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*Review paper*

# Alpha-synuclein pathobiology and therapeutic targeting in Parkinson's disease

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## Abstract

**Background and purpose:** Alpha-synuclein (α-Syn) is a presynaptic protein involved in synaptic vesicle trafficking, neurotransmitter release, and membrane remodelling. Its misfolding and pathological aggregation are central to Parkinson's disease (PD) and related synucleinopathies. This review examines how structural changes, post-translational modifications (PTMs), and interactions with proteins, lipids, metal ions, and cellular organelles regulate α-Syn function and contribute to neurodegeneration and evaluates the implications of these mechanisms for biomarker development and therapeutic targeting. **Experimental approach:** This review synthesizes and critically discusses existing literature on α-Syn structure and physiological functions, molecular interactions, aggregation pathways, cellular toxicity, and the roles of PTMs in protein homeostasis and disease progression. It also evaluates emerging diagnostic approaches, including seed amplification assays and analyses of cerebrospinal fluid, blood, and extracellular vesicles, alongside therapeutic strategies targeting α-Syn aggregation, clearance, and expression. **Key results:** The reviewed evidence indicates that α-Syn pathobiology arises from the interplay of conformational plasticity, molecular interactions, PTMs, and impaired cellular proteostasis. These processes contribute to mitochondrial dysfunction, lysosomal impairment, neuroinflammation, and the propagation of pathological α-Syn assemblies. Seed amplification assays and other molecular biomarker approaches show promise for improving detection and characterization of synucleinopathies, while immunotherapies, aggregation inhibitors, gene-based interventions, and strategies that enhance protein clearance represent potential disease-modifying approaches. However, diagnostic standardization and the demonstration of unequivocal clinical efficacy remain important challenges. **Conclusion:** Integrating α-Syn molecular biology with advances in biomarker development and therapeutic research provides a framework for understanding disease heterogeneity and identifying mechanism-based interventions. This review highlights the need to move beyond reducing α-Syn burden toward selectively preventing pathogenic conformations while preserving its physiological functions. Further validation in clinical studies is essential to establish the diagnostic utility and therapeutic effectiveness of these approaches.

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## Keywords

Protein misfolding; protein aggregation; proteostasis; synucleinopathies; disease-modifying therapies

## Introduction

Neuronal communication is fundamental to brain function and relies on the precise regulation of neurotransmitter release at chemical synapses. This process is mediated by the synaptic vesicle fusion

machinery, in which the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex plays a central role in facilitating vesicle docking and membrane fusion. Alpha-synuclein (α-Syn), an abundant presynaptic neuronal protein, is closely associated with this machinery and has been implicated in the regulation of synaptic vesicle dynamics and neurotransmitter release. Beyond its physiological functions, α-Syn is also associated with neurodegenerative disorders, particularly Parkinson's disease (PD), in which its misfolding and aggregation are linked to synaptic dysfunction and the progressive degeneration of dopaminergic neurons [1].

α-Syn, encoded by the SNCA gene (also known as PARK1 in the context of PD genetics), is a 140-amino-acid protein with a molecular weight of approximately 14.46 kDa. Structurally, it consists of three major domains with distinct physicochemical properties and functional roles. The N-terminal membrane-binding domain (residues 1-60), which contains amphipathic helices crucial for interactions with lipid membranes. The hydrophobic non-amyloid-beta component (NAC) domain (residues 61-95), which is essential for α-Syn aggregation and fibril formation. The hydrophilic C-terminal domain (residues 96-140), which has been implicated in the chaperone-like activity of α-Syn and is involved in protein-protein interactions (PPIs) [2].

α-Syn is predominantly expressed in the brain, with substantial expression in several brain regions, including the neocortex, hippocampus, substantia nigra (SN), thalamus, and cerebellum. Under pathological conditions, α-Syn can misfold and aggregate into intracellular inclusions known as Lewy bodies (LBs), which are a characteristic neuropathological feature of PD and other synucleinopathies [3]. However, α-Syn is also present in peripheral tissues, including the gastrointestinal tract and liver, suggesting its biological functions and potential pathological effects may extend beyond the brain [4].

Emerging evidence suggests that α-Syn exhibits prion-like properties, including conformational misfolding, self-propagation, and intercellular transfer, which may facilitate the recruitment and aggregation of endogenous α-Syn in recipient cells and distant brain regions. Although α-Syn does not exhibit classical prion transmission between individuals, experimental and clinical observations suggest that its pathological accumulation may involve peripheral tissues and neural pathways connecting the periphery to the central nervous system (CNS). In particular, the gut and olfactory system have been proposed as potential sites of pathological initiation, with subsequent propagation along neural pathways potentially contributing to the progression of α-Syn pathology [5-7]. These observations have prompted investigations into the gut-brain and liver-brain axes as potential routes through which peripheral α-Syn may influence CNS pathology. However, the extent to which peripheral α-Syn accumulation initiates or contributes to disease progression remains under investigation [4].

Beyond its association with neurodegenerative pathology, α-Syn participates in several physiological processes, particularly those related to synaptic function and cellular homeostasis. At presynaptic terminals, α-Syn has been implicated in regulating synaptic vesicle dynamics, neurotransmitter release, and SNARE complex assembly, although the underlying mechanisms and functional consequences depend on its molecular conformation, concentration, and cellular environment. α-Syn has also been associated with cellular responses to oxidative stress, lipid metabolism, and the regulation of dopamine homeostasis. Furthermore, emerging evidence suggests that α-Syn may influence calcium-dependent signalling through interactions with calmodulin (CaM) and participate in metabolic processes beyond the nervous system, including glucose regulation in pancreatic β-cells. These observations indicate that α-Syn functions in a context-dependent manner, with its structural properties and interactions with cellular components contributing to its diverse physiological activities [8].


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The functional properties of α-Syn are closely associated with its interactions with other biomolecules, including proteins, lipids, and metal ions. For example, its interaction with synaptobrevin-2 (also known as VAMP2) has been implicated in the regulation of SNARE complex assembly and synaptic vesicle function. In addition, interactions with calcium and membrane lipids can influence α-Syn conformational dynamics, membrane binding, and aggregation propensity. Depending on the molecular environment and interaction partners, these processes may contribute to the regulation of its physiological functions or promote conformational changes associated with pathological aggregation. Consequently, understanding the biochemical and biophysical determinants of α-Syn interactions is essential for elucidating how its molecular properties influence cellular function and contribute to disease-associated processes [9].

Given the diverse physiological functions and pathological properties of α-Syn, considerable attention has been directed toward understanding the molecular determinants underlying its involvement in PD and other synucleinopathies [3]. This review focuses on the biophysical, biochemical, and structure-function properties of α-Syn, integrating evidence on its molecular structure, conformational dynamics, physiological functions, genetic mutations, interactions with proteins, lipids, and ions, post-translational modifications (PTMs), and prion-like properties. Particular emphasis is placed on how these molecular characteristics influence α-Syn membrane interactions, synaptic activity, conformational stability, and aggregation behaviour. Rather than providing a general overview of α-Syn-associated disease mechanisms, this review examines how its structural and biochemical properties relate to physiological function and pathological aggregation. By integrating these perspectives, we aim to provide a molecular framework for understanding α-Syn biology and identifying potential therapeutic opportunities targeting its conformational transitions, molecular interactions, and aggregation-associated processes. Figure 1 summarizes the principal physiological and pathological properties of α-Syn and their relationships with its molecular interactions and cellular functions.

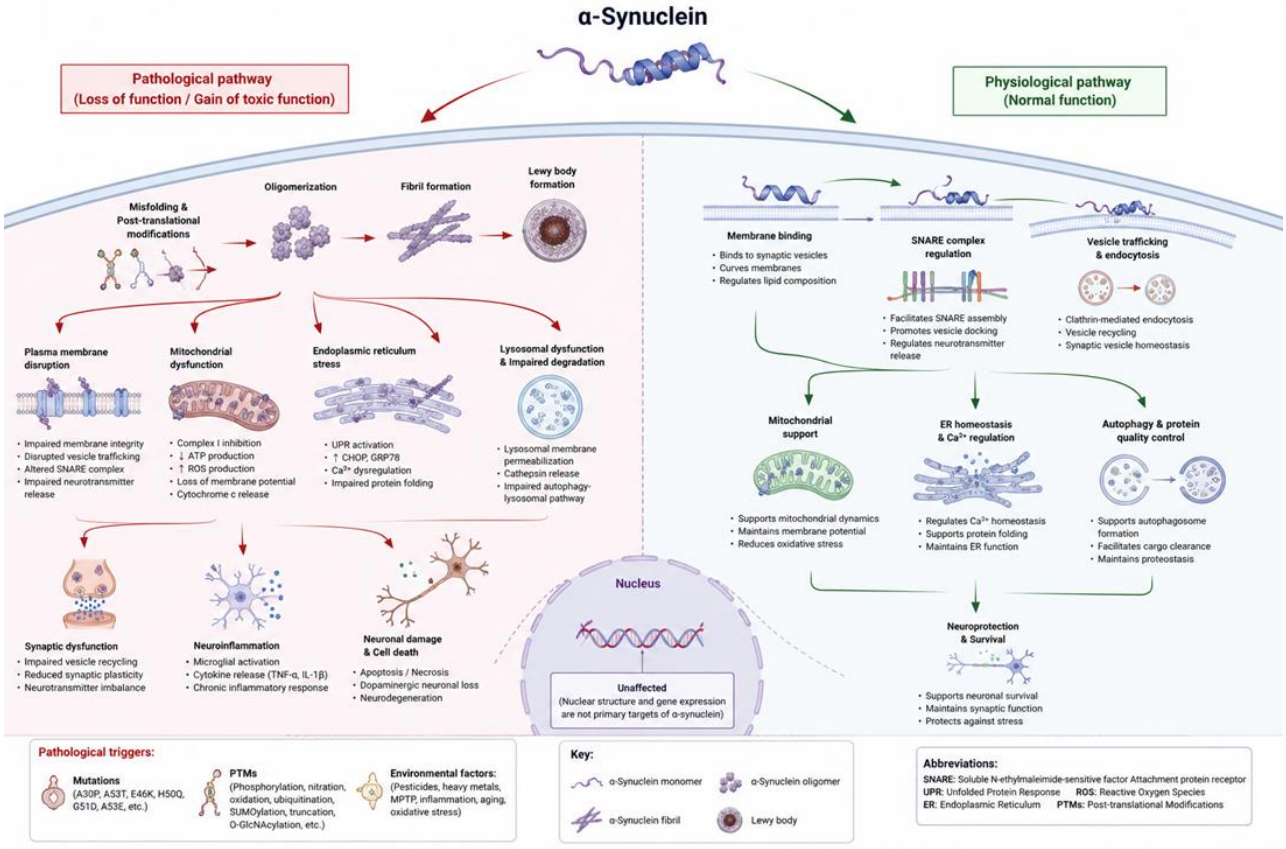



**Figure 1.** Physiological functions and pathological mechanisms of α-Syn

**Structural biology of alpha-synuclein**

The human synuclein family comprises three homologous proteins: α-Syn, β-synuclein (β-Syn), and γ-synuclein (γ-Syn). Although these proteins share structural similarities, they exhibit distinct biochemical properties and aggregation propensities. Among them, α-Syn has been extensively implicated in PD, particularly because mutations in the SNCA gene are associated with autosomal-dominant, early-onset PD and its aggregation is a characteristic feature of Lewy body pathology. α-Syn fibrils constitute a major component of Lewy bodies (LBs), which are characteristic neuropathological inclusions in PD and other Lewy body disorders [10]. In contrast, β-Syn exhibits a relatively lower propensity for aggregation and has been reported to inhibit α-Syn aggregation and toxicity in experimental models. Structural studies suggest that β-Syn differs from α-Syn in its membrane-binding region and C-terminal conformational preferences, potentially contributing to its distinct aggregation behaviour and reported protective effects [11]. γ-Syn shares several structural characteristics with α-Syn in its unbound state, including residual secondary structure and conformational flexibility, and exhibits aggregation behaviour that differs from that of β-Syn. However, its pathological significance and contribution to neurodegenerative processes remain distinct from those of α-Syn [10]. These observations suggest that differences in sequence composition, conformational preferences, and intramolecular interactions contribute to the distinct biochemical properties of the three synucleins. Investigating these structural determinants may provide insights into the molecular mechanisms underlying synuclein aggregation and its relevance to neurodegenerative disorders [12].

