

## Review of PhD. Thesis

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**Title: Proteomics analysis of aging proteins**

**Reviewer: RNDr. Václav Kašička, CSc.**, Institute of Organic Chemistry and Biochemistry,  
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The submitted PhD. thesis deals with development of new advanced analytical methods, high performance liquid chromatography (HPLC) and nano-liquid chromatography (nano-LC), both of them coupled with mass spectrometry (MS) detection, and their application for a complex proteomic analysis and characterization of aging and archaeological proteins. The topic of the thesis is up-to-date and very important, because it was not yet studied in detail and there is a lack of knowledge about the mechanism of protein aging and lack of methods for determination of D-amino acids in peptides and proteins. New knowledge in both these areas is very much needed.

The author has set four challenging aims:

1. Development of new LC-MS methods for enantioseparation of proteinogenic amino acids for the study of racemization of amino acids in the aging proteins.
2. Application of the developed LC-MS methods for analysis of the particular aging proteins, recent and old bovine and rat collagens.
3. Development of new LC-MS methods for sex estimation of ancient skeletons for the archeological, anthropological and forensic research.
4. Application of the developed methods for sex estimation of the recent and ancient individuals.

I am pleased to state that all these ambitious aims were successfully fulfilled. The advanced analytical methods have been developed and the following relevant results were achieved:

1. Using the LC-MS/MS methods, the separation of enantiomers of proteinogenic amino acids was achieved and the process of amino acid racemization, posttranslational modification and sequence degradation in the recent and aging proteins (bovine and rat collagens) could be monitored. Positive correlation between the degree of the above processes and the age of the bovine collagens was found and quantitatively assessed.
2. Minimally invasive method has been developed for a sex estimation of recent and ancient persons. It is based on minimally destructive procedure using soft protein etching and nanoLC-MS analysis of peptides released from the two sex-dependent forms of the amelogenin protein in tooth enamel. The method was successfully applied for sex estimation of various recent and medieval, adult and juvenile individuals with 100% accuracy.

It is worth to mention that the work of Marine Morvan represents a continuation of the previous successful developments of LC-MS and CE-MS methods for separation and analysis of amino acids, peptides and proteins in the working group of her supervisor, prof. Ivan Mikšík, at the Institute of Physiology of the Czech Academy of Sciences in Prague.

High quality of the results is confirmed by the fact that they were already published in two research articles and one review article in prestigious international peer-reviewed journals with relatively high impact factors, *Analytica Chimica Acta*, *Journal of Separation Science* and *Separations*. In these articles, Marine Morvan is twice the first or once the second author. This documents her substantial contribution to the achieved results. In addition, two papers with her co-authorship are currently submitted for publication.

From the formal point of view, the thesis is presented in a good and clear graphical form, with illustrative figures and tables. Publication of results in the peer-reviewed journals simplifies my role as a reviewer. However, fulfilling the role of a reviewer I have several comments, questions, and topics for the discussion:

1. The title "Proteomics analysis of aging proteins" is informationally redundant since the term "proteomics" itself already means "comprehensive analysis of proteins".
2. HCl, H<sub>2</sub>O<sub>2</sub>, DCl, and D<sub>2</sub>O are the chemical formulae, they should not be presented among the common abbreviations.
3. The introduction sections to the particular topics of the thesis are very general and very short, see e.g. section 1.1.2 Proteins and their functions containing only eight lines, section 1.2.1 Amino acids and their structures with only five lines, and section 4.2.2 Function of Collagen – four lines.
4. Some statements are too strong/general and/or incorrect, they should be corrected/explained during the defense of the thesis, see e.g.
  - a) Page 4, section 1.2.1: Amino acids consists of an amino group and a carbonyl group.
  - b) Page 4, section 1.2.1: Enantiomers have the same physicochemical properties.
  - c) Page 4, section 1.2.2: In nature, there are 20 different unmodified amino acids.
  - d) Page 7, section 1.3.3.1: In capillary electrophoresis, analytes are separated into fused silica capillaries according to their isoelectric point.
  - e) Page 7, section 1.3.3.2: In gel electrophoresis, two mechanisms for protein separation are possible. What are the other mechanism of protein separation in (gel) electrophoresis?
  - f) Page 11, chapter 3: Chiral separation is the last and most important challenge in analytical chemistry.
  - g) Page 11, chapter 3: Incoherent names of chiral selector types are used. Mixing of chemical and functional types of chiral selectors: "crown ethers, cyclodextrins, and cyclofructans" on one side and "ion exchange and Pirkle type" on the other side should be avoided.
  - h) Page 49, section 4.1.2: Addition of hydrophilic groups can increase the hydrophobicity of the protein surface.
  - i) Abstract of the paper in the journal Separations: "In this review, chromatographic and electrophoretic techniques will be recently developed (2018-2020) for enantioseparations."
5. Ad Fig. 2 in the review article in the journal Separations: Please, comment in more detail the final steps in the two approaches for determination of D-amino acid residues in peptides and proteins, i.e. "Immunological method" and "0 h Extrapolation method".
6. On page 147, in Chapter 7, it is stated that "By the enzymatic process, the racemization proceeds to free amino acids before or during peptide elongation, whereas by a non-enzymatic process, racemization proceeds on proteinogenic amino acids". Please can you comment the differences in enzymatic and non-enzymatic racemization in more detail?
7. Does the application of deuterium oxide and other deuterated chemicals guarantee that no racemization of amino acids occur during enzymatic or acid hydrolysis of peptides and proteins?
8. What are the plans of the author in her future scientific career?

