LIT-2018-5-SEE-111: **OPTO-Orai - Novel Control of Calcium Entry via Light-Sensitive Unnatural Amino Acids**

Calcium (Ca2+) ions are versatile messengers that govern many steps throughout the cell?s life. Ca2+ enters the human cell via pores in the plasma membrane, among which the Ca2+ release activated Ca2+ (CRAC) ion channel is a prominent and unique Ca2+ entry pathway. Its molecular key players are STIM1, located within cellular Ca2+ stores, and Orai, the Ca2+ selective pore in the plasma membrane. They possess critical roles in maintaining the function of the immune system, muscle cells and neurons. Defects in those proteins can cause diverse pathologies like immune dysfunction, highlighting the clinical relevance of CRAC channels. Upon intracellular Ca2+ store-depletion, STIM1 couples to the Orai channel and initiates Orai pore opening. Despite STIM1/Orai1 coupling and activation has been extensively studied recently, a detailed picture of several steps in their activation cascade is still elusive. Within this project, we aim to address this matter capitalizing innovative strategies at the border of protein engineering and biological chemistry. Via an optoproteomics approach we will establish precise spatio-temporal control of Orai channel function via light that is reversible and utilizable in vitro and in vivo. We will initially engineer a set of lightsensitive Orai1 mutants, that will be capitalized to resolve signal transmission and conformational changes upon Orai1 pore opening as well as so far unknown interaction sites for STIM1 at the molecular level. Further, light-sensitivity will be transferred to mast cell lines, a perfect system to study allergic response, via incorporation of a light-switchable Orai1 form, thus enabling precise control of cellular processes via light. In extension, we will generate an Orai1 variant, light-sensitive in the nearinfrared range required for potential medical treatments. In aggregate, this project engineers Orai1 forms that are rapidly and exactly controlled via light in order to enhance current knowledge ...