Single protein visualization of ORAI1 calcium channels with liquid-phase electron microscopy

Diana B. Peckys[1], Dalia Alansary[1], and Barbara. A. Niemeyer[1], and Niels de Jonge [2,3]

[1]Molecular Biophysics, University of Saarland, Homburg/Saar, Germany, [2]INM – Leibniz Institute for New Materials, Saarbrücken, Germany, [3]Department of Physics, University of Saarland, Saarbrücken, Germany

ORAI proteins can assemble to form Ca^{2+} channels in the plasma membrane, thus playing a crucial role in intracellular Ca^{2+} homeostasis. A still controversial question is the stoichiometry of ORAI protein subunits under resting conditions. Earlier studies supported tetra- and dimeric configurations, whereas recently, drosophila ORAI was found to be hexameric. Using liquid-phase electron microscopy (LPEM) we set out to visualize single human ORAI1 subunits, and three different concatenated, dimeric ORAI1 constructs, all supplied with hemagglutinin tags. These ORAI1 constructs were expressed in HEK cells lacking endogenous ORAI proteins (created using CRISPR/Cas9), and labeled with an anti-HA Fab and a fluorescent quantum dot nanoparticle, assuring a QD:ORAI1 labeling ratio not exceeding 1. The labeled, intact cells were kept hydrated under a graphene layer, and imaged with scanning transmission electron microscopy (STEM) detection. STEM images were automatically processed to obtain the label position coordinates. Subsequent analysis used the pair correlation function g(r), which measures any deviation from a random distribution. 298.452 labeled ORAI positions on 49 cells were analyzed revealing that ORAI1 channels at rest are present in higher order oligomers. We also show how the ORAI1 distribution but not necessarily the stoichiometry changes upon activation.