Warsaw, 01-07-2023

Dr hab. Sebastian Kmiecik, prof. UW

University of Warsaw
Biological and Chemical Research Centre,
Faculty of Chemistry,
Zwirki i Wigury 101, Warsaw
email: sekmi@chem.uw.edu.pl

Review

of the Ph.D. thesis entitled: „**Intrinsic and extrinsic determinants of the aggregation process of amyloid proteins**" prepared under the supervision of Professor Malgorzata Kotulska from Wroclaw University of Science and Technology and Dr Mounir Tarek from the University of Lorraine. Natalia Szulc's Ph.D. thesis is a joint Ph.D. thesis between the Wroclaw University of Science and Technology, Department of Biomedical Engineering in Wroclaw, Poland, and the University of Lorraine in Nancy, France.

The reviewed Ph.D. thesis is interdisciplinary in nature. It covers topics from the fields of biophysics, physical chemistry, bioinformatics, and molecular modeling. The conducted research included theoretical and experimental studies of the aggregation process and was focused on the influence of selected internal and external factors on the aggregation process of amyloidogenic peptides.

The doctoral dissertation of Mrs. Natalia Szulc is written in English language and composed of a Table of contents, Acknowledgments, Funding information, a List of publications of the candidate, Conference presentations, a List of abbreviations, the Thesis abstract (in English, French, and Polish), followed by Introduction (presenting the subject; intrinsic and extrinsic factors affecting amyloids aggregation; experimental methods in amyloid studies; computational methods in amyloid studies), followed by the section of research hypotheses, the section of Materials and Methods used in the work, followed by the

section of Results (divided into four chapters: [1] Hexapeptides, [2] Functional amyloids CsgA fragments, [3] Mutants of R4 fragments, [4] Interactions of Ab42 and hIAPP), followed by sections of Summary, Future perspectives, Bibliography, Appendix, list of figures and list of tables. In total, the Ph.D. thesis has 159 pages.

I consider the presented doctoral thesis to be well-written and the presented results to be valuable. However, I have the following questions and comments, which in my opinion are worth discussing during the defense of the doctoral thesis:

1. I have a major issue with the narrative of this work. After the introduction, the work presents and formulates four research hypotheses, which are discussed in the work in light of the obtained results. In my view, each one of the proposed research hypotheses is a broadly stated research fact, that is difficult to argue with. Couldn't each of these research hypotheses be confirmed on the basis of published scientific literature? I will not discuss each of the hypotheses here, I will focus briefly on one, hypothesis number 3 “the presence of lipid membrane affects aggregation of the native Ab42 peptide”. In recent years, at least a few studies (the result of my quick search) have been published showing that a lipid membrane affects the Ab42 aggregation process. At present, it is not a question of whether the membrane affects the aggregation process, scientists rather ask how, for example, the composition of the membrane or other factors in combination with the membrane, such as ionic conditions, may influence the aggregation mechanism. In other words, I would expect that the presented research hypotheses will define the research problem, dimension, and quality of the scope of knowledge that we want to acquire, which has not yet been known, or is not fully understood. Perhaps the presented research hypotheses should be narrowed down in some way to better describe the research results obtained and their significance against the background of the available literature.

2. In my experience, for the discussion of inter or intra- molecular interactions, the contact cutoff between alpha-carbons should be defined as around 10 Angstroms. Usually, it is set

up in the range of 8 - 12 Angstroms to correctly identify interaction patterns and not to introduce unnecessary noise (by the way the definition of contacts on alpha carbons has its drawbacks, perhaps it would be better on heavy atoms, but let's leave it at that). In section 4.3 discussing MD simulation results for the Ab42 and amylin systems, the Author wrote that "based on the contact maps, which take into account distances between alpha-carbons in amylin and Ab42, the shortest distances where assessed for about 30 Angstroms (Figure 54) … and 20 Angstroms (Figure 58)" Therefore, the mentioned shortest distances, as well as presented contact maps, suggest that there were no interactions between the molecules during the simulation, which completely changes the conclusions of the presented simulations?

3. The presented Molecular Dynamics simulations have been conducted in timescales between 1 and 8 μs (Table 9). What are the aggregation timescales for the presented systems, reported from experiments in the scientific literature? Recent reports suggest that there are two distinct physical timescales that set the number and size of aggregates (doi.org/10.1016/j.bpj.2021.04.032). The first timescale involves the fast aggregation of small clusters freely diffusing in the cytoplasm (my more specific question is: are we close to those fast aggregation timescales for the systems studied?), whereas in the second one, the aggregates are larger than the pore size of the cytoplasm and thus barely diffuse, and the aggregation process is slowed down to the timescale of minutes and beyond. How this timescale problem can be solved in simulations?