Intrinsically disordered proteins (IDPs) are characterized by the absence of a stable, well-defined tertiary structure under physiological conditions. Despite their structural flexibility, IDPs can transiently adopt secondary structures and undergo conformational rearrangements in response to interactions with other biomolecules. This structural plasticity enables them to participate in diverse cellular processes, although it can also facilitate aberrant interactions and aggregation under certain conditions [13]. α-Syn is a well-characterized IDP that predominantly exists as a dynamic ensemble of conformations in aqueous solution. Its ability to undergo structural rearrangements in response to its molecular environment is closely associated with its physiological functions and aggregation behaviour [14]. α-Syn is a 140-amino-acid protein encoded by the SNCA gene on chromosome 4 and is predominantly expressed in neuronal presynaptic terminals. Although it lacks a stable tertiary structure in aqueous solution, α-Syn undergoes substantial conformational changes upon interaction with cellular membranes and other biomolecules [14]. Its primary structure can be divided into three regions with distinct physicochemical properties: the N-terminal region (residues 1-60), the central non-amyloid-β component (NAC) region (residues 61-95), and the C-terminal region (residues 96-140) [15].

The N-terminal region contains amphipathic repeats characterized by the KTKEGV consensus sequence, which facilitates membrane association and the formation of α-helical structures upon interaction with lipid membranes. The extent of membrane binding and helix formation depends on membrane composition and the physicochemical properties of the surrounding environment. The central NAC region is hydrophobic and has a high propensity for amyloid formation, making it a critical determinant of α-Syn self-association and fibril formation. The C-terminal region is enriched in acidic and proline residues and remains predominantly disordered under physiological conditions. This region contributes to PPIs, PTMs, polyamine binding, and the regulation of α-Syn conformational behaviour and membrane association. Together, these regions provide a structural basis for the functional versatility and aggregation propensity of α-Syn [16].

In aqueous solution, α-Syn predominantly exists as a disordered monomer, although its conformational ensemble can be influenced by post-translational modifications and interactions with other biomolecules. N-


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terminal acetylation, a prevalent modification of α-Syn, influences its conformational properties and membrane interactions and has been reported to affect its aggregation behaviour under specific experimental conditions [17]. Upon interaction with lipid membranes, α-Syn can adopt α-helical conformations or, under certain conditions, undergo transitions toward β-sheet-rich structures associated with aggregation. The NAC region plays a central role in these transitions, while membrane composition, including cholesterol content, can influence α-Syn binding, conformational organization, and aggregation propensity [18]. In addition, α-Syn oligomers have been associated with membrane disruption and altered calcium homeostasis in experimental models. However, the extent to which these effects depend on oligomer structure, membrane composition, and experimental conditions remains an important consideration when interpreting their contribution to cellular toxicity. α-Syn fibrils are a major component of LBs, which are characteristic of PD and related Lewy body disorders [19].

Although α-Syn has traditionally been described as a natively unfolded protein, structural studies indicate that it is not entirely devoid of conformational organization. Small-angle X-ray scattering (SAXS) analysis has reported a radius of gyration of approximately 4 nm for α-Syn in aqueous solution. This value is larger than that typically observed for a compact globular protein of comparable molecular size, yet smaller than the dimensions expected for a fully expanded random-coil conformation. These observations support the concept that α-Syn exists as a dynamic ensemble of conformations characterized by partial compaction and transient intramolecular interactions rather than as a completely extended, disordered chain [20].

Nuclear magnetic resonance (NMR) studies have further demonstrated that, despite the absence of a stable tertiary structure, α-Syn exhibits transient secondary structure preferences and region-specific conformational dynamics. The N-terminal region, particularly residues 6-37, displays transient α-helical tendencies, while relaxation measurements have identified variations in backbone mobility across different regions of the protein. These findings indicate that α-Syn is structurally heterogeneous and that its conformational dynamics are not uniform along the polypeptide chain [16]. Paramagnetic relaxation enhancement (PRE) experiments have also provided evidence for transient long-range intramolecular contacts between the C-terminal region and the central NAC region, suggesting that interactions between distant segments contribute to the organization of the conformational ensemble [21].

Molecular dynamics (MD) simulations using the CHARMM force field have further supported the presence of transient intramolecular contacts between the C-terminal region, particularly residues 120-140, and regions spanning residues 30-100 [22]. Additional PRE experiments using site-directed spin labelling have identified transient interactions involving residues 85-95 within the NAC region and residues 110-130 within the C-terminal region. These interactions have been associated with hydrophobic residues, including M116, V118, Y125, and M127. Further studies have reported contacts between residues 120-130 and residues 105-115, as well as interactions between the C-terminal region and the N-terminal region around residue 20. Collectively, these findings support the existence of transient long-range interactions that contribute to the conformational organization of α-Syn in its monomeric state. Although these interactions have been proposed to influence aggregation propensity, their precise contribution to maintaining solubility and preventing aggregation remains dependent on the conformational ensemble and experimental conditions [13].

Electrostatic interactions are also implicated in the organization of the α-Syn conformational ensemble. The C-terminal region is enriched in negatively charged residues, whereas the NAC region contains a relatively higher proportion of hydrophobic residues and may exhibit localized positive electrostatic contributions. These differences in charge distribution have been proposed to contribute to transient interactions between the C-terminal and central regions. Polyamines, which interact with acidic residues and

can alter the electrostatic environment of α-Syn, have been reported to influence its fibrillation. Similarly, changes in the hydrophobic properties of the C-terminal region, including methionine oxidation, can alter intramolecular interactions and aggregation behaviour. These observations suggest that electrostatic and hydrophobic contributions jointly influence α-Syn conformational dynamics and aggregation propensity [13].

Changes in these intramolecular interactions may arise from mutations, PTMs, or environmental factors, including metal-ion binding, and can influence α-Syn conformational properties. Calcium binding to monomeric α-Syn has been reported to alter its intramolecular contacts and conformational organization, including interactions between the C-terminal and NAC regions. Such changes may affect the accessibility of hydrophobic regions and modify the propensity for intermolecular association. However, the relationship between these conformational rearrangements and aggregation depends on the experimental conditions and the molecular state of α-Syn [17].

Calcium has also been reported to influence the aggregation kinetics of wild-type α-Syn. One proposed mechanism involves calcium-induced changes in the electrostatic environment of the C-terminal region, which may alter its interactions with the NAC region and affect the conformational ensemble. Similarities have been proposed between calcium-induced conformational changes and those observed under acidic conditions, particularly with respect to changes in charge distribution and intramolecular interactions. Nevertheless, these mechanisms should not be considered identical, as calcium binding and pH changes can affect α-Syn through distinct molecular processes. Studies of familial α-Syn variants have additionally identified differences in long-range interactions, conformational compaction, and solvent exposure relative to wild-type α-Syn. These structural differences may contribute to variation in aggregation propensity and are discussed individually in the following sections [17].

Importantly, studies of monomeric α-Syn have indicated that calcium binding can alter the exposure of the N-terminal region and perturb interactions involving the C-terminal region. These observations support the role of the C-terminal region in modulating the conformational ensemble and solubility of α-Syn. However, the relationship between these structural changes and subsequent aggregation remains complex and may involve additional factors, including membrane interactions, intermolecular association, and the formation of aggregation-prone conformations. Understanding how these molecular interactions influence α-Syn conformational dynamics may help identify strategies for modulating its aggregation propensity. In particular, approaches aimed at stabilizing conformations associated with physiological function or interfering with aggregation-promoting interactions may provide useful directions for developing α-Syn-targeted therapeutic strategies [17].

## Functions of alpha-synuclein

### *Alpha-synuclein and oxidative stress*

α-Syn has been implicated in the regulation of cellular responses to oxidative stress through interactions with mitochondrial proteins and intracellular signalling pathways. One proposed mechanism involves cytochrome c oxidase, a key component of the mitochondrial respiratory chain, which has been identified as a potential target of α-Syn-mediated regulation. α-Syn also interacts with JNK-interacting protein 1b (JIP-1b/IB1), a scaffold protein involved in the c-Jun N-terminal kinase (JNK) signalling pathway. Under oxidative stress conditions, increased α-Syn expression has been associated with suppression of JNK signalling and reduced caspase activation, suggesting a potential role in regulating stress-induced apoptosis. Experimental studies have reported that neuronal cells overexpressing α-Syn exhibit increased resistance to hydrogen peroxide-induced oxidative stress, accompanied by changes in JIP-1b/IB1 expression and activity. These findings suggest that α-


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Syn may influence neuronal responses to oxidative stress through modulation of intracellular signalling pathways. In addition, α-Syn has been reported to inhibit lipid peroxidation in cellular membranes through interactions with phospholipids containing unsaturated fatty acids, indicating a potential relationship between its membrane-binding properties and antioxidant activity [23].

Experimental evidence further suggests that the antioxidant properties of α-Syn depend on its molecular state. Monomeric α-Syn has been reported to inhibit lipid oxidation, whereas fibrillar α-Syn exhibits reduced or absent protective activity in the experimental systems examined. The oxidation of methionine residues to methionine sulfoxide has also been implicated in the antioxidant activity of α-Syn and its interactions with reactive oxygen species. Collectively, these findings suggest that α-Syn may influence oxidative stress through multiple mechanisms, including modulation of intracellular signalling and interactions with membrane lipids. However, these effects appear to depend on its conformational state and experimental context, highlighting the importance of distinguishing the physiological properties of soluble α-Syn from those of its aggregated forms [24].

*Alpha-synuclein and lipid metabolism*

Polyunsaturated fatty acids (PUFAs) are essential components of neuronal membranes and contribute to membrane organization and cellular function. α-Syn has been implicated in lipid metabolism through interactions with lipid-associated enzymes, including acyl-CoA synthetases involved in fatty acid activation and incorporation into complex lipids. These interactions may influence the availability and metabolism of fatty acids required for neuronal membrane maintenance. Alterations in PUFA metabolism, including changes in non-esterified PUFA levels and the activities of phospholipase A2 (PLA2) and acyl-CoA synthetase long-chain family members (ACSLs), have been associated with Parkinsonian pathology. In addition, the ability of α-Syn to interact with lipids suggests that its membrane-binding properties may contribute to the regulation of lipid homeostasis and membrane stability. Oxidative modification of PUFAs can generate lipid-derived products that affect mitochondrial function and cellular integrity, while interactions between α-Syn and oxidized lipids may further influence its conformational behaviour and aggregation propensity. These observations suggest that the relationship between α-Syn and lipid metabolism involves interconnected biochemical processes that may influence neuronal function under physiological and pathological conditions [25].

*α-Synuclein and neuronal signaling*

Beyond its association with synaptic vesicles, α-Syn has been implicated in intracellular signalling pathways involved in neuronal differentiation and cellular function. One of its reported interaction partners is Rab3a, a small GTPase that regulates synaptic vesicle trafficking and neurotransmitter release. α-Syn has been shown to interact preferentially with the GTP-bound form of Rab3a on intracellular membranes, particularly synaptic vesicles. This interaction may influence α-Syn membrane association and its relationship with the Rab3a recycling machinery, which includes the GDP-dissociation inhibitor (GDI) and Hsp90-associated components. Alterations in Rab3a cycling have been associated with changes in α-Syn membrane distribution and have been investigated in the context of neurodegenerative pathology [26]. In addition, α-Syn has been implicated in signalling pathways involving extracellular signal-regulated kinase (ERK), a component of the ERK/mitogen-activated protein kinase (MAPK) pathway, which contributes to neuronal growth and differentiation. Through these interactions, α-Syn may influence intracellular signalling and transcriptional processes associated with neuronal development. However, the extent to which these effects contribute directly to neuronal differentiation remains dependent on the experimental model and the molecular context [27].

