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

Project title: Lactoferrin as a carrier of metal ions and their compounds with therapeutic potential.

1.2 Project goals

Drugs based on platinum are highly effective in the chemotherapy of wide range of cancers, but their administration is limited to intravenous injection. This mode of administration is related to many negative effects, such as high peak of drug level in the blood above the maximum tolerable concentration after dosing and later rapid decrease in the concentration of the drug below minimum therapeutic level. Therefore, the challenge is to develop a method that will allow for effective absorption of these types of drug in the gastrointestinal tract during oral administration. On the one hand, the developed method should precisely deliver the administered drugs to the intestinal epithelial cells, and on the other hand, it should minimize the side effects associated with chemical reactivity of Pt ions and their compounds as well gastrointestinal problems. One of the solutions is the use of a carrier that would be recognized by intestinal epithelial cells and, at the same time, protect the digestive system against unfavorable changes. The carrier proposed in the project for platinum compounds is lactoferrin (LTF). **Therefore, the aim of the project is obtain complexes of lactoferrin with Pt(II) and Pt(IV) chemotherapeutic agents which can be orally administrated in the treatment of cancer.**

1.3. Outline

The project will be implemented to synthesize the complex of LTF with Pt(II) and Pt(IV). During realization of the project mechanisms involved in the formation of the respective complexes will be investigated. Both modern analytical instruments and theoretical chemistry tools (quantum mechanical calculations) will be used for explanation of the nature of the binding. The nature of drug-LTF binding will also be determined through the use of spectroscopic and spectrometric techniques. The stability of obtained complexes will be verified in pH corresponding to different parts of the digestive tract. Cytotoxicity of obtained complexes will be tested on normal L929, as well cancer Caco-2, HepG2 and HeLa cell lines by MTT. Moreover, the number of apoptotic cells will be verified. Caco-2 cells that are human colon epithelial cancer cell line will be used as a model intestinal absorption of obtained complexes. These cells will be cultured as monolayer that differentiates and form tight junctions between cells. Permeability coefficients will be calculated individually for Pt, LTF and drug to fully explain the mechanism of intestine absorption. Internalization of developed complexes will be confirmed by immunocytochemistry at the level of optical and electron transmission microscopy as well by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS).

1.4. Work plan

Stages of projects	Objectives	Year			
		I	II	III	IV
1	Investigation of the metal-LTF and cisplatin-LTF binding mechanism.				
2	Verification of metal-LTF and cisplatin-LTF binding.				
3	Theoretical research on the mechanism of cisplatin-LTF binding.				
4	Analysis of biological properties of the obtained complexes.				

Figure 1. Gantt chart of project.

