BUDAPEST UNIVERSITY OF TECHNOLOGY AND ECONOMICS
FACULTY OF CHEMICAL TECHNOLOGY AND BIOTECHNOLOGY
GEORGE OLAH DOCTORAL SCHOOL

The physiological and pathological interactions of TPPP/p25 involved in the organization of the multifunctional microtubule network

Summary of PhD Thesis

Adél Szabó
MSc in Biochemical Engineering

Supervisor: Dr. Judit Oláh
Consultant: Prof. Dr. Beáta G. Vértessy

Hungarian Academy of Sciences
Research Centre for Natural Sciences
Institute of Enzymology
Cell Architecture Research Group

Budapest, 2019

Introduction and scientific background

My PhD thesis focuses on the molecular characterization of the physiological and pathological interactions of the Tubulin Polymerization Promoting Protein (TPPP/p25), which is involved in the ultrastructural organization of the multifunctional microtubule network.

The cytoskeletal microtubule network displays multiple physiological and pathological functions in eukaryotic cells, which are achieved by post-translational modifications and by associations with different partners[1]. In brain, the microtubules are of special importance since they are actively involved in the maintenance of structural polarity of neurons which is crucial for their physiological functions[1]. The thin, long axons of neurons are surrounded by oligodendrocytes, building up the myelin sheath to provide support and insulation to axons. Microtubule Associated Proteins (MAPs) and the dynamic reorganization of the microtubule network play essential roles in this process[2]. TPPP/p25, a new MAP, is expressed predominantly in oligodendrocytes, and it was identified and denoted by the Cell Architecture research group based on its function (it induces tubulin polymerization) and molecular mass (25 kDa)[3]. The group demonstrated that the disordered TPPP/p25 is the prototype of neomorphic moonlighting proteins since it displays distinct physiological and pathological functions determined by its interacting partners, without alterations at gene level[4,5].

The main physiological interacting partner of the Zn^{2+}-binding TPPP/p25 is tubulin; it induces the assembly of tubulin into microtubules, and modulates the dynamics and stability of the microtubule network by its microtubule bundling[3] and acetylation enhancing[6] activities. These functions of TPPP/p25 are achieved by its dimerization potency[7] as well as by its interactions with tubulin deacetylase enzymes (SIRT2[8] and HDAC6[6]): TPPP/p25 inhibits the deacetylase activity of these enzymes, thus increases the acetylation level of the microtubule network[6,8]. The acetylation-driven microtubule dynamics plays an important physiological role in cell division, mitotic spindle formation/degradation, regulation of projection elongation during differentiation, and also a pathological role in the formation of aggresomes[9] acting as a defensive mechanism; consequently, tubulin deacetylases are potential drug targets. Professor Manfred Jung's research group in Freiburg has recently produced highly effective, isotype-selective SIRT2 inhibitors, the "Sirtuin Rearranging Ligands" (SirReal), which act with a new mechanism[10]. The potency and the unprecedented SIRT2 selectivity of these inhibitors are based on a ligand-induced structural rearrangement of the active site unveiling a yet-unexploited binding pocket[10]. The German group has also developed a PROteolysis TArgeting Chimera (PROTAC) derivative, which is able to induce the selective proteolytic degradation of SIRT2[11].

---

[1] Conde C, Caceres A (2009) Microtubule assembly, organization and dynamics in axons and dendrites. Nat Rev Neurosci 10: 319-332.

[2] Bauer NG, Richter-Landsberg C, Ffrench-Constant C (2009) Role of the oligodendroglial cytoskeleton in differentiation and myelination. Glia 57: 1691-1705.

[3] Hlavanda E, Kovács J, Oláh J, Orosz F, Medzihradszky KF et al. (2002) Brain-specific p25 protein binds to tubulin and microtubules and induces aberrant microtubule assemblies at substoichiometric concentrations. Biochemistry 41: 8657-8664.

[4] Jeffery CJ (2011) Proteins with neomorphic moonlighting functions in disease. IUBMB Life 63: 489-494.

[5] Ovádi J (2011) Moonlighting proteins in neurological disorders. IUBMB Life 63: 453-456.