*Alpha-synuclein and calmodulin signalling*

α-Syn interacts with calmodulin (CaM), a calcium-binding messenger protein, in a calcium-dependent manner. This interaction has been demonstrated using *in vitro* photoreactive cross-linking assays and in neuronal cells expressing endogenous α-Syn and CaM. Notably, several PD-associated α-Syn mutations have been reported to preserve CaM binding, suggesting that this interaction is not necessarily abolished by disease-associated sequence alterations. *In vitro* studies have further shown that CaM binding can accelerate α-Syn fibril formation, indicating that calcium-dependent interactions may influence its aggregation dynamics. The α-Syn-CaM complex has also been implicated in the regulation of G-protein-coupled receptor kinase 5 (GRK5). In the absence of α-Syn, CaM inhibits GRK5 activity, whereas the presence of α-Syn has been associated with increased GRK5 autophosphorylation and α-Syn phosphorylation. These findings suggest that α-Syn may influence calcium-sensitive signalling by altering the functional interactions between CaM and its target proteins. The formation of higher-order α-Syn-CaM complexes under elevated calcium conditions further supports the potential relevance of this interaction to synaptic signalling and α-Syn aggregation. Nevertheless, the physiological and pathological consequences of these interactions require consideration of calcium concentration, protein abundance, and the molecular state of α-Syn [28].

*Alpha-synuclein and glucose homeostasis*

Although α-Syn is predominantly associated with neuronal function, it is also expressed in peripheral tissues, including pancreatic β-cells, where it has been implicated in the regulation of glucose-stimulated insulin secretion. Experimental studies have demonstrated that α-Syn localizes to insulin secretory granules and interacts with ATP-sensitive potassium (KATP) channels in β-cells. Immunoprecipitation experiments have identified complexes containing α-Syn and KATP channels, suggesting a potential role in regulating insulin granule exocytosis [29]. In experimental models, α-Syn overexpression suppresses insulin secretion under basal glucose conditions, whereas α-Syn deficiency increases insulin release at low and intermediate glucose concentrations. At higher glucose concentrations, this inhibitory effect is diminished, indicating that the influence of α-Syn on insulin secretion depends on the metabolic state of the β-cell. α-Syn deficiency has also been associated with alterations in β-cell morphology and the distribution of insulin granules near the plasma membrane. These findings suggest that α-Syn may contribute to the regulation of insulin secretion, particularly under basal conditions, although its precise molecular mechanism remains to be fully established [30].

Beyond its effects on insulin secretion, α-Syn has been associated with systemic metabolic regulation and insulin sensitivity. In a cohort of more than 1,100 individuals, serum α-Syn levels were inversely correlated with several metabolic parameters, including body mass index (BMI), homeostatic model assessment of insulin resistance (HOMA-IR), and immunoreactive insulin levels. In addition, α-Syn-deficient mice exposed to high-fat diets exhibited insulin resistance, particularly in adipose tissue and skeletal muscle, as assessed by euglycemic clamp analyses. α-Syn has also been reported to interact with insulin-degrading enzyme (IDE), forming complexes that may influence α-Syn aggregation and IDE proteolytic activity. These observations suggest potential links between α-Syn, insulin signalling and metabolic homeostasis. However, the relationship between α-Syn expression, insulin resistance, and metabolic disease remains complex and may differ across experimental models and physiological conditions [31].

*Alpha-synuclein and dopamine biosynthesis*

α-Syn has been implicated in the regulation of dopamine biosynthesis through interactions with tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine production. Experimental studies have reported that α-Syn interacts with TH and influences its phosphorylation at Ser40, a regulatory site associated with enzyme


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activity. TH phosphorylation at this residue is regulated by protein kinase A (PKA) and protein phosphatase 2A (PP2A). In some experimental systems, α-Syn has been associated with increased PP2A activity without corresponding changes in its expression, resulting in enhanced TH dephosphorylation and reduced dopamine production. Co-immunoprecipitation studies have further demonstrated interactions between α-Syn and PP2A, supporting a potential role for α-Syn in modulating phosphatase-dependent signalling. Pharmacological inhibition of PP2A has been reported to restore TH Ser40 phosphorylation in α-Syn-overexpressing cells, providing evidence for the involvement of this pathway in α-Syn-mediated regulation of dopamine synthesis. These findings suggest that α-Syn may influence dopaminergic signalling through changes in TH phosphorylation, although the underlying mechanisms depend on the cellular context [32].

The relationship between α-Syn and PP2A appears to be context-dependent. Whereas some studies have associated α-Syn with increased PP2A activity and reduced dopamine synthesis, others have reported that chronic α-Syn overexpression may indirectly suppress PP2A through Src kinase-dependent phosphorylation at Tyr307, a modification associated with PP2A inhibition. These apparently contrasting observations suggest that α-Syn may influence phosphatase signalling through different mechanisms depending on its expression level, molecular state, and cellular environment. Accordingly, α-Syn has been proposed to modulate dopaminergic signalling by influencing the phosphorylation status of TH. Although this regulatory relationship may contribute to dopamine homeostasis under certain conditions, its dysregulation could alter dopaminergic function and potentially contribute to processes associated with PD [33].

*Alpha-synuclein and neuronal survival signaling*

Wild-type α-Syn has been reported to influence neuronal survival through regulation of the proapoptotic kinase protein kinase C delta (PKCδ). One proposed mechanism involves modulation of nuclear factor kappa B (NF-κB) signalling and its transcriptional coactivator p300. In experimental systems, α-Syn has been associated with reduced acetylation of the NF-κB p65 subunit and decreased expression and histone acetyltransferase activity of p300. These changes have been linked to reduced NF-κB/p300-dependent transcription of the PKCδ gene and consequently lower PKCδ protein levels. The reported mechanism involves transcriptional regulation rather than changes in PKCδ mRNA or protein stability. Reduced PKCδ and p300 levels have also been observed in dopaminergic neurons of α-Syn transgenic mice, providing evidence for the potential relevance of this pathway *in vivo*. Furthermore, α-Syn-associated suppression of PKCδ has been linked to reduced PKCδ cleavage and nuclear translocation under oxidative stress conditions, including $MPP^+$ exposure. Conversely, restoration of PKCδ expression has been reported to diminish the protective effects associated with α-Syn expression. Together, these findings suggest that α-Syn may influence neuronal survival through modulation of NF-κB-, p300-, and PKCδ-associated signaling. However, these effects should be interpreted in relation to α-Syn expression levels and the experimental conditions under which they were observed [34].

*Alpha-synuclein and soluble N-ethylmaleimide-sensitive factor attachment protein receptor complex assembly*

One of the best-characterized physiological functions of α-Syn involves its participation in the regulation of synaptic vesicle dynamics and SNARE complex assembly at presynaptic terminals. Under physiological conditions, α-Syn exists in a dynamic equilibrium between predominantly disordered monomeric conformations in the cytosol and α-helical conformations associated with lipid membranes. Membrane binding induces structural rearrangements that influence α-Syn organization and its interactions with synaptic proteins. In particular, membrane-associated α-Syn has been implicated in facilitating SNARE complex assembly through interactions with vesicle-associated membrane protein 2 (VAMP2), also known as synaptobrevin-2. These

interactions may contribute to synaptic vesicle docking, priming, and fusion, thereby supporting neurotransmitter release. The proposed chaperone-like activity of α-Syn is associated primarily with its membrane-bound state and interactions with SNARE proteins rather than with the cytosolic monomer alone. Furthermore, studies of mice lacking all three synucleins have demonstrated reduced SNARE complex assembly, neurological impairments, and shortened lifespan, supporting a physiological role for the synuclein family in maintaining presynaptic function. However, these findings reflect the combined contribution of α-, β- and γ-Syn and do not establish that α-Syn alone accounts for all observed effects [35,36].

In contrast to its physiological interactions with the SNARE machinery, aggregated or oligomeric α-Syn has been associated with impaired synaptic vesicle function. Experimental studies have reported that larger α-Syn oligomers interact with synaptobrevin-2 and interfere with SNARE-mediated lipid mixing, thereby reducing neurotransmitter release. These observations suggest that changes in α-Syn conformational state can alter its interactions with synaptic proteins and influence vesicle fusion. Importantly, non-aggregated α-Syn has been reported to retain its ability to support vesicle docking and fusion under certain experimental conditions, indicating that the functional consequences of α-Syn depend not only on its abundance but also on its molecular state. Collectively, these findings support a model in which membrane-associated α-Syn contributes to SNARE complex regulation, whereas particular oligomeric species may interfere with this process. The extent to which these mechanisms contribute to synaptic dysfunction in PD remains an important area of investigation [37].

*Physiological function and pathological aggregation*

The physiological and pathological properties of α-Syn are closely linked to its structural flexibility, molecular interactions, and conformational state. At presynaptic terminals, α-Syn has been implicated in the regulation of SNARE complex assembly, membrane organization, synaptic vesicle trafficking, and neurotransmitter release. These functions may contribute to the maintenance of presynaptic homeostasis, particularly during sustained or high-frequency neurotransmission [38]. However, changes in α-Syn conformation, expression, or aggregation state can alter its interactions with cellular components and have been associated with pathological processes. α-Syn accumulation has been linked to mitochondrial dysfunction, impaired lysosomal activity, and alterations in protein degradation. In addition, interactions with mitochondrial membranes and lysosomal proteins, including glucocerebrosidase (GCase), have been investigated in relation to α-Syn-associated cellular dysfunction. Its ability to undergo conformational changes and propagate between cells has also been implicated in the spread of α-Syn pathology. These observations suggest that α-Syn-related cellular dysfunction may involve interconnected processes affecting organelle function, protein homeostasis, and synaptic activity. However, the relative contribution of these mechanisms to disease progression remains incompletely understood [39].

The duality between the physiological functions and pathological properties of α-Syn provides an important framework for investigating potential therapeutic strategies. Approaches that modulate α-Syn conformation, preserve its physiological interactions, prevent the formation of aggregation-prone species, or enhance its clearance may address different aspects of α-Syn-associated dysfunction. In particular, understanding how α-Syn interactions with membranes and SNARE proteins influence synaptic function may provide opportunities to preserve neurotransmission while limiting aggregation-associated effects. Similarly, investigating the molecular determinants of α-Syn stability, aggregation, and intracellular handling may help identify strategies for modifying its pathological behaviour. Overall, elucidating the relationships between α-Syn structure, conformational dynamics, biochemical interactions, and physiological functions is essential for


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understanding its involvement in PD and other synucleinopathies and for identifying potential molecular targets for future therapeutic development [17,38,39].

**Molecular interactions of alpha-synuclein**

α-Syn interactions are closely associated with its structural domains, which contribute to its diverse functions in the nervous system (Figure 2). The N-terminal domain (residues 1-60) contains amphipathic repeats that mediate binding to lipid membranes, particularly synaptic vesicles. These interactions are important for membrane association, and alterations in this region may impair lipid binding and influence α-Syn function. The central non-amyloid-β component (NAC) domain (residues 61-95) is hydrophobic and prone to β-sheet formation, making it a key region involved in aggregation. It also contributes to the interaction of α-Syn with lipid membranes, thereby influencing its conformation and assembly. The C-terminal domain (residues 96-140) is acidic, intrinsically disordered, and enriched in negatively charged residues. It undergoes extensive post-translational modifications (PTMs) and interacts with various proteins, metal ions, polycations, and polyamines. This region can modulate membrane interactions and is proposed to shield the NAC domain, thereby limiting aggregation under certain conditions. Together, these domain-specific interactions contribute to α-Syn's physiological functions, including the regulation of SNARE complex assembly, synaptic vesicle docking, and neurotransmitter release. Through its interactions with vesicle membranes and other proteins, α-Syn may also contribute to vesicle clustering and fusion, highlighting the importance of its molecular interactions in synaptic function [9].

