Selectivity Filter Gating in the MthK Potassium Channel and its V55E mutant

MAX PLANCK INSTITUTE FOR MULTIDISCIPLINARY SCIENCES

Dynlon

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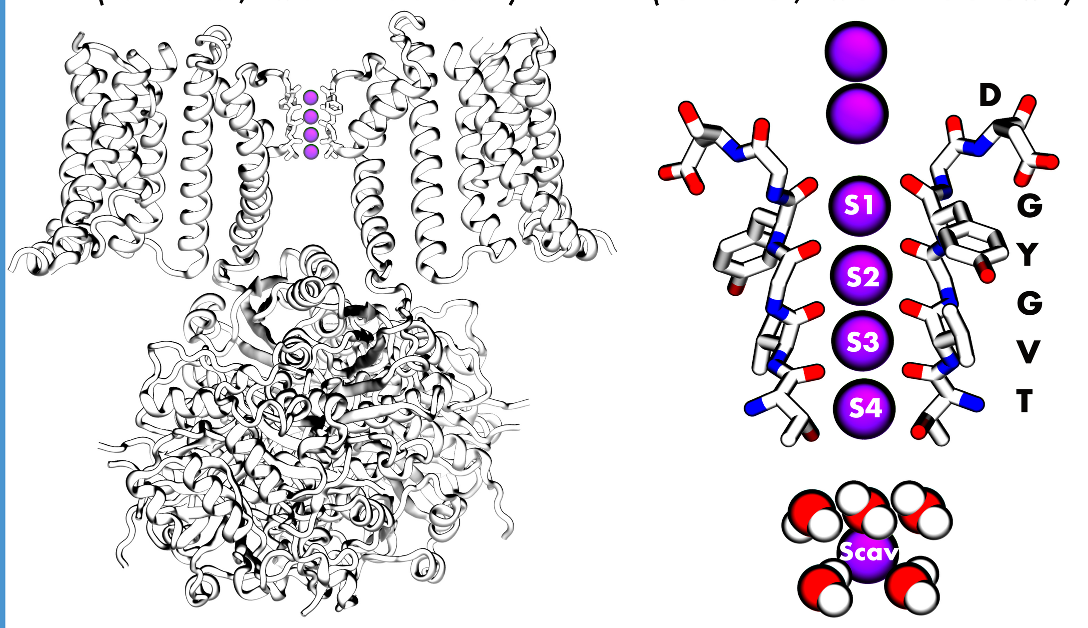
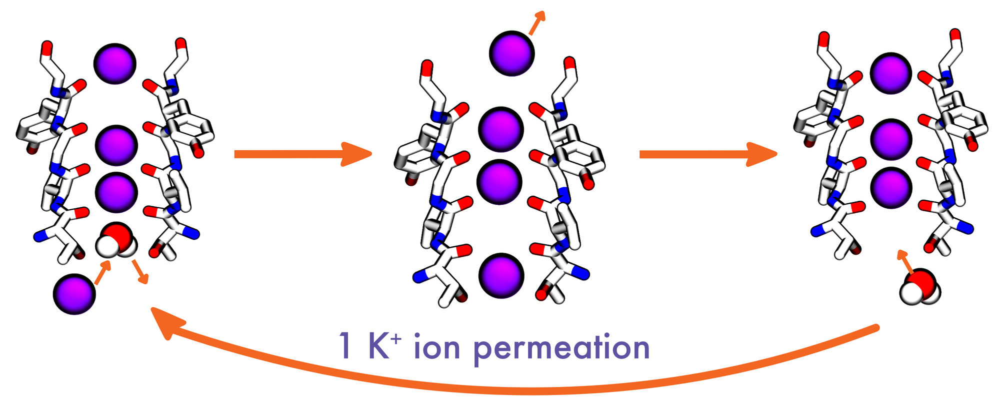
Introduction

Potassium channels facilitate the passage of K^+ ions through membranes to near-diffusion limited rates. The main structural element of potassium channels - **the selectivity filter** - is conserved in all canonical potassium channels, and yet individual channels can vary in their conduction rates by at least one order of magnitude.



