Chapter

A Potential Innovative Therapy for Parkinson's Disease: Selective Destruction of the Pathological Assemblies of Alpha-Synuclein

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Abstract

With the aging of the population, Parkinson's disease poses a serious socio-economic problem; there is no effective therapy that can arrest/revert the progression of the disease. The hallmarks of Parkinson's disease and other synucleinopathies are the disordered alpha-synuclein and TPPP/p25. These proteins have neomorphic moonlighting characteristics by displaying both physiological and pathological functions. Physiologically TPPP/p25 regulates the dynamics/stability of the microtubules and is crucial for oligodendrocyte differentiation; while alpha-synuclein is involved in neuronal plasticity modulation and synaptic vesicle pool maintenance. In healthy brain, alpha-synuclein and TPPP/p25 occur predominantly in neurons and oligodendrocytes, respectively; however, they are co-enriched and co-localized in both cell types in brain inclusions in the cases of Parkinson's disease and multiple system atrophy, respectively. The pathomechanisms of these diseases are largely unknown; the fatal species are the small, soluble homo- and hetero-associations of alpha-synuclein. These proteins with their high conformational plasticity and chameleon feature are challenging drug targets. Nevertheless, the contact surface of TPPP/p25-alpha-synuclein assemblies has been validated as a specific drug target. This new strategy with innovative impact, namely targeting the interface of the TPPP/p25-alpha-synuclein complex, could contribute to the development of anti-Parkinson drugs with unique specificity.

Keywords: alpha-synuclein, TPPP/p25, pathological assemblies, drug target, innovative therapy

1. Introduction

With the aging of society, neurological disorders have become more and more widespread resulting in serious social and economic problems. Parkinson's disease (PD) is the second most common neurodegenerative disease [1]. The etiology of this disease is initiated by unfolded/misfolded proteins, which form homologous and/or heterologous oligomers leading to the formation of aggregates and inclusions such as Lewy bodies predominantly comprised of alpha-synuclein (SYN) as histopathological hallmarks [1, 2].

At the present time, there is no proven therapy that can counteract the progression of the disease. The symptomatic therapies may reverse or slow down the progression of the symptoms, but cannot arrest/revert the neurodegenerative process [3]. The motor impairments of PD are attributed to the loss of dopaminergic neurons in the substantia nigra pars compacta; the phenotype is characterized by rigidity, resting tremor and bradykinesia [1]. The gold standard drug in the clinical practice is the L-dopa (or levodopa), precursor of dopamine, which relieves these motor symptoms by the replacement of the lost dopamine; however, large variability in drug response in terms of efficacy and adverse reactions have been observed [3, 4]. These side effects of conventional anti-parkinsonian drugs have compelled the researchers to seek novel alternatives such as gene therapy, stem cells transplants and neuroprotective agents [3]. The latest progression for the therapy suggests further opportunities (applications of antibodies, antisense-oligonucleotides and small molecules) that decrease the SYN level and its aggregation in the brain, some of them are now under clinical trials [3, 5].

One of the important factors in the PD research is related to the finding that SYN displays both physiological and pathological functions [5]; consequently, besides the specific and effective destruction of the accumulated SYN leading to the formation of its toxic assemblies/aggregations, the optimal SYN level has to be maintained/ensured for its physiological functions. In this chapter, the structural and functional potentials of SYN and Tubulin Polymerization Promoting Protein (TPPP/p25), hallmarks of PD [6], are reviewed leading to their molecular mechanism/pathomechanism in the initiation of PD and other synucleinopathies.

2. SYN and its physiological associations

SYN is an unstructured protein, prototype of the *chameleon* proteins [7]. Although the intrinsically disordered SYN is predominantly unfolded under physiological conditions, helically folded tetrameric structure or the combination of the two also have been suggested as its native structure [8]. In response to environmental changes, the disordered SYN is able to adopt significant conformational changes with different amount of secondary structures determined by pH, temperature, presence of organic solvents, membranes or specific metal ions [7, 9]. The structure of SYN have been studied in details under a plethora of distinct circumstances [10–12]. Structurally SYN comprises three regions: the N-terminal region involved in lipid binding; the highly hydrophobic central NAC region; and the acidic unfolded C-terminus, which exhibits chaperone activity and may counteract the aggregative potency of SYN [5, 13, 14] (**Figure 1**). The disordered C-terminal segment of SYN (45 aa) was found to modulate its aggregation, however, a terminal peptide (30 aa) was ineffective as a competitor in the aggregation process, which is characteristic for chameleon proteins [7].

The central hydrophobic region of SYN corresponding to residues 71–82 was found to be essential for its misfolding and aggregation, while a second critical region (residues 45–57) is of great importance in mediating β-strand to β-strand interactions in the fibril conformation [8]. Mutations are localized (18–53 aa) within the N-terminal region of SYN involved in lipid-binding. Based on comparative analysis of SYN structure, the 32–58 aa region was assigned as a crucial one to ensure the stability and secondary structure of SYN [15]. This issue is in agreement with another study which revealed the prominent role of a similar segment (39–45 aa) of the protein in membrane penetration [16]. SYN mutants with increased oligomerization efficacy are more inclined to penetrate the membrane [17].