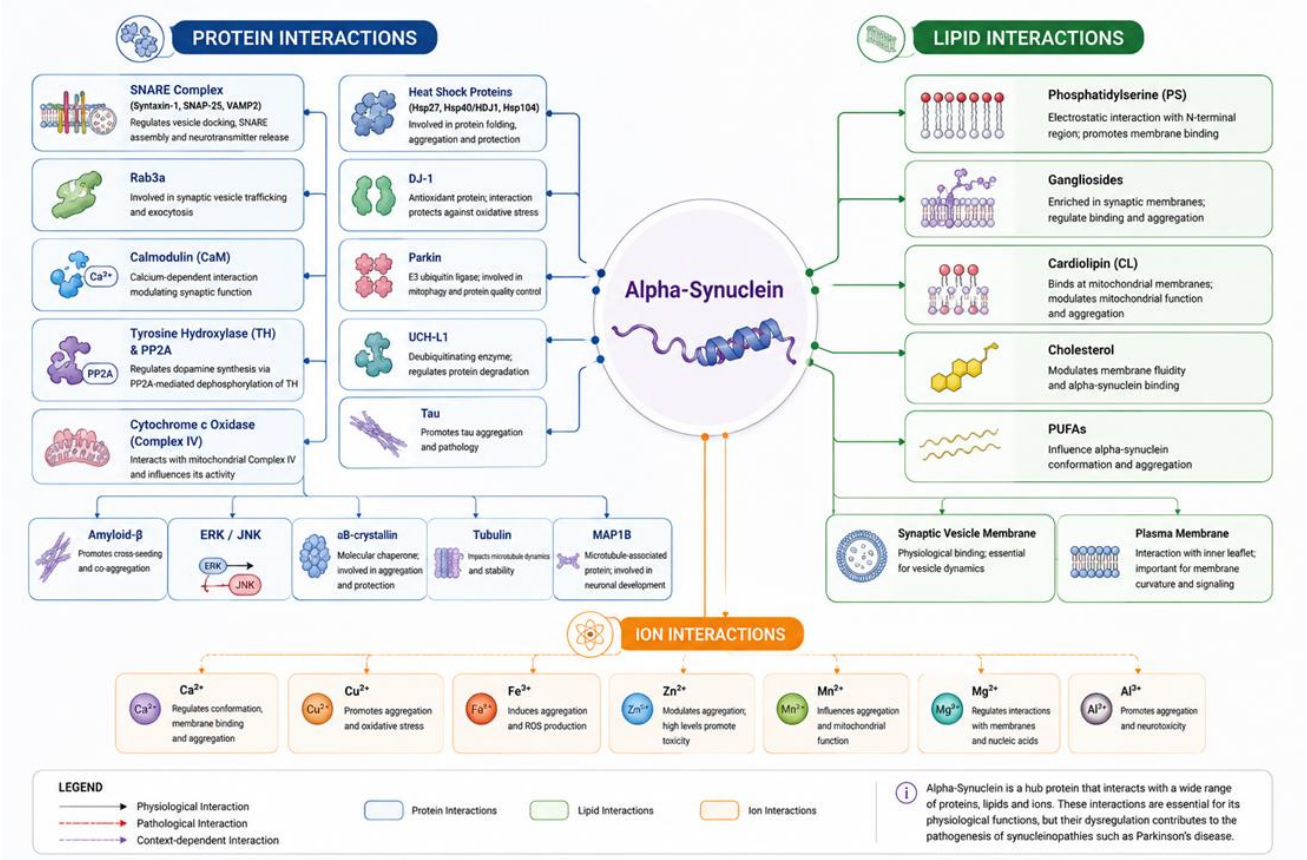



**Figure 2.** Comprehensive interaction network of α-Syn with proteins, lipids, and metal ions. α-Syn functions as a molecular hub that coordinates synaptic vesicle dynamics, membrane homeostasis, mitochondrial function, and protein quality control through diverse interactions with proteins, membrane lipids, and biologically relevant metal ions. Dysregulation of these interactions contributes to α-Syn aggregation and the pathogenesis of PD and related synucleinopathies

*Protein interactions*

α-Syn interacts with a broad range of proteins that contribute to its physiological functions and pathological effects (Figure 2). Early studies identified more than 50 individual protein partners, whereas subsequent high-throughput approaches have expanded the reported interaction network [40]. In particular, a SILAC-based proteomic analysis in dopaminergic MES cells identified 587 proteins associated with α-Syn-containing complexes. Among these, 141 proteins showed significant changes in abundance following rotenone treatment, a mitochondrial complex I inhibitor commonly used to model aspects of PD-related pathology [41]. These interacting proteins span several functional categories. Proteins associated with neurodegenerative processes include parkin [42], DJ-1 [43, 44], UCH-L1 [45], tau [46] and amyloid-β (Aβ) [47]. α-Syn also interacts with signalling proteins, including 14-3-3 [48], ERK1/2, p38 MAPK, SAPK/JNK, BAD [49], and the ELK-1/ERK-2 complex [50], implicating these interactions in cellular stress and survival pathways. Its association with cytoskeletal proteins, such as MAP1B and tubulin, further suggests a potential role in axonal transport and cytoskeletal organization [51].

Interactions with mitochondrial and metabolic proteins, including cytochrome c oxidase (complex IV) [52], TH [53], and aromatic amino acid decarboxylase [54], suggest that α-Syn may influence mitochondrial function and dopamine metabolism. α-Syn also interacts with αB-crystallin [55], phospholipase D [56], and PP2A [32], linking it to protein-folding processes, lipid signalling, and phosphorylation regulation. Additional interactions with transport-related proteins, including the dopamine transporter (DAT) [57] and Rab5A [58], as well as the calcium-binding protein calmodulin (CaM) [28], further illustrate the diversity of its molecular partners. Collectively, these interactions suggest that α-Syn participates in multiple aspects of neuronal homeostasis, while alterations in these interactions may contribute to disease-associated cellular dysfunction.

Heat shock proteins (Hsps) are involved in α-Syn homeostasis and may influence its misfolding and aggregation. Studies have reported that Hsp27, a small heat shock protein; HDJ1, an Hsp40-family member and Hsp70 co-chaperone; and Hsp104, an Hsp100-family disaggregase, can interact with monomeric α-Syn and cooperate to inhibit amyloid aggregation. Hsp27 and Hsp104 have been reported to interact primarily with the N-terminal domain, whereas HDJ1 interacts with the C-terminal region. Although these interactions are generally weak and transient, their combined activity may establish a dynamic chaperone network that influences α-Syn stability. The cooperative effects of these Hsps have been reported to exceed their individual effects, with aggregation inhibition observed at low chaperone-to-α-Syn molar ratios, including ratios as low as 1:200. These findings highlight the potential importance of coordinated chaperone activity in cellular proteostasis and suggest that disruption of this network may contribute to α-Syn accumulation in PD and related disorders [59].

An important interaction involving α-Syn is its association with VAMP2, a vesicle-associated SNARE protein involved in membrane fusion during synaptic transmission. Studies have suggested that α-Syn can facilitate SNARE complex assembly through its interaction with VAMP2, thereby supporting synaptic vesicle docking and neurotransmitter release. *In vitro* findings further suggest that α-Syn may promote vesicle docking through interactions involving both its C-terminal region and N-terminal amphipathic domain. Specifically, the C-terminal region interacts with VAMP2, whereas the N-terminal domain associates with membranes, potentially allowing α-Syn to bridge synaptic vesicles and their target membranes. However, the effects of α-Syn on SNARE complex assembly appear to depend on its concentration and conformational state [1].

Although monomeric or low-oligomeric α-Syn may support SNARE-dependent membrane fusion, α-Syn overexpression and aggregation have been associated with impaired neurotransmission in cellular and animal models of synucleinopathy. In these conditions, higher-order α-Syn oligomers have been reported to


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interact multivalently with the N-terminal region of VAMP2, potentially sequestering VAMP2 and interfering with SNARE complex formation. Such interactions may disrupt vesicle docking and fusion and contribute to synaptic dysfunction. Aggregated α-Syn may also alter the organization of synaptic vesicle pools, further affecting neurotransmission. These findings suggest that α-Syn can exert different effects on SNARE complex regulation depending on its concentration and conformation. In physiological conditions, interactions with VAMP2 and phospholipids may support vesicle docking and fusion, whereas pathological oligomerization may interfere with these processes. This context-dependent relationship provides a potential mechanistic link between α-Syn aggregation and synaptic dysfunction in PD and related neurodegenerative diseases [37].

*Lipid interactions*

α-Syn is enriched at synaptic terminals, where its interactions with lipid membranes contribute to synaptic vesicle dynamics and neurotransmitter release (Figure 2). Its N-terminal domain mediates transient and reversible association with highly curved and negatively charged membranes, including synaptic vesicles and the inner leaflet of the plasma membrane. Membrane binding promotes a conformational transition from an intrinsically disordered state to an α-helical structure. This transition is facilitated by amphipathic KTKEGV-containing repeats, whose lysine residues contribute to electrostatic interactions with acidic phospholipid headgroups, while hydrophobic residues interact with lipid acyl chains. The N-terminal domain (residues 1 to 60) mediates membrane association, particularly with phosphatidylserine- and ganglioside-enriched membranes. Together with the central NAC domain (residues 61-95), it may contribute to multivalent membrane interactions and vesicle docking [60,61].

Cholesterol and ganglioside composition can modulate α-Syn membrane-binding affinity and thereby influence its involvement in vesicle clustering and membrane fusion [61]. α-Syn also interacts with the SNARE complex, which includes VAMP2, syntaxin-1, and SNAP-25 and constitutes the core machinery for synaptic vesicle fusion [36,62]. The C-terminal region of α-Syn has been reported to interact directly with VAMP2 [63], whereas its N-terminal domain associates with the plasma membrane. These interactions may allow α-Syn to coordinate membrane association with SNARE complex assembly and contribute to vesicle docking and fusion. Thus, interactions involving the N-terminal membrane-binding region and the C-terminal VAMP2-binding region may regulate synaptic vesicle positioning and neurotransmitter release [64]. Disruption of these interactions, through mutations such as A30P or changes in the lipid environment, may affect membrane binding, SNARE assembly, and vesicle trafficking [60].

Beyond its synaptic functions, α-Syn interacts with mitochondrial membranes, particularly through its association with cardiolipin (CL), a mitochondria-enriched phospholipid. Under physiological conditions, α-Syn can interact with CL through its N-terminal domain and adopt an α-helical conformation. This interaction may contribute to mitochondrial membrane association. However, alterations in α-Syn-CL interactions have also been implicated in mitochondrial dysfunction associated with PD. Changes in CL abundance, acyl-chain composition, or oxidative state may affect membrane integrity, protein import, and mitophagy. α-Syn aggregates, including those associated with pathogenic variants such as A53T and E46K, have been reported to interact with CL-enriched membranes and induce membrane permeabilization, cytochrome c release, and mitochondrial fragmentation. Conversely, CL has also been reported to promote the conversion of pathological α-Syn oligomers into helical monomers under certain experimental conditions. These findings suggest that the consequences of α-Syn-CL interactions depend on the molecular and membrane context and may link lipid biology to mitochondrial integrity and α-Syn-associated pathology [65].

Cholesterol, a major component of synaptic vesicle membranes, can influence α-Syn membrane binding and aggregation. Although cholesterol has been reported to reduce the overall membrane-binding affinity of

α-Syn, particularly through effects involving the NAC region (residues 65-97), it may also influence the protein's membrane-bridging behaviour and vesicle clustering. The NAC region overlaps with the amyloidogenic core of α-Syn and may contribute to its sensitivity to membrane composition. Changes in cholesterol levels may therefore affect the balance between membrane-associated and unbound α-Syn and influence vesicle-vesicle interactions and SNARE complex assembly. In this context, the N-terminal domain mediates membrane association, whereas the C-terminal region interacts with VAMP2. These coordinated interactions may contribute to vesicle organization and neurotransmitter release, while alterations in lipid composition may influence both physiological regulation and pathological aggregation [18].