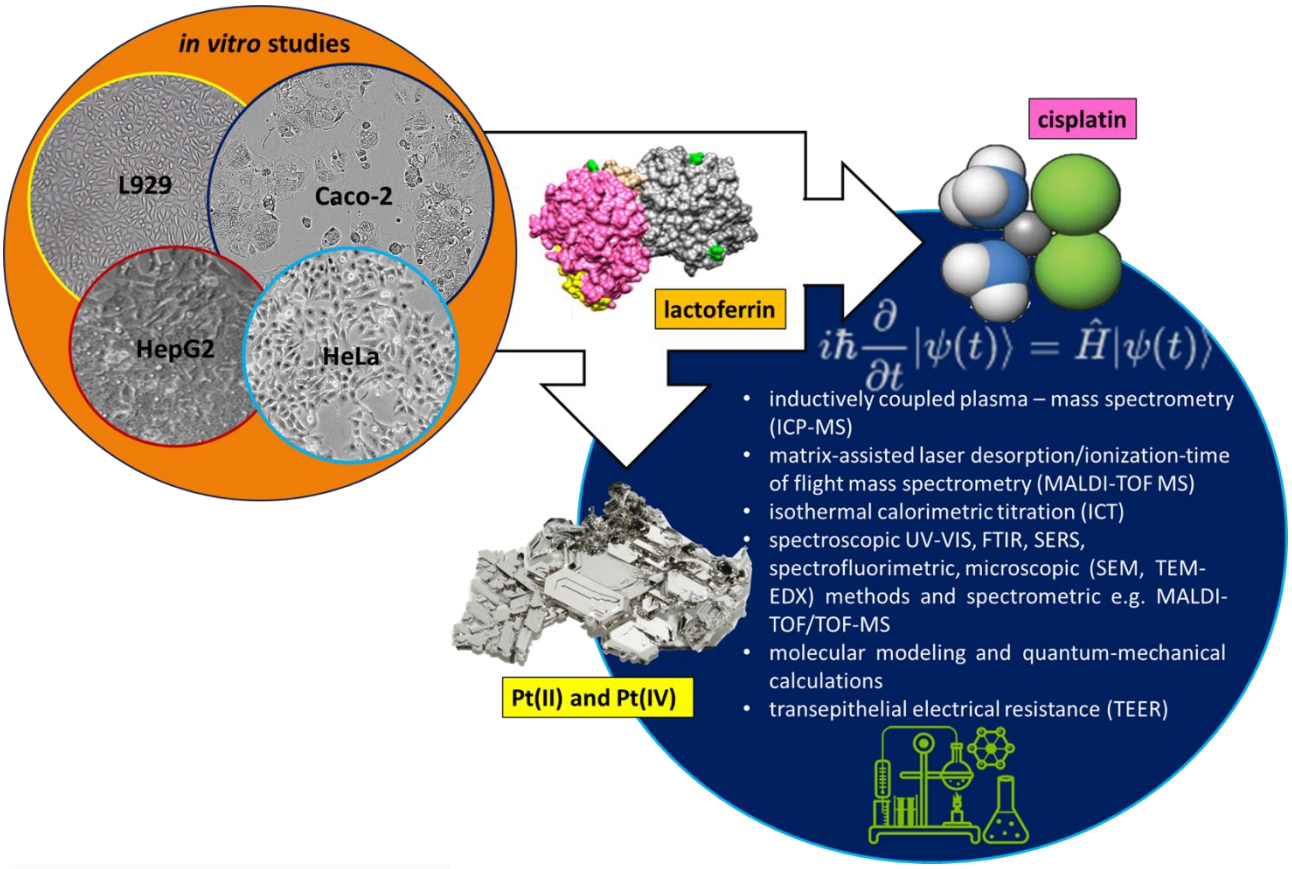

Fig. 2. Main outline of the work plan.

1.5. Literature

Alexander DB et al. Biochemistry and Cell Biology. 2017, 95(1), 1-4; **Brown** A et al. Journal of cancer science & therapy. 2019, 11(4); **Cheng** Q et al. Chem. Commun. 2015, 51, 17536-17539; **Fujimura** T et al. J Vet Med Sci. 2020, 82(11), 1648-1654; **Ganz** T. Int J Hematol. 2018, 107:7–15; **Ghosh** S. Bioorganic Chem. 2019, 88, 102925; **Karav** S. Cell Mol Biol. 2018, 64:52–7; **Kimoto** Y et al. J Vet Med Sci. 2013, 75(2), 159-164; **Liu** C et al. Inflammation. 2019, 42:2192–204; **Lepanto** MS et al. Molecules. 2019, 24:1323; **Milewska** A et al. J Virol. 2018, 92:e01933–17; **Pomastowski** P et al. J Am Chem Soc. 2016, 138, 7899–7909; **Robl** B et al. Journal of Experimental & Clinical Cancer Research. 2016, 35(1), 1-14; **Seroka** B et al. Molecules. 2020, 25(3), 655; **Thanki** et al. J Control Release. 2013, 170(1):15-40; **Zhou** P et al. Nature. 2018, 561:122–6

1.6. Required initial knowledge and skills of the PhD candidate

PhD candidate should be skilful and have creative thinking, familiar with the spectroscopic UV-VIS, FTIR, SERS, spectrofluorimetric, microscopic (SEM, TEM-EDX) methods. Candidate should also have experience in in vitro cell cultures. The experience in the sample preparation and analysis with utilization of LDI-TOF MS technique is also required. The knowledge and skills connected to the utilization of software for data processing and identification by LDI-TOF MS technique, e.g. FlexAnalysis, FlexControl, and MALDI Biotyper will be favoured.

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

PhD candidate will gain knowledge and skills in field of analytical chemistry and biochemistry. Candidate will get specialized knowledge in cell cultures, 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.