

Studijní oddělení  
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27.8.2020

Praha,

**Věc: Posudek školitele na disertační práci Tomáše Chuma s názvem: „Quantitative fluorescence microscopy techniques to study three-dimensional organisation of T-cell signalling molecules“**

Surface receptors and associated signalling molecules regulate a number of physiological and pathological processes. Deep understanding of their function is thus pre-requisite for efficient treatment of diseases. Biochemical and genetic approaches helped to determine structure, basic function and interacting partners of these molecules. The impact of localisation on protein function is often underestimated.

In his work, Tomáš Chum applied advanced quantitative fluorescence microscopy to determine the impact of structural elements of proteins on their localisation. First, he described the role of transmembrane domain (TMD) and palmitoylation of adjacent sequences on trafficking of LAT to the plasma membrane of T cells (Chum et al. J Cell Science, 2016). The work includes in depth characterisation of TMD properties involved in this process. The role of helix-breaking residues, proline and alanine, in LAT TMD was characterised in the follow up work, to which Tomas contributed by super-resolution imaging (Glatzova et al., submitted to J Mol Biol). Using high-end 3D single molecule localisation microscopy developed in collaboration with colleagues Germany and the UK, he identified the nature of palmitoylation-dependent CD4 receptor clusters and segregation of CD45 from CD4 clusters driven by nanomorphology of T cell surface (Franke, Chum et al., under review in Comms Biol). All these studies required the development of a new sample preparation method for single molecule microscopy, which will form the basis of another manuscript in near future.

During his Ph.D studies, Tomáš Chum mastered all required techniques and tools, ranging from molecular biology to advanced imaging, data analysis and adaptation of MATLAB scripts for our purposes. Importantly, he contributed to the fine tuning many methods. Without these modifications, many exciting observations would remain unrecognised. Finally, I would like to highlight his ability to build and modify home-designed microscopes. Without this skill, my team could not participate on the development of super-resolution techniques and have access to the cutting edge imaging tools.

To summarise, Tomáš Chum has evolved in a scientist capable to perform an independent research. His work will result in 4 publications (1 accepted, 2 submitted and 1 in preparation), which are summarized in his thesis. Therefore, I recommend his thesis for further proceedings and the defense.

Marek Cebecauer

Supervisor