*Ion interactions*

α-Syn interacts with several metal ions, including $Cu^{2+}$, $Fe^{3+}$, $Zn^{2+}$, $Ca^{2+}$, $Mn^{2+}$, $Al^{3+}$ and $Mg^{2+}$ (Figure 2). These interactions can influence its structural dynamics and aggregation behavior and may contribute to both physiological functions and pathological processes associated with PD. α-Syn binds metal ions through specific and nonspecific interactions involving its N-terminal and C-terminal regions. High-affinity $Cu^{2+}$ binding has been reported at the N-terminus, involving residues such as Asp-2 and Val-3, with dissociation constants (KDs) in the subnanomolar-to-nanomolar range. However, the contribution of His-50 to $Cu^{2+}$ coordination remains controversial, with some studies proposing dynamic coordination involving water molecules at physiological pH [66].

A lower-affinity $Cu^{2+}$-binding site has also been identified in the acidic C-terminal domain, with reports suggesting that up to 16 $Cu^{2+}$ ions may associate with a single α-Syn molecule under specific experimental conditions [67]. Other metal ions also interact with α-Syn. $Fe^{3+}$ has been reported to bind at two sites with micromolar affinity, whereas $Ca^{2+}$ interacts with the acidic C-terminal region, with reported K_D values ranging from 2 to 300 μM. $Zn^{2+}$, $Mn^{2+}$, and $Al^{3+}$ also interact with α-Syn, although their binding may be less site-specific [13]. The effects of metal ions on α-Syn aggregation vary according to the ion, its concentration, and the experimental conditions.

$Cu^{2+}$ has been reported to enhance α-Syn aggregation and fibrillization, potentially by stabilizing partially folded intermediates with molten-globule-like properties. $Cu^{2+}$-associated aggregation has been linked to the formation of long, thin, and interwoven fibrils and to oxidative modifications of methionine residues, which may contribute to oligomer formation [68,69]. $Fe^{3+}$ can also accelerate fibrillization and promote the formation of short, thick fibrils and stable oligomers. These $Fe^{3+}$-associated oligomers have been reported to exhibit toxicity *in vitro* and *in vivo* and may contribute to oxidative stress-related pathology [69,70].

$Ca^{2+}$ has context-dependent effects on α-Syn. It can enhance membrane interactions and aggregation and has been associated with the formation of annular oligomers. The resemblance of these structures to species isolated from the brains of patients with multiple system atrophy (MSA) has been proposed as a potential link to disease-associated α-Syn assemblies [38,69]. $Zn^{2+}$ can enhance fibrillization and has been reported to reverse $Cu^{2+}$-induced methionine oxidation, indicating that its effects may depend on the surrounding chemical environment [69]. $Al^{3+}$, although not considered a physiological metal ion in this context, has been reported to promote α-Syn fibrillization and the formation of compact, protease-resistant intermediates that can convert into fibrils under experimental conditions [69,71]. $Mn^{2+}$ can increase α-Syn secondary-structure content and accelerate fibril formation; experimental co-incubation of α-Syn with $Mn^{2+}$ has also been associated with reduced cellular viability [69]. In contrast, $Mg^{2+}$ has been reported to inhibit α-Syn aggregation and compete with aggregation-promoting metal ions, including $Cu^{2+}$, $Zn^{2+}$, $Mn^{2+}$ and $Al^{3+}$. These findings suggest a potential modulatory effect of $Mg^{2+}$ on α-Syn aggregation, although its physiological and therapeutic significance requires further investigation [69].


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The interaction between α-Syn and metal ions has been investigated in the context of synucleinopathy pathogenesis. Environmental and occupational exposure to metals, including iron, manganese, and aluminum, has been associated with PD risk. Studies have also reported altered levels of certain metals, including $Fe^{3+}$, $Zn^{2+}$ and $Al^{3+}$, in the substantia nigra of patients with PD. These observations support further investigation into how metal-dependent changes in α-Syn structure and aggregation may contribute to disease-associated processes. Overall, experimental findings indicate that some metal ions, including $Cu^{2+}$ and $Fe^{3+}$, can promote amyloidogenic pathways, whereas others, such as $Mg^{2+}$, may modulate aggregation under specific conditions. However, the implications of these interactions for disease progression and therapeutic intervention remain to be established [69].

$Ca^{2+}$ binds to the negatively charged C-terminal region of α-Syn, with a reported KDs of approximately 21 μM. This interaction can increase α-Syn affinity for lipid membranes, particularly synaptic vesicles, and may influence its role in vesicle homeostasis. However, $Ca^{2+}$ binding has also been associated with conformational changes involving the NAC domain, increased β-sheet formation, and α-Syn aggregation. *In vitro* and *in vivo* studies indicate that the effects of $Ca^{2+}$ on α-Syn depend on its concentration and the experimental context [38]. At physiological concentrations, $Ca^{2+}$ may enhance α-Syn-associated regulation of vesicle docking, priming, fusion and fusion-pore expansion. $Ca^{2+}$-dependent synaptic vesicle clustering mediated by α-Syn has been observed in rat brain-derived vesicles and ventral mesencephalic neurons, and this effect was reversible following calcium chelation. Dopamine stimulation has also been reported to promote clustering of α-Syn-positive vesicles, an effect suppressed by isradipine, a voltage-gated calcium channel blocker [72].

These findings suggest that α-Syn may act as a calcium-sensitive modulator of vesicle dynamics and that disruption of calcium homeostasis may influence α-Syn-associated neuronal dysfunction. In contrast, aggregated α-Syn has been reported to inhibit vesicle priming through a calcium-independent mechanism without affecting fusion-pore dilation. β-sheet-rich α-Syn aggregates can also disrupt calcium signalling and promote $Ca^{2+}$-dependent cell death [73]. Oligomeric α-Syn may incorporate into membranes and increase intracellular $Ca^{2+}$ levels, potentially contributing to ferroptosis in neurons derived from human induced pluripotent stem cells carrying α-Syn triplication [74].

Emerging evidence also suggests that α-Syn secretion is regulated by calcium-dependent mechanisms. In mouse striatum and cultured neurons, α-Syn secretion has been observed in response to calcium fluxes and may be modulated by GABAergic signalling. In addition, loss of ATP13A2/PARK9 function in PD patient-derived neurons has been associated with impaired lysosomal calcium homeostasis, reduced lysosomal calcium storage, increased cytosolic calcium levels and impaired lysosomal exocytosis. These alterations may contribute to intracellular α-Syn accumulation. Activation of mucolipin-1 (TRPML1), a lysosomal calcium channel, has been reported to restore exocytosis and enhance α-Syn clearance, highlighting the potential importance of lysosomal calcium regulation in α-Syn homeostasis [73,75].

Similarly, inhibition or knockdown of inositol-1,4,5-triphosphate kinase B (ITPKB), a gene associated with sporadic PD, has been reported to reduce ER-to-mitochondria calcium transfer, α-Syn aggregation and mitochondrial calcium overload. Calcium-binding proteins, including calbindin-D28k, have also been associated with neuroprotective effects. Notably, α-Syn aggregates were not detected in calbindin-positive neurons in models of dementia with Lewy bodies (DLB) and MPTP-induced PD, suggesting that calcium-buffering capacity may influence susceptibility to α-Syn pathology [76]. A summary of the major physiological and pathological interactions of α-Syn is presented in Table 1.

**Table 1.** Summary of α-Syn interactions in physiological and pathological conditions

| Interaction partner | Category | Physiological role | Pathological effect | Related disorders | Ref. |
|---|---|---|---|---|---|
| VAMP2 | Protein | Facilitates SNARE complex assembly, vesicle docking and neurotransmitter release | Oligomeric α-syn sequesters VAMP2, inhibits SNARE assembly and synaptic transmission | PD | [1,37] |
| SNARE complex (Syntaxin-1, SNAP-25, VAMP2) | Protein complex | Regulates synaptic vesicle fusion | Aggregated α-syn disrupts vesicle fusion and neurotransmission | PD | [60-64,77] |
| Hsps (Hsp27, HDJ1, Hsp104) | Chaperone proteins | Stabilize α-syn and suppress amyloid aggregation | Failure of the chaperone network favours α-syn accumulation | PD | [59] |
| Rab5a | Small GTPase | Regulates synaptic vesicle trafficking and neuronal differentiation | Rab5a recycling defects increase membrane sequestration of α-syn | PD | [58] |
| CaM | Calcium-binding protein | Mediates $Ca^{2+}$-dependent signalling | Promotes fibrillization and GRK5-mediated α-syn phosphorylation under high $Ca^{2+}$ | PD | [28] |
| TH | Enzyme | Regulates dopamine synthesis | Reduced TH activity decreases dopamine production | PD | [53] |
| PP2A | Phosphatase | Controls TH dephosphorylation and dopamine homeostasis | Dysregulated PP2A signalling contributes to dopaminergic dysfunction | PD | [32] |
| Parkin, DJ-1, UCH-L1 | Proteostasis proteins | Maintain protein quality control | Dysfunction promotes α-syn accumulation and neurodegeneration | PD | [42,43,45] |
| Tau & Aβ | Neurodegeneration-related proteins | Protein interaction network | Associated with pathological neurodegenerative processes | PD, AD | [46,47] |
| MAP1B & Tubulin | Cytoskeletal proteins | Axonal transport and neuronal structure | Cytoskeletal dysfunction may contribute to neurodegeneration | PD | [51] |
| Cytochrome c oxidase | Mitochondrial enzyme | Supports mitochondrial respiration | Mitochondrial dysfunction contributes to neuronal injury | PD | [52] |
| MAPK/ERK & JNK signalling proteins | Signalling proteins | Regulate neuronal survival and differentiation | Altered signalling contributes to neuronal dysfunction | PD | [49] |
| $Cu^{2+}$, $Fe^{3+}$, $Zn^{2+}$, $Ca^{2+}$, $Mn^{2+}$, $Al^{3+}$, $Mg^{2+}$ | Metal ions | Modulate α-syn structural dynamics | Promote conformational changes, aggregation and misfolding | PD | [13,38,66,67. 69-74,76] |
| Phosphatidylserine & gangliosides | Membrane lipids | Promote membrane binding and vesicle docking | Disturbed lipid interaction impairs synaptic function | PD | [60,61] |
| CL | Mitochondrial lipid | Supports mitochondrial membrane integrity | Dysregulation induces mitochondrial damage, membrane permeabilization and α-syn aggregation | PD | [65] |
| Cholesterol | Membrane lipid | Regulates membrane affinity and vesicle clustering | Altered cholesterol levels disturb membrane interaction and SNARE function | PD | [18] |

## Post-translational modifications of alpha-synuclein

Phosphorylation is one of the most extensively studied post-translational modifications (PTMs) of α-Syn and may influence its aggregation propensity, degradation and cytotoxicity. Among the phosphorylation sites identified in α-Syn, Ser129 (S129) is particularly prominent in pathological inclusions. Studies have reported that up to 90 % of α-Syn in Lewy bodies (LBs) is phosphorylated at this residue, whereas phosphorylation at S129 is substantially less abundant under physiological conditions. This modification is mediated by several kinases, including casein kinase II (CKII), G-protein-coupled receptor kinases (GRK2 and GRK5) and Polo-like kinase 2 (PLK2). Although S129 phosphorylation is strongly associated with pathological α-Syn inclusions in PD and related synucleinopathies, its effects on aggregation, solubility and toxicity may depend on the


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experimental and cellular context [78-81]. In contrast, phosphorylation at Ser87 (S87), which is less abundant, has been reported to inhibit fibril formation and reduce membrane interactions [82].

Although *in vitro* studies suggest that S87 phosphorylation may have protective effects against aggregation, its limited detection in pathological inclusions raises questions about its physiological relevance and contribution to disease [82]. Tyrosine phosphorylation also influences α-Syn behaviour, particularly at Tyr39 (Y39), which is targeted by the non-receptor tyrosine kinase c-Abl. Y39 phosphorylation has been associated with impaired proteasomal and autophagic clearance of α-Syn, potentially contributing to the accumulation of oligomeric species and increased toxicity. Additional phosphorylation sites, including Y125, Y133 and Y136, have been detected at relatively low levels under pathological conditions, although their precise effects on aggregation and toxicity remain incompletely characterized [78,83]. Overall, phosphorylation may influence α-Syn aggregation, oligomerization and degradation through mechanisms that vary according to the modified residue and the cellular context [84].