4. Aside from the time scale issue, I would be careful with drawing conclusions, in the context of an aggregation process (addressing 3rd hypothesis) based on a single monomer simulation (section 4.1). The stability of a single monomer vs. amyloid structure is a completely different issue (non-stabilized single-strand fragment vs. beta-sheet of amyloid). By the way, recent research suggests the opposite behavior of the monomer to that observed in the thesis: "interaction with the membrane dramatically changes the conformation of Aβ42 monomers. Moreover, membrane-bound Aβ42 proteins trigger the assembly of dimers, propagating the misfolded states of the Aβ42 molecules". (Int J Mol

Sci. 2020 Feb; 21(3): 1129, doi: 10.3390/ijms21031129). How would the Author relate to these results in the light of her own?

The above-mentioned comments and questions are aimed at getting to know the Author's views on the presented issues and initiating an interesting discussion on the defense of the dissertation.

The Author of the doctoral thesis is a co-author of several publications divided in the "List of publications" into two sections: containing some results of the doctoral thesis and not related to the thesis. Some of the results of the doctoral thesis were included in 3 publications published in well-recognized scientific journals: *Scientific Reports, International Journal of Molecular Sciences,* and *Nucleic Acid Research*. In *Scientific Reports* and *the International Journal of Molecular Sciences,* Mrs. Natalia Szulc is the first leading author.

The publication in *Scientific Reports* describes the findings from testing several bioinformatics methods for in-silico identification of amyloids, how they are robust to weak supervision, and encountering imperfect training data. The study shows that bioinformatics methods can be successfully applied to evaluate the quality of experimental data and used for their filtering. However, the fraction of mislabeled instances cannot be excessively high in the training set. As described in the co-authors' contributions in the publication, Natalia Szulc was responsible for the experimental, investigation, and writing part.

The publication in the *International Journal of Molecular Sciences* describes experimental and theoretical studies of self-aggregation of the repeat fragments of *Salmonella enterica* and *Escherichia coli*. A primary structural component of these fragments is CsgA protein. The study showed that amyloid structures of CsgA repeats are more easily formed and more durable in *Salmonella enterica* than those in *Escherichia coli*. As described in the co-authors' contributions in the publication, *Natalia Szulc was* responsible for methodology, experiment investigation, formal analysis, writing—original draft preparation, writing—review and editing, and visualization.

In a highly impactful publication in *Nucleic Acid Research,* amyloid-amyloid interactions database AmyloGraph has been proposed. In this paper, Natalia Szulc is one of 15 co-authors.

The Authors curated information from almost 200 publications, obtaining details of 883 experimentally studied interactions between 46 amyloid proteins or peptides. They also proposed a novel standardized terminology for the description of amyloid–amyloid interactions, covering all currently known types of such a cross-talk, including inhibition of fibrillization, cross-seeding and other phenomena.

In addition to these 3 publications, Natalia Szulc is the co-author of a book chapter on "Challenges in the experimental classification of amyloid peptides", published as a part of the Methods in Molecular Biology book series by Springer publishing house.

As for other papers (not related to the presented Ph.D. thesis), Natalia Szulc is a co-author of 4 publications: the second author of papers published in *Nanoscale* and *Bioelectrochemistry* and one of several co-authors of papers published in *BMC Bioinformatics* and *PloS Computational Biology*. These publications deal with the issues of rational design of nanofibrils, simulation of molecular dynamics of a biological membrane, and bioinformatics analysis of amyloid signaling motifs.

Moving on to the general assessment of the reviewed dissertation by Mrs. Natalia Szulc, I can say that it makes a significant contribution to understanding the mechanisms of amyloid protein aggregation. The thesis is unique in terms of interdisciplinarity, the Author has mastered a number of experimental techniques and methods of computational biology. Taking into account the presented doctoral dissertation, but also the scientific experience of Mrs. Natalia Szulc (co-authorship of 7 publications in total, co-authorship of a scientific monograph, active participation in 3 international conferences, co-execution of scientific grants, and finally the implementation of a doctoral dissertation as part of the cotutelle fellowship), I can state that the author is prepared for independent scientific work in the field of biomedical engineering, chemistry and supporting computational methods. The dissertation also shows the Author's ability to deal with difficult scientific problems and her extensive knowledge in the subjects of experimental and computational biology.

In summary, the dissertation presented to me for evaluation describes research at a good technical and scientific level. I believe that the dissertation meets the conditions set forth in the Act on Higher Education and Science (Dz. U. z 2018 r. poz. 1668 z późn. zm.) and with full conviction propose to admit Mrs. Natalia Szulc, M.Sc. to further stages of the doctoral process.

Dr hab. Sebastian Kmiecik, prof. UW