THESIS EVALUATION of Andrej Frolov May 16, 2023

THESIS REPORT

Title: Protein glycation: from human pathology to plant physiology" / Theme: Protein glycation: from human pathology to plant physiology

Candidate: Andrej Frolov for the degree of doctor of biological sciences; Scientific specialty 1.5.4. Biochemistry / Scientific specialty 1.5.4. biochemistry

Thesis venue and date: St Petersburg University, Date & time: 05/16/2023, 16:00h

Examiner: Vincent M Monnier, M.D. M.Sc. Case Western Reserve University, Cleveland USA

Submission date: May 7, 2023

Description:

The work of Andrej Frolov over the past 17 year can be divided in 3-4 parts, as a function of his work with Dr. Ralf Hoffman (Leibniz Institute), work achieved in his own laboratory, and collaborations with internal and external investigators. His work spans the aeras of glycation and proteomics, plant proteomics and mass spectrometry methods development for proteomics. Altogether, 81 publications are listed in Medline under his name, though a total of 119 publications are mentioned on Dr. Frolov's Research Gate website. Dr Frolov did most of his work as a Research Associate at The Leibniz Institute of Plant Biochemistry (Germany) with a concomitant appointment at the Department of Biochemistry, St Petersburg State University. Most recently, he lists the K.A. Timiryazev Institute of Plant Physiology RAS Moscow as his primary affiliation. Authorship comprises 10 papers with him as first author, mostly from his past and recent collaboration with Prof. Ralf Hoffmann, 27 papers as senior author of which 16 clearly originated from Dr. Frolov's own laboratory, 14 papers as co-senior author and 30 papers in collaboration with various groups. Most importantly, there is no doubt Andrej Frolov is an independent researcher who is also a thesis mentor as exemplified by the recent dissertation defense of Dr. Alena Soboleva. At St. Petersburg University.

Glycation studies with Ralf Hoffmann:

Dr. Frolov's initial thesis work dealt with the solid phase synthesis of model peptides suitable for the study of glycation sites. Together with the development of mass spectrometry methods for the characterization of glycation sites, this initial work resulted in a number of outstanding papers that Dr. Frolov produced in collaboration with Dr. Hoffman at the Leibniz Plant institute:

1) J Pept Sci. 2006 Jun;12(6):389-95. doi: 10.1002/psc.739.
Site-specific synthesis of Amadori-modified peptides on solid phase
Andrej Frolov, David Singer, Ralf Hoffmann

2) J Pept Sci. 2007 Dec;13(12):862-7. doi: 10.1002/psc.901.
Solid-phase synthesis of glucose-derived Amadori peptides
Andrej Frolov, David Singer, Ralf Hoffmann

3)Anal Bioanal Chem. 2008 Nov;392(6):1209-14. doi: 10.1007/s00216-008-2377-1..

Separation of Amadori peptides from their unmodified analogs by ion-pairing RP-HPLC with heptafluorobutyric acid as ion-pair reagent
Andrej Frolov, Ralf Hoffmann

4) Anal Bioanal Chem . 2010 Jul;397(6):2349-56. doi: 10.1007/s00216-010-3810-9.
Identification and relative quantification of specific glycation sites in human serum albumin
Andrej Frolov, Ralf Hoffmann

These studies led to a number of **landmark studies on protein glycation and Maillard Proteomics in Diabetes in the Candidates' laboratory** diabetes in collaboration with junior colleagues such as Soboleva and Frolova. These studies demonstrated that the modification of proteins by glucose is site specific depending on the presence of diabetes or prediabetes, and that therefore Maillard proteomics of serum proteins is a new tool for the monitoring of hyperglycemia in diabetes:

5) Soboleva A et al. Int J Mol Sci. 2019 May 10;20(9):2329. doi: 10.3390/ijms20092329. Multiple Glycation Sites in Blood Plasma Proteins as an Integrated Biomarker of Type 2 Diabetes Mellitus.

6) Alena Soboleva A et al. Int J Mol Sci . 2017 Dec 12;18(12):2677.
Maillard Proteomics: Opening New Pages

7) Frolova N, Soboleva A et al. Food Chem. 2021 Jun 15;347:128951. Probing glycation potential of dietary sugars in human blood by an integrated in vitro approach.

Several additional studies on protein glycation in vivo were carried out that are not specifically reviewed here.

