A Twist in the Tail: The Structural Basis of Kv7.2/Kv7.3 Channel Assembly through Helix D

MariaKatarina Lambourne[1,2], *Gökhan Tolun*[1,2], *Aidan Grosas*[1,2], *Lezanne Ooi*[1,2]

[1] School of Science and Molecular Horizons Institute, University of Wollongong, Wollongong, NSW 2522, Australia
[2] ARC Industrial Transformation Training Centre for Cryo-electron Microscopy of Membrane Proteins, University of Wollongong, Wollongong, NSW 2522, Australia

Epilepsy is one of the most prevalent neurological disorders, affecting around 65 million people worldwide[1]. It is characterised by recurrent seizures, the severity of which can be influenced by genetic, environmental, or unknown factors. One in three individuals with epilepsy do not respond to current treatments[2], highlighting the need for new therapeutic targets and more effective therapies.

The Kv7 family of voltage-gated potassium channels (Kv7.1–Kv7.5), encoded by *KCNQ* genes (*KCNQ1*–KCNQ5), has been implicated in epilepsy[3]. Kv7.2 and Kv7.3 assemble to form a majority of the neuronal M-channel, which regulates neuronal excitability by generating the stabilising M-current that prevents excessive firing. Mutations in the *KCNQ* genes, particularly within the transmembrane and regulatory domains of Kv7.2 and Kv7.3, are strongly associated with epileptic phenotypes.

A novel mutation has been identified in Kv7.3 within the proposed assembly domain, Helix D, arising from a glutamine to leucine substitution (Q653L). This residue lies within the distal half of the C-terminal tail, a flexible region with limited structural data, and may reveal a new mechanism affecting channel assembly and function. While Helix D in Kv7.1 and Kv7.4 promotes tetramer formation[4,5], the corresponding region in Kv7.3 has been reported to favour monomeric and dimeric states[4]. Given Helix D in Kv7.3 has a lower sequence conservation within the Kv7 family, and its key role in assembly, further investigation is needed to clarify its contribution to M-channel formation.

Here, we present the first crystal structures of the Kv7.2 Helix D domain and a heteromeric Kv7.2/Kv7.3 Helix D complex, revealing key interfaces that likely mediate subunit association. Complementary biophysical analyses, including size-exclusion chromatography, multi-angle light scattering, mass photometry, and circular dichroism spectroscopy, show that Helix D forms a dynamic assembly capable of supporting distinct oligomeric states. These findings provide a structural framework for understanding Kv7 channel assembly and set the stage for ongoing cryo-EM studies of full-length Kv7.2/Kv7.3 channels.

REFERENCES
1. Ngugi AK, Bottomley C, Kleinschmidt I, Sander JW, Newton CR. Estimation of the burden of active and life-time epilepsy: a meta-analytic approach. *Epilepsia*. 2010.
2. Chen Z, Brodie MJ, Liew D, Kwan P. Treatment Outcomes in Patients With Newly Diagnosed Epilepsy Treated With Established and New Antiepileptic Drugs: A 30-Year Longitudinal Cohort Study. *JAMA Neurol*. 2018.
3. Nappi P, Miceli F, Soldovieri MV, Ambrosino P, Barrese V, Taglialatela M. Epileptic channelopathies caused by neuronal Kv7 (KCNQ) channel dysfunction. *Pflüg Arch - Eur J Physiol*. 2020.
4. Howard RJ, Clark KA, Holton JM, Minor DL. Structural insight into KCNQ (Kv7) channel assembly and channelopathy. *Neuron*. 2007.
5. Wiener R, Haitin Y, Shamgar L, et al. The KCNQ1 (Kv7.1) COOH Terminus, a Multitiered Scaffold for Subunit Assembly and Protein Interaction. *J Biol Chem*. 2008.