[6] Tőkési N, Lehotzky A, Horvath I, Szabó B, Oláh J et al. (2010) TPPP/p25 promotes tubulin acetylation by inhibiting histone deacetylase 6. J Biol Chem 285: 17896-17906.

[7] Oláh J, Zotter A, Hlavanda E, Szunyogh S, Orosz F et al. (2012) Microtubule assembly-derived by dimerization of TPPP/p25. Evaluation of thermodynamic parameters for multiple equilibrium system from ITC data. Biochim Biophys Acta 1820: 785-794.

[8] Mangas-Sanjuan V, Oláh J, Gonzalez-Alvarez I, Lehotzky A, Tőkési N et al. (2015) Tubulin acetylation promoting potency and absorption efficacy of deacetylase inhibitors. Br J Pharmacol 172: 829-840.

[9] Kopito RR (2000) Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol 10: 524-530.

[10] Rumpf T, Schiedel M, Karaman B, Roessler C, North BJ et al. (2015) Selective Sirt2 inhibition by ligand-induced rearrangement of the active site. Nat Commun 6: 6263.

[11] Schiedel M, Herp D, Hammelmann S, Swyter S, Lehotzky A et al. (2018) Chemically Induced Degradation of Sirtuin 2 (Sirt2) by a Proteolysis Targeting Chimera (PROTAC) Based on Sirtuin Rearranging Ligands (SirReals). J Med Chem 61: 482-491.

One part of this bifunctional PROTAC molecule can specifically bind SIRT2 and the other part an E3 ubiquitin ligase complex, thus the PROTAC chemically induces the isotype-specific poly-ubiquitination, then the proteasomal degradation of SIRT2[11]. The collaboration with the German group within the frame of the EU COST EPICHEM project provided opportunity to investigate the specificity and inhibitory potency of the SirReal and PROTAC compounds.

The non-physiological expression of TPPP/p25 is coupled with distinct central nervous system diseases such as: multiple sclerosis, a demyelinization disease (altered TPPP/p25 expression level[12]); oligodendroglioma (the tumorous cells do not express TPPP/p25[13]); and certain conformational diseases, Parkinson's disease and multiple system atrophy, characterized by co-enrichment and co-localization of TPPP/p25 with α-synuclein in neuronal and glial inclusion bodies, respectively[14,15]. Besides α-synuclein, TPPP/p25 is a hallmark protein of these latter diseases[14]. However, neither α-synuclein nor TPPP/p25 can be considered as ideal drug targets by themselves since both proteins disordered and display physiological functions, which are essential for normal brain functioning, so must be preserved. Therefore, the Cell Architecture Research Group has suggested the targeting of the contact surface of their pathological complex as a new, innovative strategy for development of anti-Parkinson drugs with unique specificity; without affecting the physiological function of TPPP/p25, its regulatory effect on the dynamics and stability of microtubules[16]. Therefore, my studies also focused on the identification of the contact surfaces of the physiological and pathological complexes of TPPP/p25 (with tubulin and α-synuclein, respectively), which is essential to validate a drug target specific for the pathological process and to develop highly efficient anti-Parkinson drugs.

Previously the Cell Architecture Research Group carried out studies with "truncated" (without one or both terminal segments) mutant human recombinant TPPP/p25 and specifically synthesized peptides, which have shown that the unstructured terminal segments, especially the C-terminus (178-187 amino acids), play decisive roles in fulfilling the physiological function of the protein, i.e. its interaction with tubulin[16]. While the "core" region (147-156 amino acids) is predominantly involved in its association with α-synuclein and in the α-synuclein aggregation promoting effect of TPPP/p25[16]. The dissimilarity of these binding segments has significant innovative impact in anti-Parkinson drug research, since it enables one to selectively influence the physiological and pathological interactions. *In vivo* relevance of these findings has been proved by experiments with living CHO10 cells: the interaction of the "core" TPPP/p25 with α-synuclein resulted in their co-aggregation[16]. The more precise localization of the binding segments as well as the mapping of further possible binding sites (not only in the case of TPPP/p25, but also on α-synuclein) constitutes a significant part of my PhD thesis.

[12] Höftberger R, Fink S, Aboul-Enein F, Botond G, Oláh J et al. (2010) Tubulin polymerization promoting protein (TPPP/p25) as a marker for oligodendroglial changes in multiple sclerosis. Glia 58: 1847-1857.

