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Review of the Ph. D. thesis „Amyloid β interaction with model cell membranes – insight into the mechanism of the Alzheimer's disease etiology and a novel therapeutic approach", by Dusan Mrdenovic

The reviewed doctoral thesis was prepared in the course of International Doctoral Studies in Chemistry at the Institute of Physical Chemistry of the Polish Academy of Sciences in the Molecular Films Research group, in collaboration with the group of Prof. Jacek Lipkowski at the University of Guelph, Canada, with Prof. Włodzimierz Kutner as supervisor and Dr. Piotr Pięta as auxiliary supervisor. The PhD thesis was prepared in a classical dissertation format, arranged in six chapters, supported by the abstract, lists of abbreviations, and the references section. Information about the research funding was also included. The thesis begins with the Introduction (Chapter 1), followed by Experimental section (Chapter 2), Chapters 3, 4 and 5 presenting the Thesis results, and the final Chapter 6 which contains conclusions drawn by the Candidate from his results. The Results chapters are strictly based on a consistent set of three experimental papers, arranged in the logical and chronological order. At the time of the thesis completion the first two papers have been published and the third one submitted. The thesis was reviewed as such, but it should be noted that the third manuscript has the accepted for publication status at the time of this review. The Ph.D. candidate serves as the first author in all three papers, which assures his leading role in the multi-author studies.

In the Introduction section, Mr. Mrdenovic presented the background of his studies, providing a concise account on Alzheimer's disease (AD), biological membranes and their physicochemical models and finally the Aβ peptides and their interactions with membranes as a mechanism of neurotoxicity in AD. Overall, the Introduction is written very well, with a comprehensive general account of main issues in the first two sections and a very thorough description of the current literature of the subject in the third section. Some paragraphs of section 1.3 were somewhat repetitive, which I think reflects the Candidate's effort to comprehend and rationalize the often contradictory results present in the literature of the

subject for the apparently similar experiments. I do not raise this as a criticism. I rather mention it as a sign of sympathy of the serious effort made by Mr. Mrdenovic to make sense of the state of the art in this vigorous field of research.

The objective of the thesis was described in the fourth section of the Introduction. This section is less than one page long which I think is a correct approach. Six specific goals listed include *"(i) the mechanism of Aβ-induced permeation of physiologically-relevant model cell membranes, (ii) the type of Aβ aggregate responsible for the membrane permeation, (iii) the morphological and structural properties of the toxic Aβ form, (iv) changes in the morphological, nanomechanical, and electrical properties of the membrane because of its damaging by Aβ, (v) changes in the conformation and orientation of membrane lipids resulting from their interaction with Aβ, and (vi) testing potential inhibitors of Aβ-induced membrane permeation*." Presenting the thesis aims in such simple and direct way is a clear indication that the Candidate has a good understanding of the purpose of his study and ways and means to achieve its goals.

Naturally, the introduction to any thesis is supposed to be limited to its scope, and AD research is a very large and ever-changing area. The thesis research was focused on $A\beta_{1-42}$ ($A\beta_{42}$) presented as the most important physiological and toxic Aβ species. This opinion is well-established in the past literature, but I think that the perspective presented Introduction would benefit from mentioning about the increasing awareness of truncated Aβ species as potentially equally important agents.

The Experimental section follows as Chapter 2. I find the title of this chapter as somewhat misleading, because it presents the principles of the methods applied in the Candidate's research rather than the details of experiments. Apart from the title, this thesis chapter is a perfect piece of work, providing an excellent introduction to main methods: lipid bilayer preparation, AFM, electrochemical methods of bilayer studies, and IR including PM-IRRAS. The descriptions are full, including the mathematical apparatus, with drawings illustrating the key aspects.

The convention adopted by the Candidate in the Results chapters follows the layout of the respective research papers, with abstracts followed by brief introductions, the detailed experimental descriptions (which justifies their absence in Chapter 2), and the results and discussions, followed by conclusions. The contents of Chapters 3 and 4 have been reviewed by specialists prior to the publication in respected specialist journals. This relieves me from a detailed discussion of the quality of presented research, but I am happy to state that my own scrutiny yielded no significant criticisms regarding the experimental designs and the interpretation of the obtained data. Minor errors, such as typos and wrongly referenced

Figures are very rare, and I do not find them worth mentioning. Nevertheless, I have several points and questions of a more nature, which are provided in the penultimate paragraph of this review, where Chapter 6 (Conclusions) is discussed.

These chapters should be considered jointly as they essentially present the methodologically complementary studies on the main subject of the thesis, which is the differential interactions of $A\beta_{42}$ monomers, small and large oligomers and fibrils with the phospholipid bilayer immobilized on a solid support (mica in Chapter 3 for AFM and gold electrodes in Chapter 4 for electrochemistry and IR). The key finding confirmed by all three approaches in the respective papers is that specific $A\beta_{42}$ oligomers, composed of several monomeric units produce large pores in the membrane by extracting phospholipid molecules first from the upper (solvent-exposed) and then also from the lower leaflet of the model membrane. Judging from the size of oligomer assemblies and their irregular locations in the vicinity of the pores, established by AFM, key step of the proposed mechanism includes the formation of oligopeptide/lipid complexes, rather than a formation of a regular channel-like structure.

