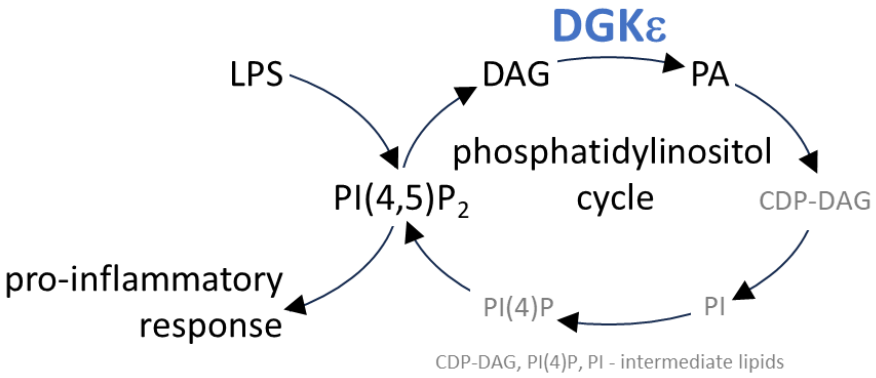
Lipids (fats) are typically associated with being an energy source, and their excess can accumulate in the body, leading to obesity. However, at the cellular level, lipids are also essential building blocks of biological membranes, and some act as signal transmitters. Signaling lipids include derivatives of phosphatidylinositol (PI), particularly $PI(4,5)P_2$ found in the plasma membrane. Under certain stimuli, $PI(4,5)P_2$ undergoes hydrolysis. Such stimuli include bacterial lipopolysaccharide (LPS), which stimulates an inflammatory response in macrophages that helps fight infection but can also lead to sepsis under certain conditions. The hydrolysis of $PI(4,5)P_2$ results in the formation of two signaling molecules that activate various cellular processes. One of these molecules is diacylglycerol (DAG), which is reconverted into $PI(4,5)P_2$ through several intermediate lipids in the so-called phosphatidylinositol cycle. Proposed project focuses on diacylglycerol kinase-ε (DGKε), which converts DAG during this cycle and is essential for macrophage responses to LPS. DGKε is also involved in lipid metabolism, and its dysfunction is associated with obesity. Additionally, mutations in the gene encoding DGKε lead to kidney disease. All these factors justify investigating the mechanisms regulating the activity of this important kinase.



The project aims to elucidate the molecular mechanisms that control the activity of DGKε in macrophages. I hypothesize that its activity is regulated by two reversible modifications: isomerization (a structural change) of one of its amino acids, proline, and palmitoylation (attachment of a lipid to the kinase molecule), which was recently discovered by our group. Additionally, the interaction of DGKε with cholesterol in the plasma membrane can also play a role in its regulation.

The research will utilize immortalized macrophage-like cell lines that lack DGKε, as well as those equipped with mutated forms of this kinase. Additionally, model cells producing various forms of the kinase along with enzymes catalyzing its aforementioned modifications will be used. The goal is to identify which proline undergoes isomerization important for DGKε activation, which enzyme catalyzes this reaction, and how it interacts with the palmitoylation of the kinase. I will also verify the hypothesis that the cholesterol-binding motif is an amino acid sequence I identified within transmembrane fragment of DGKε, near the amino acids undergoing the two studied modifications. Concurrently, I will investigate how disruption of the mechanisms regulating DGKε activity affects the pro-inflammatory response of macrophages induced by LPS.

To date, none of the proposed mechanisms regulating DGKε activity (except for the recently discovered palmitoylation) nor their biological significance have been studied. The proposed research will identify previously unknown factors that control an important pro-inflammatory pathway in macrophages. Additionally, these mechanisms likely regulate DGKε activity in other cell types and are potentially linked to the development of various diseases resulting from its dysfunction.