The aim of this project is to **obtain novel, potent, and metabolically stable zinc(II) complexes of glucagon-like peptide-1 receptor agonists** (GLP-1 RAs) and to **understand how the retro-inverso modification of GLP-1 RAs impacts their zinc(II) binding ability, structure, stability, pharmacokinetic and pharmacodynamic properties.**

Glucagon-like peptide-1 (GLP-1) is a peptide hormone able to decrease serum glucose levels by interacting with its receptor that stimulates pancreatic islet β-cells to secrete insulin, inhibits the release of glucagon, increases satiety, and delays gastric emptying. Prolonged action of GLP-1 is substantial for the treatment of diabetes, and is the reason of the **extraordinary success of the lately launched liraglutide and semaglutide (**Victoza and Ozempic, potent against both diabetes and weight loss), GLP1-RAs are being relied on as a potential 'treasure trove' of novel antidiabetic therapeutics.

Why do we want to improve and enhance the mode of action of GLP-1 RAs? Diabetes mellitus (DM) is a severe chronic metabolic disorder characterized by hyperglycaemia and impaired carbohydrate, protein and lipid metabolism, that is associated with absent or inadequate insulin secretion with or without concurrent insulin resistance. It belongs to the ten top causes of death in adults and it was estimated that in 2017 it was responsible for four million deceases globally. Type 2 diabetes mellitus is the most prevalent manifestation of DM accounting for more than 90% of cases. In 2019, it was estimated that more than 450 million people live with DM globally accounting for over 9% of adult population, and it is predicted that by 2045 the diabetic population reaches 700 million patients (almost 11% of adults). The increase in the prevalence of DM results in the dramatic increase in socio-economic costs and therefore strategies allowing to control the disease and slow down its development and progression are urgently needed.

We plan to **improve the GLP-1 based drug efficiency by coordinating it to Zn(II), which is known to exert insulin-mimetic and anti-diabetic effects**, being co-secreted together with insulin, and its presence enhances insulin response by inhibiting the insulin receptor-specific tyrosine phosphatase 1B (the receptor remains phosphorylated and thus 'senses insulin', although it is no longer there).

We plan to **overcome the main disadvantage of the (peptide-based) Zn(II)-GLP-1 RA complex – their proteolytic instability, by introducing carefully designed retro-inverso modifications**, and thus enhancing the potency of the modified drug.

Retro-inverso peptides have the **chirality of their amino acid inverted from L to D** (D amino acids cannot be recognized by common proteases of the body and will not be easily degraded) and have the **peptide sequence in reverse direction** with respect to native peptide, maintaining an **identical array of side chains** and in many cases, a similar structure. In other words, the retro-inverso modification results in a peptide in which the **side-chains are superimposable with those of the native L-peptide, but have "inverted" amide bonds and N- and C-terminal groups.**

Basing on the exciting results of our recent Sonata Bis project, we believe that zinc(II) may enhance the antidiabetic efficacy of GLP-1 RAs either by changing their local charge or structure, or by a simple synergistic effect that would sum up the efficiency of both antidiabetic agents.

We want to understand **(i) What is the impact of zinc on GLP-1 analogues? (ii) Can the main metal-peptide disadvantage, its proteolytic instability, be overcome by introducing retro-inverso modifications? and (iii) How retro-inverso modification of GLP-1RAs combined with subsequent formation of their Zn(II) complexes will influence their pharmacokinetic and pharmacodynamic properties?**

To answer these questions, we plan to: (i) design and synthesize the **modified (retro-inverso) GLP-1 analogues** (Aim 1); (ii) analyse the thermodynamics and structure of their **Zn(II) complexes** (Aim 2); (iii) define their **proteolytic stability** and define their **interactions with major serum proteins** (Aim 3); (iv) evaluate the ***in vitro* antidiabetic activity** of the newly designed retro inverso GLP-1 analogues and their zinc complexes (Aim 4) and finally (v) estimate the ***in vivo* pharmacodynamic and pharmacokinetic properties** of the most promising modified GLP-1 analogues and their Zn(II) complexes (Aim 5) and compare their efficacy to those of the corresponding native GLP-1 ligand and those of the commercially available GLP-1 RAs in order to eventually **understand the relationship between GLP-1 analogue retro-inverso modifications, their coordination chemistry, proteolytic stability, structure, thermodynamics and mode of action.**

This greatly interdisciplinary project takes our idea from the synthetic lab, through an analytical, bioinorganic one to biological studies on cell lines and eventually to *in vivo* studies on rodents. We are absolutely convinced that the results will have an impact not only on the field of the beautiful, basic bioinorganic chemistry of Zn(II)-retro-inverso peptide complexes and on the field of the inorganic biochemistry of modified (retro-inverso) GLP-1 receptor agonists, but, most importantly, will elucidate the antidiabetic potential of the newly designed complexes, and their pharmacokinetic and pharmacodynamic properties *in vivo.* Taken together, the results of the project may really be a substantial stepping stone towards finding new, enhanced, proteolytically stable antidiabetic treatments.