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REVIEW ARTICLE

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A glimpse into the structural properties of α-synuclein oligomers

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Abstract

α-Synuclein (αS) aggregation is the main neurological hallmark of a group of debilitating neurodegenerative disorders, collectively referred to as synucleinopathies, of which Parkinson's disease is the most prevalent. αS oligomers formed during the initial stages of aggregation are considered key pathogenic drivers of disease onset and progression, standing as privileged targets for therapeutic intervention and diagnosis. However, the structure of αS oligomers and the mechanistic basis of oligomer to fibril conversion are yet poorly understood, thereby precluding the rational formulation of strategies aimed at targeting oligomeric species. In this review, we delve into the recent advances in the structural and mechanistic characterization of αS oligomers. We also discuss how these advances are transforming our understanding of these elusive species and paving the way for oligomer-targeting therapeutics and diagnosis.

KEYWORDS

amyloid, oligomers, Parkinson's disease, protein aggregation, α-Synuclein

1 | INTRODUCTION

The synucleinopathies are a group of neurodegenerative disorders whose main histopathological signature is the accumulation of α-synuclein (αS) aggregates in neurons, nerve fibers, or glial cells. Parkinson's disease (PD) is the most prevalent disorder of this group of syndromes, which also includes dementia with Lewy bodies (DLB), multiple system atrophy (MSA) and juvenile onset synucleinopathy (JOS).[1,2] Additionally, αS deposits have been found in a number of patients suffering from Alzheimer's

Abbreviations: Aβ, amyloid-β peptide; cryoEM, cryoelectron microscopy; DLB, dementia with Lewy bodies; EGCG, (-)-EpiGalloCatechin-3-Gallate; fPD, familial Parkinson's disease; FRET, Forster resonance energy transfer; FTIR, Fourier transform infrared spectroscopy; IAPP, islet amyloid polypeptide; JOS, Juvenile onset synucleinopathy; MSA, multiple systemic atrophy; NAC, non amyloid-β component; P1, α-Synuclein region (36-42); P2, α-Synuclein region (45-57); PD, Parkinson's disease; SAXS, small-angle x-ray scattering; smFRET, single-molecule Förster resonance energy transfer; ssNMR, solid-state nuclear magnetic resonance; WT, wild-type; αS, α-Synuclein.

disease.[3,4] PD is the second most prevalent neurodegenerative disorder, and it is considered one of the fastest growing neurological disorders worldwide. This alarming reality has made PD the most studied synucleinopathy and great efforts are directed toward unraveling its complexities and developing effective clinical interventions.

PD etiology is considered multifactorial and involves an intricate balance between genetic, epigenetic and environmental factors affecting disease onset and progression. In spite of this, the main neuropathological hallmark of PD is the accumulation of intracellular deposits of aggregated proteins in neurons, known as Lewy bodies (LB) and Lewy neurites.[5,6] αS is the principal component of LB and its aggregation elicits toxicity and subsequent cellular death.[7,8] αS is also genetically linked to PD since hereditary single point mutations in the SNCA gene encoding αS and SNCA gene duplications lead to autosomal dominant familial Parkinson's disease (fPD).[9–17] Polymorphisms in the SNCA gene were also defined as risk factors in genome-wide analysis.[18] Consistently, injection of αS fibrils in mouse brains is reported to induce the formation of αS inclusions and a PD-like phenotype.[19,20]

Overall, αS aggregation is considered a keystone process in PD and therefore, targeting αS aggregates stands as an appealing avenue for both diagnosis and therapeutics. αS oligomers are increasingly identified as the primary effector of cellular dysfunction,[21,22] whereas fibrils can self-propagate and act as a vehicle for the prion-like spreading of the disease.[19,20] Considering our current structural knowledge about oligomers, the development of oligomer-focused strategies is still challenging. Luckily, recent advances in the last years have significantly contributed to our understanding of αS oligomers. In this manuscript, we provide an updated review of the oligomer field focused on the recent advances in the structural and biophysical properties of αS oligomers.

2 | KINETICS OF AMYLOID FORMATION

The process of amyloid fibril formation involves the transition of proteins from their soluble monomeric state to the macromolecular amyloid state. This complex process is highly dependent on the intrinsic sequence features and strongly modulated by environmental conditions. External factors such as pH, salt concentration, or the presence of lipid membranes strongly reshape the kinetic profile of amyloid formation.[23–25]

Macroscopically, in most cases, the kinetics of amyloid formation can be described as a nucleation-dependent polymerization with three well-defined phases[26] (Figure 1A): (i) A first *nucleation phase* (also known as lag phase) defined as the kinetically unfavorable association of monomers to form the first aggregation nucleus able to self-propagate. Accordingly, the addition of preformed fibrils skips this nucleation phase. (ii) The *elongation phase* in which these first nuclei elongate by monomer addition in a fast and thermodynamically favorable process that leads to exponential amyloid fibril formation. (iii) The *plateau phase* when fibril growth stops due to monomer exhaustion. These three phases result in the characteristic sigmoidal profile observed in time-dependent fibril formation experiments.