Chapter 5 was prepared on the basis of a submitted manuscript. It builds on the results of the previous two, presenting the inhibitory effect of the experimental AD drug, K-162 on the above mentioned pore-forming ability of $A\beta_{42}$ oligomers. I find the experimental data presented in this chapter to be fully convincing and the K-162 inhibition of the membrane poration to be proven under the applied experimental conditions. I am, however, concerned with the theoretical calculations of energetics of the interactions of K-162 with various $A\beta_{42}$ species, presented in Table 5.3 and visualized in Figure 5.10. These calculations carry a wealth of potential information but ought to be discussed and verified in more detail. First of all, the reactions leading to the formation of specific complexes should be written down and their reversibility or its lack should be defined. In my opinion, the suprastoichiometric effect of K-162, exemplified by only a partial effect at its 10-fold excess over the peptide, assures that at least the initial processes have a reversible character. Then, for reversible processes, the values of equilibrium constants K should be derived from the standard equation: $\Delta G = -RT\ln K$. These values should be used to compare the experimental distributions of the respective species with the theoretical considerations. I have not performed these calculations, but it seems to me that at least some values are less than realistic, e.g. the decrease of the model fibril interaction by 150 kJ/mol in the presence of K-162, whereas the 1:1 model fibril interaction with K-162 was less than 8 kJ/mol. A further point stemming from these considerations that they could help answer the important point, namely how much K-162

should be continuously present in the brain to prevent the $A\beta_{42}$ oligomers from damaging the neuronal membranes. Also, I do not see why the Candidate labels the K-162 interaction with $A\beta_{42}$ as hydrophilic, whereas K-162 is mostly hydrophobic, except of the amine at its one end. My other specific criticism to this chapter regards the assignment of $A\beta_{42}$ dimers as non-toxic, whereas such dimers have been proposed in the literature as key toxic species in AD, but according to a receptor-specific mechanism not related to the membrane.

In Chapter 6 the Candidate summarized the key results of the thesis, which in addition to the pore formation ability by oligomers, includes various additional modes of membrane destabilization by monomers and fibrils, represented by the changes of the lipid IR spectra, and the mentioned inhibition of membrane damage by K-162. In the last paragraph the Candidate lists the following eight issues stemming from his thesis:

1. What structural difference causes different AβOs to interact with the membrane differently (i.e., large AβOs fibrillate on the membrane surface, while small AβOs permeate the membrane)?

2. Do different membrane lipid compositions of membrane stimulate membrane permeation or Aβ fibrillation?

3. Does the peptide-to-lipid ratio affect Aβ-lipid interaction?

4. Are there any other membrane components, e.g., membrane proteins, that affect Aβ-membrane interaction?

5. Does K162-bound AβMs retain their beneficial neurological abilities? If not, what is the reason for this limitation? Are there any candidates that can overcome it?

6. Does the K162 inhibition efficiency depend on the K162 concentration?

7. Is it possible to provide conditions that will facilitate K162-Aβ interaction over the K162-K162 interaction, thus increasing the K162 inhibition efficiency?

8. Does the kinetics of the competing K162-K162 and K162-Aβ interactions affect the K162 inhibition efficiency?

Comparing these points with my remarks, I believe that the further analysis of results already obtained in Chapter 5 could help answer issues 5-8, while further research is indeed necessary to answer issues 1-4.

My overall assessment of the thesis by Dusan Mrdenovic is enthusiastic. I think that his finding regarding the way $A\beta_{42}$ oligomers create the large pores in the membrane is seminal. Furthermore, not only the research is excellent but also its presentation. The thesis is comprehensive, beautifully illustrated and very carefully edited. The English is almost impeccable. However, as any good research should, it created issues that warrant further

discussion and research. In addition to those raised by the Candidate himself, I would like to add a few which could be discussed during the thesis defense:

1. Why actually the lipid to peptide ratio of 20:1 was adopted throughout the thesis, and why the K-162 to peptide ratio of 10:1 was used?
2. The mechanism of poration by sucking up the lipid molecules to peptide oligomers has been demonstrated for the immobilized membrane model used in the thesis. Is it possible that such pores could be repaired by a more fluid unsupported cell membrane?
3. In some experimental procedures the peptide was mixed with lipid vesicles prior to the bilayer formation (e.g. p. 76 vs. controls in p. 85-87). Recently the effects of membrane curvature on Aβ aggregation were reported. In the light of these results, would the curvature of the vesicles affect the efficacy of the studied interactions.
4. Could the differences in the used support (mica vs. gold) and different lipid compositions affect the studied processes between Chapters 3 and 4?

To summarize the above, I would like to state that the Ph.D. thesis by Mr. Dusan Mrdenovic meets all requirements for doctoral dissertations stated in article 187 of the Republic of Poland Act of 20th July 2018 – The Bill on Higher Education and Science (Journal of Law of Poland 2018, item 1668, with changes) [art. 187 ustawy z dn. 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce, Dz. U. 2018 poz. 1668 ze zm.]. Therefore, I propose to the Scientific Council of the Institute of Physical Chemsitry, Polish Academy of Sciences in Warsaw to continue with processing the thesis of Mr. Dusan Mrdenovic, towards awarding him with the Ph. D. degree. Taking into account the crucial contributions of Mr. D. Mrdenovic to all research presented, the publication of the thesis results in peer-reviewed journals, but first and foremost the original discoveries presented. I would like to propose awarding the thesis with a distinction. I consider the finding of a new molecular mechanism of membrane damage by $A\beta_{42}$ oligomers which may be operating in Alzheimer's disease, as particularly important, because, as already preliminarily demonstrated in Chapter 5 of the thesis this mechanism may be inhibited by an appropriately designed small organic molecule, which may be developed into an AD drug.