[13] Preusser M, Lehotzky A, Budka H, Ovádi J, Kovács GG (2007) TPPP/p25 in brain tumours: expression in non-neoplastic oligodendrocytes but not in oligodendroglioma cells, Acta Neuropathol. 113: 213–215.

[14] Kovacs GG, Laszlo L, Kovács J, Jensen PH, Lindersson E et al. (2004) Natively unfolded tubulin polymerization promoting protein TPPP/p25 is a common marker of alpha-synucleinopathies, Neurobiol. Dis. 17: 155–162.

[15] Lindersson E, Lundvig D, Petersen C, Madsen P, Nyengaard JR et al. (2005) p25alpha Stimulates alpha-synuclein aggregation and is co-localized with aggregated alpha-synuclein in alpha-synucleinopathies, J Biol Chem, 280: 5703-5715.

[16] Tőkési N, Oláh J, Hlavanda E, Szunyogh S, Szabó A et al. (2014): Identification of motives mediating alternative functions of the neomorphic moonlighting TPPP/p25, Biochim Biophys Acta - Mol Basis Dis, 1842: 547-557.

Aims

At the beginning of my PhD work, it was known, predominantly based on the previous results of the Cell Architecture Research Group, that the disordered TPPP/p25 plays an essential role in modulating the dynamics and stability of the microtubule network by its microtubule bundling[3] and acetylation enhancing[6] activities. This latter function of the protein is achieved by its interactions with the tubulin deacetylase enzymes (HDAC6 and SIRT2). Since the interaction of TPPP/p25 and HDAC6 was investigated in detail previously[6]; therefore, one of my aims was to characterize the less studied interaction of TPPP/p25 and SIRT2.

Several studies have shown that TPPP/p25 is a hallmark protein of certain central nervous system diseases[14]: its interaction with α-synuclein leads to the appearance of toxic aggregates, inclusion bodies characteristic for the pathomechanism of Parkinson's disease and other synucleinopathies. Since there are no disease-modifying therapies for these neurodegenerative diseases with serious socio-economic burden, therefore this area is the subject of intensive research. Understanding of the mechanisms underlying Parkinson's disease and other synucleinopathies is of great importance in order to identify and validate a drug target, which can ultimately lead to the development of effective drug molecules that can be used in the therapy of these diseases. The interaction of the hallmark proteins and their pathological aggregation lead to the development of these disorders, therefore detailed characterization of the structural and functional properties of the hallmark proteins and their interactions are indispensable for the understanding of the pathomechanism of synucleinopathies. A significant part of my PhD thesis contributes to this research area.

Given the information presented above, my aims were as follows:

- Expression and purification of wild type and mutant human recombinant proteins (TPPP/p25, α-synuclein and SIRT2), then the structural characterization of the distinct proteins, especially the "truncated" and deletion mutant TPPP/p25 forms, with particular attention to the effect of mutations on disorder, zinc-induced structural rearrangement and dimerization ability of TPPP/p25.
- Characterization of the role of the central ("core") and terminal TPPP/p25 regions in the interaction with tubulin and stabilization/bundling of microtubules.
- Characterization of the interaction of tubulin, SIRT2 and TPPP/p25 at molecular level, including the functional consequences: the effects on TPPP/p25 induced tubulin polymerization and SIRT2 catalysed tubulin/microtubule deacetylation.
- Investigation of the effect of the new specific SIRT2 inhibitors (SirReal and PROTAC) on the deacetylase activity of human recombinant SIRT2 using various substrates (tubulin, microtubule and a synthetic fluorescence peptide).
- Identification and characterization of the interaction between TPPP/p25 and α-synuclein as pathological partners at molecular level; identification of the regions involved in the pathological complex formation in order to validate a drug target.