However, microscopically, this process is far more complex[27,28] (Figure 1B). The formation of the first nucleus involves the association of few precursors to form oligomeric species that can dissociate back to monomers or interconvert to other oligomers (that can be on- or off-pathway to the amyloid fibril) before the formation of the first elongation-competent nucleus[29–31] (Figure 1B). Then, the elongation phase not only includes monomer addition to the tips of the fibrils (Figure 1B) but also secondary pathways such as fragmentation or surface catalyzed secondary nucleation that lead to the formation of new nuclei[32,33] (Figure 1B). Fibrils can also dissociate back into monomers or release oligomers[31,34] (Figure 1B).

Although the mechanistic bases of these processes are still a subject of intense research, recent mathematic and analytical efforts thrived to describe this intricate phenomenology of associative, dissociative and structural transitions in terms of chemical kinetics and microscopic rate laws.[29,35,36] This understanding is instrumental to shed light into the aggregation process and discover new ways to fight it.[37–40] Unfortunately, this kinetic description is still waiting for structural and mechanistic information of these microscopic conversions, being this a large unmet need in the field.

3 | THE AGGREGATION MIXTURE: COEXISTENCE OF MONOMERS, OLIGOMERS, AND FIBRILS ACROSS THE AGGREGATION REACTION

Studying the species populated along an aggregation reaction is challenging. Whereas it is possible to study isolated preparations of monomeric proteins or mature amyloid fibrils at the end of aggregation reactions, intermediate species are usually low populated, transient and coexist with a mixture of diverse monomeric and aggregated forms.[29,31] When describing an aggregation reaction, it is common to think in sequential steps where we

