Investigation of the subunit stoichiometry of Orai1 channels in situ by DNA-PAINT super-resolution microscopy

Xiaolan Xu, Lusheng Gu, Hang Yang, Shuai Wang, Yuanyuan Li, Wei Ji, Tao Xu
National Laboratory of Biomacromolecules,
CAS Center for Excellence in Biomacromolecules,
Institute of Biophysics, Chinese Academy of Sciences
15 Datun Road, Chaoyang District, Beijing, 100101, China
E-mail: xu_xl@163.com

Keywords: subunit stoichiometry, Orai1 channels, DNA-PAINT

Abstract: Ca^{2+} release-activated Ca^{2+} channels, formed by Orai1 subunits and gated by STIM1, play an essential role in the immune system. However, the channel subunit stoichiometry has drawn considerable disagreement, single molecule bleaching assay [1,2] and early electrophysiological data of tandem multimeric Orai1 construct [3] suggested that Orai1 channels were tetramers, against the hexameric stoichiometry revealed by the crystal structure of Drosophila Orai channel [4]. Based on our newly developed single molecule localization super resolution microscopy-repetitive optical selective exposure (ROSE) technique [5], here we incorporated unnatural amino acid-KPN into the surface of GFP nanobodies, site-specifically labeled them with DNA docking strand by click reaction, imaged the GFP rigidly fused to the dimer concatemer of Orai1 in Orai1 knockout HEK293 cells, then collected images under super resolution microscope ROSE.Our results indicated that the rigid linker between Orai1 C-terminal and GFP didn't disturb the membrane location of Orai1, and DNA-oligomers were site specifically labeled to GBP-KPN. Our work paved the way to investigate the subunit stoichiometry of Orai1 channels.

References:

[1] Ji W; Xu P; Li Z; Lu J; Liu L; Zhan Y; Chen Y; Hille B; Xu T, and Chen L, "Functional stoichiometry of the unitary calcium-release-activated calcium channel,"*Proc Natl Acad Sci U S A*, 105(36):13668-73 (2008).

[2] Penna A; Demuro A; Yeromin AV; Zhang SL; Safrina O; Parker I, and Cahalan MD, "The CRAC channel consists of a tetramer formed by Stim-induced dimerization of Orai dimers,"*Nature*, 456(7218):116-20 (2008).

[3] Mignen O; Thompson JL, and Shuttleworth TJ, "Orai1 subunit stoichiometry of the mammalian CRAC channel pore," *J Physiol*, 586(2):419-25 (2008).

[4] Hou X; Pedi L; Diver MM, and Long SB, "Crystal structure of the calcium release-activated calcium channel Orai," *Science*, 338(6112):1308-13 (2012).

[5] Gu L; Li Y; Zhang S; Xue Y; Li W; Li D; Xu T, and Ji W, "Molecular resolution imaging by repetitive optical selective exposure," *Nat Methods*, 16(11):1114-1118 (2019).