

# **Towards the Molecular Mechanisms of Ligand Activation in Kv7.5 Potassium Ion Channels**

*Thomas Ficker<sup>1,2</sup>, Aidan Grosas<sup>1,2</sup>, Lezanne Ooi<sup>1,2</sup>, Gökhan Tolun<sup>1,2</sup>*

<sup>1</sup>*Molecular Horizons and School of Science University of Wollongong, Wollongong, Australia*

<sup>2</sup>*ARC Industrial Transformation Training Centre for Cryo-electron Microscopy of Membrane Proteins, Wollongong, Australia*

The regulation of neuronal excitability is essential for processes such as learning, memory, and cognition, as well as for the control of muscle contraction. A key protein family involved in this regulation is the Kv7 voltage-gated potassium channel family, including Kv7.5. Mutations in Kv7.5 are linked to epilepsy, neuropathic pain, and other neurological disorders. While pharmacological modulators of Kv7 channels hold therapeutic promise, the structural basis of ligand activation of Kv7.5 remains poorly understood. This question is of particular importance since Kv7 subunits are highly conserved, making selective targeting challenging and raising the risk of off-target effects.

To address this, we expressed Kv7.5 homotetramers in Expi293F cells using the BacMam expression system, purified them via a GFP-nanobody resin, and started structural characterization in both apo and ligand-bound conditions using cryo-electron microscopy (cryo-EM). Four known modulatory ligands were selected for structural analysis, with the aim of comparing their binding interactions and conformational effects on channel gating. In parallel, patch-clamp electrophysiology and molecular docking will be used to complement the structural data and link observed conformational changes to functional modulation.

Through these studies, we hope to gain structural insight into the mechanisms of ligand-dependent activation of Kv7.5. Establishing this framework will be an important step towards understanding subtype-specific modulation within the Kv7 family and could ultimately help with the design of selective therapeutics for neurological disorders.