Experimental design

- **Protein expression:** The wild type and mutant TPPP/p25, SIRT2 and α-synuclein proteins were produced by recombinant DNA technology and expressed in *E. coli*. Tubulin was prepared from bovine brain.
- **Protein purification:** The proteins were purified by affinity or ion-exchange chromatography, then the purity of the protein was verified by polyacrylamide gel electrophoresis containing sodium dodecyl sulfate (SDS-PAGE), the protein concentration was determined by spectrophotometry.
- **Structural studies:** To compare the structure of the different protein forms and to register the possible structural changes (dimerization, folding), circular dichroism (CD), fluorescence spectroscopic (using 8-anilinonaphthalene-1-sulfonicacid (ANS) hydrophobic fluorescence probe), and sandwich ELISA (enzyme-linked immunosorbent assay) measurements were performed.
- **Characterization of protein-protein interactions:** The potential heteroassociations of the studied proteins were detected and characterized by CD spectroscopy, affinity chromatography, Pepscan and ELISA assays, as well as pelleting experiments (in the case of samples containing tubulin). For the determination of protein segments involved in the interactions, we used the deletion mutant and/or "truncated" proteins or forms produced by limited proteolysis as well as synthetic peptides.
- **Study of tubulin polymerization and microtubule ultrastructures:** The functional consequences of TPPP/p25 mutations or the SIRT2-TPPP/p25 interaction concerning the tubulin polymerization promoting activity of TPPP/p25 were tested by turbidity measurement, as the polymerization of tubulin into microtubules can be monitored through changes in absorbance at 350 nm. The tubulin polymerization induced by the various TPPP/p25 forms was also tested by pelleting experiments and the formed microtubule ultrastructures were visualized by electron microscopy in some cases.
- **Enzyme activity measurements:** The activity of SIRT2 deacetylase was tested on one hand using tubulin (or microtubule) as a natural substrate by Western blot using acetylated α-tubulin (K40) antibody. On the other hand, SIRT2 activity was also measured using a synthetic fluorescent substrate with a commercially available kit (BPS Bioscience, 50087). By means of these methods, we investigated the effect of the above mentioned new, specific SIRT2 inhibitors (SirReal) and a SIRT2-specific PROTAC compound acting with a new mechanism on the deacetylase activity of the protein.

Results and possible applications

TPPP/p25 is an intrinsically unstructured protein, the disordered N- and C-terminal segments (45 and 44 amino acids) of the protein are straddling a 130 amino acid long highly flexible region[17]. Mutant TPPP/p25 forms due to the truncation of one or both terminal segments and/or deletion of the previously suggested tubulin/α-synuclein binding sites[16] were produced in *E. coli.* After expression of the various truncated and deletion mutant TPPP/p25 species, I characterized the structure of these protein forms with CD spectroscopy. The CD and ANS fluorescence spectroscopy (which is suitable to specifically detect the hydrophobic surface of molten globule proteins but not that of unfolded or folded ones) experiments revealed that all TPPP/p25 forms containing the zinc-finger motif can specifically bind the bivalent zinc ion (3). The ability of Zn^{2+}-binding is of great importance, because in myelinating oligodendrocytes, where TPPP/p25 is endogenously expressed, the intracellular Zn^{2+} concentration has been found to be relatively high (50 μM) (7). According to the previous results of our research group, the Zn^{2+}-induced structural rearrangement in the wild type TPPP/p25 promotes its dimerization resulting in increased tubulin polymerization promoting activity (7). TPPP/p25 tends to oligomerize[7,18]: both *in vitro* and *in vivo* systems the protein occurs primarily in dimeric form (under some circumstances, oligomeric forms of higher molecular weight are also present), which is stabilized through disulfide bonds[7] (2). Sandwich ELISA experiments have shown that all TPPP/p25 forms, except for the recombinant fragments, are capable of microtubule-independent dimerization, which is a key element in the physiological function of the protein (2).

The main physiological interacting partner of TPPP/p25 is tubulin/microtubules[3]. ELISA and sedimentation experiments with tubulin and the truncated TPPP/p25 forms provided evidence for the key role of the disordered terminal segments of TPPP/p25 not only in its interaction with tubulin, but also in the stabilization and bundling of microtubules (2). The interactions of TPPP/p25 with tubulin and the tubulin deacetylase SIRT2 enzyme (as a potential anti-cancer drug target) were proved by the above mentioned biophysical and biochemical methods using human recombinant proteins (1). CD spectra revealed structural differences too: the CD spectrum of TPPP/p25 corresponds to that characteristic for unstructured proteins, while tubulin and SIRT2 behaved as globular ones (1). The differential CD spectra of TPPP/p25 with tubulin or SIRT2 indicated significant structural rearrangements due to the heteroassociations (1). Binary and ternary complexes of TPPP/p25, tubulin and SIRT2 could be formed, which was underlined and quantified by ELISA experiments (1).