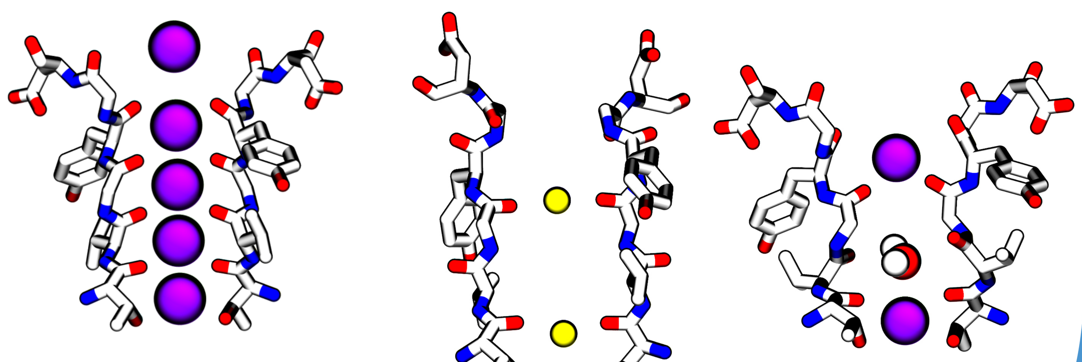
Ion permeation

Recent combined experimental and computational studies [1,2] suggest that potassium ions permeate through the channel via the so-called 'direct knock-on' mechanism. The 'direct knock-on' mechanisms provides a framework to study ion conduction in potassium channels and its regulatory mechanisms in greater detail.



Selectivity Filter Gating

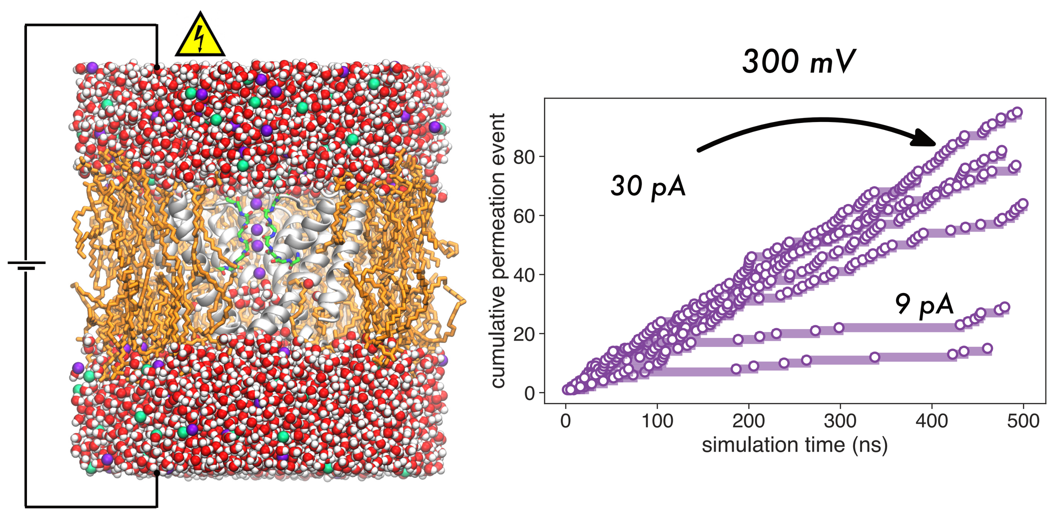
Apart from enabling rapid and selective ion permeation, the selectivity filter is a gate in many potassium channels: it undergoes conformational transitions that allow or halt ion permeation in a process called inactivation or selectivity filter gating [3].



Methods

in silico electrophysiology

We use *in silico* electrophysiology Molecular Dynamics (MD) based simulations, to obtain a clear view into ion permeation on at the atomistic scale. We start from high resolution structures of potassium channels and apply an electric field (membrane voltage), akin to electrophysiological measurements, that leads to individual ion permeation events. Counting these events results in measured ionic currents.



References

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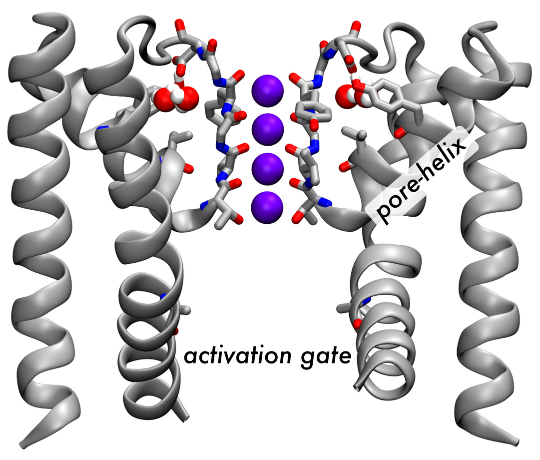
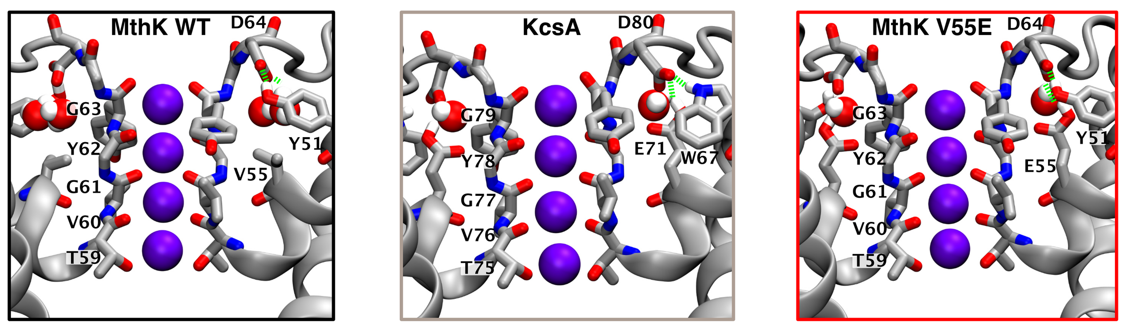
V55E mutation destabilizes the open state of MthK in experiments

