**MASTER THESIS**

Identification of the inter-subunit interactions that maintains the trimeric structure of P2X receptors by using computational modeling.

**ABSTRACT**

ATP-gated cationic P2X receptors assemble as homomers or heterotrimers from a repertoire of seven subunits, P2X1-P2X7. Based on our previous biochemical experiments and available high-resolution structures, relevant intersubunit contacts are restricted to the large extracellular domain, flanked at both ends by a membrane spanning domain, TM1 and TM2. From our previous work, biochemical and functional data sets of an alanine scanning mutagenesis study is available that covers in blocks of 4-6 residues the entire rP2X1 receptor ectodomain. Some of the block mutations seem to weaken the intersubunit contacts, while others lead to complete aggregation of the rP2X1 subunits in the endoplasmic reticulum. Comparison of our biochemical data with the contact points predicted by the X-ray structure of the zebrafish P2X4 receptor led us to hypothesize that weakening of the subunit-subunit interaction indicates a hit in the assembly interface, while aggregation may indicate misfolding of the mutant subunit.

The goal of my thesis is to solve this issue through computational modeling. My working hypothesis is that an interaction site present in both the apo-closed and open states of a P2X receptor is important for maintaining its trimeric state. Currently, I plan to run MD simulations with GROMACS on the homology models of rP2X1 and hP2X4 in both the closed and open states. I will consider intersubunit interactions present during the complete simulations as hits. I will further validate the hits by RMSD and free energy calculations and, in collaboration, by native PAGE analysis of the Xenopus oocytes expressed P2X receptor mutants.