

Cryo-EM Structure of a Voltage-Gated Potassium Channel Kv3.1 In Complex with A Novel Potentiator Reveals a New Binding Site Suggesting The Mechanism Of Action

Yun-Ting Chen¹, Mee Ra Heo¹, Xin-Jun Zhan³, James Kostas¹, Yuxing L¹, Richard Kraus¹, Vincent Santarelli¹, Yacob Gomez-Llorente¹, Daniel Klein¹, Anthony Ginnetti¹, Michael Marino¹, Shawn Stachel¹, Andrii Ishchenko¹

¹*Merck and Co., Inc*

yun-ting.chen@merck.com

Voltage-gated K⁺ (Kv) channels play a crucial role in the management of neuronal excitability. Among them, the Kv3.1 channels mediate action potential repolarization being broadly expressed across the nervous system with fast-spiking interneurons in the neocortex, hippocampus, striatum, cerebellum, and the auditory brainstem. The malfunction of Kv3.1 channels in interneurons has been linked to the pathophysiological mechanism of schizophrenia. It has been hypothesized that positive modulators of these channels could restore normal function and high-frequency firing in those pathologies. In this study, we present a 3 Å resolution cryo-EM structure of the Kv3.1 channel bound to an internally developed small-molecule positive modulator. The comparison of the ligand-bound structure with the apo state sheds light on the structural determinants of the compound's unique mechanism of action. Notably, the compound-bound structure has revealed a new mechanism of ion conductivity potentiation by stimulating the interaction of the S5-S6 loop with the voltage sensor domain, consistent with the observed shift in voltage-dependent activation produced by these compounds. The binding mode observed in the structure provides a structural basis for the biophysical properties of this type of compounds, in particular slower deactivation rate, inability to respond to high-frequency inputs, and channel inactivation. Our structures also suggest the synergetic role of lipids, in particular cholesterol, in the formation of the ligand binding site and the potential modulation of the compound binding through this mechanism.