Ubiquitination is another important PTM involved in the regulation of α-Syn aggregation and degradation. Several lysine residues, including K6, K10, K12, K21, K23, K32, K34, K43, K45, K96 and K102, as well as the N-terminal methionine (M1), have been reported as ubiquitination sites. This modification is mediated, in part, by the E3 ubiquitin ligases seven in absentia homolog 1 (SIAH-1) and the carboxyl terminus of Hsp70-interacting protein (CHIP) [85,86]. SIAH-1 promotes mono- and di-ubiquitination of α-Syn, particularly at K12, K21 and K23, as well as at additional sites, including K10, K34, K43 and K96. Unlike ubiquitination pathways that facilitate protein degradation, SIAH-1-mediated ubiquitination has been associated with increased α-Syn aggregation and cytotoxicity. Monoubiquitinated α-Syn has been reported to accumulate in inclusion bodies, particularly in dopaminergic neurons, and may contribute to LB formation. SIAH-1 also interacts directly with α-Syn and has been observed to colocalize with it in pathological inclusions. In experimental studies, suppression of SIAH-1 expression reduced α-Syn monoubiquitination and aggregate formation, supporting a potential role for this ligase in α-Syn accumulation [86,87].

In contrast, CHIP has been associated with protective effects through its involvement in α-Syn degradation. CHIP functions as a co-chaperone and ubiquitin ligase and can regulate α-Syn turnover through proteasomal and lysosomal pathways. Through its tetratricopeptide repeat (TPR) domain, CHIP interacts with Hsp70 and promotes proteasomal degradation of misfolded or oligomeric α-Syn species (O-α-Syn). Its U-box domain has also been reported to facilitate lysosomal targeting of α-Syn. CHIP has been detected in intracellular α-Syn inclusions and LBs. Experimental overexpression of CHIP has been associated with reduced α-Syn abundance, decreased oligomer formation, and reduced inclusion accumulation, suggesting a potential role in limiting α-Syn-associated toxicity [85,88]. Together, these findings indicate that the effects of ubiquitination on α-Syn are context-dependent. SIAH-1-mediated ubiquitination has been associated with aggregation and toxicity, whereas CHIP-mediated ubiquitination may promote α-Syn turnover and cellular protection. These effects depend on the ubiquitination machinery and the conformational state of α-Syn, including soluble, oligomeric and aggregated species [85-88].

Nitration is another PTM of α-Syn that has been detected in LBs and other α-Syn-containing inclusions associated with PD and related synucleinopathies. Nitration has been reported primarily at tyrosine residues Y39, Y125, Y133 and Y136 and, less frequently, at S129. Studies using purified nitrated α-Syn have provided insight into its potential effects on aggregation. Nitrated monomeric and dimeric α-Syn, but not nitrated oligomers, have been reported to accelerate fibril formation by unmodified α-Syn when present at low concentrations. The incorporation of these nitrated species into growing fibrils suggests that they may influence early stages of fibrillogenesis [89].

Although nitrated α-Syn monomers do not readily form fibrils independently, they can influence the aggregation of unmodified α-Syn when the two species are present together. Nitration at Y39 has also been associated with reduced α-Syn binding to lipid membranes. This effect may result from impaired α-helical formation in the presence of vesicles, a structural transition normally associated with membrane binding. Because membrane association contributes to the physiological functions of α-Syn, disruption of this interaction may alter its conformational behaviour and aggregation propensity. Nitration has also been reported to preserve the chaperone-like activity of α-Syn under certain experimental conditions. However, nitrated α-Syn is degraded more slowly by the 20S proteasome and calpain I than unmodified α-Syn, suggesting that nitration may affect protein clearance. Collectively, these findings indicate that nitration can influence α-Syn aggregation, membrane interactions and proteolytic degradation, although the extent of these effects may depend on the nitration site and the molecular state of the protein [89].

Truncation is another PTM-related process that can substantially alter the structure, aggregation behaviour and toxicity of α-Syn in synucleinopathies, including PD. Truncated α-Syn species have been detected in LBs, suggesting their potential involvement in pathological processes. Calpain I and neurosin have been identified as proteases involved in α-Syn cleavage [90,91]. Calpain I, a calcium-dependent cysteine protease, cleaves soluble α-Syn near or within its central hydrophobic domain, including the NAC region. The resulting fragments have been reported to be unable to fibrillize independently. Interestingly, soluble fragments generated by calpain I can inhibit fibril formation by full-length α-Syn, suggesting a potential aggregation-suppressing effect under these conditions [90].

However, calpain I may have different effects depending on the conformational state of α-Syn. When it cleaves preformed α-Syn fibrils, including wild-type and nitrated fibrils, it generates C-terminally truncated fragments that retain fibrillar structure and can promote co-assembly with soluble full-length α-Syn. Thus, calpain I-mediated cleavage may inhibit aggregation of soluble α-Syn while promoting aggregation-related processes involving pre-existing fibrils. This context-dependent behaviour highlights the importance of the substrate conformation in determining the effects of proteolytic processing [90].

Neurosin, a serine protease expressed in the brain, also cleaves α-Syn at several sites. One reported cleavage site is between Lys80 and Thr81 within the NAC region (residues 61-95), which is important for α-Syn aggregation. Cleavage within this region has been reported to inhibit α-Syn polymerization and may therefore represent a potential protective mechanism. However, other truncation events that preserve the NAC domain while removing portions of the C-terminal region may increase aggregation propensity and contribute to pathological processes [91].

Phosphorylated α-Syn and the PD-associated A30P mutant have been reported to exhibit increased resistance to neurosin-mediated degradation. These findings suggest that PTMs and disease-associated mutations may influence proteolytic processing and α-Syn turnover, potentially contributing to protein accumulation [91]. Overall, the effects of α-Syn truncation vary according to the protease involved, the conformational state of α-Syn and the cleavage site. Truncated species may either suppress or promote aggregation and may also alter protein degradation, highlighting the context-dependent consequences of proteolytic processing in PD [90,91].

SUMOylation, the conjugation of small ubiquitin-like modifier (SUMO) proteins, has also been investigated as a regulator of α-Syn aggregation and degradation in PD. The SUMO E3 ligase PIAS2 has been reported to promote α-Syn SUMOylation, particularly at lysine residues K96 and K102. This modification has been associated with increased α-Syn aggregation through more than one mechanism. First, SUMOylation may directly influence α-Syn conformational behaviour and inclusion formation. Second, it may interfere with


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ubiquitination by E3 ligases, including SIAH and Nedd4, thereby affecting proteasomal and lysosomal degradation. These effects may contribute to the accumulation of SUMOylated α-Syn and the formation of cytoplasmic aggregates and LB-like inclusions [92].

SUMOylation has also been associated with the release of α-Syn into the extracellular space, raising the possibility that it may influence processes involved in the spread of α-Syn pathology. Disease-associated α-Syn mutants have been reported to exhibit increased SUMOylation compared with wild-type α-Syn, alongside changes in aggregation propensity and cellular toxicity. In PD brain tissue, elevated PIAS2 expression and increased levels of SUMO1-conjugated α-Syn have been reported, particularly in LBs within the substantia nigra. These observations suggest an association between altered SUMOylation and α-Syn accumulation, although they do not by themselves establish a causal role in disease progression [92]. In experimental studies, inhibition of SUMOylation using agents such as ginkgolic acid has been reported to restore α-Syn degradation by relieving inhibition of the ubiquitin-proteasome system (UPS). These findings identify SUMOylation as a potential regulator of α-Syn turnover and a candidate for further investigation. However, its contribution to PD progression and its therapeutic relevance require additional validation [92].

O-GlcNAcylation is a dynamic and reversible PTM in which a single N-acetylglucosamine (GlcNAc) moiety is attached to serine or threonine residues by O-GlcNAc transferase (OGT). In PD and other synucleinopathies, this modification has been investigated for its potential protective effects against α-Syn aggregation [93]. Nine O-GlcNAcylation sites have been identified in α-Syn from human and mouse brain tissues, including T33, T59, T64, T72, T75, T81 and S87. Site-specific studies have reported that O-GlcNAcylation at T72 produces particularly strong inhibition of α-Syn aggregation, while modification at S87 has also been associated with anti-aggregative effects, although to a lesser extent [93]. Biochemical and cellular studies suggest that O-GlcNAcylation can reduce α-Syn aggregation, alter aggregate structure and attenuate the cytotoxicity of extracellular α-Syn fibrils. These findings may be relevant to proposed mechanisms of cell-to-cell propagation of α-Syn pathology, although the extent to which these effects occur *in vivo* remains to be established [93].

Furthermore, α-Syn modified at multiple O-GlcNAc sites has been reported to inhibit aggregation of unmodified α-Syn through a dominant-negative effect. This inhibitory activity has also been observed in experiments involving mutant α-Syn species with increased aggregation propensity. Despite these findings, O-GlcNAcylated α-Syn may be difficult to detect using conventional pan-O-GlcNAc antibodies, potentially limiting its identification in experimental studies. Overall, the available findings suggest that O-GlcNAcylation can suppress α-Syn aggregation under certain conditions. However, further investigation is needed to establish the physiological significance of this modification and determine whether modulation of OGT activity or O-GlcNAcylation could provide a viable strategy for reducing α-Syn-associated toxicity in PD [93].

N-terminal acetylation (NTAc) is a common, generally irreversible PTM that occurs on many eukaryotic proteins. In α-Syn, N-terminal acetylation is the predominant physiological form found in neurons. Unlike several other PTMs, NTAc has not been consistently associated with a substantial increase in the pathological behaviour of α-Syn. Biophysical studies, including solid-state NMR spectroscopy, have reported that NTAc can increase α-Syn binding affinity for synaptic-like lipid membranes without substantially altering its structure or topology in the membrane-bound state. Experimental findings have also suggested that N-terminal acetylation has limited effects on α-Syn aggregation propensity, although its effects on membrane interactions may vary according to the lipid environment and experimental conditions. Thus, N-terminal acetylation appears to be important for regulating physiological membrane interactions, while its direct contribution to pathological aggregation remains uncertain [94].

**Alpha-synuclein as a biomarker**

*Cerebrospinal fluid*

Cerebrospinal fluid (CSF) is widely used in the investigation of biomarkers for Parkinson's disease (PD) because of its proximity to the CNS and its potential to reflect neuropathological changes. Numerous studies have investigated CSF α-Syn levels as diagnostic and prognostic biomarkers for PD. However, despite accumulating evidence, findings have been inconsistent across cohorts and analytical methods [95].

Early and subsequent studies detected α-Syn in CSF using immunoassays, including ELISA and Luminex. Many reported significantly lower total α-Syn concentrations in patients with PD than in healthy controls (HCs) and individuals with neurological disorders not associated with synucleinopathy. However, other studies found no significant differences, particularly those involving smaller cohorts or alternative detection methods, such as immunoprecipitation and immunoblotting. This variability may reflect preanalytical factors, differences in assay sensitivity, epitope masking associated with PTMs, inconsistencies in sample handling, and variation in antibody specificity. Although total CSF α-Syn has been proposed as a potential biomarker, its diagnostic specificity remains limited, particularly for distinguishing PD from other synucleinopathies, including DLB and MSA. Furthermore, associations between total α-Syn levels and disease progression or severity have been inconsistent. Some studies have reported modest associations between reduced total α-Syn levels and disease severity, whereas others have found no significant relationship [96,97].