I also characterized the functional effects of SIRT2; Western blot analysis revealed that the presence of TPPP/p25 elevated the tubulin acetylation level due to the inhibition of SIRT2 activity. SIRT2 inhibited the TPPP/p25-induced formation of intact-like microtubules independently of its deacetylase activity as demonstrated by turbidimetry and electron microscopy. However, the effect of SIRT2 depends on the ultrastructure of microtubule network: the TPPP/p25-assembled microtubules were resistant against the depolymerization and deacetylation effects of SIRT2 added afterwards indicating that certain acetylation sites are not accessible to SIRT2 (1).

The potency and specificity of new chemical SIRT2 inhibitors (SirReal) synthetized by our German collaborative partners were tested and characterized[10]. The inhibitory potency of the chemical SirReal compounds and TPPP/p25 was similar and additive; the simultaneous addition of the protein and the synthesised inhibitors resulted in microtubule hyperacetylation that counteracts the uncontrolled cell division (1) (Figure 1). A PROteolysis TArgeting Chimera (PROTAC)[11] derivative of a SirReal compound enhanced the microtubule acetylation level by the selective

[17] Zotter A, Bodor A, Oláh J, Hlavanda E, Orosz F et al. (2011) Disordered TPPP/p25 binds GTP and displays Mg(2+)-dependent GTPase activity. FEBS Lett 585: 803-808.

[18] DeBonis S, Neumann E, Skoufias DA (2015) Self protein-protein interactions are involved in TPPP/p25 mediated microtubule bundling. Sci Rep 5: 13242.

proteolytic degradation of SIRT2. The advantage of this new inhibitory mechanism is the high specificity coupled with the lack of unwanted side effects. These issues make this chemical compound to be a potential anti-cancer drug-like agent[11].

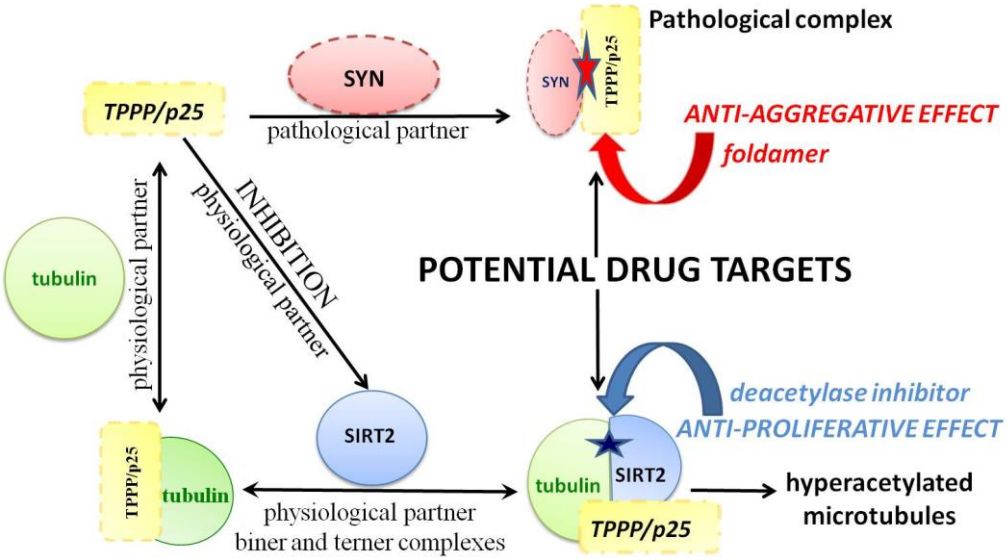

Figure 1: Scheme of the multiple interactions of TPPP/p25 and therapeutic potencies.

