Abstract – PhD Thesis – Christian Bartling

Neurodegenerative diseases are characterized by the loss of neurons in certain regions of the central nervous system. Alzheimer’s disease (AD) is the most common form of dementia and characterized by the formation and accumulation of amyloid plaques and tau tangles. The amyloid plaques are deposits of the neurotoxic amyloid β (Aβ) peptide, formation, aggregation, and accumulation of which are believed to be important for AD pathology. The intracellular interaction between the Munc-18-interaction (Mint) protein and the amyloid precursor protein (APP) is involved in the amyloidogenic processing of APP leading to the formation of Aβ and hence is thought to be of relevance to AD. This hypothesis is supported by Mint knockout mice, exhibiting an age-dependent delay of Aβ plaque formation, as well as by up-regulation of Mint proteins in Aβ plaques of AD patients. In addition, Mint proteins also interact with presenilin-1, the active subunit of the γ-secretase complex cleaving APP to form Aβ. This suggests that Mint proteins might serve as a putative target to reduce Aβ levels. However, the functional details underlying the Mint/APP interaction remain unclear and to date no potent ligand targeting Mint has been developed.

Here, we first established a structure activity relationship (SAR) for an APP peptide fragment interacting with the phosphotyrosine binding (PTB) domain of Mint2. Through a comprehensive study of APP, we characterized the APP/PTB binding interface, including amide to ester (A-to-E) substitutions to study the backbone hydrogen bond network. Furthermore, we incorporated non-canonical amino acids and performed lactam cyclizations, enabling the design of a cyclic and metabolically stable peptide, KSL-221036, binding to Mint2 with nanomolar affinity. We demonstrated that a cell-permeable version, KSL-227102, reduces Aβ formation in neurons. To further optimize these ligands, in particular to enable in vivo applications, we initiated the development of a second generation protein-protein interaction (PPI) inhibitor. We evaluated novel cyclic scaffolds of APP employing different side-chain to side-chain cyclization methods and applied a high-throughput screening platform (μSPOT) to map the APP binding motif and perform positional and substitutional scans including side-chain to side-chain cyclizations.

In summary, the results presented in this thesis shed light on the functional role of Mint2 examining its impact on the amyloidogenic processing of APP. We present a first generation peptide inhibitor of the Mint2/APP interaction reducing Aβ formation neurons. This supports us to pursue Mint2 as a putative drug target in AD. We are confident that the initiated development of a second generation inhibitor will further elucidate the impact of Mint2 on Aβ formation in vivo, ultimately contributing to a better understanding of the underlying pathological mechanisms in AD.