The aim of this project is to **obtain novel, potent and metabolically stable metal complexes of antimicrobial clavanin retro-inverso peptide-based analogues** and to understand **how the retro-inverso modification impacts metal binding ability and antimicrobial mode of action**.

Why do we want to study this problem? The inspiration for this project comes from the increasing antimicrobial drug resistance – the European Comission claims, that if no new classes of antimicrobial frugs will be developed, then in 2050, almost 50 mln deaths worldwide will be caused by antimicrobial resistance. This drastic vision makes novel, effective treatments actively sought. Where should we look for possible solutions? Because of the general lack of resistance towards antimicrobial peptides (AMPs), they are being relied on as a potential 'treasure trove' of novel classes of therapeutics. However, their biggest disadvantage, **proteolytic instability**, severely limits their clinical use.

We plan to solve this problem by using **retro-inverso peptides**. Retro-inverso peptides have reversed sequences and chirality with respect to their parent molecules; at the same time, they maintain an identical array of side chains and, in some cases, a similar structure. The presence of D-amino acids, that results in an inverted chirality, makes them less prone to proteolytic degradation, overcoming the main disadvantage of peptide-based drugs – their **lack of stability**. To maximize the similarity to their native analogues, in retro-inverso peptides, the D-amino acids are introduced in the sequence in a reverse direction. This 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.

It seems that **both AMPs and retro-inverso peptides are a thus potential treasure trove for discovering novel, safe drugs with enhanced half-lives and an increased potential as new drugs**. In the scope of this project, we plan to use both strategies to enhance the antimicrobial potency of metal-clavanin C complexes.

One of the most exciting results of our NCN Sonata Bis project was the explanation of the impact of the coordination of Zn(II) to clavanin C (a His-rich AMP from hemocytes of the tunicate *Styela clava*) on the complex structure, thermodynamics and antimicrobial mode of action. We show details of how **Zn(II)** (and, to a smaller extent, also Cu(II)) **changes clavanin C's structure and drastically enhances its antimicrobial properties** and explain the impact of non-metal binding sequences on clavanins' antimicrobial activity.

We plan to **enhance the proteolytic stability of metal-clavaninc C complexes** and thus **enhance their biological activity via introducing retro-inverso modifications.** To the best of our knowledge, not only the coordination chemistry of retro-inverso antimicrobial clavanins, but the **coordination chemistry of retro-inverso peptides in general have never been studied before**; we know nothing about the impact of the reversed, D-amino acid based sequence on the peptide's metal binding mode, metal selectivity, structure and thermodynamic stability.

We will (i) **design and synthesise the modified (retro(-inverso)) clavanin (ModClav) analouges** (Aim 1); (ii) analyse the **thermodynamics and structure** of their Zn(II) and Cu(II) complexes (Aim 2); (iii) define their **biological activity** (MIC and cytotoxicity of modClav ligands and their metal complexes on regular cell lines, Aim 3); (iv) evaluate the **proteolytic stability of ModClav metal complexes** and compare them to those of the corresponding wild-type forms (Aim 4) in order to eventually **understand the relationship between clavanin (retro(-inverso) modifications, their coordination chemistry, proteolytic stability, structure, thermodynamics and mode of action** which will allow us to (v) **design chimeric ModClav metal complexes, covalently linked to common antibiotics or targeting molecules** (Aim 5).

The impact of the results we plan to obtain will have an effect on the research field on at least several levels: (i) first, it will be a **large input into the general knowledge of the beautiful, basic bioinorganic chemistry of the (not yet explored(!)) retro-inverso peptide complexes with Zn(II) and Cu(II)** – we will **explain how the difference in chirality impacts the coordination abilities of retro-inverso peptides** in general; second (ii), it will allow us to **understand the inorganic biochemistry of ModClavs**, molecules that are actually relevant for biology; third (iii), it might really be a **stepping stone towards finding new, proteolytically stable antimicrobial treatments**.