Medical University of Graz

Biophysical characterization of light-activatable peptidomimetic inhibitors for CRAC channel

Summary

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Supervisors:	Dr. Pedro Alejandro Sanchez Murcia Dr. Bernadett Bacsa
Availability:	This position is available.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 15, 2022 00:00 and March 28, 2022 23:59 (Europe/Zurich)

Description

Background:

Calcium ion is the most widely used intracellular messenger in eukaryotic cells and controls myriad of different biological processes.1,2 The primary calcium entry route via plasma membranes in non-excitable cells involves calcium-release activated calcium (CRAC) channels. CRACs are localized in the endoplasmic reticulum – plasma membrane junctions. The CRAC channel complex is composed of two molecular key components: the highly calcium selective plasma membrane channel Orai1 and the calcium sensor protein stromal interaction molecule 1 (STIM1), which is embedded in the endoplasmic reticulum.3-5 This protein complex interacts in respond to calcium depletion of the endoplasmic reticulum. Abnormal CRAC channel activity has been associated with a number of human disorders including immunodeficiency, autoimmunity and acute pancreatitis.6

Hypothesis and Objectives:

In this project, we aim to identify new peptidomimetic scaffolds with inhibitory properties for future therapeutic interventions targeting the CRAC channel complex. The proposed work will utilize a tightly coordinated interdisciplinary approach, which combines computational methods with advanced organic and peptide synthesis as well as experimental biophysical techniques. The identification of STIM1 and Orai1 proteins as key components of CRAC channels provides an opportunity to screen for and desing drugs that can modulate channel activity and help to better understand and manipulate calcium signalling pathways. Up to present, only few CRAC channel inhibitors have reached human clinical trials.

Methodology:

After initial simulation of STIM1-based macrocyclic derivatives of different size in complex with the C-terminal domain of Orai1, the best virtual candidates (peptidomimietics) will be synthesized and chemically characterized. In the following step, the peptidomimetics will undergo comprehensive biophysical characterization by employing Ca2+-imaging/influx and patch clamp techniques. At least one Stim-derived and/or Orai1-derived peptidomimetic with inhibitory activity will be delivered. Additionally, preparation and biophysical characterization of light-activatable peptidomimetics for precise spatiotemporal control of the CRAC complex is planned.

The succesfull PhD candidate will join our multidisciplinary and international team (Austria/Hungary) and her/his PhD-thesis will deal with the biophysical characterization of the novel peptidomimetic inhibitors of the CRAC complex prepared within this project. In particular, the PhD candidate will characterize the peptidomimetic inhibitors employing fluorescensce live cell imaging in combination with molecular biology tools (i.e. Ca2+ imaging/influx, fluorescence resonance energy transfer (FRET) microscopy), and will implement a nuclear factor of activated T cells translocation assay. Furthermore, electrophysical patch clamp recordings will be carried out. Computational techniques like molecular dynamics simulations and free energy calculations will broad the PhD training.

References:

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3. Muik, M.; Fahrner, M.; Derler, I.; Schindl, R.; Bergsmann, J.; Frischauf, I.; Groschner, K.; Romanin, C., A Cytosolic Homomerization and a Modulatory Domain within STIM1 C Terminus Determine Coupling to ORAI1 Channels. J Biol Chem 2009, 284 (13), 8421-8426.
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5. Wang, Y.; Deng, X.; Zhou, Y.; Hendron, E.; Mancarella, S.; Ritchie, M. F.; Tang, X. D.; Baba, Y.; Kurosaki, T.; Mori, Y.; Soboloff, J.; Gill, D. L., STIM protein coupling in the activation of Orai channels. Proc Natl Acad Sci U S A 2009, 106 (18), 7391-7396.
6. Stauderman, K. A., CRAC channels as targets for drug discovery and development. Cell Calcium 2018, 74, 147-159.