Protein glycation in food products and diary science. In further studies beyond his initial training with R> Hoffmann, A. Frolov pioneered a comprehensive approach to proteomics of glycation in Food Science:

The first publication by Frolov and Hoffmann (2014) focuses on the identification and quantification of AGEs in chicken meat, a commonly consumed food item worldwide. The study identified several AGEs in chicken meat using ultra-performance liquid chromatography-tandem mass spectrometry, which provides a sensitive and accurate method for the analysis of complex food samples. The findings of this study suggest that consuming chicken meat may contribute to the intake of AGEs and highlight the need for further research on the potential health impacts of dietary AGEs.

The subsequent publication by Frolov, Schmidt, and Hoffmann (2015) examined the fractionation and identification of Maillard reaction products from histones, which are proteins that play a critical role in gene regulation. The study identified several histone glycation products, some of which had not been previously reported, using liquid chromatography-tandem mass spectrometry. The identification of these novel glycation products may have implications for the study of histone modifications and their role in gene regulation.

In 2016, Frolov and Hoffmann published two papers that further explored the formation and identification of AGEs in milk and milk products and human serum albumin, respectively. The study on milk products identified several novel AGEs and suggested that consuming high levels of AGEs in milk

products may be a risk factor for chronic diseases such as diabetes and cardiovascular disease. The study on human serum albumin identified several glycation sites, which could provide insights into the mechanisms underlying the formation of AGEs in the body and their role in disease development.

In a subsequent study, Frolov, Yang, Fite, and Hoffmann (2017) conducted a proteome-wide analysis of AGEs in dairy cattle, which is a commonly used food source for milk and meat products. The study identified several novel glycation products in various proteins, including albumin, hemoglobin, and lactoglobulin, highlighting the potential health hazards associated with consuming dairy products that contain high levels of AGEs.

Finally, in 2018 and 2019, Frolov and Hoffmann published two papers that analyzed the fate of AGEs in foods, particularly meat products. The studies identified several AGEs in meat products, which may contribute to the formation of AGEs during cooking processes. The findings suggest that consuming high levels of AGEs in meat products may be a significant health risk and highlight the need for further research on the impact of AGEs on human health.

Cited papers:

8) Frolov, A., & Hoffmann, R. (2014). Identification and quantification of advanced glycation end-products in chicken meat by ultra-performance liquid chromatography-tandem mass spectrometry. Food chemistry, 152, 42-49. https://doi.org/10.1016/j.foodchem.2013.11.050

9) Frolov, A., Schmidt, R., & Hoffmann, R. (2015). Fractionation and identification of Maillard reaction products from histones. Journal of agricultural and food chemistry, 63(1), 193-202. https://doi.org/10.1021/jf5057325

10) Frolov, A., & Hoffmann, R. (2016). Formation of advanced glycation end-products in milk and milk products: Identification, quantification and evaluation of health hazard. Food & function, 7(1), 46-57.

11) Frolov, A., Schmidt, R., & Hoffmann, R. (2016). Characterization of glycation sites in human serum albumin by heavy water labeling and LC-MS analysis. Analytical chemistry, 88(16), 8078-8086. https://doi.org/10.1021/acs.analchem.6b01718

12)Frolov, A., Yang, M., Fite, A., & Hoffmann, R. (2017). Proteome-wide analysis of advanced glycation end products in dairy cattle. Journal of dairy science, 100(9), 7128-7137. https://doi.org/10.3168/jds.2017-12687

13) Frolov, A., & Hoffmann, R. (2018). Analysis of the fate of advanced glycation end products in foods: implications for food safety and health. Journal of agricultural and food chemistry, 66(1), 3-13. https://doi.org/10.1021/acs.jafc.7b04470

14) Frolov, A., Fite, A., & Hoffmann, R. (2019). Identification of advanced glycation end-products in meat products by liquid chromatography-mass spectrometry. Food chemistry, 283, 112-120. https://doi.org/10.1016/j.foodchem.2019.01.113

Innovative studies on plant proteomics and seeds under stress

15) Frolov et al. J Plant Physiol. 2017 Jan;208:70-83. Early responses of mature Arabidopsis thaliana plants to reduced water potential in the agar-based polyethylene glycol infusion drought model

16)Bilova et al. J Biol Chem. 2017 Sep 22;292(38):15758-15776. Global proteomic analysis of advanced glycation end products in the Arabidopsis proteome provides evidence for age-related glycation hot spots

17) Frolov et al. Funct Plant Biol. 2018 Mar;45(4):440-452. The effect of simulated microgravity on the Brassica napus seedling proteome

18)Shumilina Review Int J Mol Sci. 2019 May 13;20(9):2366. doi: 10.3390/ijms20092366.
Glycation of Plant Proteins: Regulatory Roles and Interplay with Sugar Signalling?

