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Report on the manuscript submitted by Ms Natalia SZULC with a view to obtaining the degree of Doctor in Biomedical Engineering at Wroclaw University and Doctor in Chemistry at University of Loraine

Ms Natalia SZULC presented a detailed manuscript entitled «Intrinsic and extrinsic determinants of the aggregation process of Amyloid proteins». The work was carried out in two laboratories, at the University of Loraine in the LPCT laboratory under the supervision of Dr. Mounir Tarek and at the University of Wroclaw in the Department of Biomedical Engineering under the supervision of Prof. Malgorzata Kotulska.

The manuscript comprises four parts presenting the framework of the study, the methodologies used and the results obtained and their interpretation. The whole is clearly presented, with well-chosen, high-quality illustrations. The aim of the project is to demonstrate that the prediction of amyloid structures using bioinformatics methods is still far from being complete and robust. The work reported by Ms Natalia SZULC highlights the variations that small experimental modifications (pH, salts, point mutation, etc.) can produce in amyloid structures. Obtaining reliable prediction methods is a major challenge, for example to assess the effect of molecules that inhibit fibrillation. This is a major public health issue, as there is still no effective treatment for patients suffering from Alzheimer's or Parkinson's disease.

Part 1 is the introduction. It presents a very brief presentation of amyloid peptides and proteins, their aggregation process and kinetics. Particular emphasis is placed on the various factors that can induce changes in aggregation, such as threshold, concentration, solvent, pH, buffers, temperature and protein-protein interactions. The references are numerous and well chosen. The chapter also briefly presents the biophysical methods classically used to characterise amyloid peptides and proteins: Circular dichroism spectroscopy, Infrared spectroscopy (ATR-FTIR or FTIR microscopy), Raman spectroscopy (FT-Raman) and fluorescence of Thioflavin T (ThT), Electron microscopy and Atomic Force Microscopy (AFM). The advantages, disadvantages and specific features of each method are summarised and are very useful. In the same way, the computational methods are listed but few details are given about the limitations of each method. For non-specialists like me, it's difficult just by reading the few lines of presentation to know what each of these methods is based on. Of course there are references, but it would have been interesting to have a table giving the specific features of each method and their limitations, as was done for the experimental methods. This part ends with a presentation of the various objectives and issues to be addressed.

The second part is the description of the materials and methods: the peptides used, the different protocols used to study the peptides.

The third and most important part is the presentation of the results. Four studies were carried out and are presented one after the other 1 (four chapters).
Chapter 1 describes the study of the aggregation of short peptides (6 residues). 34 hexapeptides were selected on the basis of whether or not they were predicted to form amyloidogenic or non-amyloidogenic assemblies (prediction by bioinformatics methods). Infrared spectroscopy was used to monitor the ability of compounds to form amyloid fibers. It is regrettable that only the amide I range (1700-1600 cm^{-1}) is presented, the amide I/amine II ratio can be an indication of fiber formation, particularly in a D_2O medium. Was there a full exchange? The analysis of the results refers to data presented in a publication and supplementary files. It would have been more pleasant for the reader to attach the publication of which Ms. Natalia SZULC is co-author in the thesis manuscript in annex for example. The conclusion given following the analysis of the results is that "minor deviation in experimental conditions strongly influence the validity of a protein classification as an amyloid".

From my point of view, a single biophysical method is not enough to prove the amyloid character of a peptide. It would have been interesting for example to observe the same samples in TEM.

Chapter 2 and 3 describes the study of the aggregation of longer peptides (23 amino acids). In these chapters, various sequences of the functional amyloid CsgA are studied, as well as the effect of point mutations in R4 fragment. The objective is to assess whether the prediction made by the various bioinformatics programs is in line with the results of experimental methods to describe the amyloid character or not. Several biophysical methods are used to characterise assemblies, CD, ATR-FTIR, FT-Raman, TEM and fluorescence. The results of the experimental part are well described and discussed. On the other hand, results from the various bioinformatics programs are not discussed at all. For the program FolAmyloid or Waltz predicts a non-amyloid sequence classification while the programs Aggrescan and ArchCandy predicts them as amyloid. It would have been interesting for non-specialists to have an explanation of these important differences.

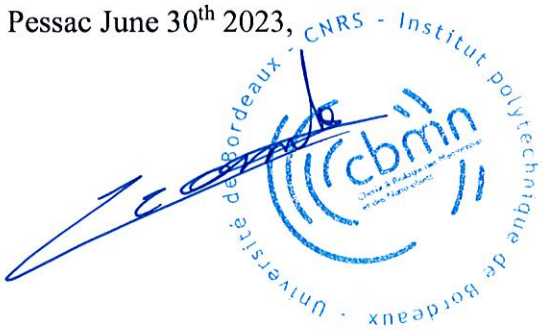
Chapter 4 reports a rather different study. The focus was on the Aβ1-42 and hIAPP peptides and their cross talk interacting with membrane. Using simulation methods (MD) and AFM, Ms. Natalia SZULC demonstrated the impact of the Aβ peptide on DOPC/DPPC model membranes. The simulation predicts strong stabilisation of the peptide linked to the formation of a transmembrane helix. The AFM images show that the interaction of the Aβ1-42 peptide with the membranes is preferential in the fluid phase, as already demonstrated by other authors. It would have been interesting to use a method such as polarised ATR-FTIR to identify, if possible, the damage induced on the lipids and the structure of the Aβ1-42 peptide in the membrane. An interesting result is the modification of the interactions by the presence of the IAPP peptide. No AFM image of the impact of hIAPP on the membranes is shown, this is important to be sure that the peptide alone has no influence on the DOPC/DPPC bilayer. AFM images reveal significant changes, but the structure of the formed elements cannot be determined. Additional relevant analyses are proposed to go further in the understanding of the observed mechanisms.

The manuscript ends with a discussion recalling all the results and conclusions.

In conclusion, on reading this manuscript, it seems indisputable that Ms Natalia SZULC has done a very important work. I can imagine that in the context of the health crisis, it can't have been easy to be between two laboratories and two countries. The manuscript is of excellent quality in its presentation of the results and their interpretations with detailed argumentation linked to a strong knowledge of the literature in the field. This work has already given rise to 4 publications of which Ms Natalia SZULC is co-author (2 as first author). It is noted that during her thesis work Ms Natalia SZULC also participated in other works not presented in this manuscript, she is co-author of 4 other publications. It is a remarkable assessment of its results and expertise.

Consequently, I express a very favorable opinion on the oral defense of the thesis of Ms Natalia SZULC, which can be defended as is, in view of obtaining the degree of Doctor from the University of Loraine and of the University of Wroclaw.

Pessac June 30th 2023,



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