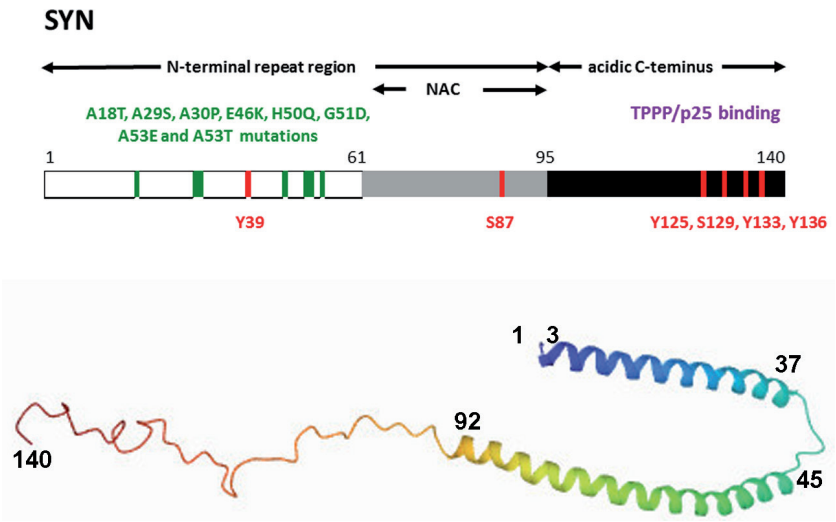


Figure 1.
Schematic representation of SYN. Familial mutations related to PD (green), and phosphorylation sites (red) are indicated. 3D structure of the human micelle-bound alpha-synuclein determined by NMR (DOI: 10.2210/pdb1XQ8/pdb) [14].

In normal brain, SYN binds to the surface of synaptic vesicles [5, 18]. Although it is highly disordered when isolated in solution; however, its micelle-bound form displays a partial helical structure that could be formed into curved α-helices [14, 19] (**Figure 1**). In spite of the data accumulated so far, the physiological function of SYN is still unclear in details. Membrane bound conformations of SYN are likely mediate its physiological function including the modulation of neuronal plasticity, synaptic vesicle pool maintenance, and dopamine metabolism [5, 13]. Moreover, it has been proposed that it can function as a microtubule regulatory protein (dynamase) [20–22]; as a disordered hub protein it also interacts with at least 50 ligands and other proteins [23].

The role of molecular chaperones in the regulation of the physiological function of SYN has been recently reviewed [24]. These interactions reduce the amount of free SYN in the cells and thus prevent its structural transition towards pathological states. Heat shock proteins (Hsp) are molecular chaperones that assist in proper conformational binding of proteins; they display protective effect against their toxicity and counteracts aggregation [25, 26]. SYN interacts with Hsp90 and Hsp70 as shown by co-immunoprecipitation [27]. The modulation of the proteolytic degradation of SYN by inhibiting Hsp90 function or by promoting Hsp70 function resulted in enhanced degradation of the aggregated protein. In fact, this issue has been suggested for treatment of PD against SYN toxicity [25]. Small molecules, which either directly interact with SYN or modulate molecular chaperones, were found to decrease SYN aggregation in vitro or in some animal models of PD; however, there is no clinical proof for their efficacy yet [25].

3. SYN mutations and pathological assemblies

SYN was the first identified causative gene of familial PD, all identified mutations can be found in the N-terminal region that affect the oligomerization, fibrillation and/or aggregation of SYN leading to the formation of the toxic species, see [1, 5, 6] and references therein. Until now, the following mutants have been

identified to be involved in PD: A18T, A29S, A30P, E46K, H50Q, G51D, A53E and A53T [28] (**Figure 1**). There are mutants (A18T, A29S, E46K, H50Q and A53T) that increase SYN aggregation, others (G51D and A53E) slow down its aggregation; while the A30P mutation increases the oligomerization, yet hinders the fibrillation [29, 30]. G51D mutation, although the slowest to aggregate, is the most potent of the known early onset mutations supporting the hypothesis that increased lifetime of smaller oligomers can impart toxic effects [8]. The post-translational modifications, such as Ser129, Ser87 and Tyr125 phosphorylation, could also display various effects on the SYN assembly. The phosphorylation of SYN on Ser129 is negligible in normal brain, but it is the dominant form in Lewy bodies [31]. However, the effects of these modifications on the drug/ligand binding of SYN have not been clarified yet.

Two cellular pathways are involved in SYN clearance trying to maintain its physiological protein level: the ubiquitin-proteasome system (UPS) [32] and the autophagy-lysosomal pathway [33–35]. UPS is involved in proteolytic degradation of short-lived, damaged and misfolded protein; while the degradation of the long-lived and aggregated protein as well as that of the damaged organelles are achieved by macroautophagy (autophagy) and the selective chaperone-mediated autophagy (CMA) [36–38]. Macroautophagy degrades cellular waste through the fusion of the autophagosomes, carrying the material, with the lysosomes containing hydrolyses. Whereas CMA degrades soluble cytosolic proteins containing a specific CMA motif related to the pentapeptide KFERQ. The cytosolic chaperone heat-shock cognate 70 kDa protein (Hsc70) recognizes this motif, then it delivers the targeted protein to the lysosomes, and after binding to the lysosomal-associated membrane protein 2A (LAMP-2A), the targeted protein is translocated into the lysosomal lumen.