The other main objective of my research is related to the innovative strategy suggested by the Cell Architecture Research Group. The strategy concerns the identification and validation of such a drug target, which may contribute to the design and synthesis of specific anti-Parkinson agents. This new, innovative strategy was based on the following observations: i) both α-synuclein and TPPP/p25, hallmark proteins of synucleinopathies, displays essential physiological functions, therefore cannot be considered as ideal drug targets; ii) the formation of their pathological complex leads to aggregation and finally development of inclusion bodies; iii) in normal brain α-synuclein is expressed in neurons, while TPPP/p25 occurs predominantly in oligodendrocytes; however, both proteins are accumulated and co-localized in inclusion bodies characteristic for Parkinson's disease and multiple system atrophy. Therefore, the Cell Architecture Research Group has suggested the targeting of the TPPP/p25-α-synuclein pathological complex, namely its contact surface, as a new, innovative strategy in order to impede the formation of the fatal oligomers under pathological conditions, when these proteins are co-localized in neurons and/or in oligodendrocytes[16] (Figure 1). Consequently, the identification of the contact surfaces of the physiological and pathological complexes of TPPP/p25 (with tubulin and α-synuclein, respectively) is essential in order to validate an efficient, specific anti-Parkinson drug target (Figure 1).

The binding segments of both TPPP/p25 (59-62, 147-156) and α-synuclein (126-140), which are dominantly involved in the formation of the pathological contact surface and may serve as drug targets, have been identified using deletion mutant human recombinant protein species by the above mentioned biophysical, biochemical and immunological methods (complemented with the results of the NMR measurement of the interaction of DT TPPP/p25 and α-synuclein). ELISA and affinity chromatography experiments with deletion mutants of TPPP/p25 to test the specificity of the binding segments surprisingly revealed that the removal of different 10-25 amino acid (aa) segments of the wild type TPPP/p25 reduces its binding potency to α-synuclein in different extents, depending on the nature of deletions, but does not prevent it (3). These results indicate that the binding segment(s) could be replaced by other segments due to the high conformational plasticity of TPPP/p25. This phenomenon was termed as "*Neomorphic Chameleon"* feature indicating that alteration at gene level (deletions) does not results in functional alteration (3). However, this was not the case for α-synuclein mutants; the dominant, irreplaceable role of its C-terminus (126-140 segments) in the formation of the pathological complex was unambiguous (Figure 2).

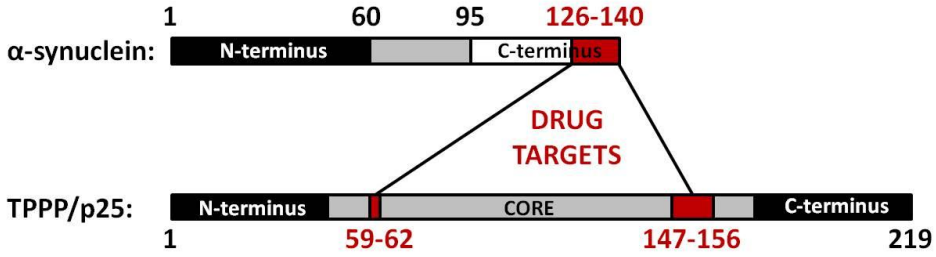

Figure 2: Identified segments of the contact surfaces of TPPP/p25-SYN complex.

The binding segments may function as a possible lead compound for development of foldamer-type drugs (3-4) (Figure 1). Peptidomimetics are new tools in the discovery of small-molecule inhibitors of protein–protein interactions. Peptide-based aggregation inhibitors hold significant promise for future Parkinson-therapy owing to their high selectivity, effectiveness, low toxicity, good tolerance, low accumulation in tissues, high chemical and biological diversity, possibility of rational design, and highly developed methods for analysing their mode of action, proteolytic stability (modified peptides), and blood–brain barrier permeability[19,20].

[19] Vagner J, Qu H, Hruby V (2008) Peptidomimetics, a synthetic tool of drug discovery. Curr Opin Chem Biol 12: 292–296.
[20] Goyal D, Shuaib S, Mann S, Goyal B (2017) Rationally Designed Peptides and Peptidomimetics as Inhibitors of Amyloid-β (Aβ) Aggregation: Potential Therapeutics of Alzheimer's Disease. ACS Comb Sci 19: 55–80.

