Structure, Inhibition and Regulation of Two-pore Channel TPC1 from *Arabidopsis thaliana*

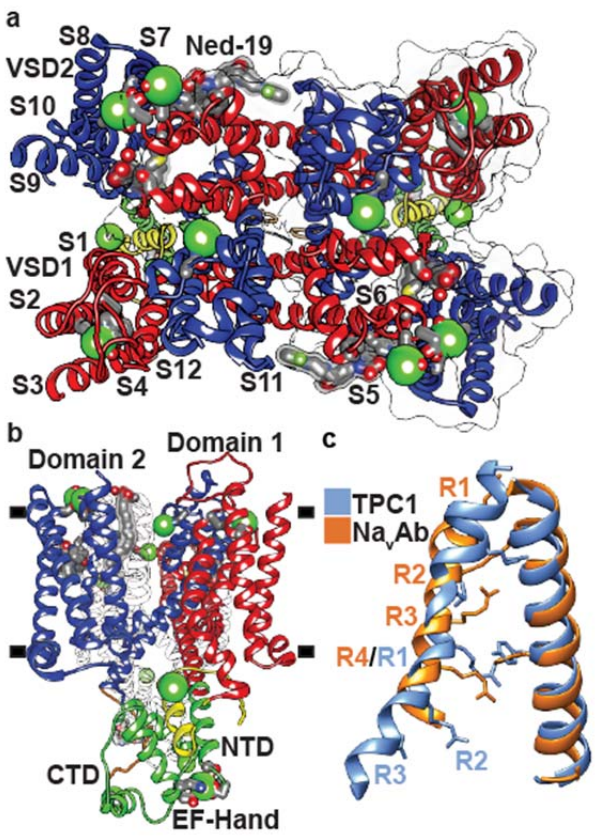
Using macromolecular crystallography beam lines at SSRL and the ALS scientists Alexander F. Kintzer and Robert M. Stroud at the University of California, San Francisco (UCSF) determined the structure of the first intracellular, voltage-gated, transmembrane protein ion channel, called two-pore channel 1 or TPC1, with an allosteric inhibitor Ned-19 bound to the outside of the channel. Their structure was determined by anomalous diffraction from 9 heavy-atom derivatives that relied on collaboration with the SSRL team at Beam Line 12-2.

This diffraction tour-de-force provided the first structure of a voltage-gated transmembrane channel with its voltage sensor domain in the 'resting state' at 2.87 Å resolution, published in the March 10, 2016 issue of *Nature*. Previous ion channel structures observed voltage-sensing domains in the active conformation (at zero voltage as necessary in the crystal), and as required by their physiology. Since TPC1 is in a resting-state at zero voltage, the structure reveals for the first time how voltage sensors move to couple transmembrane electric potential, to opening of a central ion channel.

Two-pore channels (TPCs) are voltage- and ligand-gated ion channels that reside in endolysosomes, capsular-shaped compartments made of membrane inside cells that are the primary mechanism of entry of receptor-bound ligands, nutrients, and viruses.

TPCs, by their action in regulating the endolysosomal pH, potential, and ion concentrations, determine the fate of the endolysosome, and of their contents.

Viruses like Ebola gain entry to the cell by co-opting the endolysosomes and borrow a cholesterol receptor Niemann-Pick C1 in the endolyso-



Overview of the TPC1 Structure. **a**, Top view from the luminal side onto the membrane plane and **b**, side view from the right side with the perpendicular to the membrane plane vertical of the TPC1 structure labeled by domain and transmembrane helices. Dashes denote approximate endolysosomal, membrane, and cytosolic boundaries. The positions of Ca^{2+} ion binding sites (green), bound lipids, and Ned-19 are shown. **c**, Structure of the resting-state AtTPC1 VSD2 (blue, PDBID 5DDQ) and activated-state Na_vAb VSD (orange, PDBID 3RVY). Voltage sensing arginines labeled R1-R4. See **www.msg.ucsf.edu/stroud/** for movie of the transition between resting and active states of VSD2.

some, to attach, and then fuse the viral membrane envelope with the endolysosomal membrane to allow viral RNA to enter the cytoplasm for replication. Ned-19 and other drugs that block the TPCs channel cure 50% of Ebola-infected animals by blocking the normal ion conductance of TPCs that favors fusion of the viral and endolysosomal membranes. The structure of TPC1 in complex with Ned-19 reveals a surprising mechanism of 'allosteric blockade' from the outside of the channel complex.

See **www.msg.ucsf.edu/stroud/** for details and a movie of the VSD transition from the resting to active state.

Primary Citation

A. F. Kintzer and R. M. Stroud, "Structure, Inhibition and Regulation of Two-pore Channel TPC1 from *Arabidopsis thaliana*", *Nature* **531**, 258 (2016), DOI: 10.1038/nature17194.

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SSRL is primarily supported by the DOE Offices of Basic Energy Sciences and Biological and Environmental Research, with additional support from the National Institutes of Health, National Institute of General Medical Sciences.