Genetic and post-mortem studies have suggested that modifications occur in both macroautophagy and CMA in the case of PD [39]. Mutations or post-translational modifications of SYN can also affect its turnover by CMA, such as the A30P and A53T mutants, related to familial cases of PD, which are not efficiently degraded through CMA, they can bind LAMP-2A, but are not internalized inside the lysosomes [40]. The protein level of the LAMP-2A, a key CMA marker, can be decreased in the substantia nigra of PD brains as compared to controls [41], while its protein level correlates with increased SYN accumulation in the affected PD brain regions.

The inhibition of the chaperone-SYN interaction facilitates the binding of SYN forming amphipathic helix into the lipid bilayer of the mitochondria membrane leading to membrane disruption [24]. SYN interaction with mitochondria occurs at higher protein expression or impaired chaperone-SYN ratio; therefore, the pathological conditions result in the failing of its CMA-derived proteolytic degradations [24]. Therapeutic strategies aiming to increase the SYN degradation through activation of these clearance pathways have thus been deeply explored in order to re-establish physiological levels of the protein and prevent its accumulation in PD [25, 42, 43]. The most interactions of SYN with mitochondria occur in cells in the case of oxidative stress [44] that can promote SYN aggregation associated with mitochondrial dysfunction [45, 46]. The localization of the enriched SYN on the mitochondrial membrane can produce destructive effect. Cellular oxidative stress is known to be a common factor driving synucleinopathy progression [44, 47].

Under oxidative stress conditions DJ-1, a cellular protease, is translocated from the cytoplasm to the mitochondria [48, 49]. DJ-1 is able to interact with both monomeric and oligomeric SYN counteracting its oligomerization propensity [50]. The crucial role of DJ-1 to control the aggregated SYN in proximity of mitochondria is also reflected by the fact that DJ-1 has been found in the proximity of Lewy bodies [51, 52]. Mutations within DJ-1 associated with PD reduce the capacity of DJ-1 to

prevent toxic SYN assemblies [53–55]. The ability of DJ-1 to inhibit SYN aggregation appears to be dependent on the oxidation of its Cys106 residue (Cys_{106}-SO_2^- form) [50, 56, 57]. SYN overexpression activates CMA by elevating the levels of LAMP-2A; however, DJ-1 deficiency suppressed this effect. Experiments with DJ-1 knockout (KO) mice and DJ-1 siRNA knockdown SH-SY5Y cells confirmed that DJ-1 deficiency increased the accumulation and aggregation of SYN in both models, by accelerating the degradation of LAMP-2A, a lysosome-associated membrane protein. DJ-1 deficiency also downregulated the level of lysosomal Hsc70 [52]. These findings provide evidence for the molecular interaction between PD-related proteins via the CMA pathway.

In recent years, emerging evidence points out that microglial and astrocytic dysfunction may also play an important role in the pathogenesis of PD [40]. Several genes associated with PD are also expressed in glial cells, displaying comparable or even higher levels than in neurons, which are also involved in inflammatory response, oxidative stress, lysosomal and mitochondrial function, and autophagy. Perturbations in DJ-1 may alter different glial processes that can impact neuronal survival, DJ-1-deficient microglia displayed elevated intracellular reactive oxygen species and nitric oxide leading to increased dopaminergic neurotoxicity [58]. Recently it has been suggested that primary cortical astrocytes form DJ-1 KO mice may provide decreased neuroprotection to surrounding neurons due to alterations in pro-inflammatory mediator expression [52].

The cytoskeletal microtubule system plays a crucial role in several physiological and pathological processes which is achieved by the decoration of this filamentous network with proteins/enzymes as well as post-translational modifications [59]. The microtubule associated proteins/enzymes regulate these intracellular processes such as cell division, differentiation, autophagy, intracellular trafficking and aggresome formation by modulating microtubule dynamics and stability. Destabilization of the microtubule network, low tubulin acetylation levels and axonal transport deficits have been observed in PD [60, 61]. SYN has been described as a microtubule dynamase [21] and it also interacts with microtubule stabilizing proteins such as tau and TPPP/p25 [22, 59]. SYN binds within the microtubule-binding domain of tau and may promote its hyperphosphorylation resulting in impaired axonal transport [62]. The microtubule associated tau and TPPP/p25 stabilize the microtubule network [22, 59].

4. TPPP/p25, a multifunctional microtubule associated protein

Physiologically, TPPP/p25 modulates the dynamics and stability of the microtubule network by bundling the microtubules and enhancing the tubulin acetylation due to the inhibition of tubulin deacetylases [59, 63]. In normal brain, TPPP/p25 is expressed in oligodendrocytes (OLGs) and a key factor in the growth of projections in the course of differentiation requested for the axon ensheathment [64]. Therefore, the optimal endogenous TPPP/p25 level plays key physiological functions in the formation of differentiated OLGs, which are key players in myelin sheath formation.

