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Determinants of alpha-synuclein pathogenesis in Parkinson's disease

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Alpha-synuclein and Parkinson's disease: Neuronal damage and inflammation caused by the aggregation of alpha-synuclein (α-syn) are central to a group of disorders known as synucleopathies, which includes Parkinson's disease (PD), dementia with Lewy bodies, and multiple system atrophy, among others. PD, the most common synucleinopathy, is the second most prevalent neurodegenerative disease after Alzheimer's disease, and it is the fastest growing. Its primary hallmark is the degeneration of dopaminergic neurons in the substantia nigra pars compacta, disrupting the communication with the striatum. This has adverse motor and nonmotor effects, with the most prominent symptoms being tremors, rigidity, instability, and gait difficulties. While most patients have a late onset (60+ years), certain dominant genetic mutations in the gene encoding α-syn are associated with earlier onset. Despite the severity and prevalence of the disease, no treatments that halt or modify the pathology progression exist, with available therapies providing only symptomatic relief for motor symptoms (Vázquez-Vélez and Zoghbi, 2021).

α-Syn is a 140-residue intrinsically disordered protein widely expressed throughout the brain. Although its precise physiological function remains unknown, it has been reported to have an active role in the synapse, aiding in the maintenance of the presynaptic compartment and regulating the release of dopamine vesicles. Unlike globular proteins, α-syn aggregation does not result from destabilization and unfolding, as most of the protein is already exposed to the solvent under normal conditions. In pathogenesis, α-syn self-assembles into neurotoxic oligomers and low molecular weight protofibrils. These species can later evolve into amyloid fibrils, responsible for the cell-to-cell transmission of aggregated species, ultimately leading to extensive neurotoxicity and cell damage in the brain (Nielsen et al., 2024). Recent research has highlighted the complementary role of exosomes and microglia in the neuronal transmission of α-syn pathogenic species. Exosomes carrying pathogenic α-syn engage with microglia through Toll-like receptor 2, triggering microglial activation and facilitating the transfer of aggregated α-syn species from microglia to neurons (Xia et al., 2021). Aggregated forms of α-syn can also bind to the Fc gamma receptor IIB on microglia, impairing the clearance of aggregates. Additionally, microglia and astrocytes act synergistically, transitioning to a pro-inflammatory phenotype in response to α-syn pathogenic species (Harackiewicz and Grembecka, 2024).

Properties of alpha-synuclein regions: α-Syn consists of three distinct regions, as illustrated in **Figure 1A** and **B**: N-terminal, nonamyloid component (NAC), and C-terminal; each exhibits distinct charge and hydrophobic properties linked to their respective aggregation and pathogenesis roles. The N-terminal is mildly basic (net charge of +4) and hydrophobic, especially in the so-called P2 region. The NAC domain is almost neutral in charge (–1) and highly hydrophobic. In contrast, the C-terminal is highly acidic (net charge of –12) and hydrophilic. Numerous variants and mutants have been generated and studied to assign each region's role in α-syn pathogenesis, which we will briefly review in this perspective.

The N-terminal region, encompassing residues 1–60, is central to α-syn pathogenesis. Different truncation variants within the first 40 residues lead to significant changes in aggregation kinetics and amyloid morphology. Notably, residues 14–35 appear crucial for monomer recruitment into fibrils, as shown by cross-seeding incompatibilities observed in truncation variants involving this segment (McGlinchey et al., 2021). Mutagenesis studies on the complete α-syn sequence showed that, despite most of the N-terminus being weakly involved in the core of mature α-syn amyloid fibrils extracted from diseased brains, mutations in this region had a comparable impact on fibril formation than mutations on the NAC domain and a much higher impact than those in the C-terminus (Chlebowicz et al., 2023). This region also seems to be involved in pathogenesis through liquid-liquid phase separation, a phenomenon by which α-syn partitions into denser phases and increases its local concentration (Thrush et al., 2024). While the exact role of liquid-liquid phase separation in α-syn aggregation remains uncertain, elevated local concentrations facilitate inter-chain interactions, potentially driving amyloidogenicity.

Within the N-terminus, two arbitrarily delimited segments, P1 and P2, have been identified as key modulators or "master controllers" of α-syn aggregation (Doherty et al., 2020). Deletion of the 7-residue P1 (ΔP1) or the 13-residue P2 (ΔP2) sequences dramatically reduces *in vitro* α-syn aggregation, whereas the ΔP1–ΔP2 deletion completely prevents amyloid formation. This inhibitory effect was also demonstrated *in vivo* in *Caenorhabditis elegans* models of PD. Interestingly, most familial mutations associated with α-syn pathology occur within P2 (E46K, H50Q, G51D, A53T/E/V), highlighting the region's conformational sensitivity to mutation.

