing  $\alpha$ Syn [221]. These macrocyclic strategies expand the peptide toolbox beyond linear sequence mimetics by exploiting conformational preorganization and multivalent display to engage dynamic aggregation intermediates [222].

Cationic poly-arginine peptides such as R18D (an 18-mer of D-arginine) reduce both intracellular  $\alpha$ Syn aggregation and neuronal uptake of  $\alpha$ Syn seeds—by 38% and 78%, respectively—in primary cortical neurons, and also diminish monomer aggregation in cell-free assays, highlighting combined anti-seeding and anti-aggregation effects [223]. Notwithstanding these preclinical signals, translation is constrained by short in vivo half-life, blood–brain barrier permeability, and potential off-target interactions; current solutions include sequence/structure optimization (e.g.,  $\beta$ -sheet breakers [224], D-residue incorporation [225], and macrocyclization [226]), computational epitope scaffolding to improve conformational selectivity, and peptidomimetic design to enhance pharmacokinetics and CNS delivery [227].

TAT- $\beta$ Syn-Degron is a rationally engineered, cell-penetrating peptide that selectively binds endogenous  $\alpha$ Syn and directs it to the proteasome for targeted degradation, thereby reducing intracellular aggregates and ameliorating motor deficits in PD mouse models [228,229]. This construct incorporates three functional domains: a TAT sequence for efficient cellular uptake, a  $\beta$ -synuclein-derived binding motif for specific recognition of  $\alpha$ Syn, and a degron tag to recruit proteasomal machinery, enabling clearance of pathogenic species. Preclinical studies demonstrated significant reductions in  $\alpha$ Syn burden and associated neuropathology, supporting its potential as a disease-modifying strategy. However, translational challenges remain, including short systemic half-life and limited blood–brain barrier penetration, underscoring the need for advanced delivery platforms or chemical modifications to enhance CNS bioavailability [228,229].

#### 7.5. PTMs

PTMs exert a pivotal influence on  $\alpha$ Syn homeostasis and aggregation dynamics. Monoubiquitination of  $\alpha$ Syn by the E3 ligase seven in absentia homolog (SIAH) facilitates its proteasomal degradation but paradoxically also promotes aggregation in dopaminergic cells, indicating a context-dependent role [230]. Conversely, USP9X-mediated deubiquitination reverses this modification, stabilizing  $\alpha$ Syn and thereby increasing its aggregation propensity [231]. In addition, SUMOylation catalyzed by PIAS2 enhances  $\alpha$ Syn stability and fosters pathological fibril formation, while reciprocal regulation between SUMOylation and ubiquitination determines degradation efficiency [232]. Engelender et al. are developing a peptide-based therapeutic designed to inhibit  $\alpha$ Syn SUMOylation, a modification that promotes aggregation and neurotoxicity. In vivo studies using nigral injection of AAV2/1 encoding  $\alpha$ Syn A53T demonstrated that treatment with this

peptide significantly improved motor performance and reduced both nigral neurodegeneration and levels of S129-phosphorylated  $\alpha$ Syn [233].

#### 7.6. Proteolysis-Targeting Chimeras

Proteolysis-targeting chimeras (PROTACs) represent a rational approach to eliminate pathogenic  $\alpha$ Syn by leveraging the ubiquitin-proteasome system. Arginine-based PROTACs utilize a bifunctional design in which one moiety binds the E3 ubiquitin ligase UBR1, while a benzothiazole-aniline group engages  $\alpha$ Syn, forming a ternary complex that facilitates polyubiquitination and subsequent proteasomal degradation [234]. This catalytic mechanism enables repeated cycles of target elimination, offering a potentially efficient strategy for reducing intracellular  $\alpha$ Syn burden. However, limitations include restricted proteasomal capacity to degrade large aggregates, incomplete specificity for  $\alpha$ Syn, and poor blood–brain barrier (BBB) penetration, necessitating structural optimization and advanced delivery systems [235].

