Prof. dr hab. Marta Pasenkiewicz-Gierula *[professor, Ph.D., postdoctoral degree holder]*
Faculty of Biochemistry, Biophysics and Biotechnology
Jagiellonian University
Krakow

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Review
of the doctoral dissertation of Mr. Nguyen Truong Co, M.Sc.,
entitled "A study of the factors governing the mechanism of protein aggregation by using computer simulation"

The formation of amyloid deposits (plaques) in the brain causes fatal degenerative diseases. These diseases lead to dementia and are an individual tragedy as well as a serious social problem. Amyloid deposits are formed as a result of protein aggregation – in the case of Alzheimer's disease, amyloid beta (Aβ) peptides, which are the products of digestion of the amyloid precursor protein (APP), are aggregated. The factors affecting this aggregation and the structures of highly ordered fibrils resulting from aggregate fusion are known, but the mechanism and causes of amyloid formation remain unclear. There are also no methods of preventing the formation of deposits and treating diseases caused by them. Therefore, learning about the mechanisms of both their creation and disintegration is a highly pressing and current problem.

The doctoral dissertation presented to me for review is devoted to model studies of the influence of various factors on the formation of amyloid deposits and their breakdown. The study described in it was performed using computer methods. These methods make it possible to predict the behavior of the modeled system and, despite the simplicity of the model, can provide important information about the molecular mechanisms of the aggregation process. The doctoral dissertation is based on three articles published in prestigious journals, which means that each of them was positively assessed by at least two competent reviewers. The involvement of the doctoral student in these publications is significant and amounts to 85%, 35%, and 50%, respectively.

The doctoral dissertation of Nguyen Truong Co, M.Sc., was conducted at the Department of Theoretical Physics of the Institute of Physics of the Polish Academy of Sciences under the supervision of prof. dr hab. Mai Suan Li *[professor, Ph.D., postdoctoral degree holder]*. It consists of five main parts and also contains important background information.

The first part is an introduction (*Introduction*), which presents the motivation of the study, its importance for basic research, and potential therapeutic applications.

The second part is a literature review (*Literature Review*). It presents the current views on the mechanisms of the formation of amyloid deposits, the influence of internal and external factors on this process, as well as the methods and results of its study. Particular attention was paid to the problem of peptide aggregation on surfaces of different roughness. The third part (*Computational Methods*) describes the computer methods and models used in the papers on which the doctoral dissertation is based. The computational methods are mainly the Monte Carlo (MC) method on a grid with the Metropolis algorithm and variants of molecular dynamics simulation (MD). Surface models of different roughness on which peptides can aggregate are described in detail, and the energetic and structural criteria of the resulting aggregates are given.

Chapter 4 (*Summary of Publications*) has three subsections; each of them discusses the next of the three articles on which the dissertation is based.

Subsection 4.1 is an introduction to modeling studies on the effect of surface roughness on polypeptide aggregation. These studies are described in Co, N.T.; Li, M.S. *Effect of Surface Roughness on Aggregation of Polypeptide Chains: A Monte Carlo Study*. Biomolecules 2021, n, 596 (Ph.D. student participation is 85%). The study of the surface effect on peptide aggregation is highly justified, as there are many different surfaces in the tissue, for example, cell membranes with a different protein-lipid composition. Using a simple bead model of both the peptide and the surface and the MC method on the grid, the authors of the paper showed that the type of surface influences both the rate of aggregation and the structure of the generated aggregate. In the case of a smooth surface, the peptide aggregation time depends on the energy of interaction of the peptides with the surface. When the interactions are weak or strong, the aggregation time increases with the interaction energy, and it decreases for intermediate energies. In the case of a rough surface, for weak and strong interactions, the aggregation time increases with the surface roughness, and the very high roughness completely inhibits aggregation. For indirect effects, the chart of aggregation time versus surface roughness has a minimum.

Computer studies carried out in this paper, in addition to confirming previous experimental and simulation studies, predicted the dependence of peptide aggregation time on surface roughness, unknown from experimental studies, for the intermediate energy of peptide interactions with the surface, the graph of which is similar in shape to the chain curve (*U-shape*). This means that for certain surface roughness, the aggregation time is shorter than for lower and higher roughness.

