FWFP27641: **Gating of the Orai channel complex involves outer regions surrounding the pore**

$Ca^{2+}$ release-activated $Ca^{2+}$ (CRAC) channels represent one of the most prominent $Ca^{2+}$ entry pathway into the cell. They control diverse cellular signaling processes ranging from cell proliferation, gene expression and T-cell activation. Two proteins, the endoplasmic $Ca^{2+}$ sensor, STIM1, and the $Ca^{2+}$ selective Orai1 channel, forming a hexameric complex, are sufficient to fully reconstitute CRAC channels. Coupling of STIM1 C-terminus to Orai, via bridging Orai1 N- and C-terminus, is hypothesized to induces a conformational change to the open state. Here, we aim to uncover mechanistic steps how STIM1 activates the Orai channel. Specifically, we will address if STIM1 docking alters i) the orientation of the C-terminal strands and in the following ii) TM helix interactions leading to iii) an open pore conformation. Therefore we will employ a combined approach of patch-clamp, FRET and cysteine crosslinking studies complemented by molecular dynamic (MD) simulations and NMR measurements. Primarily, MD simulations and in vitro experiments together with site-directed mutagenesis aim to elucidate relevant residues and/or intramolecular interactions in TM3/TM4 involved in gating. Our initial experiments revealed indeed that mutation of specific residues in TM2 (H134), TM3 (V181) and TM4 (P245) induce constitutive, $Ca^{2+}$-selective currents potentially via interactions with neighbouring TM helices. Hence, these amino acids maintain wild-type Orai1 in the closed state and might be directly affected by STIM1 binding to Orai C-terminus thus inducing channel opening. In this context we will investigate if STIM1 coupling alters the orientation of the C-termini and in turn the conformation of outer helical structures by comparing Orai1 wild-type with constitutively active mutants as well as STIM1-activated Orai1. Moreover, as preliminary experiments point to distinct key residues in the intracellular loop2 and the TM3 of Orai1 and Orai3 channels maintaining them in the closed state, we will evaluate our hypothesis that the two channels undergo different conformational changes upon their activation. Hence, we aim to elucidate isoform specific gating mechanisms. In summary, these studies aim at uncovering the molecular mechanism of STIM1-induced conformational changes in Orai1 helices surrounding the pore leading to its open state. Thus, fundamental insight into Orai1 TM reorientations from the closed into the open state will be provided.