1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and workplan)

Project title: Exploring the mechanisms of metal ions binding with ovotransferrin, ovalbumin, and lysozyme: a new generation of protein nanocomplexes

1.1.Project goals

The project aims to address the gaps in current knowledge about metal-protein interactions, and reveal their implications for biomedicine and materials science. The insights gained could potentially transform the way we think about and use metal ions in various applications, e.g. as antimicrobial and anticancer agents.

1.2. Outline

A hen's egg is a complete material needed for the life of the future organism, which means that both the yolk and the protein contain all the high-value substances. Eggs are primarily a very good source of complete, easily digestible protein. Ovalbumin is the main protein of the hen egg white and the earliest isolated in pure form[1]. It constitutes 54% of all proteins in a hen's egg. Ovalbumin, belonging to the group of phosphoglycoproteins, is composed of 385 amino acid residues with a molecular weight of 44.5 kDa. Ovotransferrin constitutes 12-13% of all proteins in the protein. It is a glycoprotein composed of 686 amino acid residues with a molecular weight of 77.7 kDa and an isoelectric point of 6.111. It has disulfide bridges in the N-domain and 9 in the C-domain, which contributes to high stability of the protein[2]. Ovotransferrins belong to the transfer family, a group of proteins that reversibly bind iron in various biological fluids. Lysozyme has the ability to inactivate viruses by creating insoluble complexes with their DNA, and also enhances the synthesis of interferon[3]. The antibacterial activity of lysozyme is the highest against strains of Gram-positive bacteria (e.g. B. stearothermophilus, C. thermosaccharolyticum)[4].

The metal ions (Me^{n+}) are important modulators of the many biological processes and are essential for nearly all aspects of metabolism. Despite the wide array of theoretical knowledge about metal - protein binding, processes occurring at interfaces and the resulting effects such as nanoparticle/nanocomposite formation or protein aggregation are often undervalued and have been inadequately discussed in literature.

Zinc (Zn^{2+}), silver (Ag^{+}), and ruthenium (Ru^{4+}) are transition metals that play significant physiological roles, but can also exhibit potent cytotoxicity. Zinc's interaction with proteins is complex, with a multitude of potential binding sites and implications for protein function. However, the specific chemistry of zinc, particularly its aquacomplexes, is often overlooked in research, which can lead to misconceptions about its role in biological systems[3,4]. Silver, used since ancient times for its healing properties, is used extensively today in bactericidal formulations. It has demonstrated strong antimicrobial activity against many pathogens,

including drug-resistant strains[5,6]. Ruthenium, despite having no known essential biological role, is garnering interest due to its potential as an antitumor agent. Its complexes are being developed into anticancer drugs with potent therapeutic properties, show high affinity to nucleic acids, suggesting potential interference with processes like translation. They also bind to transferrin and albumin *in vitro*, hinting at potential intracellular protein targets. Further exploration of ruthenium's binding to proteins could provide valuable insights into its biological interactions and potential therapeutic applications[7]. Understanding the interactions between these transition metals and proteins is crucial not only for analytical chemistry but also for designing new types of drugs and medical treatments. This research aims to fill gaps in our understanding of metal-protein interactions, which will have wide-ranging implications for fields including biomedicine, nanotechnology, and materials science.

1.3. Work plan

1. Preparation and Characterization of Proteins: Isolate lysozyme, ovalbumin, and ovotransferrine from hen eggs using precipitation methods and affinity chromatography system, PAGE and MADLI

2. Perform the immobilization processes of Zn^{2+}/Ag^{+}/Ru^{4+} ions on proteins in water solutions using the sorption method.

3. Thermodynamics of Metal-Protein Binding: Carry out a batch experiment using ICP-OES/MS to study the kinetics of metal cations binding to proteins. Determine the effect of pH, ionic strength on the binding process and study sorption isotherms.

