Determination of ion channel subunit stoichiometry by visualizing single molecules using STEM

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A range of cell functions depend on the dynamics of intracellular Ca^{2+} signals regulated by calcium channels in the plasma membranes of cells. Of key importance for studying their function is knowledge about their stoichiometry, i.e. how these ion channel-forming protein complexes are assembled from their subunits. However, such knowledge is only available from pooled material extracted from many cells. We present a new microscopy approach capable of visualizing the locations of individual subunits within intact cells in liquid. Cells expressing the proteins of interest are first labeled with fluorescent nanoparticles of high atomic number, and then studied with correlative fluorescence microscopy and scanning transmission electron microcopy (STEM) in a liquid environment, thus remaining in their liquid environment [1]. Two different ion channel proteins were explored. 1) Anoctamin-1, also known as Transmembrane Member 16A (TMEM16A), a voltage-sensitive calcium activated chloride channel, expected to form a channel from a pair of proteins. A streptavidin binding tag was included in an extracellular loop of the protein for binding of a nanoparticle, i.e. a streptavidin-conjugated quantum dot. 2) The Orai1 channel, a store operated Ca^{2+} channel of which many open questions exist about its stoichiometry and the possible existence of complexes involving also other members of the Orai family. To render Orai proteins accessible to QD labeling HA-tagged protein analogs were expressed. Statistical analysis of distances between the labeled proteins was used to examine the most probable configuration of these channels.

[1] D.B. Peckys, N. de Jonge, Microsc. Microanal. 20, 346-365, 2014.6 (2014).