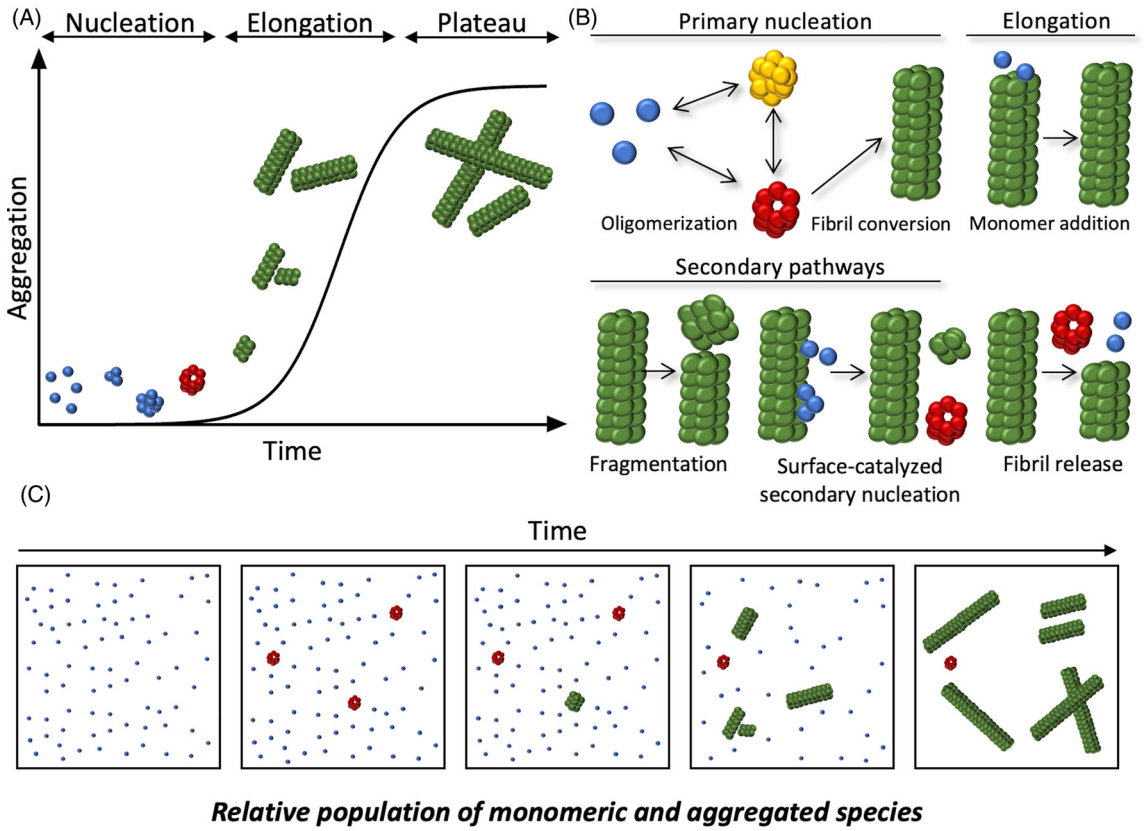

FIGURE 1 (A) Illustration of the sigmoidal aggregation profile representing the three macroscopic phases of amyloid formation. Monomers, oligomers and fibrils are represented in blue, red and green, respectively. (B) Microscopic reactions occurring in amyloid formation. (C) Schematic representation of the different species coexisting in an aggregation reaction.

have monomers, then oligomers, small fibrils and finally the amyloid fibrils. One should, however, keep in mind that these species are populated simultaneously and at different concentrations, creating a mixture here referred to as "aggregation soup" (Figure 1C).

Once the aggregation reaction begins, oligomeric species—a heterogenous mixture of structurally diverse oligomers- start forming and accumulating up to 10% of the total protein.[29] During this period, oligomers coexist with >90% of monomeric protein. After the formation of the first fibrillar nucleus and the beginning of the elongation phase, monomers, oligomers, and fibrils coexist at different concentrations until monomer exhaustion in the steady phase. Therefore, for most of the aggregation reaction, several species are simultaneously populated. On top of that, oligomers are often in equilibrium with monomers which implies that monomer exhaustion causes oligomer dissociation.[29]

It is then highly challenging to identify, quantify, characterize or isolate intermediate species. They are difficult to purify due to the excess of other species and in the process a significant part of the population dissociates back into monomers or progresses to fibrillar aggregates. Likewise, the contribution of low populated oligomers is usually not reflected in ensemble averaged experimental techniques, hidden by the monomer signal.

In this scenario, many of the advances we have witnessed in the last decade relied on two pillars; the use of single-molecule approaches to quantify and characterize low-populated species, and the development of protocols that allow the preparation of isolated oligomeric samples for structural characterization. For instance, the kinetic analyses discussed in the previous section stem from oligomer quantification using single-molecule approaches. Overall, the aggregation soup concept illustrates the challenges and constrains of studying amyloid oligomers.

4 | GENERAL PROPERTIES OF AMYLOID OLIGOMERS

In the amyloid field, the term "oligomer" is commonly used to englobe a myriad of relatively low molecular weight prefibrillar aggregates populated during the nucleation phase. The specific aggregation determinants of each particular amyloidogenic protein, together with the

multiplicity of pathways that can lead to amyloid formation, result in an extremely high level of structural diversity in the oligomer universe. This heterogeneity is reflected in terms of size (i.e., dimers, tetramers, high order multimers), β-sheet content (from fully disordered to amyloid-like contents), degree of solvent exposed hydrophobicity, amyloid dye binding, relative life-time in the aggregation reaction (transient or more persistent) and elongation capacities.[29,41,42] On a practical level, it implies that it is intrinsically difficult to define common structural and physicochemical traits shared by all oligomeric species, which contrast the well-defined features of the amyloid state.

Despite their diversity, there are two key features of oligomers that help to explain the increasing association of oligomers with cellular dysfunction. (i) Compared to fibrils composed of thousands of monomer copies, oligomers have a much lower molecular weight and therefore a higher diffusion coefficient. Their higher diffusivity implies a higher chance to establish aberrant interactions with cellular components, easier cell-to-cell transmission and cellular uptake.[43] (ii) Fibrils are at a thermodynamic global minimum in the free energy landscape, being very stable and difficult to disaggregate.[44,45] This also implies that they are less prone to interact with other cellular components compared to metastable oligomers that display higher dynamism and might expose hydrophobic regions to the solvent.

From a kinetic perspective, amyloid oligomers have been shown to be critical intermediates in the formation of amyloid fibrils.[29] In various amyloidogenic proteins, the relative concentrations of monomers, oligomers and fibrils can be fitted to a model of oligomer-mediated fibril formation that proves their kinetic importance in the reaction.[29] These analyses also demonstrate that oligomers are nonfibrillar species that need a structural transition to maturate into amyloid fibrils before being able to rapidly grow by monomer addition. This is not a unidirectional process, and the majority of oligomers do not evolve into fibrils; they dissociate back to monomers or persist in the oligomeric state due to a relatively high kinetic stability. Even though in the literature, the term "oligomer" is also assigned to any small fibrillar and prefibrillar aggregates,[41] this kinetic analysis provides a stricter definition of amyloid oligomers as nonfibrillar and non-capable of elongation metastable intermediates, that better reflects the type of oligomers discussed here.

