



According to the WHO data, in 2019 907 million people worldwide were struggling with mental health problems, with anxiety and depression the most common. These problems are reflected in the global consumption of antidepressant and antiepileptic drugs, which is constantly growing. The global antidepressant market was valued at \$17.02 billion in 2022 and is growing at an annual rate of 7.90% in 2023, which means that the market is expected to reach \$36.41 billion in 2032. Psychotropic drugs are available as products with a variety of formulations and forms of administration. However, the most important ingredient in all commercial pharmaceutical products is the pharmaceutically active ingredient (eng. API). Current methods for synthesizing APIs are very complicated, consisting of many different steps, and require the use of

expensive catalysts and toxic solvents, and the resulting products often require additional purification steps. Therefore, due to the increasing amount of antidepressant and antiepileptic drugs used by the public every year and the need to reduce their price and make the synthesis process more sustainable and environmentally friendly, it is necessary to develop new and efficient protocols for their synthesis. **Hence, the main research objective of the presented project is the design and manufacture of advanced multienzymatic cascade systems for application in the synthesis of active pharmaceutical ingredients (APIs), mainly with antidepressant and antiepileptic activity. In addition, an attempt will be made to carry out API synthesis reactions in microreactors to construct multienzymatic cascade bioreactors.**

It should be emphasized that the scientific problems to be solved by this project involve important social and scientific issues and require effective solutions. In addition, there is a lack of literature data on the use of multienzymatic cascade systems with improved stability and robustness in the synthesis of APIs, particularly those with antidepressant and antiepileptic effects. Moreover, data on API synthesis in microreactors with heterogeneous biocatalysts are also very limited. Therefore, the novelty of the planned work is undeniable, and the lack of effective answers to the problem posed makes the scientific challenges to be solved fully justified. To carry out efficient enzymatic synthesis of APIs, a number of scientific problems must be solved, among which the most important are the design and production of multi-enzymatic cascade biocatalysts with improved catalytic properties, including stability and reusability, and a reduced number of reaction steps. To solve the above challenges, the planned tasks will include the design, through modelling and artificial intelligence (AI) techniques, of enzyme structures, followed by their production using modified microorganisms, and the manufacture of synthetic enzymes. All biocatalysts will be characterized, and then, using the obtained enzymes, multienzymatic immobilization will be modeled and carried out to produce cascade systems that, after characterization and optimization, will be used in the synthesis of APIs in microreactors. In addition, a variety of innovative solvents will be tested to improve the efficiency of the process and make it environmentally friendly, including ionic liquids (ILs) and deep eutectic solvents (DES). The long-term utility of the generated biocascades in the construction of one-pot flow microreactors will also be investigated to achieve high process speed and desired enantioselectivity. The presented project is interdisciplinary in nature and covers areas such as biotechnology, biology, biocatalysis, materials science, chemical technology, and chemical synthesis. The data obtained from the planned tasks, after careful evaluation, can significantly enrich the current state of knowledge in all these disciplines. The final results of the presented proposal will provide new, previously undescribed knowledge and solutions for the production of multienzymatic cascade systems for their application in one-pot cascade processes of API synthesis in microreactors. **The developed research methodologies and established theoretical basis will be a measurable outcome of the project with conference presentations and technical reports, as well as articles published in recognized scientific journals. In addition, the evaluation of technological assumptions for efficient biosynthesis of antidepressant drugs has a major social impact, as it provides environmentally friendly solutions for the production of value-added chemicals with desired therapeutic effects. Finally, the research conducted will provide valuable data on the production and application of advanced multienzymatic cascade systems and the development of enzyme immobilization techniques to enhance the practical application of biocatalysts.**