Scope of the thesis

The general goal of the thesis investigations was to apply newly designed and generated ABPs in fundamental research on lysosomal glycosidases. Described is the characterization of a large number of novel ABPs targeting various glycosidases as well as a broad scale of applications for ABPs in research.

Chapter 1 briefly reviews current research on Gaucher disease and applications of activity-based probes (ABPs) targeting lysosomal glucocerebrosidase (GBA). It provides detailed protocols for *in vitro* and *in situ* visualization of active GBA molecules.

Chapter 2 reports on the use of a suite of ABPs in a gel-based competitive activity-based protein profiling (cABPP) approach to investigate the *in vivo* glycosidase targets of the widely applied GBA inhibitor conduritol B epoxide (CBE), and its close structural analogue cyclophellitol (CP). Off-target glycosidases are identified for both cyclitols, and the selectivity windows for selective GBA inactivation by both inhibitors are assessed.

Chapter 3 demonstrates the use of novel modified CPs for selective GBA inactivation and generation of neuropathic Gaucher zebrafish models. These new compounds containing a biphenyl or adamantly group installed at the C8 position of CP, and when orally administered the adamantyl compound selectively and potently inactivates GBA in visceral organs and brain of adult zebrafish.

Chapter 4 reports on the biochemical and structural investigation of CP aziridine ABPs toward GH31 α-glucosidase and GH2 β-glucuronidase. These ABPs allow mechanism-based inactivation/labeling of their target glycosidases *in vitro* and in cells. This enables the profiling of the endogenous active enzymes across biological samples, including fibroblasts from Pompe patients for diagnostic purposes.

Chapter 5 documents the design and characterization of CP aziridine ABPs towards GH39 α-L-iduronidase, the enzyme deficient in Hurler syndrome (MPS 1). These compounds exhibit

mechanism-based labeling of iduronidase, allowing detailed structural investigation on the conformational itinerary of the cleavage reaction. In addition, the Cy5 ABP assists monitoring of cellular uptake of pre-labeled therapeutic iduronidase by fluorescent microscopy.

Chapter 6 presents the development and characterization of CP aziridine ABPs targeting GH38 α-mannosidases or GH2 β-mannosidase. For this purpose use was made of SDS-PAGE-based fluorescence detection, measurement of inhibitory potency and kinetic studies, structural analysis, and chemical proteomics. Obtained data on labeling potency and glycosidase selectivity for both sets of compounds, provide a basis for application in mannosidase ABPPs across a range of biological samples as well as their use in screening of compound libraries for inhibitors.

Chapter 7 investigates the labeling mechanism, potency, inhibition kinetics, and glycosidase targets of the β-galactose configured CP aziridine compounds and ABPs. This allows the activity-based profiling of both lysosomal β-galactosidase (GLB1) and galactocerebrosidase (GALC) in biological samples. Chemical proteomics also identified other GLB1-like proteins, whose biological functions and implication in GM1-gangliosidosis or Krabbe disease should be explored in the future.

In the **General discussion and future prospects** section, the design and applications of cyclophellitol and cyclophellitol aziridine ABPs reacting with lysosomal glycosidases are reviewed, with a focus on their application in LSD research. The present limitations of current ABPs and future prospects are discussed.

This thesis is concluded with **Appendices** that include a **List of publications** and ***Curriculum vitae*** of the author.