Diabetes is an enormous threat to global health. It has been long known it manifests itself among others by impaired functions of β-cells, but regeneration of β-cell mass is a therapeutic goal that to date has not been achieved. In this project, we plan to investigate a therapeutic strategy to normalize the levels of Pancreatic and duodenal homeobox factor-1 (PDX-1), which is a critical protein for insulin production and for β-cell differentiation and survival.

PDX-1 is a target of another protein called SPOP, that mediates its degradation. SPOP is responsible for linking of Pdx1 to a Cullin-3 E3 ubiquitin-protein ligase, thus causing its degradation. It is known, that disrupting this process leads to improved glucose homeostasis by normalization of β-cell function, survival and mass. Therefore, targeting the turnover of PDX-1 appears as a promising therapeutic option to improve glucose homeostasis in diabetic patients.

Recently, first molecules inhibiting SPOP were reported as potential kidney cancer therapeutics. However, no research has been conducted yet on the potential effects of these compounds on β-cells, which renders this topic previously unexplored. In our preliminary work initiating this project we used these published inhibitors to test SPOP-PDX-1 protein-protein interaction (PPI) as a target for diabetes treatment. Prof. Oliver Plettenburg's team at Leibniz University Hannover (LUH) synthesized both reported and novel molecules that produce enhanced effects. These were then examined by dr Anna Czarna's group at the Malopolska Centre of Biotechnology (MCB) in biological assays. The results of our experiments indicated a **substantial increase of insulin levels in isolated pancreatic islets, highlighting the promise of this approach for beta cell health.**

In this multidisciplinary project, we aim to extend our preliminary results by **validating PDX-1-SPOP inhibitors as a promising mechanism leading to the observed, highly desired functions of pancreatic islets**. First, we aim to enhance the potency of our the compounds using insights from structural biology and molecular modelling. We will then validate the compounds at various levels, including cellular models and human organoids in the MCB lab through chemoproteomics. Additionally, we will develop another peptidomimetic series of inhibitors. This will be done using structural data on the Pdx1-SPOP interaction in dr Maciej Dawidowski labs at the Medical University of Warsaw (WUM). Another key focus of this project is the targeted delivery of SPOP inhibitors to β-cells, using GLP-1 receptor-mediated internalization to ensure specificity and overcome possible cell penetration challenges. This approach, involving carefully designed peptides, will be conducted at LUH. Additionally, the scientists from Helmholtz Zentrum Munich (HMGU) will complement our efforts with structural biology analysis of the interacting molecules.

The biological studies planned in this proposal will utilize mouse and rat β-cell lines, dispersed islets and human islet organoids, progressively increasing in complexity. Finally, we intend to conduct exploratory *in vivo* studies using promising compounds in rodent models of diabetes, advancing our research towards practical therapeutic applications. Our ultimate goal is to thoroughly establish SPOP inhibition as a groundbreaking target for diabetes treatment. This uncharted territory, if successful, could pave the way for innovative strategies in developing curative antidiabetic drugs. Ultimately, our research aims to demonstrate whether safeguarding PDX-1 protein levels can effectively prevent β-cell apoptosis and loss, a crucial milestone in the journey towards a therapy against diabetes**.**