Abstract of doctoral thesis entitled: "Neurotoxic ligands interactions with insects membrane proteins"

Mosquitoes and other insects spread a number of dangerous diseases, including malaria, denga, or yellow fever, and the increasing range of their occurrence due to climate change poses a growing threat. Insects also destroy approximately 20% of crop yields, which poses a tremendous challenge in ensuring the food security of the growing world's population. The main method of limiting the spread of pest insects is the use of repellents and insecticides that interact with the nervous system proteins. However, commonly used chemicals lose their efficacy due to the growing resistance. Therefore, it is necessary to understand the molecular mechanisms of action of the available chemicals and the resistance to them to develop new methods of insect control.

The aim of this study was to perform a detailed analysis of the physicochemical interactions between selected neurotoxic ligands and two target proteins: (1) muscarinic receptors, responsible for recognizing olfactory stimuli, and (2) voltage-gated sodium channels, which play a crucial role in neural conduction and are a molecular target of the most commonly used insecticides. The results are presented in three articles included in the doctoral thesis.

Firstly, using computer simulations of docking and molecular dynamics on a scale of hundreds of nanoseconds, we contributed to expanding the knowledge of allosteric pathways of structural signal propagation in the muscarinic receptor (Article I). Ligand binding induces the allosteric pathway of conformational changes that correspond to the function of the target protein. The models of insect proteins built in this study, as well as the model of the cell membrane in which they are anchored, enabled the examination of differences in the action of ligands on human and insect receptors, which is crucial for designing selective agents. Additionally, the class of photoactive ligands proposed here can serve as an excellent tool in research on neural conduction.

Secondly, we compared the binding of four peptide toxins from sea anemones to the voltage-gated sodium channel of the cockroach *Periplaneta americana*. The results of molecular modeling are consistent with the electrophysiological experiments presented in Article II. A better understanding of the fast inactivation process and its inhibition by neurotoxins can contribute to the development of selective insecticides, as well as new analgesics.

Finally, based on the analysis of the dissociation pathways of a sodium channel blocker insecticide DCJW, we identified its' entry route into the mosquito channel inner pore. We also explained the role of the mutation causing resistance to this class of neurotoxins, as well as the molecular basis of the increased toxicity of the metabolite compared to the pre-insecticide. We also identified the amino acid residues that constitute the binding site of two groups of insecticides – channel blockers and pyrethroids. Mutations in these residues may be involved in the mechanism of cross-resistance. The results are presented in a form of a preprint to the manuscript that is in review (Article III).

In summary, the results of computer modeling presented in the doctoral thesis significantly broaden the knowledge of the molecular mechanisms of action of repellents and insecticides. Simulations at the scale of individual atoms enabled the examination of the response of target proteins to the binding of neurotoxic ligands, contributing to a better understanding of biophysical processes such as fast inactivation of the voltage-gated sodium channel or activation of muscarinic receptor. These findings may aid in the development of new insecticides and painkillers with improved effectiveness and selectivity.

Beata Nikles