

## Dissertation assessment „Investigation and inhibition of $\alpha$ -synuclein aggregation “

**Candidate: Kseniia Afitska, MSc.**

**Supervisor: Dmytro Yushchenko, PhD**

The presented thesis focuses on  $\alpha$ -synuclein (AS), an intrinsically disordered protein that under physiological conditions is implicated in regulation of synaptic vesicle mediated protein trafficking, while its misfolding into amyloid fibrils is linked to the development of Parkinson's disease. The candidate investigated in detail mechanisms of AS oligomerization/fibrilization and designed and evaluated a series of inhibitors of AS fibrilization.

The thesis is written in English in an abridged format (80 pages including references; 177 pages with reprints/supplements) and is based on five publications in recognized international peer-review journals (two of them in revision), where Kseniia is the first author in three of them. Furthermore, the candidate co-authored one additional publication not included in the thesis. Overall, the thesis is very well written with minimum typos and errors. From the formal point of view, however, I would prefer putting all chapters together and only then insert reprints of manuscripts to simplify orientation within the text.

The thesis and related original publications clearly document that during her PhD training Kseniia mastered a number of experimental techniques including molecular biology methods (cloning, mutagenesis), heterologous protein expression and purification as well as biophysical measurements (CD and fluorescence spectrometry). She also contributed substantially to manuscript preparation demonstrating thus her competence in presenting and disseminating her findings to the scientific community. The fact that publications presented here underwent a successful peer-review process in internationally recognized journals confirms the high quality and originality of candidate's research. It also simplifies today's defense as it would be somewhat redundant to re-analyze results already accepted.

*I would like the candidate to elaborate on the following issues/questions:*

1. What is the current status of your *J Med Chem* and *J Mol Biol* manuscripts (in revision upon thesis submission)?
2. Kinetics of AS fibrilization are followed by the increase of Thioflavin T fluorescence. As the dye interacts directly with fibrils it could likely influence their stability/dynamic behavior. Could you please

comment on this issue and elaborate if alternative techniques exist to monitor AS oligomerization/fibrilization?

3. Protein-based inhibitors reported in your thesis could be used to disrupt AS fibrils *in vitro*, but efficient targeting to the central nervous system would be needed. Could you please briefly summarize approaches that are used for the CNS targeting of protein-based therapeutics?

4. Are there any special considerations (advantages/disadvantages) for heterologous expression and purification of intrinsically disordered and aggregation prone proteins, as compared to proteins with defined 3D structures?

**In conclusion, the PhD thesis by Kseniia Afitska fulfills all criteria required for the successful defense of this work. Therefore, it is my pleasure to fully recommend the candidate to be awarded a PhD degree.**

**Závěrem můžu konstatovat, že předkládaná dizertační práce splňuje všechny požadavky Oborové rady Biochemie a proto ji doporučuji k obhajobě.**

Vestec, December 8, 2019

RNDr. Cyril Bařinka, PhD  
Laboratoř strukturní biologie  
Biotechnologický ústav AV ĀR  
Průmyslová 595, Vestec  
telefon: +420-325-873-777  
e-mail: [cyril.barinka@ibt.cas.cz](mailto:cyril.barinka@ibt.cas.cz)  
<http://lsb.avcr.cz/>