This scenario also indicates that the oligomer to fibril transition is a rate-limiting step that supposes a major barrier to fibril formation and therefore, it constitutes a privileged target for therapeutic intervention in amyloid-linked diseases. By targeting a small population of oligomers and blocking this step, the kinetic productivity of amyloid formation will be severely reduced.

Unfortunately, we currently have very limited structural information on amyloid oligomers. The cylindrin β-barrel constitutes the first high resolution evidence of a globular oligomer but only corresponds to a 11-residue protein fragment, having thus limited biological relevance.[46] Aβ42 (Amyloid-β peptide) tetramers and octamers formed in the presence of a detergent (Dodecylphosphorylcholine) have also been determined by NMR but it is not clear if they represent a relevant conformation or have a kinetic role in the aggregation.[47] Thus, to date, there is no atomic structure of a natural full-length oligomer in solution. The dynamic and transient nature of oligomers generally precludes a high-resolution characterization, even if certain structural traits have been extracted from solid-state nuclear magnetic resonance (ssNMR), Fourier transform infrared spectroscopy (FTIR), small-angle x-ray scattering (SAXS), cryoelectron microscopy (CryoEM) and atomic force microscopy analysis.[48–50] For instance, it is worth noting that a significant number of amyloidogenic proteins have been reported to form oligomers with a doughnut-like shape, including Aβ, αS, IAPP and serum amyloid A.[51–53]

Regarding their implication in disease, oligomers are thought to be important pathogenic species in the onset and progression of amyloid related disorders. Generally, oligomers are associated with gain-of-toxicity activities such as membrane disruption,[54] induction of oxidative stress,[55] proteasome impairment[56] and mitochondrial dysfunction.[57] Accordingly, oligomeric forms of different amyloidogenic proteins have been identified in the tissue of patients suffering from neurodegenerative disorders and in correlation with cellular dysfunction.[21,22,58–61] Yet, it is not clear if oligomers can propagate the disease in a prion-like manner as amyloid fibrils do.[19] Considering this, it is now hypothesized that oligomers are the primary cytotoxic agents, whereas amyloid fibrils are responsible for disease spreading; a framework in which oligomer formation occurs via fibril surface catalysis or amyloid disaggregation is attracting increasing attention.[34,62]

5 | α-SYNUCLEIN DOMAINS AND AGGREGATION

αS primary sequence is typically divided in three domains according to their differential physicochemical properties and activities (Figure 2A). The N-terminal domain[1–60] is an amphipathic and lysine-rich region that participates in membrane interactions adopting a helical conformation[63] (Figure 2B). The NAC (Non amyloid-β component)