TPPP/p25 is an intrinsically disordered protein without a well-defined 3D structure, whose middle, highly flexible CORE region is straddled by the unstructured N- and C-termini [65] (**Figure 2**). Two human gene sequences have been identified, which encode homologous proteins displaying approximately 60% identity with TPPP/p25. These proteins are N-terminal-free forms denoted as TPPP/p18 and TPPP/p20 [66]. The similarity of TPPP/p25 to TPPP/p20 is manifested in their intrinsically disordered characteristics and association to microtubules [66]. 3D

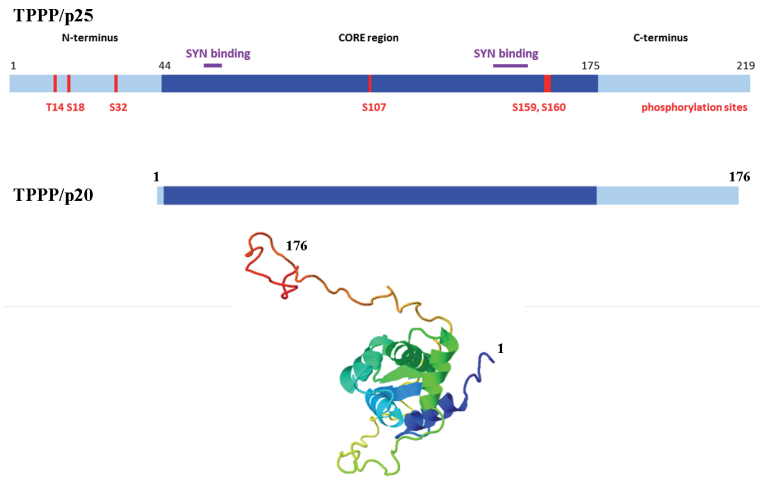


Figure 2.
Schematic representation of TPPP/p25. Phosphorylation sites (red) are indicated. 3D structure of the homologous TPPP/p20 determined by NMR (DOI: 10.2210/pdb2JRF/pdb) [67].

structure of TPPP/p20, but not TPPP/p25, has been determined by NMR (from TPPP/p20 the unfolded N-terminal tail of TPPP/p25 is missing) [67]. TPPP/p20 is involved in developmental processes of the musculoskeletal system [68], and surprisingly, not in neurodegenerative, rather in cancerous processes due to its modulation of the cell proliferation, see [59] and references therein.

TPPP/p25 occurs in monomeric and dimeric forms, the dimeric form displays enhanced tubulin polymerization promoting activity [69]. The UPS is the major system responsible for the elimination of the disordered TPPP/p25 suggested by the finding that MG132, a well-established inhibitor of proteasome, enhanced the intracellular TPPP/p25 level [70, 71]. The stabilization of TPPP/p25 against the proteolytic degradation is resulted from the structural changes of the protein coupled with its dimerization which is essential for the maintenance of the stability of the myelin sheath.

The forms of plasticity of synapsis within the OLG lineage as well as the connection of the OLG and myelin dysfunction in neurodevelopmental disorders with cognitive symptoms have recently been described [72]. The OLG precursor cells proliferate and some of them differentiate. A subset of these new OLGs integrates into sheaths on unmyelinated axon segments. In this process, TPPP/p25 could be a key player since its endogenous expression is involved in the differentiation of the dividing progenitor cells under post-transcriptional control [64]. In the course of this process, the plasticity of the myelin sheath might be modified.

Recently in has been shown that TPPP/p25 KO mice have shorter lamellar microtubules, and consequently shorter and thinner myelin sheaths [73]. Cultured TPPP/p25 KO OLGs also displayed additional aberrant features, including more proximal branches, mixed microtubule polarity and accumulation of myelin basic protein mRNA. In the brain of these mice, decreased myelination have been observed, although no gross differences were found in neurofilament staining, indicating that axonal tracts and neuronal morphology is largely intact [73]. Concerning the behavior of TPPP/25 KO mice, their anxiety behavior has been similar as in the case of wild type mice, however, they lack fear responses. Deficits in fear-conditioning, which is a memory dependent task, as well as in spatial memory tests support possible short-term memory defects [74]. Experiments with TPPP/p25 KO mice that exhibit hypomyelination with aberrant myelin sheaths and motor coordination deficits have suggested that microtubule nucleation outside the

cell body at Golgi outposts by TPPP/p25 is critical for the elongation of the myelin sheath [73]. In fact, elevation of the TPPP/p25 level was detected in rat brain in the course of aging [75], however, it is unclear whether it is due to increased demand or aberrant accumulation. The latter issue may be related to the development of different neurological disorders such as Alzheimer's disease (AD), PD, multiple system atrophy (MSA) and diffuse Lewy body disease (DLBD); however, increased TPPP/p25 level was detected with remyelinating lesions in the case of multiple sclerosis [76].

