According to the World Health Organization (WHO), antimicrobial resistance (AMR) is among the top ten global public health concerns. As healthcare systems continue to be strained by SARS-CoV-2, antimicrobial resistance (AMR), often referred to as the “silent pandemic,” is escalating, costing society billions. Antimicrobial resistance (AMR) is a phenomenon whereby bacteria evolve in such a way as to resist the action of drugs, making them apparently ineffective. The main mechanisms of resistance that bacteria use are changes in membrane permeability to antibiotics, limiting the uptake of a drug, modifying a drug target, inactivating a drug, and actively effluxing a drug. On the basis of predictive statistical models, in 2019 there were an estimated 4.95 million deaths associated with bacterial AMR, including 1.27 million deaths attributable to bacterial AMR. Among the most critical "alarm pathogens" capable of evading antibiotic action are several prominent species, including **E**nterococcus faecium, **S**taphylococcus aureus, **K**lebsiella pneumoniae, **A**cinetobacter baumannii, **P**seudomonas aeruginosa, and **E**nterobacter spp. (ESKAPE).

Antimicrobial peptides (AMPs) are an excellent candidate to overcome antibiotic resistance; they demonstrate a broad-spectrum antimicrobial activity. To overcome AMR, recent strategies have focused on using nanoparticles to deliver encapsulated antibiotics directly to bacterial targets. In recent years, there has been considerable advancement in research focused on lipid nanoparticles (LNPs), particularly regarding the development of non-lamellar LNPs for next-generation nanomedicine. However, the number of LNPs that have received approval for clinical use remains limited, primarily consisting of liposome-based formulations. In our project, we propose the use of non-lamellar lipid carriers, cubosomes, as carriers of peptide antibiotics. A cubosome consists of a curved lipid bilayer, forming the walls of a system of water channels. This structure makes the internal surface of the crystal huge (approx. 400 $m^2/g$) and can accommodate a large amount of the drug.

This project aims to evaluate the effectiveness of AMPs encapsulated in LNPs as a therapeutic strategy against bacterial infections. AMPs have significant limitations as drugs, including their poor chemical and physical stability, which impacts clinical translation. Natural AMPs are prone to chemical and enzymatic degradation in vivo, leading to low oral absorption. Encapsulation within LNPs can improve therapeutic efficacy by increasing circulation time and antimicrobial action. We will functionalize LNP surfaces to enhance interaction with bacterial membranes. In our project, we aim to answer two primary questions: first, can we enhance the antibacterial efficacy of existing peptide antibiotics by encapsulating them in cubosomes that are modified on their surface with groups that have an affinity for bacterial membranes? Second, what are the mechanisms and kinetics of interaction between these carriers and both model and biological bacterial membranes? Finding answers to these questions could lead to the development of clinical applications for lipid carriers in the fight against bacterial infections.

Immobilization of AMPs in lipid carriers may improve therapeutic efficacy by prolonging circulation time and antimicrobial activity. To enhance interactions with bacterial membranes, we will appropriately functionalize the surface of LNP. In our research, we will focus on bacterial models from the ESKAPE group. We will select antibiotics that destabilize negatively charged bacterial cell membranes, such as polymyxin B, nisin, and colistin. Our study aims to determine the effects of surface-functionalized lipid nanoparticles (LNPs) on both model and biological membranes. To conduct this research, we will employ a variety of techniques, including confocal laser scanning microscopy (CLSM), small-angle X-ray scattering (SAXS), electrochemical methods, and fluorescence spectroscopy. The combination of these methods will enable us to assess the kinetics of interactions between LNPs loaded with drugs and the bacterial membrane. In the final stage of our study, we will evaluate the effects of these carriers on biological membranes in vitro. We anticipate that our results will enhance fundamental knowledge regarding the interactions between LCPs and bacterial cells, as well as the impact of antimicrobial peptide (AMP)-doped lipid nanoparticles on bacteria. This research will allow us to select an effective system with antibacterial properties.