4. Nature of Metal-Protein Binding: Determine functional groups and post-translational modifications involved in the binding process using instrumental techniques (spectroscopic, microscopic methods, and spectrometric LDI).

5. Mechanism of Metal-Protein Binding: Conduct theoretical research on aggregation, speciation, or nanocomposites formation using molecular modelling and quantum-mechanical calculations.

6. Application of Metal-Protein Composites: Test the antimicrobial activity of the composites against clinically relevant and drug-resistant pathogens. Perform cytotoxicity assays as well as on the cancer cell lines.

7. MALDI/NALDI Approach: Utilize MALDI/NALDI techniques for characterization of metal-protein interactions.

1.4. Literature

1. Pomastowski P, Sprynskyy M, Buszewski B. The study of zinc ions binding to casein. Colloids Surfaces B Biointerfaces. 2014;120. doi:10.1016/j.colsurfb.2014.03.009

2. NATURAL FOOD ANTIMICROBIAL SYSTEMS. CRC Press; 2019. https://www.routledge.com/Natural-Food-Antimicrobial-Systems/Naidu/p/book/9780367398453. Accessed December 14, 2022.
3. Sugahara T, Murakami F, Yamada Y, Sasaki T. The mode of actions of lysozyme as an immunoglobulin production stimulating factor. Biochim Biophys Acta. 2000;1475(1):27-34. doi:10.1016/S0304-4165(00)00041-6
4. Malicki A, Trziszka T, Szpak M, Jarmoluk A, Janik P, Źródłowska-Danek J. Technological and microbiological aspects of the lysozyme effect on poultry meat durability. Przem Chem. 2011;90(5).
5. Buszewski B, Railean-Plugaru V, Pomastowski P, et al. Antimicrobial activity of biosilver nanoparticles produced by a novel Streptacidiphilus durhamensis strain. J Microbiol Immunol Infect. 2018;51(1):45-54. doi:10.1016/j.jmii.2016.03.002
6. Pomastowski P, Sprynskyy M, Žuvela P, et al. Silver-Lactoferrin Nanocomplexes as a Potent Antimicrobial Agent. J Am Chem Soc. 2016;138(25):7899-7909. doi:10.1021/jacs.6b02699
7. Kwong W-L, Lam K-Y, Lok C-N, Lai Y-T, Lee P-Y, Che C-M. A Macrocyclic Ruthenium(III) Complex Inhibits Angiogenesis with Down-Regulation of Vascular Endothelial Growth Factor Receptor-2 and Suppresses Tumor Growth In Vivo. Angew Chemie Int Ed. 2016;55(43):13524-13528. doi:10.1002/anie.201608094

1.5. Required initial knowledge and skills of the PhD candidate

PhD candidate should be skilful and have creative thinking, familiar with the separation (SDS-PAGE, GC-MS, LDI) and other instrumental techniques (FTIR, SERS, UV-VIS, ICP-OES/MS). Candidate should also have experience in microorganisms isolation, culturing and identification. The experience in the sample preparation and analysis with utilization of LDI-TOF MS technique is also required. Knowledge in field of nanomaterials synthesis will be highly honoured. The knowledge and skills connected to the utilization of software for data processing and microorganisms identification by LDI-TOF MS technique, e.g. FlexAnalysis, FlexControl, and MALDI Biotyper will be favoured.

1.6. Expected development of the PhD candidate's knowledge and skills

PhD candidate will gain knowledge and skills in field of microbiology, analytical chemistry and colloids science. Candidate will get specialized knowledge in microbial identification, metal-protein complex synthesis and their physicochemical characterization by separation and other instrumental techniques.

Moreover, the skills of analytical and statistical data processing will be developed during PhD study. During the study student will be able to present obtained data in form of high-impact factor publication. Moreover, the possibility to present posters and oral presentations at domestic and international conferences will be ensured. As part of the PhD project, it is planned to develop new technological solutions with a high level of creativity, legally protected by a patent.