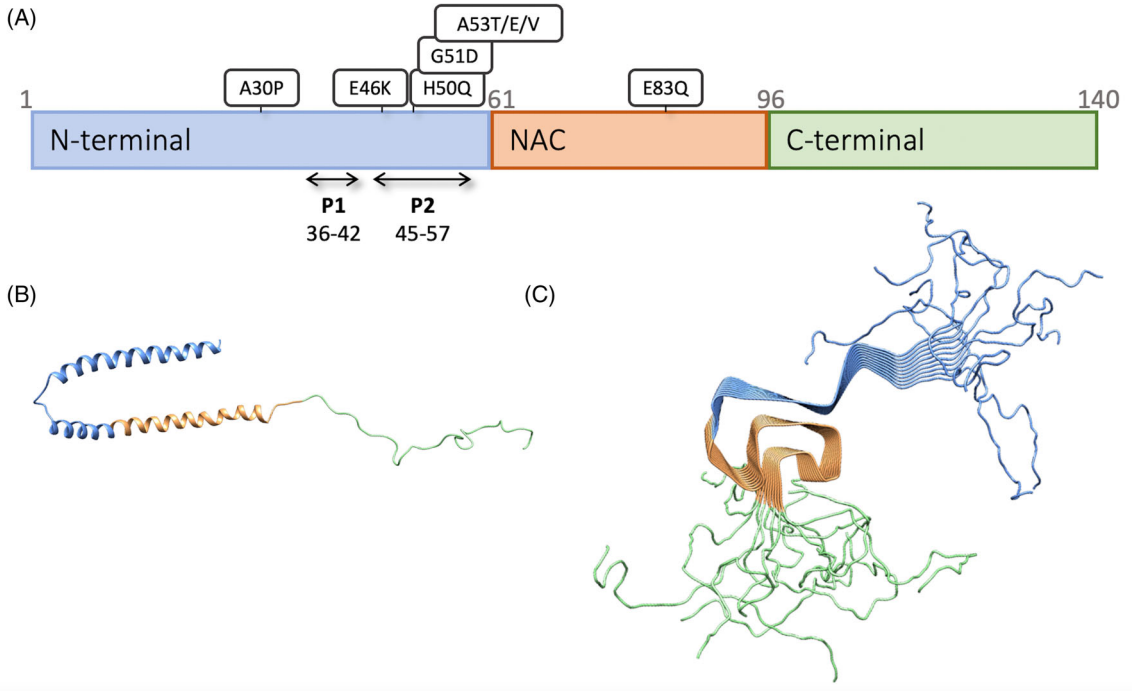

FIGURE 2 α-Synuclein sequence and structure. (A) Schematic domain organization of αS. fPD mutations and P1 and P2 regions are indicated. (B) Structure of a micelle-bound αS monomer (PDB: 1XQ8). (C) Structure of an αS fibril determined by solid-state nuclear magnetic resonance (PDB: 2N0A). The three domains are color-coded according to (A).

region (61–95) has a marked hydrophobic character and can also be incorporated into the helical structure in the membrane bound state.[63] Finally, a very flexible C-terminal domain that is highly anionic and remains disordered both in solution and in the membrane bound state.[63,64] αS contains seven imperfect repeats (KTKEGV) covering the N-terminal and part of the NAC region, responsible for the hydrophobicity patterning driving membrane binding.[65] Charge distribution is uneven across the sequence with +4 in the N-terminal, −1 in the NAC and −12 in the C-terminal.

This asymmetric organization of the αS biochemical properties is also related to its aggregation. The NAC domain is considered the principal element driving αS aggregation (Figure 2C), being essential and sufficient for amyloid fibril formation.[66] This region also forms the core of αS fibrils.[67,68] However, full-length αS aggregation requires the contribution of other regions. The N-terminal domain is gathering increasing attention due to recent reports identifying regions modulating amyloid formation. The extreme N-terminal residues,[1–11] are fundamental for monomer recruitment to oligomers and fibrils, actively participating in surface-catalyzed secondary nucleation.[69,70] Accordingly, extreme N-terminal truncations delay amyloid formation. It was recently reported that 2 N-terminal segments, named P1[36–42] and P2,[45–57] act as master regulators of amyloid formation; P1 deletion or single alanine substitutions at positions Y39 and S42 inhibit amyloid formation.[71,72] The effect of P2 deletion is milder but acts synergistically with P1. This renewed interest in the modulatory N-terminal domain also stems from the fact that most single point mutations associated with familial PD (fPD) map into this region (Positions 30, 46, 50, 51, 53), while only the recently identified mutation at position 83 is in the NAC domain[16] (Figure 2A). In contrast, the C-terminal tail acts as a solubilizing element due to its extremely anionic character.[73,74] C-terminal truncations or charge compensation by pH or ionic strength increase the aggregation propensity.[75,76]

In solution, monomeric αS is an intrinsically disordered protein that populates an ensemble of compact states with a smaller radius of gyration than expected if it was an extended random coil.[77,78] This compaction is driven by long range interactions involving distinct domains, with identified contacts between the N- and C-terminal domain and both of them with the NAC.[78–80] These tertiary contacts are thought to be fundamental to maintain αS solubility in the cell by shielding the hydrophobic residues of the amyloidogenic NAC region. This protective mechanism -similar to burying the hydrophobic core in globular proteins- explains why αS can accommodate such a high amyloidogenic load and justifies the chaperoning activity of the C-terminal domain.

6 | α-SYNUCLEIN OLIGOMERS

The observation of small spherical and/or annular oligomers in αS aggregation reactions in both Wild-type (WT) and fPD-associated variants more than 20 years ago prompted the hypothesis that these species may have an important role in cellular dysfunction in PD[52,81] (Figure 3A). The annular shape of these oligomers suggested that they may behave as pore-forming bacterial toxins and cause membrane permeabilization. These annular oligomers have also been observed in post-mortem biopsies from patients with MSA.[58] Yet, their study and characterization using conventional techniques has been hampered by their low-population and transient nature. Many approximations were developed to trap oligomers of diverse morphologies through the use of chemical compounds, covalent crosslinking or modification of the solvent conditions (detailed review in[82]), but it was unclear to which extent those trapped oligomers recapitulate the properties of those populating standard aggregation reactions.