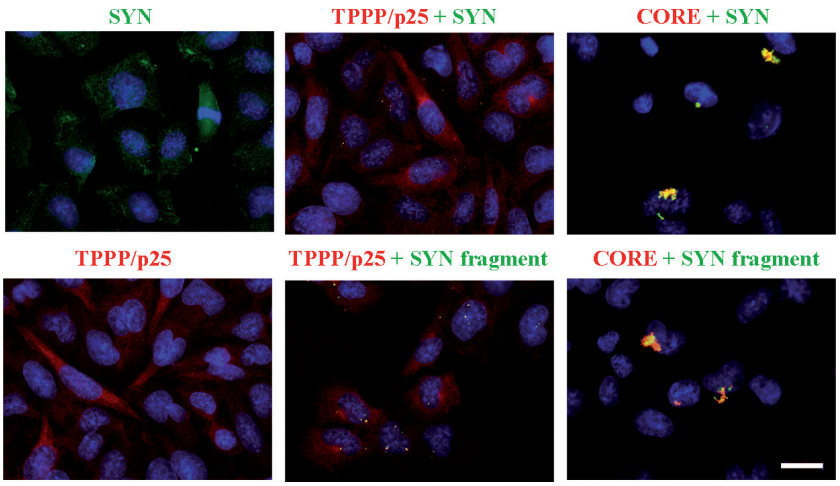
5. From TPPP/p25-SYN interaction to their co-localization in Lewy body

TPPP/p25, as a *moonlighting* protein, performs distinct functions under physiological and pathological conditions without alterations at gene level [77]. This feature of TPPP/p25 manifested primarily itself in its association with SYN, the hallmark of PD. Pathologically, TPPP/p25 interacts with SYN resulting in its oligomerization/aggregation [78]. Studies with various truncated and deletion mutants of the human TPPP/p25 produced by recombinant techniques revealed significantly reduced, but not abolished interaction with SYN [79, 80]. These findings indicated that the lack of identified binding segments of the wild type TPPP/p25 could be replaced by other segments [81]. Although it has been well-established that SYN is also a disordered protein; notwithstanding, the *neomorphic chameleon* characteristic was introduced for TPPP/p25 to indicate the distinction of the two disordered proteins. Namely, the modifications of TPPP/p25 at gene level is able to maintain its associative potency [81]; in contrast to this, the deletion of the last 20 amino acid residues of SYN abolished its interaction with TPPP/p25 [80].

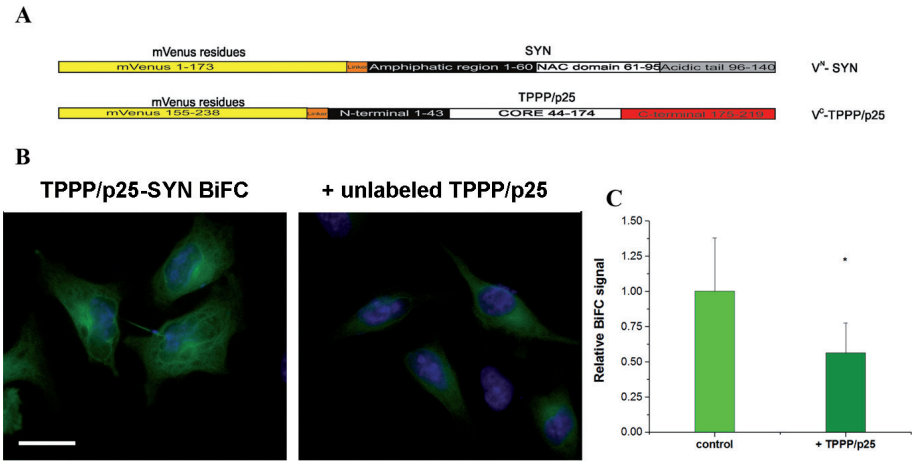
The unfolded SYN and TPPP/p25 are expressed distinctly in neurons [82, 83] and OLGs [64, 75], respectively, in healthy brain; however, they are co-enriched and co-localized in pathological inclusions in the cases of PD and MSA [84]. The interaction of SYN and TPPP/p25 has been proven at atomic, molecular and cellular levels as well as in post-mortem brain tissues [6, 59]. Short peptide fragments have been produced by proteolytic degradation of the interacting proteins as well as by chemical synthesis based upon the interface segments identified experimentally using the wild type proteins [79, 80]. The interactive and aggregative potencies of the wild type and truncated forms of SYN and/or TPPP/p25 were visualized by immunofluorescence microscopy (**Figure 3**). Massive co-aggregation of the two hallmark proteins were achieved by the contact surface-containing fragments instead of the full proteins.

The interaction of TPPP/p25 with SYN has been extensively characterized at atomic, molecular, cellular and tissue levels using wild type and mutant human recombinant proteins and living human cell models [6, 59, 85]. The interaction of SYN and TPPP/p25 in living cells was visualized by immunofluorescent confocal microscopy coupled with Bifunctional Fluorescent Complementation (BiFC) technology using mVenus vectors [81]. The immunofluorescence images presented in **Figure 4** verify the hetero-association of TPPP/p25 and SYN at cellular level; the hetero-association (green fluorescence) is reduced due to the addition of unlabeled TPPP/p25 as a competitor, which provides evidence for the dynamic and specific association of the two disordered proteins [81].