Subsection 4.2 is devoted to the issue of the dependence of the peptide aggregation rate on its secondary structure. This part of the research is described in Thu, T.T.M. *et al.*

Aggregation rate of amyloid beta peptide is controlled by beta-content in monomeric state. J. Chem. Phys. 2019, 150, 225101 (Ph.D. student participation is 35%). In this paper, *the replica exchange molecular dynamics (REMD) simulation* method was used. The simulations were carried out with implicit consideration of water on almost all-atom monomer models of the Aβ peptide composed of 42 amino acid residues (Aβ42) and its 19 mutants. The structures generated in the simulations differed in the content of β-strands. Each of the obtained structures was assigned an experimentally determined relative aggregation rate (in relation to the Aβ42 aggregation rate). The comparison of the structures with the numbers showed a strong dependence of the peptide aggregation rate on the β-strand content in their structure. The novelty of the paper is the prediction that this relationship can be described by both linear and exponential functions. However, it is not possible to unequivocally state whether the relationship is linear, as suggested by the experimental results, or exponential since the dispersion of points around the fitted curve is similar in both cases.

Subsection 4.3 is devoted to the issue of the thermal disintegration of peptide aggregates. The study and its results are described in the article Co, N.T. *et al*. *Heat-induced degradation of fibrils: Exponential vs. logistic kinetics*. J. Chem. Phys. 2020, I52, 115101 (Ph.D. student participation is 50%). A bead and all-atom peptide model was used in these studies. In the bead model simulated at several temperatures by the MC method, the decay during intra- and inter-chain contacts was recorded in aggregates composed of 10, 16, and 28 molecules. Peptide aggregates in the all-atom model were subjected to MD simulations with explicit consideration of water at three temperatures. Two aggregates were built; one of 10 molecules of the Aβ42 peptide with the first 36 residues removed (Aβ37-42) and the other of five molecules of the Aβ42 peptide with the first 16 residues (AβI7-42) removed. In the MC simulations of the bead model, the results were obtained which, following the experimental results, predict that the thermally induced degradation of aggregates as a function of time is described by a double exponential function for each tested temperature. It was also predicted that the change in aggregate size over time, caused by thermal dissociation of individual peptides, is a logistic function for the tested temperatures. This function, above the critical size of the aggregate, i.e. initially, decreases slowly, and below this value, i.e. for longer times, it decreases quickly. Aggregate size changes are slower when peptide re-aggregation is possible. The MD simulations of the all-atom model showed a similar functional dependence of the change in aggregate size over time due to thermal dissociation of individual peptides as the MC simulations with re-aggregation.

The double exponential time dependence of the thermal destabilization of the peptide aggregate predicted in the MC simulations is consistent with the experimental results. Such a dependence

is the result of breaking interactions between peptides due to the deformation of their β structures. On the other hand, the experimental results indicated a single exponential time dependence of the aggregate size change due to the thermal dissociation of peptides. This dependence was valid for long times. The novelty of the discussed paper was the demonstration that the entire time course of the process of thermal dissociation of peptides from the aggregate is described by a logistic function. The authors showed that below a certain size of the remaining aggregate, i.e. for long times, this function behaves as a single exponential function, which is consistent with the experimental results.

Chapter five (*Conclusions and Future Work*) summarizes the most important results and also includes plans for future research.

Both the publications on which the doctoral dissertation of Nguyen Truong Co, M.Sc., is based, as well as the dissertation itself are written very well – the information contained in the texts is brief but comprehensive and interesting. The authors of the works, as well as the author of the dissertation, made every effort to present the objectives, methods, and results of the research to the reader in a clear manner. There is no ambiguity in these papers. In addition, the papers are very carefully illustrated, which perfectly complements and explains the verbal information.

From the editorial point of view, I found a few mistakes and typos in the text, but they did not change the meaning of the information provided. I have no background to judge the correctness of the English language in which the thesis is written, but while reading the thesis, I had the impression that the doctoral student is fluently and correctly using this language.

The Act on academic degrees and academic title requires that the doctoral dissertation be an original solution to a scientific problem, demonstrate the candidate's general theoretical knowledge in a given scientific discipline, and the ability of the doctoral student to conduct independent research work. Based on the doctoral dissertation presented to me by Nguyen Truong Co, M.Sc., I can state that he can independently and skillfully solve the scientific problems presented to him and that he has general and detailed theoretical knowledge in the field of biophysics, as well as skills in the use of advanced computational methods for studying biomolecular systems. Therefore, I am asking the Scientific Council of the Institute of Physics of the Polish Academy of Sciences to admit Nguyen Truong Co, M.Sc., to the next stages of the doctoral dissertation.

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