Chapter 1

Introduction

1.1 Background

G protein-coupled receptors (GPCRs) are the largest family of membrane receptors in the human genome, responsible for transducing extracellular signals into intracellular responses. They are activated by diverse ligands, such as hormones, neurotransmitters, and lipids, and play a critical role in regulating cellular responses (Lorente et al., 2025). GPCRs are also one of the most important classes of therapeutic targets, with approximately one-third of marketed drugs acting through GPCR-mediated mechanisms (Cheng et al., 2023). However, despite their importance, many aspects of GPCR signaling remain unclear, particularly in terms of the spatial regulation of signals beyond the plasma membrane.

In recent years, there has been growing interest in endomembrane and endosomal signaling, primarily in Gs- and Gi-coupled receptors (Calebiro et al., 2025). Traditionally, GPCR signaling was thought to be a transient event confined to the plasma membrane, where receptor activation leads to desensitization and internalization. However, emerging evidence suggests that GPCRs can continue to signal from intracellular compartments, including early endosomes. This ongoing signaling from distinct compartments results in distinct cellular responses, a concept referred to as spatial bias. Understanding this spatial regulation of GPCR signaling is crucial, as it has significant implications for the specificity, duration, and efficacy of pharmacological interventions (Calebiro et al., 2025; Crilly & Puthenveedu, 2020).

GPCRs can activate one or several heterotrimeric G proteins from 4 distinct families ($G\alpha_{s/olf}$, $G\alpha_{i/o}$, $G\alpha_{q/11}$ and $G\alpha_{12/13}$). $G\alpha_{q/11}$ or simplified as Gq henceforth, is the main focus in this study, and is responsible for Ca^{2+} mobilization in the phosphoinositide signaling pathway (Kamato et al., 2015). GPCRs have been reported to activate Gq at early endosomes and for some of these receptors, a

physiological response (pain) has been attributed to this compartmentalized Gq signaling (Jensen et al., 2017; Latorre et al., 2022; Retamal et al., 2026). This shows that Gq signaling from endosomes produces a downstream signaling, required to generate a cellular response.

Gq-coupled receptors stimulate phospholipase Cβ (PLCβ), which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2; abundant at the plasma membrane), generating the second messengers diacylglycerol (DAG) and inositol trisphosphate (IP3) (Sassmann et al., 2010). At early endosomes, phosphatidylinositol 3-phosphate (PI3P) is the most abundant phosphoinositide, but it is not a suitable substrate for PLCβ, as it does not conform due to steric constraints into the enzyme's catalytic site (Fisher & Lyon, 2020). Interestingly, phosphatidylinositol 4-phosphate (PI4P), another phosphoinositide, is also present at early endosomes (Hammond et al., 2009). Furthermore, previous studies have shown that PI4P can serve as a substrate for PLCβ at the plasma membrane and Golgi (de Rubio et al., 2018; Smrcka et al., 1991). PI-273, a selective inhibitor of PI4KIIα (which generates PI4P at endosomes), is 200x more selective than the PIKIIIα isoform, which is responsible for producing PI4P at the plasma membrane (Li et al., 2017). By selectively inhibiting endosomal PI4P production, PI-273 can help dissect the role of PI4P in DAG production at endosomes, further illuminating the spatial dynamics of GPCR signaling.

1.2 Objective

The main objectives of the study are:

1.2.1 To monitor the real-time production of DAG at the plasma membrane and early endosomes upon activation of Gq-coupled receptors reported to activate Gq at early endosomes in absence or presence of PI-273 using NanoBiT-based biosensors.

1.2.2 To visualize the localization and dynamics of DAG at early endosomes and evaluate the impact of PI-273 on its production in these intracellular compartments by confocal microscopy.

1.3 Hypothesis

It is hypothesized that the following outcomes would be observed in this study:

1.3.1 Endosomal DAG production will be negatively impacted by the depletion of endosomal PI4P levels by PI-273, while DAG production at plasma membrane should not be impacted by this inhibitor.

1.3.2 Upon stimulation of Gq-coupled receptors, they will internalize into early endosomes leading to endosomal DAG production. Good colocalization of receptor, early endosome marker, and DAG reporter is expected following agonist stimulation. In presence of PI-273, we expect to observe less colocalization involving the DAG reporter.