

**Referee's review on a master thesis of Bc. Barbora Svobodova, BSc., a student of the joint degree master program of Biological Chemistry**

**Bc. Barbora Svobodova, BSc.** submitted for defence a master thesis entitled "CRAC channel gating – Involvement of Orai1 transmembrane regions and STIM1" to obtain the academic degree Master of Science in the master study of Biological Chemistry ("a joint degree").

Barbora has worked out a master thesis of the high quality with regards to both aspects – its formal elaboration as well as its content which has brought excellent and very promising results of very currently topic and demanding assignments. A subject of Barbora's thesis has approached mechanism of  $\text{Ca}^{2+}$  ions trafficking across plasma membrane (through Ora1 = CRAC / $\text{Ca}^{2+}$  - released-activated  $\text{Ca}^{2+}$  channel/) in tight association with STIM1, dimeric proteins inserted in ER membrane. Laboratory of Prof. Romanin has focused on trafficking systems for  $\text{Ca}^{2+}$  ions as the topic itself must be considered as one of crucial from physiological point of view.  $\text{Ca}^{2+}$  ions play fundamental role in signalling - transduction of signals from plasma membrane to intracellular compartments/organelles including nucleus.  $\text{Ca}^{2+}$  ions play their role under strong regulatory mechanisms to keep a  $\text{Ca}^{2+}$  content/concentration in cytosol (inside of the cell) strictly under control – to avoid any problems non-desirable amount of  $\text{Ca}^{2+}$  in a free space of inner environment of the cell. To get  $\text{Ca}^{2+}$  into the cell cytosol a coupled of Orai1 and STIM1 (through a junction with ER membrane) is needed, afterwards the SERCA molecular structure (Sarcoplasmic/endoplasmic reticulum  $\text{Ca}^{2+}$  ATPase) delivers  $\text{Ca}^{2+}$  ions across ER membrane into ER lumen. A role of such structurally and functionally system of  $\text{Ca}^{2+}$  trafficking has been found as extremely demanding from physiological point of view – for instance a lack of  $\text{Ca}^{2+}$  transportation ability after putting Orai1 out of service in immune cells causes severe combined immune deficiency (SCID) in humans.

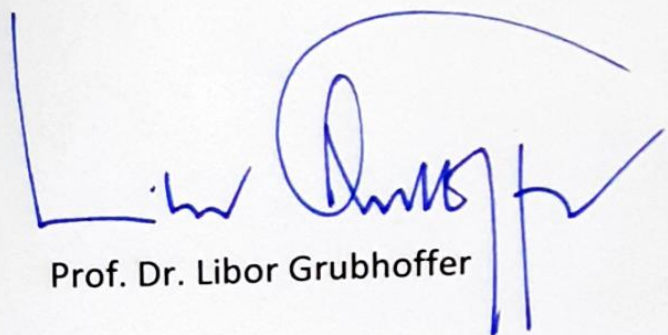
Barbora's aim was to prove a role of TM (transmembrane) domain of Orai1 (regarding site amino acid mutations) on physiological function of the  $\text{Ca}^{2+}$  gate. Barbora managed several modern techniques of molecular biology, preparative biochemistry, as well as physiology of membrane transportation. She carried out successfully cloning of genes coding Orai1, STIM1 as well as Ora1-STIM1 chimers, and transfected human embryonic cell kidney cells (HEK293) to get a functional model for  $\text{Ca}^{2+}$  electrochemical measurements.

I have put two questions to the author:

- 1) What is the function of glycans in SAM domains of STIM1 located beneath of ER membrane. Is there a convincing evidence for the occurrence of glycans there – which type of glycans might be there?
- 2) PS domain of the STIM1 protein is a domain enriched in Pro/Ser – could it be a potential/real site of glycosylation, if so which type of glycosylation?

In conclusion, I am pleased to state that Bc. Barbora Svobodova, BSc. submitted a master thesis of very high quality, she has elaborated such demanding piece of currently topic subject in membrane biochemistry and biophysics, I do believe that some of results she achieved could be published or will be published after a bit more experimental effort.

Therefore, I would like to suggest that Barbora's master thesis after successful defence deserves to be appraised as Excellent (in Czech university scale of grades) or Very Good (in Austrian university scale of grades).



Prof. Dr. Libor Grubhoffer

In České Budějovice, June 30<sup>th</sup>, 2013