Effective cell-to-cell communication enables the optimal functioning of the entire organism. Numerous specialized protein receptors present in the cell membrane of all cells bind signaling molecules secreted by other cells and receive stimuli from the environment. Stimulated receptors activate various intracellular signaling pathways that determine the appropriate response of the cells.

Adhesion G protein-coupled receptors (aGPCRs) are a recently distinguished group of GPCRs activated by membrane proteins and extracellular matrix proteins. aGPCRs regulate a number of important processes, including cell migration, proliferation, and differentiation. aGPCR mutations as well as their overexpression underlie the development of diseases of the nervous, immune, and circulatory systems, as well as the development and progression of various types of cancer. A characteristic feature of an extracellular region of aGPCR is the presence of a GAIN domain (GPCR autoproteolysis-inducing), which contains the site of autoproteolysis, which occurs during aGPCRs maturation in the endoplasmic reticulum. This process generates two fragments: an N-terminal- (NTF) and a 7-transmembrane C-terminal (CTF) fragment. These fragments remain non-covalently associated and the complex is exposed on the cell surface. Ligand binding to the receptor's NTF leads to conformational changes in the NTF-CTF complex, which results in the disruption of non-covalent bonds and dissociation of the NTF. As a consequence, the intramolecular agonist present in the CTF structure is exposed resulting in aGPCR activation.

Recent studies indicate that NTF release may occur as a result of the activity of certain proteases. However, it is unclear: (i) whether this is a common phenomenon or limited to a small number of aGPCRs, (ii) what proteases are involved in this process, (iii) what is the effect of this proteolysis – activation or inactivation of receptors.

The preliminary results of our research show that one of aGPCRs, namely Cirl, is proteolytically processed by unknown enzymes in a certain area of the brain of *Drosophila melanogaster* larvae, and that some mammalian aGPCRs may be substrates for proteases from the ADAM (A Disintegrin And Metalloprotease) family – ADAM17 or ADAM10. The substrates of these proteases include, among others: membrane precursors of growth factors and cytokines as well as receptors and adhesion molecules. The ADAM-mediated shedding of extracellular fragments of these proteins strongly modulates their biological activity and affects cellular processes such as proliferation and differentiation. The changes in activity of ADAM17 and ADAM10 are observed in many pathological conditions, including chronic inflammation and cancer.

The aim of the project is to investigate the process of releasing NTF fragments of aGPCRs due to proteolysis by external proteases. Two research strategies will include: (i) identifying the proteases involved in Cirl NTF release and investigating the *in vivo* physiological significance of this process using the *D. melanogaster* model, and (ii) identifying the ADAM17 and ADAM10 substrates among those mammalian aGPCRs that have a documented role in the development of pathological conditions and assessment of the impact of proteolysis on their biological activity. For the identified substrates, the ADAM17 and ADAM10 cleavage site will be determined, as well as the dependence of ADAM17- and ADAM10-mediated proteolysis on the presence of accessory proteins (iRhom, tetraspanins) and prior GAIN autoproteolysis. Moreover, the results will answer the question about the implications of the NTF shedding on the functionality of aGPCRs: whether it is an additional mechanism exposing the intramolecular ligand that activates aGPCRs, or rather a process leading to their inactivation by removing N-terminal ligand binding domains. The studies will also explain whether the released aGPCR ectodomains can act as inhibitors of the interactions between membrane aGPCRs and their ligands.

If indeed ADAM10 and/or ADAM10 do regulate the biological activity of aGPCRs then these proteases may emerge as new therapeutic targets in the treatment of diseases caused by impaired aGPCR activation.