#### 7.7. Autophagy-Targeting Chimeras

Autophagy-targeting chimeras (AUTOTACs) provide an alternative degradation pathway by redirecting  $\alpha$ Syn aggregates to autophagosomes [236]. ATC161, a fusion of the aggregation inhibitor Anle138b and the ZZ domain of p62, selectively targets aggregated  $\alpha$ Syn for autophagic clearance without affecting monomeric species [237]. In  $\alpha$ SynPFF mouse models, ATC161 reduced cell-to-cell transmission of  $\alpha$ Syn and improved striatal pathology, supporting its therapeutic potential. Currently in Phase I trials in South Korea, AUTOTACs address some limitations of PROTACs by exploiting lysosomal degradation; however, challenges such as BBB permeability and aggregate size remain critical barriers to clinical translation [238].

#### 7.8. Activators of Autophagy

Autophagy-enhancing agents have emerged as potential disease-modifying strategies in PD by promoting clearance of misfolded  $\alpha$ Syn. Trehalose, a disaccharide, activates autophagy through both mTOR-dependent and mTOR-independent pathways and has completed Phase I trials for sporadic and LRRK2-associated PD [239]. Metformin induces autophagy via AMPK activation and is currently in Phase II trials for PD, with epidemiological studies linking its use to reduced dementia risk [240,241]. Rapamycin, an mTOR inhibitor, enhances autophagy and has shown neuroprotective effects in mild cognitive impairment and AD, though PD-specific trials remain lacking [242]. Lithium, acting through an mTOR-independent mechanism, has been associated with a 77% reduction in PD risk in observational studies [243], but clinical trials for PD have not yet been initiated [244].

Despite promising mechanisms, these compounds present significant translational challenges. Lithium is related to renal and cardiac toxicity [245]. Rapamycin's immunosuppressive profile limits long-term use, while metformin's gastrointestinal side effects and variable CNS bioavailability complicate dosing strategies [246,247]. Furthermore, indiscriminate activation of autophagy may lead to degradation of essential proteins, underscoring the need for selective modulators and biomarker-driven trials to optimize therapeutic benefit while minimizing adverse effects.

#### 7.9. Activators of the Proteasome

Currently, no compound has demonstrated robust activation of the proteasome for therapeutic purposes in PD. Preclinical efforts have explored agents that indirectly enhance proteasomal activity, such as selective phosphodiesterase inhibitors (e.g., roflumilast), which increase intracellular cAMP and upregulate proteasome function [248]. Other candidates include modified tetramethylpyrazine [249] and fluspirilene [250], identified through high-throughput screening for proteasome stimulators [251]. While these molecules show modest effects on proteasomal activity in vitro, their efficacy in reducing  $\alpha$ Syn aggregates remains limited, and none have advanced to clinical trials. Major challenges include insufficient potency, off-target interactions, and poor blood–brain barrier

penetration, highlighting the need for rational design of proteasome activators with improved specificity and pharmacokinetics.

## 8. Contradictory Points

### 8.1. Neurodegeneration Without Misfolded Protein Aggregates

Neurodegeneration can occur independently of protein misfolding, as demonstrated by hippocampal sclerosis (HS), which is characterized by severe gliosis and neuronal loss in the CA1 region of the hippocampus. HS may present as an isolated pathology or co-occur with other neurodegenerative conditions, including AD, frontotemporal dementia, temporal lobe epilepsy, multiple sclerosis, ALS, and TDP-43 proteinopathy [252]. Clinically, HS is associated with progressive memory impairment and cognitive decline, although at a slower rate compared to AD. These observations underscore that neuronal loss and gliosis alone can drive cognitive deterioration, highlighting that misfolded protein aggregates are not an obligatory feature of all neurodegenerative processes.

### 8.2. LBs: Beyond $\alpha$ Syn Aggregates

Although  $\alpha$ Syn is the predominant component of LBs, these inclusions represent highly complex pathological structures. Proteomic analyses have identified more than 300 proteins within LBs, including molecular chaperones, components of the ubiquitin-proteasome system, and markers of axonal injury, indicating that Lewy pathology involves widespread disruption of proteostasis and neuronal integrity [253–255]. Advanced correlative light and electron microscopy studies have revealed that LBs are not homogeneous fibrillar aggregates but rather crowded membranous environments enriched with fragmented organelles, lipid membranes, autophagosomes, and lysosomes, reflecting severe impairment of intracellular trafficking and degradation pathways [256]. Experimental models further demonstrate that  $\alpha$ Syn seeding initiates a cascade of secondary pathological events, culminating in the recruitment of diverse cellular components and the formation of complex LB-like inclusions. Critically, Mahul-Mellier et al. showed that the process of LB maturation—rather than  $\alpha$ Syn fibrillization alone—is a major driver of neurodegeneration, underscoring that Lewy pathology is a multifactorial phenomenon involving protein misfolding, organellar dysfunction, and failure of cellular clearance mechanisms [257].