The hetero-association induced by the excess SYN and TPPP/p25 results in the appearance of massive aggregates [79–81]. The co-enrichment and co-localization of TPPP/p25 and SYN specific for synucleinopathies were established in post-mortem human brain tissues of patients with PD and other neurological disorders (**Figure 5**). TPPP/p25 is enriched in filamentous SYN bearing Lewy bodies of PD



01 **Figure 3.**
Intracellular co-enrichment and co-localization of wild type SYN and TPPP/p25 as well as their fragments in CHO10 cells [80]. Uptake of SYN and/or TPPP/p25 by CHO10 cells from the medium following their premixing as detected by immunofluorescence microscopy. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (blue). Scale bar: 5 μm.



02 **Figure 4.**
Dynamic association of TPPP/p25 with SYN in living HeLa cells as visualized by BiFC technology [81]. (A) Scheme of BiFC constructs for co-transfection of TPPP/p25 and SYN. (B): Visualization of the association of mV^N-SYN and V^C-TPPP/p25 (green). Effect of the unlabeled TPPP/p25 on the association of TPPP/p25 with SYN (BiFC) signal. Bar: 10 μm. (C) Quantification of the relative BiFC signal.

03 and DLBD, as well as in glial inclusions of MSA [84]. In contrast to synucleinopa-
04 thies, no co-localization was found between TPPP/p25 and phosphorylated tau in
05 inclusions of Pick's disease (PiD), progressive supranuclear palsy (PSP), and corti-
06 cobasal degeneration (CBD). It is worth noting that clustered immunoreactivity of
07 TPPP/p25 was found along filaments of unstructured but not compact neurofibril-
08 lary tangles in the case of AD. Based on these findings TPPP/p25 was suggested to
09 be a novel marker of alpha-synucleinopathies [84].
10 Co-immunoprecipitation analysis carried out on HEK293T and oligodendroglial
11 KG1C cell lines with ectopically expressed SYN and TPPP/p25 corroborated the
12 specific interaction of the two proteins; moreover, TPPP/p25 is able to induce SYN
13 oligomerization [86, 87]. Recently an oligodendroglial cell model of MSA has been
14 studied, in which after the overexpression of TPPP/p25 and uptake of human

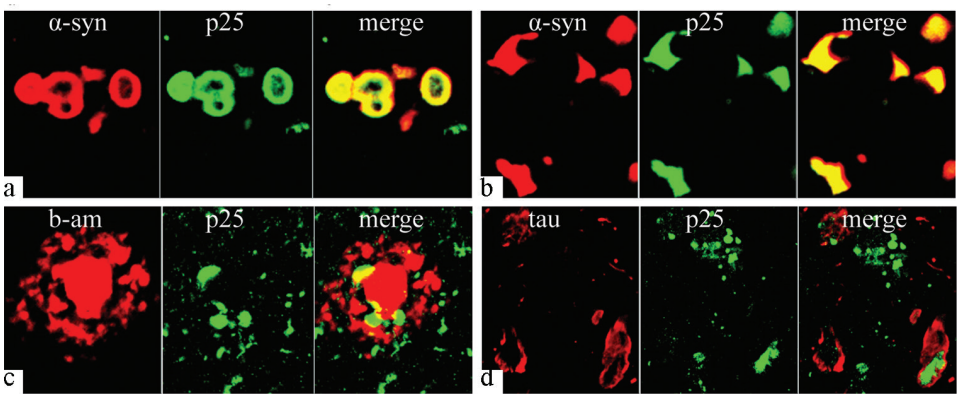


Figure 5.
SYN and TPPP/p25 in post-mortem brain samples in the cases of PD (a), MSA (b), DLBD and AD (c) and AD (d), respectively [84].

pre-formed SYN fibrils, the cells formed insoluble, highly aggregated, pathological assemblies [86]. Mavroeidi and his co-workers have also revealed that these assemblies resulted in the disruption of the microtubule and myelin networks [86] indicating the toxic potential of the pathological TPPP/p25-SYN assembly. In addition, the formation of the glial cytoplasmic inclusion was suggested due to the endogenously expressed hallmark proteins. In the case of MSA, early relocation of TPPP/p25 (from the myelin sheath and the nucleus to the cytoplasm) has been observed [88, 89].

In normal brain SYN and TPPP/p25 are expressed predominantly in neurons and in OLGs, respectively [64, 75, 82, 83]. However, these two hallmark proteins are co-enriched and co-localized in Lewy bodies and glial cytoplasmic inclusions characteristic for PD and MSA, respectively [84, 87, 90]. The intra- and extracellular transmission of SYN forms between neurons as well as between neurons and OLGs in the case of PD and MSA has been established [91, 92]. In addition, the presence of both proteins in the extracellular space has been reported inasmuch as their occurrence in the cerebrospinal fluid (CSF) [93–95], their cellular uptake from the medium were also detected [79, 96]. Consequently, the cell-to-cell transmission as a pathological situation can be mimicked in cells models such as HeLa by taking up SYN and/or TPPP/p25 from the medium [79–81].