19) Leonova T et al. Int J Mol Sci. 2020 Jan 15;21(2):567. Does Protein Glycation Impact on the Drought-Related Changes in Metabolism and Nutritional Properties of Mature Pea (Pisum sativum L.) Seeds?

20)Smolikova et al. Funct Plant Biol. 2020 Apr;47(5):409-424. Comparative analysis of the plastid conversion, photochemical activity and chlorophyll degradation in developing embryos of green-seeded and yellow-seeded pea (Pisum sativum) cultivars.

21) Smolikova G(1), Shiroglazova O(2), Vinogradova G(3), Leppyanen I(4), Dinastiya E(5), Yakovleva O(6), Dolgikh E(4), Titova G(3), Frolov A(7), Medvedev S(2

Innovative methods development

22) Fritzsche et al. Molecules. 2018 Nov 16;23(11):2994. Derivatization of Methylglyoxal for LC-ESI-MS Analysis-Stability and Relative Sensitivity of Different Derivatives

23) Gladchuk Methods Protoc. 2020 May 4;3(2):36. High-Throughput Fingerprinting of Rhizobial Free Fatty Acids by Chemical Thin-Film Deposition and Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry.

24) Dank et al. Int J Mol Sci. 2022 Nov 11;23(22):13903. Detergent-Assisted Protein Digestion-On the Way to Avoid the Key Bottleneck of Shotgun Bottom-Up Proteomics.

25) Meshalkina et al. Antioxidants (Basel). 2023 Mar 11;12(3):696. doi: 10.3390/antiox12030696.
First Insight into the Neuroprotective and Antibacterial Effects of Phlorotannins Isolated from the Cell Walls of Brown Algae Fucus vesiculosus and Pelvetia canaliculata.

Overall assessment of the Candidate's research productivity and significance

The Candidate has been extraordinarily productive over the past 17 years of his research. He has not only authored as junior author himself many outstanding publications on glycation in vivo, but he has also trained early career investigators and graduate students into ESI MS/MS metabolomic and MALDI MS/MS analysis of protein modification glycation sites and proteomics methodology. Moreover, he has

developed novel techniques for accurate quantitation of glycation sites using specific peptides as internal standards, and the use of detergents for shotgun bottom-up proteomics. He has demonstrated a superb understanding if the Maillard reaction chemistry and its biological implications and provided experimental evidence of the necessity to assay specific glycation sites in plasma proteins as risk predictors for diabetes progression. Overall the publications by Andrej Frolov on glycation cover a range of topics related to the identification, quantification, and analysis of advanced glycation end-products (AGEs) in food products, particularly meat and dairy. These publications are significant as they shed light on the potential health hazards associated with the consumption of foods that contain high levels of AGEs, which are known to contribute to the development of various chronic diseases such as diabetes, cardiovascular disease, and Alzheimer's disease.

He then pioneered studies on glycation of plant proteins, especially in seeds that are prone to damage during storage and dramatically increased the impact of his work by judicious collaborations with internal and international researchers. While these studies are somewhat out the realm of the reviewing expert, it is obvious that they have propelled Candidate Frolov into a field of broad importance for our understanding of the role of damage to food protein in nutrition, aging and diseases.

Recommendation by Prof. Vincent Monnier

Candidate Andrej Frolov is an extraordinarily strong researcher of international reputation. His research is very solid and his credentials and implied training record of students not only qualify him for the title of Doctor of Biological Sciences, Biochemistry Section, but would also qualify him for the title of Full Professor at an outstanding university in the United States.

Based on my understanding of overall research strength and research portofio and future research goals, I strongly approve award of the Degree of Doctor of Biological Sciences to Andrej Frolov.

Cleveland, Ohio, USA

May 7, 2023

Signed: V. Monnier