2 Aims

Despite its importance in PI3Kγ signaling, the regulatory p101 subunit is considerably less well characterized than the catalytic p110γ subunit. Recent work has fostered the concept that p101 plays a crucial role in the activation of PI3Kγ downstream of GPCRs in a cellular context, where it binds to G$\beta\gamma$ subunits and thereby recruits p110γ to the plasma membrane (Brock *et al.*, 2003). Although p101 is known to interact with both p110γ and G$\beta\gamma$, the underlying structural determinants of these interactions are unresolved.

Therefore, this study aimed first at elucidating the domain structure of p101. To this end, deletion mutants with various N- and C-terminal truncations were analyzed in order to identify the regions within the primary structure of p101 that are responsible for binding to p110γ and G$\beta\gamma$. The resulting domain information was then to be used for identifying novel p110γ binding proteins and G$\beta\gamma$ effectors.

Surprisingly, a protein was identified that resembles p101 with respect to both interaction domains. The second aim of this thesis was thus to clone and characterize this potentially novel regulatory subunit of p110γ and to begin to unravel the relative impact of both regulatory subunits on PI3Kγ signaling.

The novel regulatory subunit termed PI3Kγ adapter protein of 87 kDa (p87$^{\mathrm{PIKAP}}$) was also included in the analysis of the interactions involved in the function of PI3Kγ regulatory subunits. In the third part, peptide SPOT libraries were used to identify linear peptide epitopes that confer affinity towards p110γ and G$\beta\gamma$. Additionally, it was attempted to obtain heterodimeric PI3Kγ of sufficient purity for crystallization screens.