Architecture of MthK WT



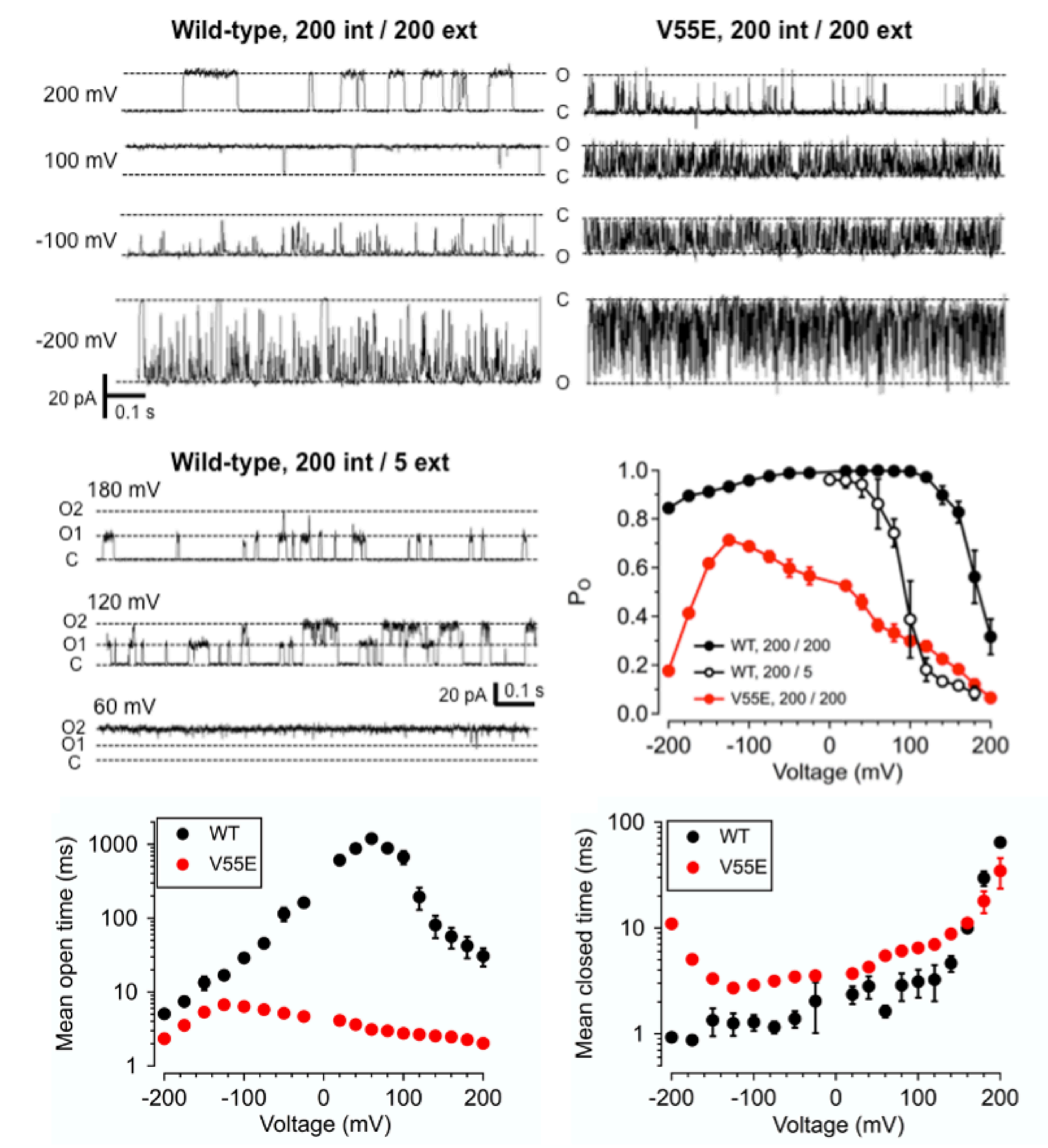
MthK is a model potassium channel gated by Ca^{2+} ions. We have previously established that MthK displays an exquisite coupling between the lower **activation gate** and the selectivity filter [4]. Here, we investigate how a **pore-helix** mutation in MthK affects its conduction and gating properties.