To address the limitations of measuring total α-Syn, researchers have increasingly investigated O-α-Syn and PTM-specific forms, particularly α-Syn phosphorylated at serine 129 (pS129). Several studies have reported increased CSF O-α-Syn levels and elevated O-α-Syn/total α-Syn ratios in patients with PD, suggesting that these measures may provide greater diagnostic discrimination than total α-Syn alone. Elevated O-α-Syn levels have also been reported in patients with PD and dementia, indicating potential utility in disease stratification [98].

Similarly, pS129, a major pathological PTM enriched in LBs, has emerged as a candidate CSF biomarker. Mass spectrometry and immunoassay-based studies have confirmed the presence of pS129 in CSF, with some reporting higher levels in patients with PD than in controls and individuals with non-synucleinopathy disorders. In some studies, the pS129/total α-Syn ratio was significantly elevated in PD, suggesting that this measure may provide additional information beyond absolute pS129 or total α-Syn concentrations. Nevertheless, associations between CSF pS129 levels and disease severity have generally been weak or absent in both cross-sectional and longitudinal studies [99].

The simultaneous measurement of multiple α-Syn species may improve diagnostic performance. For example, combining the O-α-Syn/total α-Syn and pS129/total α-Syn ratios has been reported to improve discrimination between PD and controls. Similarly, integrating α-Syn measures with other biomarkers, including β-glucocerebrosidase activity, tau and Aβ42, may further improve diagnostic specificity, particularly when distinguishing PD from MSA and other atypical parkinsonian syndromes [100].

Despite these promising findings, several challenges limit the clinical application of CSF α-Syn biomarkers. These include substantial interstudy variability, the absence of standardized assays and reference standards, the use of antibodies with incompletely characterized specificity, and differences in α-Syn aggregation states and PTMs that may interfere with epitope recognition. Moreover, although CSF sampling provides access to biomarkers associated with CNS pathology, lumbar puncture is invasive and may be unsuitable for routine screening or large-scale population studies [101].


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The development of ultrasensitive techniques, including single-molecule arrays (Simoa) and α-Syn seed-amplification assays, such as real-time quaking-induced conversion (RT-QuIC) and protein misfolding cyclic amplification (PMCA), has enabled the detection of low-abundance α-Syn species and seeding activity. These approaches have demonstrated promising sensitivity for detecting misfolded α-Syn in CSF and may contribute to early diagnosis and the assessment of disease-related outcomes, including cognitive decline. However, their diagnostic and prognostic performance requires further validation in well-characterized longitudinal cohorts [101].

*Plasma*

Because blood sampling is minimally invasive and readily scalable, peripheral blood represents an attractive source for developing α-Syn-based biomarkers for PD. However, despite extensive investigation, findings have remained inconsistent, reflecting both biological complexity and analytical limitations [96].

Studies investigating α-Syn concentrations in plasma and serum have reported conflicting results. Some have found increased total α-Syn or O-α-Syn levels in patients with PD compared with healthy controls, with certain studies also reporting associations with clinical severity [102]. Conversely, other investigations have observed no significant differences or even reduced α-Syn levels in PD [103]. These discrepancies may reflect differences in sample processing, assay sensitivity and, particularly, the susceptibility of plasma measurements to contamination by α-Syn released from RBCs during haemolysis. More than 99 % of blood α-Syn is localized within erythrocytes, and even limited haemolysis can substantially affect measured plasma concentrations. In addition, inadequate control of platelet contamination, differences among analytical platforms (ELISA, Western blot and Luminex), and the absence of standardized preanalytical procedures have contributed to variability across studies [104].

Consequently, the diagnostic performance of plasma total α-Syn remains limited. Studies have reported sensitivities of approximately 48 to 53 % and specificities of approximately 69 to 85 % [96]. Plasma pS129 and O-α-Syn have also been investigated, but findings remain inconsistent. Some studies reported elevated levels of these species in PD, whereas others found no significant differences from controls or no association with disease severity. Longitudinal studies have likewise reported changes in plasma pS129 over time, although the observed patterns have not been consistent across cohorts [105].

Given the substantial contribution of RBCs to circulating α-Syn, several studies have investigated erythrocyte-associated α-Syn species as potential biomarkers. These approaches include measurements of total, aggregated, oligomeric and PTM-specific α-Syn in RBC membranes and cytosol. Increased total and aggregated α-Syn levels in RBC membranes, together with elevated cytosolic pS129, have been reported in patients with PD compared with healthy controls [106]. Similarly, RBC O-α-Syn levels have been reported to be higher in PD than in MSA and healthy individuals. In animal models, RBC α-Syn levels have also shown a modest correlation with brain α-Syn levels, suggesting a possible relationship between peripheral and central α-Syn pathology [107].

Other studies have reported increased α-Syn dimers, altered ratios of proteinase K-resistant species, and elevated levels of oxidized, nitrated, or SUMOylated α-Syn in erythrocytes from patients with PD [108]. Despite these findings, RBC-based biomarkers have several limitations. Differences in RBC counts, cell age, comorbidities, sample preparation and analytical methods may influence α-Syn measurements. The lack of standardized reference values and variability in detection techniques remain important barriers to clinical implementation [96].

An alternative approach to reducing interference from RBC-derived α-Syn is the measurement of α-Syn in neuron-derived extracellular vesicles (NDEVs) isolated from plasma or serum. These vesicles may carry CNS-derived α-Syn and potentially provide information about its molecular state. Recent studies have reported elevated α-Syn levels in extracellular vesicles (EVs) enriched for neuronal markers, including L1CAM, in patients with PD compared with controls [109]. Increased EV-associated α-Syn has also been reported during prodromal disease stages, suggesting potential utility for early detection. Combining α-Syn measurements with other EV-associated proteins, such as clusterin or tau, has been proposed to improve diagnostic accuracy. Although EV-based assays are promising, their clinical translation requires standardized isolation and characterization procedures, validation in large longitudinal cohorts and improved reproducibility [96].

*Saliva*

Among peripheral biofluids, saliva represents an accessible and non-invasive source for biomarker investigation in PD. Its suitability for repeated sampling and potential use in routine clinical assessment have increased interest in salivary α-Syn. However, studies measuring total and oligomeric α-Syn in saliva have produced inconsistent findings.

Several investigations have reported reduced total salivary α-Syn levels in patients with PD compared with healthy controls, suggesting potential diagnostic value [110]. Conversely, O-α-Syn levels and the O-α-Syn/total α-Syn ratio have been reported to be significantly elevated in PD, particularly in patients with bradykinesia- and rigidity-dominant phenotypes. Some studies have also observed positive associations between these measures and disease duration [111]. These findings raise the possibility that changes in total α-Syn and its oligomeric forms may reflect alterations in its distribution or aggregation state. However, this interpretation requires further validation. Genetic factors may also influence salivary α-Syn measurements. One study found no significant difference in total salivary α-Syn between patients with PD and controls but reported elevated O-α-Syn in PD and associations between salivary α-Syn levels and specific SNCA single-nucleotide polymorphisms (SNPs), rather than disease duration or severity [112].

A meta-analysis reported a significant association between increased salivary O-α-Syn and PD, whereas total salivary α-Syn was not consistently reliable for diagnostic purposes. Variability across studies may reflect differences in analytical platforms, including ELISA, Luminex and Western blot; cohort size; disease-stage heterogeneity; and, particularly, saliva collection and processing protocols. Subsequent studies have also reported conflicting findings [113]. Some investigators found no significant differences in α-Syn levels between PD and control groups when analysing unstimulated saliva supernatants or cellular fractions, whereas others reported reduced α-Syn levels using ELISA-based methods. These discrepancies highlight the need for standardized saliva collection, processing and normalization procedures, as well as validation in larger cohorts [104].

In addition to whole saliva, the major and minor salivary glands have been investigated as potential sites of α-synuclein pathology. Immunohistochemical studies have detected α-Syn aggregates in labial salivary glands in approximately 60 % of patients with PD and in submandibular glands in up to 100 % of cases. These findings suggest that salivary gland pathology may reflect α-Syn-associated disease processes. However, the diagnostic utility of glandular biopsies remains limited by their invasive nature, sampling variability and differences in detection methods.

Overall, saliva represents a promising and patient-friendly source for α-Syn biomarker investigation in PD, although the current evidence remains heterogeneous. O-α-Syn appears to provide more consistent diagnostic information than total salivary α-Syn in the studies described here. Nevertheless, standardized


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collection and analytical procedures, together with validation in larger and well-characterized cohorts, are required before salivary α-Syn can be considered for routine clinical use [114].

*Peripheral tissue*

PD is increasingly recognized as a disorder involving both the CNS and peripheral nervous system. Accordingly, the detection of pathological α-Syn in peripheral tissues has attracted interest as a potential diagnostic and prognostic approach. Aggregated, oligomeric and phosphorylated α-Syn species have been reported in tissues including the skin, salivary glands, gastrointestinal mucosa, olfactory epithelium and autonomic ganglia. Some of these findings have been associated with non-motor symptoms that may precede the clinical diagnosis of PD [104].

Skin biopsy is among the most accessible approaches for investigating peripheral α-Syn pathology. Immunohistochemical and confocal imaging studies have reported increased α-Syn deposition in cutaneous sympathetic nerve fibres, particularly in patients with advanced PD and prominent autonomic symptoms [115]. O-α-Syn detected using proximity ligation assays has also been reported to be elevated in patients with PD, including individuals in prodromal stages and monozygotic twins discordant for PD [116]. Similarly, pS129 has been detected in sympathetic nerve fibres in patients with PD and has been investigated for its potential to distinguish PD from related disorders, including MSA and essential tremor [117]. Furthermore, α-Syn aggregation and seeding activity assessed using techniques such as RT-QuIC and PMCA have been reported in skin biopsies and autopsy samples from patients with PD. These findings support continued investigation of skin as a potential source of peripheral α-Syn biomarkers [118].

Non-motor symptoms, particularly gastrointestinal dysfunction, may precede the onset of motor symptoms in PD by several years. This observation has motivated extensive investigation of the enteric nervous system (ENS). Early studies reported LBs and α-Syn aggregates in the esophagus, colon and sympathetic ganglia [119]. Subsequent investigations identified α-Syn pathology in Auerbach's and Meissner's plexuses, including phosphorylated α-Syn [120]. Aggregated α-Syn has also been detected in gastrointestinal mucosa during the prodromal phase of PD, raising the possibility that peripheral α-Syn pathology may precede or accompany CNS involvement. These observations have contributed to the proposed "gut-first" hypothesis, which suggests that pathological α-Syn may spread from the gastrointestinal tract toward the CNS through the vagus nerve. However, the presence of gastrointestinal α-Syn pathology alone does not establish a peripheral origin of PD or confirm the direction of pathological spread [121].

Several studies have reported a higher frequency or intensity of α-Syn pathology in colonic and rectal mucosal biopsies from patients with PD than in controls [122]. Nevertheless, findings remain inconsistent. Some studies have detected α-Syn immunoreactivity in aged controls, found no significant differences between PD and non-PD groups, or observed increased phosphorylated α-Syn without corresponding changes in total α-Syn [120,123]. These discrepancies may reflect differences in SNCA genotype, biopsy location and technique, detection methods and disease stage [124]. Although a systematic review and meta-analysis reported an association between gastrointestinal α-Syn pathology and PD, the diagnostic performance of gastrointestinal biopsies remains dependent on the sampling and detection methods used [125].

The submandibular gland has also emerged as a potential site for detecting α-Syn pathology. Several autopsy- and biopsy-based studies have reported pS129 and O-α-Syn immunoreactivity in patients with PD, with limited or absent staining in some control and MSA groups [126,127]. In certain studies, Lewy pathology was detected in all examined PD cases, suggesting potentially high diagnostic performance in those cohorts. However, results have not been uniformly positive. Chahine *et al.* detected α-Syn immunoreactivity in 56.1 %

of PD cases using monoclonal antibodies, although specificity was reported to be 100 % [128]. Similarly, Kang *et al.* found no significant difference in total α-Syn content between PD and control groups but reported elevated O-α-Syn in PD, further suggesting that oligomeric forms may provide more disease-relevant information than total α-Syn [112]. α-Syn levels in the submandibular gland have also been reported to vary with specific SNCA genetic variants. In addition, RT-QuIC-based detection of pS129-associated seeding activity in frozen or paraffin-embedded submandibular tissue from PD cases has provided further evidence supporting investigation of this tissue as a potential biomarker source [129].