The mechanism of this process is unclear yet, however, the liberation of the endocytosed materials in the cytoplasm by the mechanism of "endosomal escape" to reach autophagic vacuole has been proposed [97]. This mechanism could take place in the case with the exogenously applied SYN and/or TPPP/p25. Endocytosis has a special relevance in the brain, because of its involvement in neurotransmitter and neurotrophic signaling. Since neuronal cells are highly polarized, they require a highly specialized and complex endocytic machinery. Alterations in this complex system have also been described in PD [40]. Besides conventional endocytosis, exosomal transport, receptor-mediated internalization, passive diffusion, or even direct penetration of the plasma membrane have been suggested as possible pathways for SYN uptake [98].

6. Innovative strategy for PD and MSA therapy

In a recent review Devos and co-workers have reported that "Despite decades of successful preclinical neuroprotective studies, no drug has then shown efficacy

in clinical trials." [99]. According to this and other publications, effective neuroprotective therapy is still an unmet need both in PD and MSA. Symptomatic treatments are available, although MSA patients usually show poor l-Dopa responsiveness [100]. Concerning possible disease-modifying therapies, the following strategies are under clinical trials: targeting SYN pathology such as active and passive immunization, anti-aggregative small molecules, RNA interference techniques and an increase in SYN clearance, intervening neuroinflammation or neuronal loss (by stem cells) [25, 92, 100–102]. Remyelinating molecules are also being tested in clinical trials in the case of MSA, since this disease is accompanied by myelin loss as well [100].

A recently reported innovative strategy is based upon the effective and specific inhibition/destruction of the pathological TPPP/p25-SYN complex/assembly by peptide fragments of the partner proteins [81]. The highly flexible foldamers that can recognize oligomers and proteins are among potential therapeutics. These foldamers are endowed with variable pharmacokinetic properties; nonetheless, their constructions with suitable recognition surfaces are still challenging; they have to display contiguous recognition surface or long sequences with broadly distributed recognition contacts, see [103] and references therein. Foldamer-based protein mimetics have been designed by following the principles of multivalent biomolecule-recognizing ligands [103, 104]. In fact, the fragment-based foldamer approach displays unnatural protein mimetics that are capable of specific molecular recognition and inhibition of multifunctional target.

The recognition that the TPPP/p25-derived SYN aggregation is involved in the pathomechanism of the synucleionopathies, but not in that of the tauopathies, underlined that the TPPP/p25-SYN complex is a potential drug target [79–81]. However, the complex as a whole could not be considered as an optimal drug target since both proteins display physiological functions as well, but the interface of their complex occurring only under pathological conditions was proposed to be an excellent target. Thus, the interface of the complex of the two hallmark proteins has been validated at molecular and cellular levels [79–81]. The binding segments of TPPP/p25 involved in its interaction with SYN was identified (147–156 aa) [79–81] (**Figure 6**). The interface has been considered as a potential drug target, which is found to be distinct from the physiological TPPP/p25-tubulin one (178–187 aa). These findings showed the role of the middle, CORE region of TPPP/p25 in the formation of the pathological TPPP/p25-SYN complex; in addition, the stable complex was created by the interaction between the two unstructured proteins with sufficient avidity. Thus, short peptide fragments by targeting the interface of the pathological complex could function as potential anti-Parkinson agents.

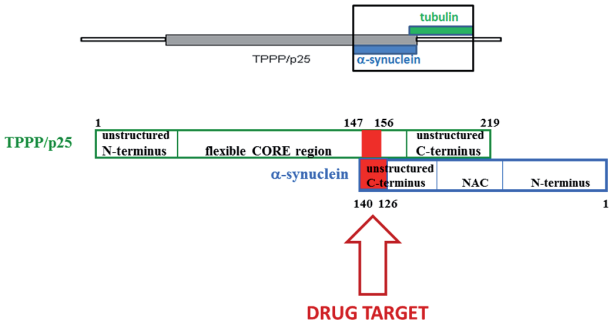


Figure 6.
Distinct segments of TPPP/p25 involved in the physiological (tubulin) and pathological (SYN) interactions. Identified interface segments of the pathological TPPP/p25-SYN complex [6, 79].

The effectiveness of these fragments can be tested by in vitro competition experiments by ELISA using human recombinant proteins and by BiFC approach coupled by fluorescence microscopy [79–81]. The inhibition of the direct association of SYN with TPPP/p25 can be visualized and the inhibitory effect of the fragments can be quantified by the reduction of the fluorescence signal (green). This two-steps-assay seems to be applicable to screen potential drug-like molecules for their anti-Parkinson activity. The innovative interface-targeting methodology allows one to further develop it to disease-related/unrelated interface targeting.

The recognition of the endogenous expression of SYN and TPPP/p25 in neurons and OLGs, respectively, offers opportunity for the selective influence of PD and MSA such as disease-dependent interface targeting of the pathological TPPP/p25-SYN complex. Let us consider the specific interface-targeting fragments that can inhibit and/or destruct the TPPP/p25-SYN assemblies. The nature of the interface fragments for elimination of the pathological complex has to be determined by their origin (TPPP/p25 or SYN fragments).