A subsequent study demonstrated that P1 and P2 are necessary to transition low-molecular-weight oligomers into fibrils. These regions adopt distinct structural conformations in neurotoxic oligomers compared to the functional monomeric form while remaining accessible and targetable (Santos et al., 2024). This finding clarifies prior observations that helical amphipathic peptides interacting with P1 and P2 selectively recognize pathogenic oligomers and fibrils without interacting with non-toxic oligomers or monomeric α-syn (Santos et al., 2021). Together, these insights point towards the P1 and P2 regions being prime pharmacological targets in the treatment of PD. Indeed, recently, a synthetic single-domain antibody (Sybody) that binds to P1 was demonstrated to inhibit α-syn amyloid formation (Gialama et al., 2024). This binder was optimized to bind to residues 36–42 using ribosome display, and its anti-amyloidogenic effect was evaluated using a Thioflavin-T assay, simultaneously selecting for both strong binding and significant delay in aggregation kinetics. As before, targeting this region enabled selective interaction with α-syn oligomers and fibrillary species, inhibiting both primary and secondary nucleation processes.

The NAC domain (residues 61–95) is the most hydrophobic region of α-syn. It is inherently amyloidogenic and sufficient to drive α-syn aggregation, with the deletion of residues 71–82 within the NAC domain effectively preventing α-syn aggregation *in vitro* (Giasson et al., 2001). The critical role of the NAC domain in α-syn aggregation is further supported by the fact that β-synuclein, a highly homologous protein with a 12-amino acid variation in the central NAC region, does not aggregate and is absent from Lewy inclusions (Biere et al., 2000).

To showcase the central role of the NAC domain in α-syn fibril formation, we examined which regions are consistently found in the fibril cores of amyloid structures deposited in the Protein Data Bank. **Figure 1C** highlights these core regions (blue) and the more flexible, less structured stretches (gray). The analysis reveals that the P1–P2-NAC regions, and to a lesser extent, other segments of the N-terminus, are rigid and almost universally present in these structures, regardless of whether formed *in vitro* or isolated from patient brain samples. In

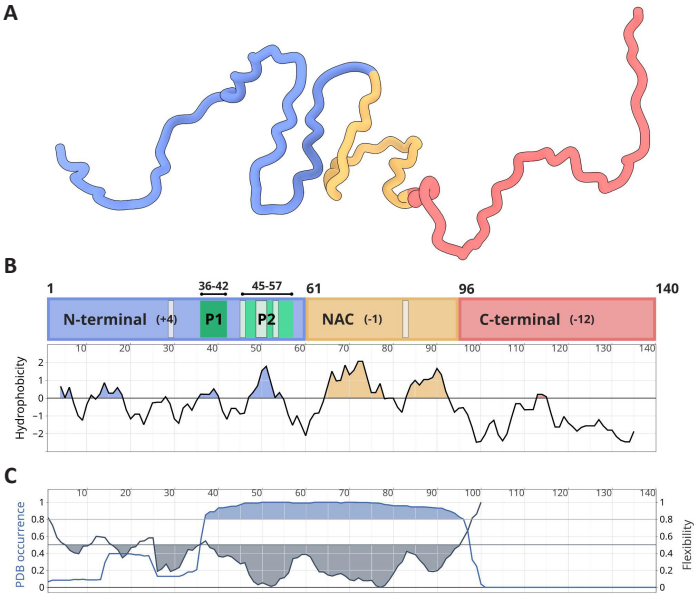

Figure 1 | **A snapshot of alpha-synuclein (α-syn) structure and properties.**
(A) Representation of α-syn structure, colored by their three regions (N-terminal (1–60, blue), NAC (nonamyloid component; 61–95, ochre), and C-terminal (96–140, red)). (B) Analysis of α-syn sequential properties. α-Syn's net charge (at pH 7) is calculated for each region. Additionally, the hydrophobicity of the sequence is presented below. To highlight the hydrophobic character, the area under the curve and above a hydrophobicity of 0 is colored by the designated colors for each region. Regions P1 (36–42) and P2 (45–57) are highlighted in bright and pale green, respectively. Familial mutation sites are highlighted in gray; from N to C-terminus: A30P, E46K, H50Q, G51D, A53T/E/V, and E83Q. (C) Analysis of α-syn amyloid Protein Data Bank (PDB) structures. In blue, we present the fraction of residues in the amyloid PDB structures; residues having over 80% PDB coverage are shaded in blue. The gray line represents the flexibility of those positions (normalized B-factor), with regions of normalized flexibility under 50% shaded in gray. As a general trend, the most hydrophobic regions are also the most rigid components within the cores of α-syn fibers, highlighting the critical role of non-polar interactions in maintaining fibril structure and stability. The hydrophobicity of the sequence was calculated using Kyte-Doolittle's hydropathy scale. α-Syn amyloid structures were compiled from AmyloidAtlas before 1 October 2024 (109 out of 111 structures, excluding nuclear magnetic resonance to standardize flexibility analysis).

stark contrast, the C-terminus is notably absent in non-nuclear-magnetic-resonance experimental structures. Despite the NAC domain's critical role in α-syn aggregation, its limited accessibility in both the oligomeric and fibrillary states makes it difficult to target. However, a peptide designed to interact with the so-called NACore (residues 69–77) has shown efficacy in blocking seeded polymerization by preventing fibril elongation at the ends (Sangwan et al., 2020).