Single molecules techniques boosted the field by allowing quantitative and qualitative analysis of the populations of oligomers formed during aggregation reactions. Under standard aggregation conditions (Neutral pH, 37°C and under agitation), two populations of oligomers (named type-A and type-B) are sequentially populated during αS lag phase, showing distinct structural arrangements, as evidenced by their different single-molecule Förster resonance energy transfer (smFRET) signature[31] (Figure 3B). smFRET efficiency is inversely related to the sixth power of the distance between the fluorescence labels, being a sensitive method to distinguish different oligomer's conformations. The initially formed type-A oligomers have a low-FRET-efficiency, are protease sensitive and devoid of cellular toxicity. Type-A oligomers later convert into protease resistant, high-FRET-efficiency type-B oligomers that elicit high cellular toxicity. Recently, Choi and coworkers exploited smFRET to observe the formation of type-A oligomers and their conversion to type-B oligomers inside neurons, which confirmed the biological relevance of these oligomers.[21] Type-B oligomers are then considered disease relevant oligomers that can be assembled by αS under physiological conditions.

Oligomers with similar smFRET signatures than type-B can also be released from amyloid fibrils.[31] Notably, such oligomer release has been shown to account for fibril toxicity indicating, that fibrils can act as reservoirs of these toxic assemblies.[34]

Leveraging smFRET information, Chen et al. developed a new strategy to obtain stable and kinetically trapped oligomers structurally analogous to type-B (named type-B* to reflect their kinetically trapped nature).[48] Type-B* oligomers show the same smFRET signature as type-B, indicating an equivalent molecular architecture, and have the same annular morphology (Figure 3C). Type-B* oligomer preparations only require a lyophilization step of monomeric αS followed by resuspension in phosphate-buffered saline, incubation for 20 h

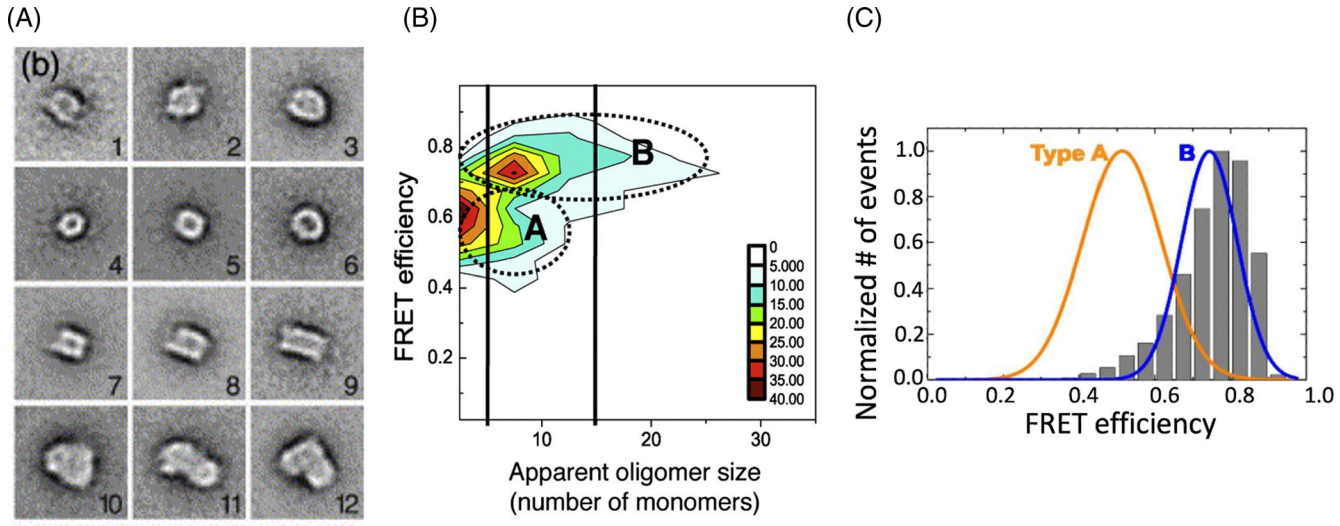

FIGURE 3 α-Synuclein oligomers. (A) Microelectron micrographs of αS oligomers. Reproduced with permission from Ref. [81]. (B) 2D plot of the number distribution of oligomers after 60 h of incubation showing their FRET efficiency distributions (vertical lines corresponding to apparent oligomer sizes of 5- and 15-mer). Two oligomer populations (named A and B) can be identified. Reproduced from Ref. [31]. (C) smFRET efficiency distribution of type-B* oligomers (shown in gray bars) and the two main oligomeric species (type-A and type-B) found during α-synuclein fibril formation. Reproduced with permission from Ref. [48].