As illustrated in the scheme (**Figure 7**), in the case of PD the inclusions are formed predominantly in neurons, SYN, and not TPPP/p25, is expressed endogenously in these cells; in this situation a TPPP/p25 fragment can be effective to destruct the pathological complex in neurons without displaying side effects. Conversely, for the treatment of MSA, when the inclusions are formed in OLGs that express endogenously TPPP/p25, it is expected that SYN-based fragments could be effective to diminish the co-assemblies of SYN and TPPP/p25 in OLGs and no unwanted side effect occurs. These issues are based upon the recognition that TPPP/p25 is enriched in Lewy bodies of neurons exclusively in the case of PD, while SYN accumulates in OLGs in cytoplasmic inclusions according to the etiology of MSA.

PD, DLBD and MSA have some common features such as inclusion bodies comprised of SYN and TPPP/p25 as well as decline in motor, cognitive, behavioral and autonomic functions. However, these diseases may be distinguished based on affected cell types and brain structures, the relative onset and prognosis [105, 106]. Cognitive impairment precedes parkinsonism in the case of DLBD, while PD dementia starts 1 year or more after the diagnosis of PD; DLBD patients show more profound cognitive impairments. Approximately ~30% of MSA patients also suffers from cognitive impairment, in particular executive dysfunction. The hippocampus is one of the most vulnerable brain regions affected

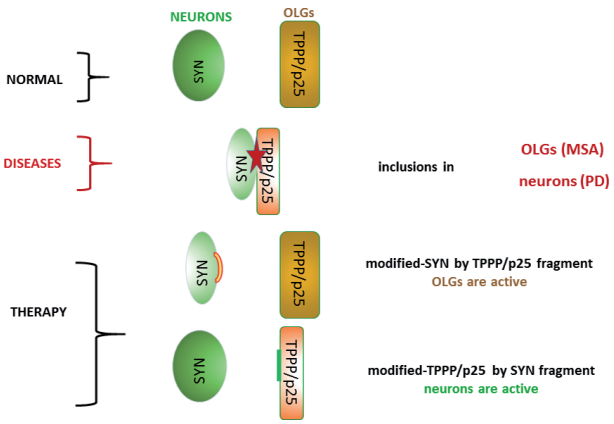


Figure 7.
Disease-dependent interface targeting of the pathological TPPP/p25-SYN complex. Targeting the interface by SYN or TPPP/p25 fragments for MSA and PD therapies.

by synucleinopathies, and its dysfunction may result in cognitive deficits and depression. Oligomerization/aggregation of SYN was found to induce deficits in synaptic transmission and hippocampal neurogenesis, which may contribute to the appearance of cognitive deficits. Short-term memory defects have also been observed in TPPP/p25 KO mice, which exhibit hypomyelination [73, 74]. Recently it has been proposed that OLGs and myelin sheaths play crucial roles in memory and learning [72].

Clinically, the differentiation between PD and MSA is challenging, especially at the early stages of diseases [107]. In contrast to PD, no causal SYN mutations for MSA have been found to date. However, neuropathological hallmarks of both MSA and PD could be observed in the case of the G51D SYN mutant [108]. Two possible scenarios have been proposed to explain the origin of SYN in OLGs and SYN accumulation in glial cytoplasmic inclusions characteristic for MSA brains: either OLGs overexpress SYN under pathological conditions or they take up the neuronal protein from their environment, such as CSF [98]. The latter one, the cell-to-cell transmission has been proven. Recent studies have suggested that the SYN structures/aggregates formed in the cases of different synucleinopathies are distinct that could contribute to the discrimination between PD and MSA [107]. Nevertheless, it is important to notice that the aggregated structures amplified from CSF were similar to those ones amplified from the brain [107]. Biomarkers in CSF, such as phosphorylated/total tau, SYN and β-amyloid$_{1–42}$, can be useful to distinguish PD or MSA patients from healthy controls, and SYN and total-tau could also be used to distinguish between MSA from PD [109]. The analysis of the hallmark TPPP/p25 occurring in the CSF and inclusions of patients might provide more unambiguous information about the nature of synucleinopathies.

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Conflict of interest

The authors declare no conflict of interest.

Abbreviations

SYN	alpha-synuclein
AD	Alzheimer's disease
BiFC	bifunctional fluorescent complementation
CSF	cerebrospinal fluid
CMA	chaperone-mediated autophagy
CBD	corticobasal degeneration
DLBD	diffuse Lewy body disease
Hsc70	heat-shock cognate 70 kDa protein
Hsp	heat shock proteins
LAMP-2A	lysosomal-associated membrane protein 2A

MSA	multiple system atrophy
OLG	oligodendrocyte
PD	Parkinson's disease
PiD	Pick's disease
PSP	progressive supranuclear palsy
TPPP/p25	Tubulin Polymerization Promoting Protein
UPS	ubiquitin-proteasome system

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