Medical University of Graz

Protein purification and cryo-EM of TRPC3 channel

Summary

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Supervisors:	PD Dr. Rainer Schindl Dr. Oleksandra Tiapko
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:

TRPC3 is a non-selective cation channel which is highly expressed in brain. Accumulating data indicates that TRPC3 is a crutial pacemaker in hippocampus, cerebellum and substantia nigra. Newly discovered functional role establishes TRPC3 as a potential target for therapeutic intervention. However, there are only a few modulators of this channel available. To precisely design new therapeutic ligands, it is of high importance to gain knowledge about TRPC3 protein structure and, consequently, its gating mechanism. Recently, two TRPC3 structures were revealed by cryo electron microscopy (cryo-EM). Nonetheless, neither of them captured open pore architecture. Hence, the open conformations is the critical missing piece of information to solve the TRPC3 gating puzzle. This knowledge is essential for comprehension of the molecular function of TRPC3 and definition of possible ligand binding sites to control this channel is health and, especially, disease.

Hypothesis and Objectives:

We propose to succeed in capturing open pore structures of TRPC3 by stabilizing and synchronizing the channels at a high open probability using photochromic ligands and in addition by preserving their lipid environment during the protein preparation for cryo-EM.

Methodology:

We will screen two expression systems for suitability to maintain TRPC3 in open conformation. We will utilize photopharmacological tools for a temporal control over TRPC3 gating during protein extraction and cryo-EM. Structural details obtained from cryo-EM will be followed by structure-guided mutagenesis and evaluated in a multifunctional live cell approach combining Ca2+ imaging and electrophysiology.

References:

Lichtenegger, M.; Tiapko, O.; Svobodova, B.; Stockner, T.; Glasnov, T. N.; Schreibmayer, W.; Platzer, D.; Cruz, G. G.; Krenn, S.; Schober, R.; et al. An optically controlled probe identifies lipid-gating fenestrations within the TRPC3 channel. Nature Chemical Biology 2018, 14 (4), 1–9 DOI: 10.1038/s41589-018-0015-6.

Tiapko, O.; Shrestha, N.; Lindinger, S.; Guedes de la Cruz, G.; Graziani, A.; Klec, C.; Butorac, C.; Graier, W. F.; Kubista, H. et al. Lipid-independent control of endothelial and neuronal TRPC3 channels by light. Chemical Science 2019, 10 (9), 2837–2842 DOI: 10.1039/c8sc05536j.

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