Because anosmia can occur early in PD, the olfactory mucosa has also been investigated as a potential site for detecting α-Syn pathology. However, findings have been inconsistent. Some postmortem studies identified α-Syn-positive neurites in patients with PD and, in certain cases, in controls. In contrast, other studies reported no detectable α-Syn in antemortem olfactory mucosal biopsies from patients with PD, raising questions about the reliability of this tissue for diagnostic purposes [127].

α-Syn pathology is not restricted to the skin, salivary glands, or gastrointestinal tract. Lewy-type inclusions have also been reported in several peripheral structures, including the sympathetic ganglia, vagus nerve, sciatic nerve, cardiac plexus, laryngeal nerves and genitourinary tissues. Reported frequencies include approximately 80 % in sympathetic ganglia, 73 % in the vagus nerve and 50 % in the sciatic nerve. These observations indicate extensive peripheral involvement in PD but also highlight the difficulty of identifying a single optimal tissue for biopsy-based diagnosis.

Overall, peripheral tissues represent a promising source for α-Syn biomarker discovery and may provide information relevant to early detection, disease monitoring and the investigation of peripheral pathology. Among the tissues examined, colonic submucosa and submandibular glands have shown relatively consistent signals in some studies, particularly when pS129 and O-α-Syn are assessed. However, differences in methodology, genetic variation, disease heterogeneity and sampling procedures remain important sources of uncertainty. Standardized protocols, multicenter validation and approaches integrating molecular pathology with clinical phenotyping are needed to establish the diagnostic value of peripheral tissue α-Syn biomarkers [101].

*Retina*

The retina, as an extension of the CNS, provides a potential window into neurodegenerative processes, including those associated with PD. Endogenous α-Syn is expressed in retinal neurons and has been reported to be particularly abundant in the inner plexiform layer, as initially described by Surguchov *et al.* [130] and subsequently confirmed by Beach *et al.* [131]. Given the frequency of visual disturbances in PD, including reduced contrast sensitivity, impaired colour discrimination and changes in visual processing speed, retinal α-synuclein pathology has attracted interest as a potential biomarker reflecting broader CNS involvement.

Non-invasive techniques, including optical coherence tomography (OCT), electroretinography (ERG) and visual evoked potentials (VEPs), have increasingly been used to assess retinal structure and function in patients with PD [132]. Studies have reported thinning of the retinal nerve fibre layer (RNFL), changes in ganglion cell layer thickness and abnormal retinal responses. These alterations may reflect neurodegenerative changes affecting the retina and have been investigated in relation to α-Syn pathology. However, the extent to which these structural and functional changes specifically reflect retinal α-Syn aggregation remains uncertain [133]. Animal models of PD have also demonstrated α-Syn accumulation in retinal tissue, providing experimental evidence supporting further investigation of retinal pathology in PD [134].

In humans, evidence for retinal α-Syn pathology remains more limited, although several studies have reported encouraging findings. One study identified α-Syn aggregates in retinal tissue from patients with PD


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and associated these deposits with visual dysfunction and retinal structural abnormalities [135]. Beach *et al.* [131] detected pS129 in nerve fibres at the inner retinal surface of PD eyes but not in control eyes. In a detailed immunohistochemical investigation, Ortuño-Lizarán *et al.* [136] also confirmed pS129 in PD retinas, reporting deposits resembling LBs and Lewy neurites in ganglion cell bodies and their axonal and dendritic processes. Notably, total α-Syn immunoreactivity did not differ significantly between PD and control retinas in that study, whereas pS129 distinguished PD cases, highlighting the potential importance of PTM-specific detection in retinal pathology.

Conversely, Ho *et al.* [137], using postmortem enucleated eyes, detected α-Syn immunoreactivity in both PD cases (67 %) and controls (50 %) but found no specific evidence of Lewy pathology. This finding illustrates the limitations of total α-Syn as a retinal biomarker, particularly because α-Syn is physiologically present in healthy retinal tissue.

Taken together, the retina represents an interesting potential source for non-invasive biomarker development in PD, although substantial challenges remain. While postmortem studies have identified pS129 and α-Syn aggregates in PD retinas, *in vivo* detection of pathological α-Syn in the retina is not currently established. Differences in tissue quality, fixation procedures and antibody specificity also complicate comparisons across studies. Overall, retinal α-Syn pathology, particularly pS129-associated pathology, may reflect disease-related processes and could be relevant to the visual abnormalities observed in PD. However, larger human studies, development of imaging methods capable of detecting pathological α-Syn *in vivo*, and standardized detection protocols are required before its clinical utility can be established [96].

Collectively, these biomarker platforms differ in their biological sources, analytical approaches, accessibility and potential clinical applications. A comparative overview of their biological sources, detection methods, clinical significance, strengths and limitations is provided in Table 2.

**Table 2.** Summary of α-Syn biomarkers and their clinical applications.

| Biomarker source | Main α-Syn species | Detection methods | Clinical significance | Advantages | Limitations | Ref. |
|---|---|---|---|---|---|---|
| CSF | Total, oligomeric and phosphor-rylated α-Syn | ELISA, RT-QuIC, PMCA | Supports early diagnosis and disease monitoring | Reflects central nervous system pathology | Lumbar puncture is invasive | [95-97, 99-101] |
| Plasma | Total and oligomeric α-Syn | ELISA-based immunoassays | Potential peripheral biomarker | Easily accessible and minimally invasive | Blood cell contamination may affect results | [96, 102-108] |
| RBCs | Total and aggregated α-Syn | Immunoassays | May improve diagnostic performance | High α-Syn content | Limited disease specificity | [106,107] |
| NDEVs | Oligomeric and phosphorylated α-Syn | Immunoassays | Reflect neuronal pathological changes | High diagnostic potential | Isolation and standardization remain challenging | [109] |
| Saliva | Total and oligomeric α-Syn | ELISA | Non-invasive biomarker candidate | Simple and inexpensive collection | Variable biomarker concentration | [104-110, 114] |
| Skin biopsy | Phosphorylated α-Syn | Immunohistoche mistry | Detects peripheral α-Syn pathology | High diagnostic accuracy | Requires tissue biopsy | [115-118] |
| Retina | Retinal α-Syn deposition | Retinal imaging / histological analysis | May identify early neurodegenerative changes | Potentially non-invasive approach | Requires further clinical validation | [95, 130-137] |
| Colon | Aggregated/phosp horylated α-Syn | Immunohistoche mistry | Indicates prodromal pathological changes | Supports early disease detection | Invasive tissue sampling | [101, 119-122] |
| Olfactory mucosa | Aggregated α-Syn | RT-QuIC / immunoassays | Useful for early diagnosis | Easily accessible tissue | Limited clinical standardization | [127] |

## Conclusions

α-Syn occupies a distinctive position at the interface of neuronal physiology and neurodegeneration. Under physiological conditions, its conformational flexibility and interactions with synaptic vesicles, cellular membranes and other molecular partners contribute to the regulation of synaptic vesicle trafficking, neurotransmitter release and neuronal homeostasis. However, alterations in its conformational and functional equilibrium, arising from genetic mutations, PTMs, changes in lipid and metal ion interactions, impaired proteostasis, or environmental stressors, may promote the formation of oligomeric and fibrillar assemblies associated with PD and other synucleinopathies. These observations highlight the importance of understanding how the molecular properties of α-Syn influence both its physiological activities and its pathological behaviour.

Advances in structural, biophysical and biochemical research have expanded our understanding of α-Syn beyond its characterization as an aggregation-prone protein. Its intrinsically disordered nature, conformational dynamics, membrane-binding properties and interactions with cellular proteins collectively contribute to its diverse biological activities. Moreover, the effects of distinct conformational assemblies, cellular environments and PTMs on α-Syn behaviour underscore the complexity of its involvement in neuronal function and neurodegeneration. Rather than representing a single pathogenic mechanism, α-Syn-associated processes appear to involve interconnected molecular events whose contributions may vary according to cellular context and disease stage.

Recent developments in biomarker research and therapeutic strategies have further emphasized the clinical relevance of α-Syn molecular heterogeneity. Seed amplification assays, extracellular vesicle analyses and the investigation of distinct α-Syn species have provided promising approaches for improving the detection and characterization of synucleinopathies. In parallel, immunotherapies, aggregation-modulating compounds, gene-based approaches and strategies targeting proteostasis and lysosomal function are being investigated as potential therapeutic interventions. Nevertheless, their clinical significance and ability to modify disease progression require further validation, and no approach has yet established unequivocal disease-modifying efficacy across the broader spectrum of synucleinopathies.

Future research should therefore extend beyond the reduction of total α-Syn levels and consider how its physiological functions can be preserved while pathogenic conformations and interactions are selectively targeted. Integrating structural biology, biophysical and biochemical analyses, advanced disease models, multi-omics approaches and clinically validated biomarkers may help clarify the molecular determinants of α-Syn function, aggregation and disease-associated heterogeneity. Ultimately, a more comprehensive understanding of the relationships between α-Syn structure, conformational dynamics, molecular interactions and cellular function will be essential for elucidating its contribution to neuronal homeostasis and neurodegeneration and for guiding the development of mechanism-based therapeutic strategies for PD and other synucleinopathies.

## Abbreviations

| | |
|---|---|
| α-Syn | Alpha-synuclein |
| Aβ | Amyloid-beta |
| BBB | Blood-brain barrier |
| CaM | Calmodulin |
| CHIP | Carboxyl terminus of Hsp70-interacting protein |
| CKII | Casein kinase II |
| CL | Cardiolipin |
| CNS | Central nervous system |
| CSF | Cerebrospinal fluid |
| DAT | Dopamine transporter |


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| | |
|---|---|
| DLB | Dementia with Lewy bodies |
| DJ-1 | Parkinsonism-associated deglycase (PARK7) |
| ER | Endoplasmic reticulum |
| ERK | Extracellular signal-regulated kinase |
| EV | Extracellular vesicle |
| GABA | Gamma-aminobutyric acid |
| GBA (GCase) | Glucocerebrosidase |
| GRK | G protein-coupled receptor kinase |
| HC | Healthy control |
| Hsp | Heat shock protein |
| ITPKB | Inositol-1,4,5-trisphosphate 3-kinase B |
| KD | Dissociation constant |
| LBs | Lewy bodies |
| LRRK2 | Leucine-rich repeat kinase 2 |
| MAPK | Mitogen-activated protein kinase |
| MPTP | 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine |
| MSA | Multiple system atrophy |
| NAC | Non-amyloid-β component |
| NDEVs | Neuron-derived extracellular vesicles |
| O-GlcNAc | O-linked β-N-acetylglucosamine |
| OGT | O-GlcNAc transferase |
| PD | Parkinson's disease |
| PIAS2 | Protein inhibitor of activated STAT2 |
| PLK2 | Polo-like kinase 2 |
| PMCA | Protein misfolding cyclic amplification |
| PP2A | Protein phosphatase 2A |
| PPIs | protein-protein interactions |
| PTMs | Post-translational modifications |
| RBCs | Red blood cells |
| RT-QuIC | Real-time quaking-induced conversion |
| SAPK | Stress-activated protein kinase |
| SIAH-1 | Seven in absentia homolog 1 |
| SILAC | Stable isotope labelling by amino acids in cell culture |
| Simoa | Single-molecule array |
| SNARE | Soluble N-ethylmaleimide-sensitive factor attachment protein receptor |
| SNAP-25 | Synaptosomal-associated protein 25 |
| SUMO | Small ubiquitin-like modifier |
| TH | Tyrosine hydroxylase |
| TRPML1 | Transient receptor potential mucolipin 1 |
| UCH-L1 | Ubiquitin C-terminal hydrolase L1 |
| UPS | Ubiquitin-proteasome system |
| VAMP2 | Vesicle-associated membrane protein 2 |

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