The C-terminus of α-syn is highly flexible and has a marked negative charge. Although it does not participate in the amyloid core, it imparts a key property to aggregated species: a negatively charged, "fuzzy" outer shell (Santos et al., 2021, 2024). This charged shell prevents lateral association of aggregates and reduces monomer interactions through electrostatic repulsion. Thus, truncations at the C-terminus increase aggregation propensity, with the extent of aggregation enhancement directly proportional to the degree of truncation. Additionally, it has been demonstrated that C-terminally truncated α-syn promotes pathological accumulation of the full-length protein, and these truncated forms are enriched in protein deposits in PD patients (Sorrentino et al., 2020). The high aggregation tendency of these truncated forms has hindered the identification of effective binders to inhibit amyloidogenesis, although a recent small molecule has shown promising results (Peña-Díaz and Ventura, 2024). While the C-terminus is the most immunogenic region of aggregated species and is frequently targeted by therapeutic antibodies, their effectiveness in reducing aggregation remains limited (Nielsen et al., 2024).

Discussion and perspective: α-Syn is a disordered protein with three distinct regions defined by their physicochemical properties: N-terminus, NAC, and C-terminus. While disordered polypeptides are generally thought to adopt an extended and random structure, α-syn can transiently populate a set of metastable states with local structuration. This dynamic behavior is integral to α-syn's pathogenicity, as it modulates the transition between functional and aggregation-prone states.

The sequential features of each α-syn segment are closely related to their amyloidogenic properties. The NAC domain drives fibril formation, whereas the N and C-terminal regions play regulatory roles. On the one hand, the N-terminal region is essential for oligomer-to-fibril transition, especially the P1–P2 regions, which also concentrate most familial mutations. These two pieces of evidence suggest that tightly controlling this conformational conversion is critical to pathogenesis. Conversely, the C-terminal seems to have a gatekeeping role in reducing α-syn aggregation propensity through electrostatic repulsion and entropic exclusion.

While the aggregation of α-syn is a hallmark of PD pathology, to date, no standardized clinical assay currently utilizes α-syn as a biomarker for diagnosing or tracking disease progression in patients. Nevertheless, two potential diagnostic methods are under development. The first involves the detection of phosphorylation at serine 129, a modification shown to accumulate significantly in Lewy bodies (Oueslati, 2016). The second approach exploits the polymerization of existing aggregates in peripheral tissues or fluids using recombinant monomeric α-syn as a substrate (seeding amplification assays; Kovacs et al., 2024).

Despite the many efforts to prevent α-syn aggregation using small molecules, they have encountered substantial obstacles. First, the target is highly dynamic, precluding using a rational design approach based on the existence of defined binding pockets. Second, organic molecules are much smaller than the aggregated species they aim to target, making it challenging to block their large surfaces and preclude inter-protein interactions. These challenges have shifted the focus toward alternative therapeutic strategies, including protein-based binders such as antibodies.

Two anti-α-syn monoclonal antibodies to treat PD recently entered clinical trials: cinpanemab (Biogen, Cambridge, MA, USA) and prasinezumab (Roche, Basel, Switzerland), with different sequential targets within α-syn. Cinpanemab has been described to bind residues 1–10 with 800-fold higher affinity for aggregated species over monomeric α-syn and was identified after screening human memory B-cell libraries from healthy elderly subjects. Prasinezumab binds to the C-terminus of aggregates; it was derived from a murine monoclonal antibody that reduced α-syn neural accumulation and behavioral decline in animal models. While cinpanemab was discontinued in Phase 2 Clinical Trials in February 2021 because it did not meet its primary and secondary efficacy endpoints, prasinezumab trials are ongoing, with modest efficacy. Despite these limited outcomes, another immunotherapy to treat Alzheimer's disease, lecanemab (Biogen), has reached the market in certain world regions, with some associated security concerns. This monoclonal antibody binds to oligomeric species, thus differing mechanistically from the monoclonal antibodies used to date in PD that broadly target aggregated species.

The evidence presented in this perspective illustrates the deep mechanistic insights needed to advance anti-amyloidogenic therapies, which is behind the current lack of effective treatments for these prevalent yet incurable diseases. Potential therapeutic strategies include small molecules, peptides, and antibodies, with binding selectivity and affinity increasing along this progression. However, the challenges associated with brain penetration also escalate in the same order. Given the accessibility and differential structural features of the P1 and P2 regions in oligomeric and amyloid forms — distinct from those in monomeric α-syn, they represent new targets for inhibiting α-syn aggregation while minimizing off-target interactions with monomeric α-syn. Thus, strategies targeting these regions hold the potential for greater efficacy than current approaches, and we envision that they may enable the future development of disease-modifying treatments for PD.

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