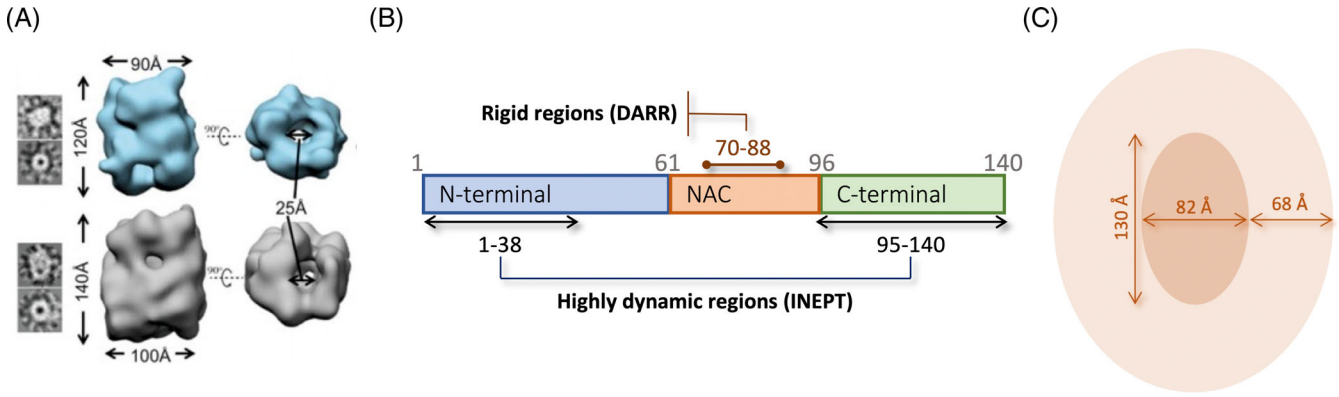

FIGURE 4 Structural features of α-synuclein oligomers. (A) CryoEM 3D reconstruction of type-B* oligomers. Two size populations are represented in blue and gray. Reproduced with permission from Ref. [48]. (B) Schematic representation of ssNMR assignments in type-B* oligomers as reported in Ref. [49]. (C) SAXS reconstruction of type-B* oligomers. Adapted from Ref. [74].

at 37°C and centrifuge-based separation. Purified type-B* oligomer preparations are stable for days, contain around 90% of the sample in the oligomeric state -the rest is monomeric- and can be produced at yields sufficient for structural characterization (1% of total initial protein). Importantly, type-B* oligomers reproduce the cellular toxicity observed in type-B oligomers, being commonly used as the model αS oligomers in cellular and animal-related experiments.[19,48,83,84]

Additionally, the inhibitory molecule (-)-EpiGalloCatechin-3-Gallate (EGCG) has been used to produce kinetically trapped type-A* oligomers with a smFRET signal equivalent to type-A, being used as non-toxic counterparts of type-B* oligomers.[49] Unfortunately, EGCG mechanism of action is unclear, restraining the relevance of the structural and biophysical information that can be obtained.

7 | STRUCTURAL FEATURES OF TYPE-B* KINETICALLY TRAPPED α-SYNUCLEIN OLIGOMERS

An essential requirement for structural studies is the isolation of well-defined and stable oligomeric samples. For this reason, type-B* oligomers have emerged as the most extensively investigated model for αS oligomers characterization.

The analysis of type-B* oligomers revealed critical structural features of these toxic assemblies. Reconstruction of type-B* oligomers by cryoEM rendered a cylindrical shape architecture with a central hollow core[48] (Figure 4A). Two populations of different sizes were identified in agreement with analytical sedimentation experiments. These oligomers had an average of 19 or 29 αS monomers respectively and dimensions ranging from 120 to 140 Å in length and 90 to 100 Å in diameter. The central cavity had approximately 25 Å. Since disordered segments are averaged out in cryoEM reconstructions, SAXS measurements of type-B* oligomers were essential to characterize the outer shell of disordered tails that surrounds the denser core of the oligomer[74] (Figure 4B). The anionic C-terminal tail of αS, known to be intrinsically disordered in the oligomer, gives a negative character to this unfolded fuzzy coat. This outer unstructured corona is not visible by EM but contributes to a significant part of the oligomer volume and protects them from lateral associations.[85] Such repulsion between oligomers' anionic fuzzy coats probably explains why these oligomers can be manipulated at the high concentrations required for cryoEM or ssNMR without undesired associations.

Analysis of αS type-B*oligomers using FTIR spectroscopy revealed ≈ 35% of β-sheet content with a predominantly antiparallel geometry that contrast the parallel β-sheet observed in all αS polymorphs.[48] This orientation was recurrently observed in other oligomers formed by αS and several other amyloidogenic proteins, including the cylindrin β-barrel.[46] This apparent structural contradiction is, however, in line with the here discussed nonfibrillar nature of oligomers (Section 4)[29]; the parallel β-sheet signature in αS amyloids arises from the contacts between different fibril layers and such contacts are not expected in a nonfibrillar oligomer. Consistently, αS type-B*oligomer preparations show marginal Thioflavin-T binding.[48] Together, the kinetic and thermodynamic metastability of αS oligomers can be explained by the energy barrier imposed by the structural divergence between the contacts sustaining the oligomer and fibril structure.

