AGGREGATION OF RECOMBINANT TAU PROTEIN ISOFORM 2N4R DEPENDENCE ON DIFFERENT ENVIRONMENTAL CONDITIONS

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Neurodegenerative diseases are one of the most widely spread disorders in the world. Sadly, despite the intensive research, the understanding of the disease mechanisms is quite moderate and all available therapies are only symptomatic. Alzheimer's disease has attracted the most of attention, because it is the most common disorder affecting around 50 million people worldwide and this number is expected to increase in the near future. It was determined that the neurofibrillary tangles formed from microtubule-associated protein Tau are the hallmark of this disease and other tauopathies. Therefore, it is imperative to understand the mechanisms affecting this process and to find the best way to tackle them. However, in order to carry out such experiments it is important to obtain Tau protein with good yield and purity. In this work, we used SUMO-fusion technology [1], to produce Tau isoform 2N4R, which allowed to reduce the time of purification and resulted in higher protein purity and yield.

For further experiments polyanion heparin has been used as amyloid-like protein aggregation inducer *in vitro*. In order to understand mechanism behind the complexity of protein Tau aggregation, different conditions were examined. Since protein Tau is classified as intrinsically disordered protein, the aggregation rate is highly dependent on different pH values changing its total net charge and solubility due to high proportion of polar and charged amino acids in protein sequence [2]. Further, high sodium chloride concentrations are expected to heavily affect aggregation rate [3]. Also, we presume that pre-formed protein Tau fibrils can induce aggregation without using polyanion heparin what would explain misfolding of monomeric protein Tau in healthy recipient neuron cells in the brains [4].

All performed aggregation kinetics were followed using Thioflavin T fluorescence assay at a range of recombinant Tau protein and heparin concentrations. Formed protein fibrils were imaged using atomic force microscopy.

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[3] Goto, Y., Adachi, M. et al., Salt-induced formations of partially folded intermediates and amyloid fibrils suggests a common underlying mechanism, Biophysical reviews, 10(2), 493–502 (2018).
[4] Nizynski, B., Nieznanska, H. et al. , Amyloidogenic cross-seeding of Tau protein: Transient emergence of structural variants of fibrils, PloS one, 13(7) (2018).