Antibiotics have been used since World War II to treat bacterial infections. However, these drugs have become less and less effective. Due to antibiotic overuse in humans and livestock, bacteria have developed resistance to known antibiotics. Therefore, many bacterial infections cannot be eradicated with currently available drugs. Unfortunately, the pace of designing new antibiotics is still way too slow to solve this timely and critical issue.

One difficulty in designing new antibiotics is connected with their delivery to bacterial cells. Bacteria protect their cytoplasm with a complex cell envelope, and most antibiotics need to pass through this envelope to perform their role. In general, bacteria do not allow inside compounds that are not necessary for bacterial growth or metabolism. One approach is to use the Trojan Horse strategy and connect the antibiotic to a molecule that is naturally absorbed by bacteria and, as a result, would carry this antibiotic to the cell. Unfortunately, such carriers are specific to the type of bacteria and delivered compound, so they are difficult to identify or design. Moreover, the mechanism of delivery needs to be well understood to wisely predict the carrier.

For carriers, we will use molecules that are naturally involved in bacterial iron transport. Iron is required for bacterial growth, but in the environment it is present in an insoluble form. As a strategy to retrieve iron, bacteria secrete iron-chelators, called siderophores. These natural compounds scavenge for iron and bind it with high affinity. Next, such ferric-siderophore complex is brought back to the cell through a set of siderophore-specific outer-membrane receptors. Thus, siderophore mimics seem to be good candidates for carriers of active compounds into bacteria. **We plan to use siderophores and their transport systems to deliver molecules to bacterial cells.**

The molecules that we plan to deliver are short oligonucleotides. They will be designed to bind to bacterial RNA observing the Watson-Crick base pairing rules. By such complementary binding, oligonucleotides will block the production of an essential protein and, as a result, inhibit bacterial growth. However, since natural oligonucleotides are unstable, **we plan to use a synthetic oligomer called peptide nucleic acid (PNA)**. PNA oligomers efficiently bind natural RNA, and are not degraded in cells.

Unfortunately, bacteria do not take up oligonucleotides, including PNA, from the environment. Thus, our strategy is to **design PNA with antibacterial properties and covalently connect it with a siderophore carrier to deliver PNA to bacterial cells**. Computer-aided design of the siderophore-like carrier will assure that it is recognized by a siderophore-specific outer-membrane receptor and retrieved to the cytoplasm at the same time “pulling in” the PNA molecule.

Importantly, iron metabolism plays crucial roles in bacterial infections; bacteria encounter iron-depleted conditions in the host, and need to secrete and take up ferric-siderophores. To our advantage, natural antibiotics, sideromycins, which are composed of a siderophore part connected to the antibiotic, use a similar strategy to pass through the bacterial membrane barrier.

In summary, we will **design, synthesize and test siderophore-PNA conjugates composed of a siderophore-analog as a carrier and antibacterial PNA sequence.** Our aim is to find a non-invasive way to deliver antibacterial PNA to Gram-negative bacterial cells using bacterial iron-transport systems and perform simulations of this transport at atomistic details.