Theses

1. After expression of various truncated and/or deletion mutant as well as the wild-type TPPP/p25 species in *E. coli*, I characterized the structure of the isolated proteins with CD spectroscopy. CD and ANS fluorescence spectroscopy experiments revealed that all TPPP/p25 forms containing the zinc-finger motif can specifically bind the bivalent zinc ion, which induces structural rearrangement, the formation of a partially folded molten globule structure in the protein (3). Sandwich ELISA experiments showed that all mutant TPPP/p25 forms, with except for the recombinant fragments, are capable of microtubule-independent dimerization, which is a key element in the physiological function of TPPP/p25 (2).

2. ELISA and sedimentation experiments with tubulin and the truncated TPPP/p25 forms provided evidence for the key role of the disordered terminal segments of TPPP/p25 not only in its interaction with tubulin, but also in the stabilization and bundling of microtubules (2).

3. The possible hetero-associations among tubulin – TPPP/p25 – SIRT2 were detected and characterized by CD spectroscopy, affinity chromatography and ELISA experiments, which revealed the formation of binary and ternary complexes (1).

4. Western blot analysis revealed that the presence of TPPP/p25 elevated the tubulin acetylation level due to the inhibition of SIRT2 activity. SIRT2 inhibited the TPPP/p25-induced formation of intact-like microtubules independently of its deacetylase activity as demonstrated by turbidimetry and electron microscopy. However, the TPPP/p25-assembled microtubules were resistant against the depolymerization and deacetylation effects of SIRT2 added afterwards (1).

5. The potency and specificity of new chemical SIRT2 inhibitors (SirReal) were tested on tubulin as the natural substrate of SIRT2 by Western blot, within the frame of an international collaboration. A PROteolysis TArgeting Chimera (PROTAC) derivative, which can enhance the microtubule acetylation level by the selective proteolytic degradation of SIRT2, was also examined. The inhibitory potency of the chemical SirReal compounds or PROTAC and TPPP/p25 was similar and additive; the simultaneous addition of the protein and the synthesised inhibitors/degrader resulted in microtubule hyperacetylation that counteracts the uncontrolled cell division (1).

6. Binding studies with the deletion mutants of TPPP/p25 concerning the previously suggested (8) and the newly identified (3) α-synuclein binding segments revealed the high conformational plasticity, the *"Neomorphic Chameleon"* feature of TPPP/p25 (3). Our results indicated that the 59-62 and the 147-156 amino acid segments of the wild type TPPP/p25 have prominent potency in the formation of the pathological complex (3).

7. The studies performed with ELISA and affinity chromatography proved that the removal of the C-terminus of α-synuclein prevented its interaction with TPPP/p25, in the case of α-synuclein the 126-140 amino acid segment was identified as a key region for TPPP/p25 binding (4). The obtained results were underlined by living cell experiments carried out in our research group, the validation of the binding segments as drug targets was performed at molecular and cellular levels using α-synuclein fragments as possible lead compounds for the development of foldamer-type drugs (3-4).

Publications

I. Original publications related to the dissertation

(1) **Szabó A**, Oláh J, Szunyogh S, Lehotzky A, Szénási T, Csaplár M, Schiedel M, Lőw P, Jung M, Ovádi J: Modulation of microtubule acetylation by the interplay of TPPP/p25, SIRT2 and new anticancer agents with anti-SIRT2 potency. SCIENTIFIC REPORTS 7(1):17070. (2017) IF: 4,259; Independent Citation: 2; Author Contribution: 80%

(2) Oláh J, Szénási T, Szunyogh S, **Szabó A**, Lehotzky A, Ovádi J: Further evidence for microtubule-independent dimerization of TPPP/p25. SCIENTIFIC REPORTS 7: 40594. (2017) IF: 4,259; Independent Citation: 1; Author Contribution: 70%

(3) Szénási T, Oláh J, **Szabó A**, Szunyogh S, Láng A, Perczel A, Lehotzky A, Uversky VN, Ovádi J: Challenging drug target for Parkinson's disease: Pathological complex of the chameleon TPPP/p25 and alpha-synuclein proteins. BIOCHIMICA ET BIOPHYSICA ACTA-MOLECULAR BASIS OF DISEASE 1863(1): 310-323. (2017) IF: 5,476; Independent Citation: 3; Author Contribution: 70%

