

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

Project title: Lactoferrin receptors as new route for microelements supplementation

1.2 Project goals

Mineral deficiencies negatively affect billions of people around the world, putting a serious strain on well-being and economic productivity. The most commonly diagnosed deficiencies concern iron and zinc. However, deficiencies in calcium, magnesium, selenium and fluoride are also reported to contribute significantly to the health burden (Zohoori and Duckworth, 2020). Multiple micronutrient supplements of iron and zinc were added to the WHO's List of Essential Medicines in 2019 (WHO, 2019).

Most orally administered drugs and supplements enter to the circulatory system by passage from the enterocyte layer of the intestinal tract to the blood capillaries. However some compounds due to poor water solubility and/or membrane permeability need carriers to improve their bioavailability. Many studies showed that intact form of human or bovine lactoferrin is absorbed by human small intestinal cells derived from the Caco-2 cell line, this process depends on the presence of receptors on apical membrane of cells. The presence of a small 105 kDa lactoferrin receptor that play a key role in internalization of this protein through clathrin mediated endocytosis and facilitate absorption of iron was confirmed inter alia by Liao et al. (2007).

Therefore, the aim of the project is to obtain complexes of lactoferrin with iron, zinc and magnesium as well with their chelates (diglicinates) and to verify their transport to Caco-2 cells and further use as supplements.

1.3. Outline

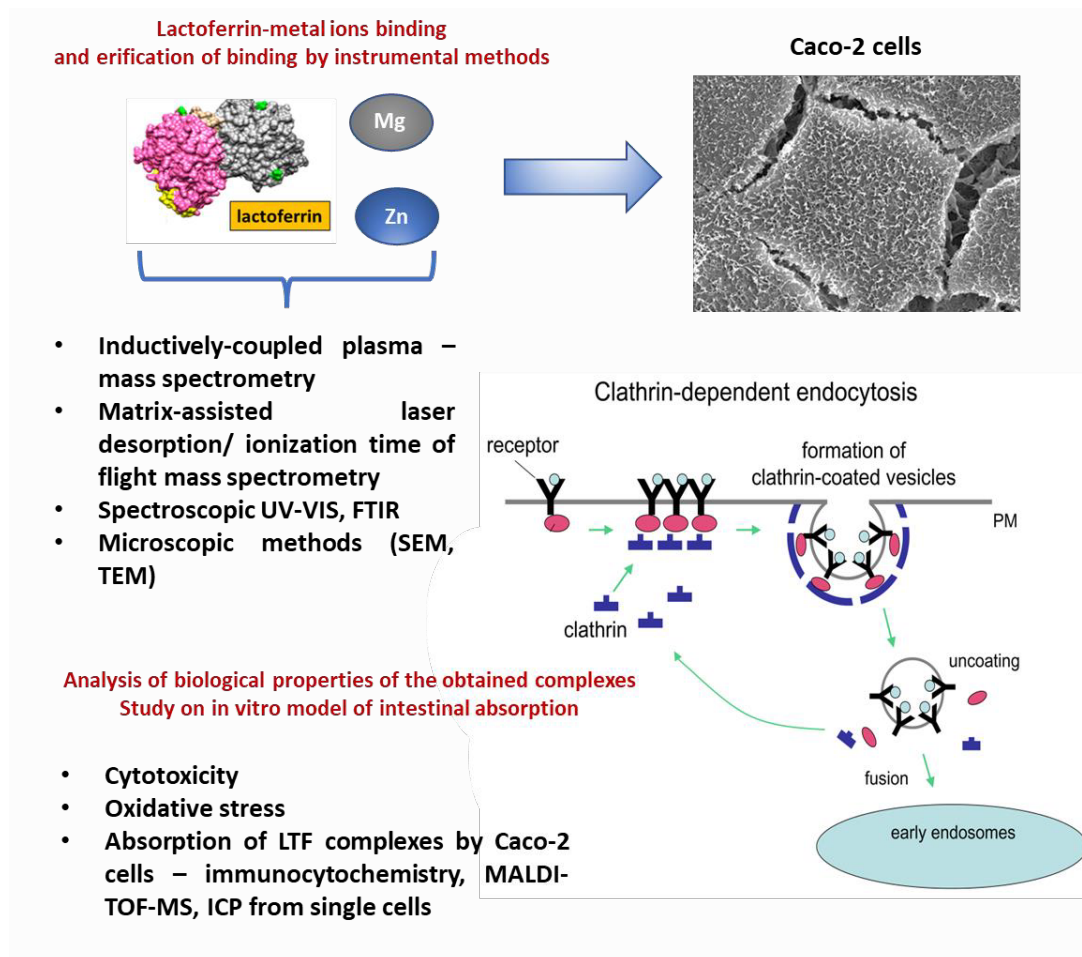
The project will be implemented to synthesize the complex of LTF with iron, zinc and magnesium as well with their chelates. Verification of metal-LTF binding using instrumental methods will be performed by spectroscopic UV-VIS, FTIR, SERS, microscopic (SEM, TEM-EDX) methods and spectrometric e.g. MALDI-TOF/TOF-MS. Cytotoxicity of obtained complexes will be tested on L929, Caco-2, and HepG2 cell lines by MTT. Caco-2 cells that are human colon epithelial cancer cell line will be used as a model of intestinal absorption. The SC-ICP-MS analysis will allow to determine the content of the tested element in a single cell, including the determination of the concentration of the measured element in individual cells, the distribution of concentrations in the analyzed cells and the number of cells containing the tested element. Internalization of developed complexes will be confirmed by immunocytochemistry. The presence of LTF in Caco-2 cells will be also confirmed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). This technique will also allow for comparison of metabolome control cells and cells treated with obtained complexes.

1.4. Work plan

Stages of projects	Objectives	Year			
		I	II	III	IV
1	Investigation of LTF with iron, zinc and magnesium as well with their chelates (diglicinates) binding				
2	Verification of binding by instrumental methods				
3	Analysis of biological properties of the obtained complexes, i.e. cytotoxicity, oxidation				

Figure 1. Gantt chart of project.

Fig. 2. Main outline of the work plan.



1.5. Literature

Liao Y. et al. *Comp Biochem Physiol A Mol Integr Physiol.* 2007, 148(3), 584-90; Zohoori FV and Duckworth RM (eds): *Monogr Oral Sci.* Basel, Karger, 2020, 28, 32-47.

1.6. Required initial knowledge and skills of the PhD candidate

PhD candidate should have completed a basic course in cell biology and biochemistry, be skilful and have creative thinking, familiar with the spectroscopic UV-VIS, FTIR, 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.

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. During the study student will be able to

present obtained data in form of high-impact factor publication. 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.