### 8.3. Mixed Pathologies

Autopsy studies reveal that  $\alpha$ Syn pathology co-occurs in more than 50% of AD cases, often alongside tau aggregates, which are also frequently detected in Lewy body disease [253,258]. These concomitant pathologies complicate diagnostic accuracy and therapeutic development, as overlapping molecular signatures may obscure disease-specific biomarkers and treatment targets [259]. Clinically, co-pathologies are associated with accelerated cognitive decline and a more aggressive disease course compared to cases with isolated pathology [260,261].

Large-scale histopathological and proteomic studies confirm that mixed proteinopathies are frequent in aged brains; however, the prevalence and distribution of co-occurring protein seeds remain incompletely characterized across different neurodegenerative conditions [262]. Importantly, even low-level protein aggregation appears to be nearly ubiquitous in advanced age, suggesting that cases entirely devoid of misfolded proteins are rare [259].

Fein et al. demonstrated that approximately 24% of  $A\beta$ -positive synaptic terminals also contain phosphorylated tau, with the strongest co-localization observed in hippocampal regions, suggesting a synaptic interface for dual pathology [263]. Complementing this, Aksman et al. used a data-driven approach to map progression patterns and confirmed the existence of amyloid-first and tau-first subtypes, while highlighting their interaction within hippocampal circuits [264].

Using ultrasensitive seeding assays and large-scale neuropathological analyses, Manca et al. demonstrated that tau seeds are present in prodromal stages and occur across multiple neurodegenerative disorders, including Lewy body disease and other tauopathies [265]. This early appearance of tau pathology suggests that misfolded tau may act as an initiating or accelerating factor in disease progression, particularly when combined with  $\alpha$ Syn or other protein aggregates. The frequent co-occurrence of tau and  $\alpha$ Syn inclusions complicates diagnostic interpretation and may contribute to more severe cognitive impairment and faster clinical decline compared to cases with isolated pathology.

#### 8.4. Rethinking Protein Aggregation

The persistent failure of anti-aggregation therapies in clinical trials for PD and AD underscores the need to reassess the amyloid hypothesis [266]. Despite thousands of reported aggregation inhibitors, no disease-modifying therapy has succeeded clinically. This gap may reflect oversimplified models and reductionist approaches that fail to capture the complexity of aggregation in vivo, where protein misfolding occurs within a dynamic cellular environment rich in lipids, organelles, and PTMs [267,268].

Advances in cryo-EM reveal that fibrils generated in vitro differ significantly from those isolated from diseased brains, both structurally and biochemically [175,269]. LBs and amyloid plaques are not homogeneous fibrillar deposits but complex assemblies of truncated proteins, lipids, and organelles. Moreover, polymorphism in  $\alpha$ Syn and tau aggregates correlates with distinct seeding activities and possibly clinical heterogeneity, challenging the notion of a single toxic species [270].

PTMs such as phosphorylation, ubiquitination, and truncation are abundant in pathological aggregates and act as regulators rather than mere markers. Evidence suggests that phosphorylation often delays aggregation, while C-terminal truncation of  $\alpha$ Syn promotes fibril compaction and Lewy body maturation [257,271]. These findings imply that PTMs orchestrate aggregation dynamics and interactions with cellular degradation systems, influencing neurotoxicity.

Data from neuronal models indicate that fibril formation alone is insufficient to induce neurodegeneration; toxicity emerges during the transition to Lewy body-like inclusions, which disrupt mitochondrial function, synaptic integrity, and protein homeostasis [257]. This process-centric view suggests that therapeutic strategies should target early aggregation and inclusion formation rather than solely removing mature deposits, as damage likely occurs during intermediate stages.

