Visualizing single subunits of ORAI channels with STEM to study stoichiometry dependence on activation status

Diana B. Peckys [1], Niels de Jonge [3, 4], Barbara A. Niemeyer [1], and Dalia Alansary [1]

[1]Molecular Biophysics, Saarland University, Homburg/Saar, [3]INM – Leibniz Institute for New Materials, and [4]Department of Physics, Saarland University, Saarbrücken, Germany

The dynamics of intracellular Ca^{2+} signals govern a wide variety of cellular functions. Especially for long lasting processes cells rely on the so-called store-operated Ca^{2+} entry pathway. STIM1 proteins in the endoplasmic reticulum (ER) sense a decrease of the Ca^{2+} concentration, then react by clustering and trapping of ORAI1 proteins, located in the plasma membrane, to form functional Ca^{2+} channels in close apposition to the ER. ORAI channel stoichiometry may thus change during different functional states (i.e. at rest, and during channel activation). The assembly and stoichiometry of ORAI channels remains a matter of debate, and dimeric, tetrameric as well as hexameric assembly was reported. To solve this question we reached out for the visualization of single ORAI ion channel subunits, by using HA-tag labeling with fluorescent nanoparticles in combination with a novel correlative light- and electron microscopy technique [1]. Contrasting conventional approaches using extraction of proteins from their native environment of the plasma membrane we thus studied ORAI1 stoichiometry in intact cells in their liquid state. Therefore we first generated cells without endogenous ORAI1 expression, using the CRISPR/Cas9 approach, and different HA-tagged ORAI1 constructs, including concatenated ORAI1 proteins, which allow us to calculate our labeling efficiency. Labeled cells at rest and after activated Ca^{2+} influx, were then subjected to recording of STEM images with 2 nm resolution. Finally, statistical analysis of automatically measured distances between thousands of individually labeled proteins, was used to study ORAI1 stoichiometries under different conditions.

[1] D.B. Peckys, et al, Microsc. Microanal. 22(4), 902-912, (2016).