The solvent-exposed hydrophobicity of αS type-B* oligomers exceeds that of the fibrils and monomers and has been proposed to account for oligomer promiscuous

interactions and subsequent cellular toxicity.[48,86] Type-B* oligomer toxicity has also been associated with membrane binding, known to be mediated by the N-terminal domain and impaired by extreme N-terminal deletion and the genetic A30P mutation associated with fPD. Additionally, type-B* oligomers induced cellular toxicity has been associated with lipid binding, metal interactions and Ca^{2+} dysregulation.[49,55,87]

The rigid core of the oligomer is formed by residues 70–89 as probed by magic-angle spinning ssNMR ^{13}C–^{13}C dipolar-assisted rotational resonance[49] (Figure 4C). Highly mobile regions corresponding to residues 1–38 and 95–140 were assigned using insensitive nuclei enhanced by polarization transfer (Figure 4C). Based on this analysis, only 15% of the αS sequence is sufficiently rigid for ssNMR characterization, illustrating the structural heterogeneity and fuzziness of αS oligomers. Thus, a significant portion of the αS sequence populates a continuum of disordered and partially folded states that surround the core of oligomers. Overall, this structural dynamism is the major impediment in the structural analysis of oligomers, constraining these efforts to low-resolution analysis.

8 | IMPACT OF GENETIC MUTATIONS IN OLIGOMER STRUCTURE

Genetic mutations associated with fPD (A30P, E46K, H50Q, G51D, and A53T) have been reported to affect oligomers structure, generating oligomer polymorphism.[88] Whereas the overall structural features of these mutants in terms of size and antiparallel β-sheet content are relatively similar, with only slightly different population distributions or relative β-sheet content, the most remarkable difference observed in this work was captured by circular dichroism spectroscopy. G51D oligomers presented a batch-dependent α-helical contribution that contrasted with the β-sheet rich conformation observed in the WT. Such structural variation was associated with decreased solvent exposition of hydrophobic regions. In A30P and E46K oligomers a certain deviation toward helical structures can also be intuited.[88] A30P has also been reported to reduce oligomer binding to lipids.[49] This conformational heterogeneity evidences certain plasticity in αS oligomers while preserving an antiparallel β-sheet core. Noteworthy, G51D oligomers displayed higher cellular toxicity on SH-SY5Y cells, decoupling the previously established association between hydrophobicity and toxicity. Together with the previous observation that G51D mutation reduces oligomer's membrane disruption properties,[89] it can be proposed that oligomer toxicity is not unequivocally associated with membrane disruption.

9 | CONCLUSION

Decoding how oligomers are assembled and identifying the specific contacts that stabilize them, and the αS regions engaged in these interactions is pivotal, not only for disease-related implications, but also to understand the intricate biology of αS and its interplay with evolution. Likewise, obtaining mechanistic and structural information on oligomer to fibril transitions could delineate a therapeutically addressable process to block amyloid progression. Type-B* oligomers have become the benchmark for structural and functional characterization of αS oligomers, being an invaluable model system to address these questions.

However, the field is marked by debate, primarily because the reasons behind the kinetic trapping of these oligomers remain ambiguous. Additionally, questions arise about how accurately they replicate αS oligomers generated in standard aggregation reactions. Consequently, it is imperative to validate the significance of type-B* oligomers in offering insights into disease-relevant αS oligomerization. Noteworthy, it has been demonstrated that molecular binders designed toward type-B* oligomers also bind oligomers that aren't kinetically trapped, inhibiting their transformation into fibrils.[90] This indicates that type-B* oligomers are suitable -or at least "sufficient"—models for understanding the structural biology of αS oligomers and developing molecular binders relevant for therapeutics and diagnostic purposes.

In conclusion, we are optimistic that the continuous advances in structural biology techniques and machinery, combined with a deeper understanding of oligomers' properties will allow us to access higher resolution information. We are confident that such progress will serve as a cornerstone in forging novel oligomer-oriented therapies and diagnostic tools.

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CONFLICT OF INTEREST STATEMENT

SV, IP and JS have submitted a patent protecting the use of helical peptides for PD therapy and diagnosis. Request number: EP20382658. Priority date: July 22, 2020.

DATA AVAILABILITY STATEMENT

No new data has been generated in this Review article.

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