Steroids are a group of chemical compounds with a characteristic system of four aliphatic rings (steroid ring system) and a diverse range of biological function. We can distinguish structural steroids, such as cholesterol, that are important component of the cell membrane and regulatory steroids, that determine human secondary sex characteristics (e.g testerosterone, progresterone or estrogenes). Some steroids are also a nutrition source of gut or soil bacteria.

Ubiquity and functional diversity of steroids in human organisms results with their application as therapeutics. They are used in treatment of many illnesses, such as asthma and immunological disorders (e.g. Crohn's disease, rheumatoid arthritis) or even tumor disease. In the context of this project especially interesting are anti-inflammatory corticosteroids and anabolic androgenic steroids used in treatments of conditions associated with a loss of muscles (e.g. after prolonged immobilization of a patient or severe traffic accidents). The latter group of compound is also frequently misused by culturists and sportsmen as doping agents.

There is a constant need for development of novel, economically efficient and environmentally friendly methods of steroid's synthesis. Due to wide range of steroids application in medicine there is a high demand on their production. Although organic chemistry provides various protocols for steroid synthesis, these methods are frequently very complex, environmentally hazardous and as a result expensive.

This is where the biotechnology comes to play. The production and modification of the aforementioned compounds can be conducted with help of isolated bacterial enzymes or even specialized strains of microorganisms. A perfect example of such enzyme is the cholest-4-en-3-on Δ^1-dehydrogenase (AcmB), an object of the project study. The enzyme was isolated from *Sterolibacterium denitrificans*, bacteria growing in sewer sludge on cholesterol. The isolated enzyme can be used for production of 1-dehydrosteroids (i.e. anti-inflammatory drugs or anabolic steroids). However, good knowledge of the (bio)catalyst features is a prerequisite for its efficient application in the industrial process. This means we want to know the enzyme's structure, starting from composition of the enzyme active site where reaction takes place, up to quaternary protein organization. Moreover, the full characterization of the catalytic features (such as temperature optimum, pH, ionic strength, identification of various substrate) is crucial for determination of factors responsible for enzyme phenomenal catalytic power.

In our project we aim at a deep down understanding of the AcmB catalyst. Therefore, we are interested not only in its structure of general catalytic characteristics. We want to understand the catalytic mechanism of the reaction down to a molecular level. The studies we undertake will be carried with help of the overexpression system that produces huge quantities of AcmB in a genetically modified bacteria. We will try to crystalize the enzyme and solve its structure. Furthermore, the enzyme will be mutated to test our hypothesis on the amino acids crucial for the catalytic process. Moreover, the catalytic tests with steroids labeled with isotopic markers will be used as a validation of the mechanistic hypothesis. The experimental studies will be confronted with theoretical modeling of reaction pathway. Quantum chemical (DFT) and Nobel Prize awarded QM:MM modeling techniques will be used to investigate the catalytic reaction carried out by AcmB. Up to date no theoretical study for ketosteroid dehydrogenases has been conducted. Therefore, a combined use of both experimental and advanced theoretical approaches will provide a new quality to studies of that enzyme through synergetic effect of both approaches. Finally, our studies will shed light on secrets of catalytic mechanism of a whole class of the very important enzymes, which are used in modification of steroid drugs.