PhD thesis Abstract by Ingvar Rúnar Möller

Integral membrane proteins connect the cell to its surroundings and serve a crucial role in biological processes such as neuronal signalling. One such protein family is the neurotransmitter:sodium symporters (NSS). In humans, expressed in the central nervous system (CNS) as well as other tissues, the serotonin transporter (SERT) is responsible for the termination of the neuronal signalling brought on by its respective monoamine neurotransmitter molecule, serotonin (5-HT). The transporter achieves its function through rapid reuptake of the neurotransmitters from the synaptic cleft into the presynaptic nerve terminal where they are packed into vesicles. SERT serves as a target for therapeutic drugs as its dysregulation has been connected to the establishment of neurological diseases such as obsessive-compulsive disorder and depression. In this PhD-thesis, hydrogen-deuterium exchange (HDX) mass spectrometry (MS) was used to probe the conformational dynamics of NSS proteins in distinct solution-phase states. In the first part of the thesis, the effect of ligand binding on the conformational dynamics of the human SERT was investigated. Our results indicated SERT adopts an inward-facing conformation in the presence of 5-HT, as well as when it was in the presence of K+ or ibogaine, while adopting an outward-facing conformation when subjected to competitive inhibition from either S-citalopram or cocaine.

In the second part of the project, the enzymatic-digestion of SERT and three other integral membrane proteins was optimized through the screening of quench buffer additives and aspartic proteases, compatible with HDX-MS. Digestion of SERT and the closely related dopamine transporter from *Drosophila melanogaster,* with immobilized rhizopuspepsin resulted in the highest sequence coverage. The commonly used porcine pepsin was better suited for the digestion of the leucine transporter (LeuT) from *Aquifex aeolicus*, and a Cl-/H+ exchange transporter from *Escherichia coli*.

In the third part of the project, the effect of two related detergents on the conformational dynamics of LeuT, a prokaryotic homologue to SERT, was evaluated. Solubilized in maltose neopentyl glycol-3, the conformational dynamics of LeuT were stabilized compared to when the transporter was solubilized in the commonly used dodecyl maltoside. Nevertheless, LeuT underwent similar stabilization upon binding substrate independent of the detergent.