

Parkinson's disease – expression and characterization of the misfolding protein α -Synuclein



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Introduction

Parkinson's disease (PD) is a neurodegenerative protein misfolding disease, which cause loss of memory and motor function. There has not been determined a definitive cause of PD. However, aggregations of proteins in the affected neurons, called Lewy bodies (LBs), have become a known hallmark of PD^{2,3}. LBs composed mainly of aggregated α -Synuclein, TAU-protein and ubiquitin¹.

Aim: The aim of this project was to identify hot-spots for misfolding, using *In silico* prediction. Then, to express and purify α -Synuclein and explore the protein's fold and oligomerization activities via NMR-spectroscopy.

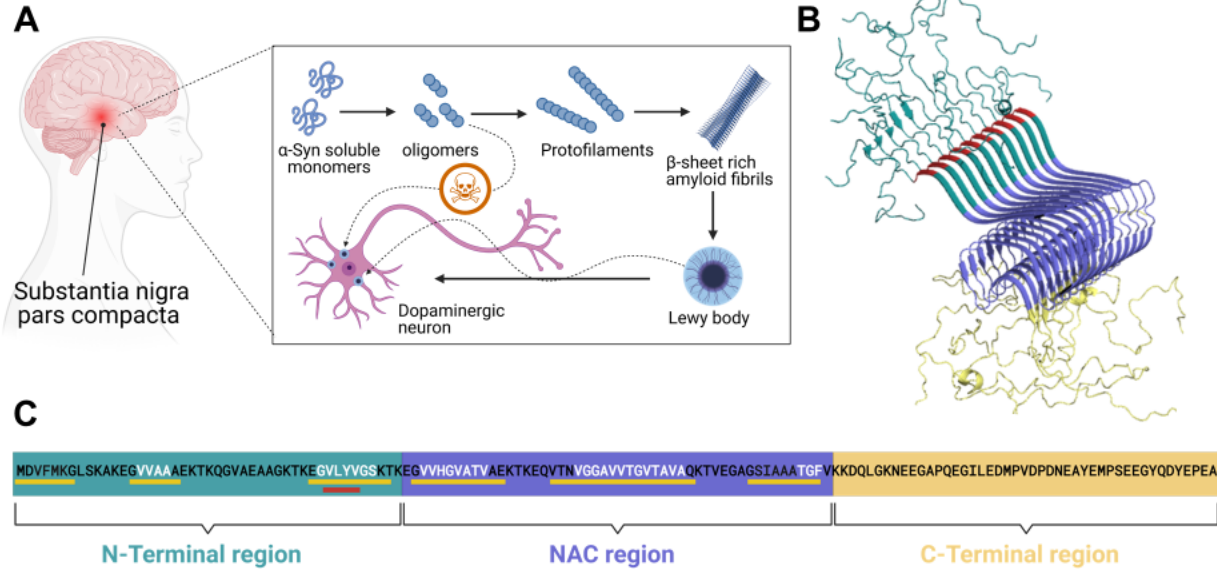


Figure 1. Aggregation of α -Synuclein occurs through a nucleation-polymerization process. A) Amyloid fibrils are found in LBs mainly located in dopaminergic neurons in the *substantia nigra pars compacta*, which is a region of the brain involved in coordinating movement. The presence of LBs are associated with neuronal apoptosis and neurodegeneration^{1,2}. Figure created in BioRender.com. B) Solid-state NMR structure of a fibril of full-length human α -Syn (PDB id: 2N0A). Figure illustrated with PyMOL. C) *In silico* prediction of amyloid-forming tendencies in α -Syn (140 amino acids)³.

Workflow and Methods

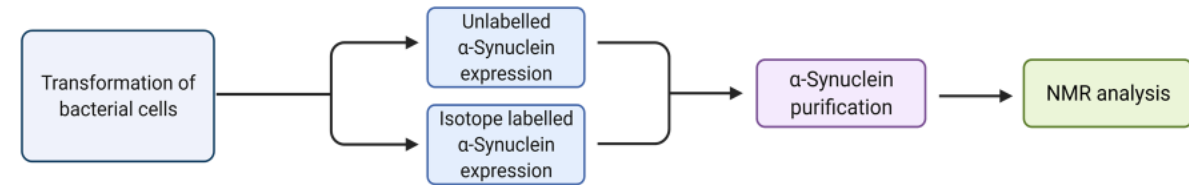


Figure 2. The work process from α -Synuclein recombinant expression to NMR-analysis.

Results

Expression and purification of α -Synuclein

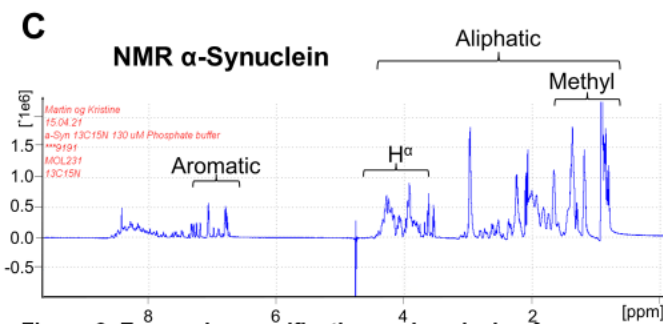
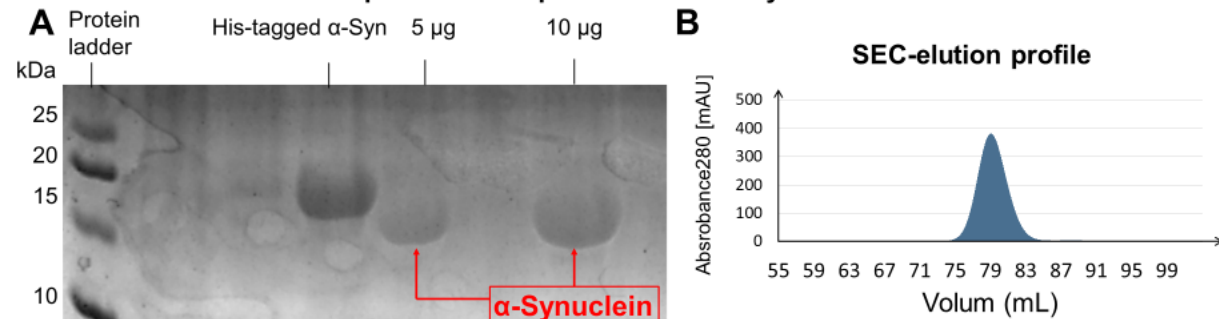


Figure 3. Expression, purification and analysis of α -Synuclein. A) SDS-page with His-tagged and pure α -Synuclein. B) SEC-profile with the purification results. C) NMR-spectrum showing α -Synuclein exhibiting the expected fold.

Conclusion

- α -Synuclein protein was expressed, purified and analysed with NMR.
- Analysis confirmed it to be a monomeric, intrinsically disordered protein
- Future perspectives; determine the importance of α -Synuclein's different misfolding stages, relative to PD.

References:

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3. Tsolis AC, Papandreou NC, Iaconomidou VA, Hamodrakas SJ. A Consensus Method for the Prediction of "Aggregation-Prone" Peptides in Globular Proteins. 2013 [cited 2021 Feb 15]; Available from: www.plosone.org