Given the heterogeneity of aggregates and co-pathologies, a single-target approach is unlikely to succeed. Future strategies should combine aggregation inhibitors, PTM modulators, and enhancers of proteostasis while addressing neuroinflammation and metabolic dysfunction [272]. Embracing complexity—rather than avoiding it—will be key to developing effective disease-modifying therapies.

## 9. Future Studies

Several critical questions remain unresolved. When do self-propagating assemblies acquire clinical relevance? Defining the temporal threshold between silent seeding and symptomatic disease requires longitudinal studies integrating seed amplification kinetics (e.g., RT-QuIC lag phases) with neuroimaging markers of network atrophy and clinical conversion rates. Another priority is clarifying whether a single structural strain can account for clinicopathological heterogeneity, or if mixed conformers drive divergent phenotypes. This is particularly relevant given the frequent co-occurrence of proteoforms—such as 3R/4R tau and  $\alpha$ Syn—in both primary synucleinopathies and tauopathies, where dual seeding activity reaches near-universal prevalence in Lewy body disease cases examined [72,180].

Future research should also dissect the biochemical complexity of seeds, including PTMs and their impact on amplification efficiency and regional vulnerability. PTM-resolved cryo-EM and proteomics could reveal structural fingerprints that distinguish disease subtypes and predict

propagation patterns [273]. Additionally, the role of LLPS and hydrogel-like transitions in vivo remains unexplored. While in vitro studies show that condensates mature into highly resistant amyloid states, direct evidence for hydrogel-to-Lewy body conversion in brain tissue is lacking [4]. Advanced imaging and biorthogonal labeling in animal models should address whether these transitions occur physiologically and influence aggregate resilience.

The failure of single-target approaches—such as aggregation inhibitors or immunotherapies—underscores the complexity of neurodegenerative diseases. These disorders are driven not only by misfolded protein accumulation but also by systemic proteostasis collapse, neuroinflammation, and metabolic dysfunction. Targeting aggregation alone often fails because toxic intermediates, impaired clearance pathways, and inflammatory cascades remain unchecked, allowing pathology to progress despite partial reduction of fibrils. Future strategies should adopt a multi-target paradigm that combines aggregation inhibitors with proteostasis enhancers (e.g., autophagy activators, proteasome modulators) and neuroinflammation modulators (e.g., microglial phenotype regulators, inflammasome inhibitors). Such combinatorial interventions address upstream and downstream drivers of neurodegeneration, restoring protein homeostasis while mitigating secondary damage. Integrating these approaches with biomarker-guided patient stratification and systems-level modeling may overcome translational barriers and deliver durable disease modification.

Finally, the discovery of w-tau, a human-specific isoform generated by intron 12 retention, introduces a natural anti-aggregation mechanism. Its polycationic segment antagonizes polyanion-driven assembly, and reduced levels in AD brains suggest a protective role [135,274]. Structural characterization of w-tau and development of peptide mimetics could yield biomimetic strategies to competitively inhibit nucleation sites within the microtubule-binding region. These directions, combined with strain-aware modeling in iPSCs [275] and yeast systems [246], will enable mechanistic dissection of aggregation pathways and accelerate precision-targeted interventions.

## 10. Conclusions

Proteostasis is a fundamental requirement for neuronal integrity across both neurodegenerative and select non-neurodegenerative movement disorders. Protein clearance systems operate in a highly integrated manner to maintain proteome stability. Aging progressively impairs these mechanisms, while aggregation-prone proteins exploit this vulnerability, initiating a self-amplifying cycle of dysfunction. Misfolded proteins not only accumulate but also propagate through prion-like mechanisms, achieving disease-level burden and driving network-wide pathology. Their ability to seed, amplify, and maintain structural fidelity underlies distinct clinical phenotypes and experimental transmissibility. These features, combined with the interdependence of clearance pathways, underscore the complexity of targeting aggregation and proteostasis therapeutically, demanding multifactorial strategies rather than single-target interventions.

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## Abbreviations

The following abbreviations are used in this manuscript:

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
A $\beta$	Amyloid-beta
CNS	Central nervous system
HD	Huntington's disease
LB	Lewy bodies
LLPS	Liquid-liquid phase separation
MSA	Multiple system atrophy
PD	Parkinson's disease
PTMs	Post-translational modifications
UPS	Ubiquitin-proteasome system
$\alpha$ Syn	$\alpha$ -Synuclein

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