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Kseniia Afitska: Investigation and inhibition of α-synuclein aggregation REVIEWER'S REPORT

The Ph.D. thesis submitted by Kseniia Afitska consists of Introduction, Results presented as a commented list of publications, Discussion, short Conclusions, and Appendix including supporting information of the attached papers. Such organization of the texts gives the reader an opportunity to get familiar with the scientific background and state of the art of the field, and to read the results in the form of original papers, with short but sufficient comments, and without unnecessary repetitions. Quality of the text is high, without errors and typos. I have found only two formal features somewhat distracting: (i) research aims presented on p. 33 as an itemized list (I would prefer a more readable fluent text), and (ii) placement of Supporting Information as an Appendix, separated from the corresponding papers (it would be much more convenient if SI followed each publication immediately).

As the title of the thesis says, the aim of the Ph.D. project was to investigate α-synuclein aggregation, accompanying Parkinson's disease, and to test the possibilities to inhibit this undesired process. The results document that the supervisor provided the Ph.D. candidate an ideal working environment and that she was able not only master a broad range of experimental techniques, but also summarize the results in articles published in respected journals. The four already published papers (two with the candidate being the first author) represent a firm ground for the successful defense of the work, another paper is currently under revision. I appreciate that the work does not only explore a hot area closely relates to a serious health problem, but is also based on solid and careful biophysical analysis of the studied interactions.

I would like to ask the candidate to answer the following questions.

1. How do the cryo-EM structures (page 22) correlate with the results of investigation of the C-terminal extension (page 39)? At the first glance, the C-terminus is relatively unrestricted in the fibrils according to cryo-EM.

2. I did not fully understand the argument in the last sentence of Section 1.4.2. Why the high affinity of anle138b to the fibrils contradicts the assumed ability of anle138b to target α-synuclein oligomers?

3. Could the candidate comment the effect of arachidonic acid and anionic detergents on the critical concentration of α-synuclein fibrillization (p. 702 of the BBA paper)?

4. What is the significance of the α-synuclein behavior at sub-micromolar concentration, considering the approximately 20 μM concentration of α-synuclein in the synapse (p. 708 of the BBA paper)?

5. Could the candidate comment more the unexpected finding that C-terminal truncation makes α-synuclein dimers more efficient inhibitors? Is the use of dimeric inhibitors a more promising route than a search for better bulky groups to be attached to the C-terminus?

6. What is the proteolytic stability of the designed inhibitors and what approach should be used to deliver the inhibitors to the neuronal target?

Considering the scientific content and quality of the thesis, I recommend Kseniia Afitska to be awarded the PhD degree.

Lukáš Žídek