(4) Szunyogh S, Oláh J, Szénási T, **Szabó A**, Ovádi J: Targeting the interface of the pathological complex of α-synuclein and TPPP/p25. BIOCHIMICA ET BIOPHYSICA ACTA-MOLECULAR BASIS OF DISEASE 1852: 2653-2661. (2015) IF: 5,158; Independent Citation: 3; Author Contribution: 40%

(5) **Szabó A**: Új stratégia a Parkinson-kór kutatásában – Reményt adó fehérjék? ÉLET ÉS TUDOMÁNY LXXIII. 5. 137-139. (2018) Author Contribution: 100%

II. Other publications

(6) Oláh J, Szénási T, **Szabó A**, Kovács K, Lőw P, Stifanic M, Orosz F: Tubulin binding and polymerization promoting properties of TPPP proteins are evolutionarily conserved. BIOCHEMISTRY 56 (7): 1017-1024. (2017) IF: 2,938; Independent Citation: 2; Author Contribution: 80%

(7) Lehotzky A, Oláh J, Szunyogh S, **Szabó A**, Berki T, Ovádi J: Zinc-induced structural changes of the disordered TPPP/p25 inhibits its degradation by the proteasome. BIOCHIMICA ET BIOPHYSICA ACTA-MOLECULAR BASIS OF DISEASE 1852(1):83-91 (2015) IF: 5,158; Independent Citation: 0; Author Contribution: 50%

(8) Tőkési N, Oláh J, Hlavanda E, Szunyogh S, **Szabó A**, Babos F, Magyar A, Lehotzky A, Vass E, Ovádi J: Identification of motives mediating alternative functions of the neomorphic moonlighting TPPP/p25. BIOCHIMICA ET BIOPHYSICA ACTA-MOLECULAR BASIS OF DISEASE 1842(4): 547-557. (2014) IF: 4,882; Independent Citation: 2; Author Contribution: 50%

III. Presentations related to the dissertation

III/1. Oral presentations (author's name underlined)

Szabó A, Oláh J, Szunyogh S, Lehotzky A, Szénási T, Ovádi J
Double life of the multifunctional disordered TPPP/P25: physiological function
22-24. 09. 2017. EpiChemBio and MuTaLig COST actions joint meeting (Porto, Portugal)

Szabó A, Oláh J, Szunyogh S, Lehotzky A, Szénási T, Ovádi J
A többfunkciós TPPP/p25 fehérje fiziológiás és patológiás kölcsönhatásai: Út specifikus anti-Parkinson molekulák fejlesztéséhez
02. 02. 2017. Oláh György Doktori Iskola XIV. Doktoráns Konferencia (Budapest)

Szabó A, Oláh J, Szunyogh S, Lehotzky A, Szénási T, Ovádi J
A rendezetlen TPPP/p25 fehérje fiziológiás és patológiás kölcsönhatásainak jellemzése: Út anti-Parkinson molekulák fejlesztéséhez
15-17. 04. 2016. Tavaszi Szél Konferencia (Budapest)

Szabó A, Oláh J, Szunyogh S, Lehotzky A, Szénási T, Ovádi J
TPPP/p25, egy új agy-specifikus fehérje moonlighting funkciói
04 – 05. 04. 2014. VIII. Szent-Györgyi Albert Konferencia (Budapest)

III/2. Co-authored posters (author's name underlined)

Szunyogh S, Szénási T, Oláh J, **Szabó A**, Lehotzky A, Ovádi J
Double life of the multifunctional disordered TPPP/p25: pathological function
EpiChemBio and MuTaLig COST actions joint meeting, Porto, Portugal, 22-24. 09. 2017.

Oláh J, Szénási T, **Szabó A**, Szunyogh S, Lehotzky A, Ovádi J
A new innovative strategy to validate drug target for Parkinson's disease
13th International Conference on Alzheimer's and Parkinson's Diseases, Vienna, Austria,
28. 03. 2017.– 02. 04. 2017.

Oláh J, Tőkési N, Szunyogh S, **Szabó A**, Lehotzky A, Ovádi J
Tubulin Polymerization Promoting Protein is a prototype of neomorphic moonlighting proteins
The Biological and Biomedical Consequences of Protein Moonlighting, Charles Darwin House, London, UK, 29-30. 07. 2014.