## Conclusion

To summarize, the general evaluation of the thesis of MSc. Marine Morvan is very positive. The thesis brings new significant results and represents a valuable contribution to the development of new chiral separation methods and their application for determination of D-amino acids in aging and archaeological proteins. The author proved a very good knowledge of the studied topics. She showed the ability of elaboration of new analytical techniques and their creative application. For this reason, I recommend to accept the thesis of Marine Morvan for the defense and to consider the thesis as a basis for awarding her with the scientific degree PhD.



Faculty  
of Science

Genius loci ...

#### Dissertation review

**Author:** Marine Morvan, M.Sc.  
**Title:** Proteomic analysis of aging proteins  
**Supervisors:** prof. Ing. Ivan Mikšík, DrSc., assoc. prof. Ing. Petr Česla, Ph.D.,  
assoc. prof. Ing. Tomáš Čajka, Ph.D.  
**Opponent:** assoc. prof. RNDr. Petr Bednář, Ph.D.

Author focused her thesis on analysis and characterization of aged and archaeological proteins. This is interesting topic with a high potential in archaeology, heritage science, forensic research and practice as well as in medicine, food production and storage.

The dissertation is written as a set of published articles supplemented with a commentary. In introductory part, author describes structure of proteins, general processes occurring during their ageing, post-translation modifications, chirality and description of analytical methods used in proteomics with the emphasis on chromatography and electrophoresis. Last introductory chapter very briefly deals with the analysis of ancient materials. In the last mentioned chapter I somewhat lack at least the most important literary sources.

Aims of the thesis cover a wide scientific scope including chiral separation of amino acids, analysis of aging collagens and approaches for sex estimation of ancient human remains using proteomics including possibilities to minimize its invasivity. The research work included in the thesis was published in several papers. In two of them, Marine Morvan is the first author (Separations 8 (2021) 112; Anal Chim Acta 1262 (2023) 341260). First paper is a comprehensive review on contemporary chiral analysis of proteins and peptides covering advances in the period 2018 – 2020. The second one is an interesting and original study of aging of collagen that includes amino acids racemization, post-translation modifications and sequence degradation. In this work, the authors were able to locate the positions of D-amino acids in peptides prepared by enzymatic hydrolysis of aged collagens. Besides, Marine Morvan is co-author of a paper dealing with the utilization of tooth enamel peptide analysis for sex estimation (J. Sep. Sci 2023; 2300183). Another two co-authored papers that are currently under review validate and apply the proteomic method to remains of humans from Early Medieval centre of Mikulčice. The obtained results are very interesting and utilizable in number of applications.

The thesis is written in English, it is clearly prepared and practically without grammatical errors and typos. It has a good graphic level.

I have one question for discussion: The presented studies proved the reliability of the minimally invasive proteomic method for the sex estimation of ancient humans. Would it be possible to adapt the method to determine the sex of animals? What problems can be expected in such a potential application?

The presented dissertation clearly meets the criteria for this type of qualification works in terms of quality, scope and applicability of the results. Therefore, **I recommend the dissertation for defense**. In the event of its successful defense, **I recommend the award of the academic title "Doctor", abbreviated as Ph.D., to Marine Morvan** in accordance with Section 47 of the Act on Higher Education No. 111/98 Sb.

In Drnovice, October 6<sup>th</sup>, 2023

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