Selectivity filters of MthK WT and MthK V55E



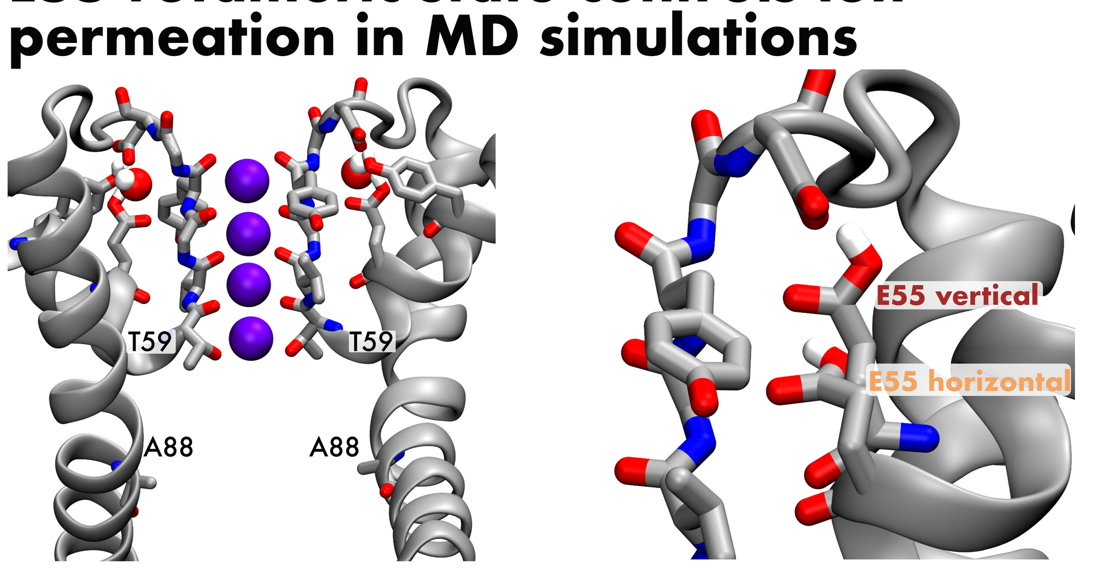
We study MthK WT and MthK V55E channels. MthK V55E has a valine in the pore-helix replaced by a protonated glutamate, a unique residue, found in another model potassium channel KcsA, known to play a key role in inactivation.

Electrophysiology of MthK WT and MthK V55E

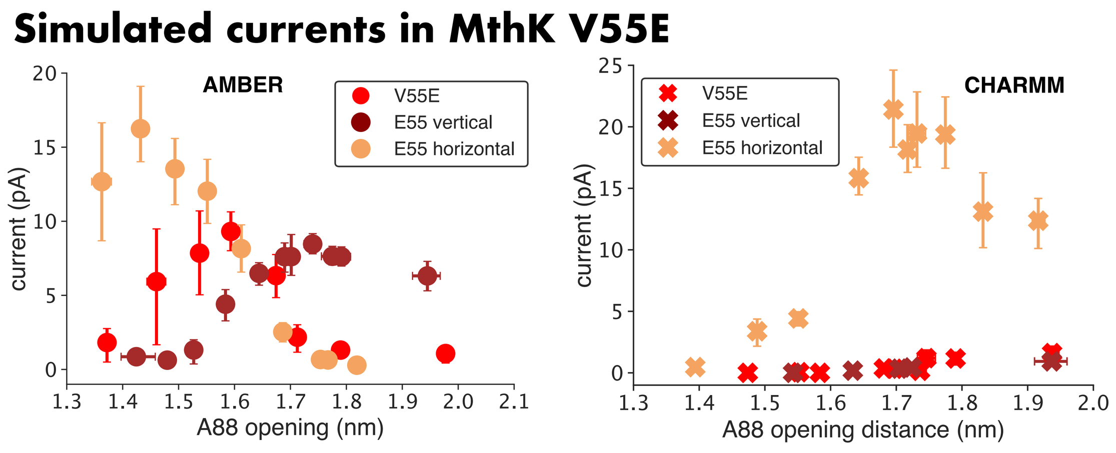


Experiments show lower open probability (Po) of MthK V55E, due to decreased stability of the open state.

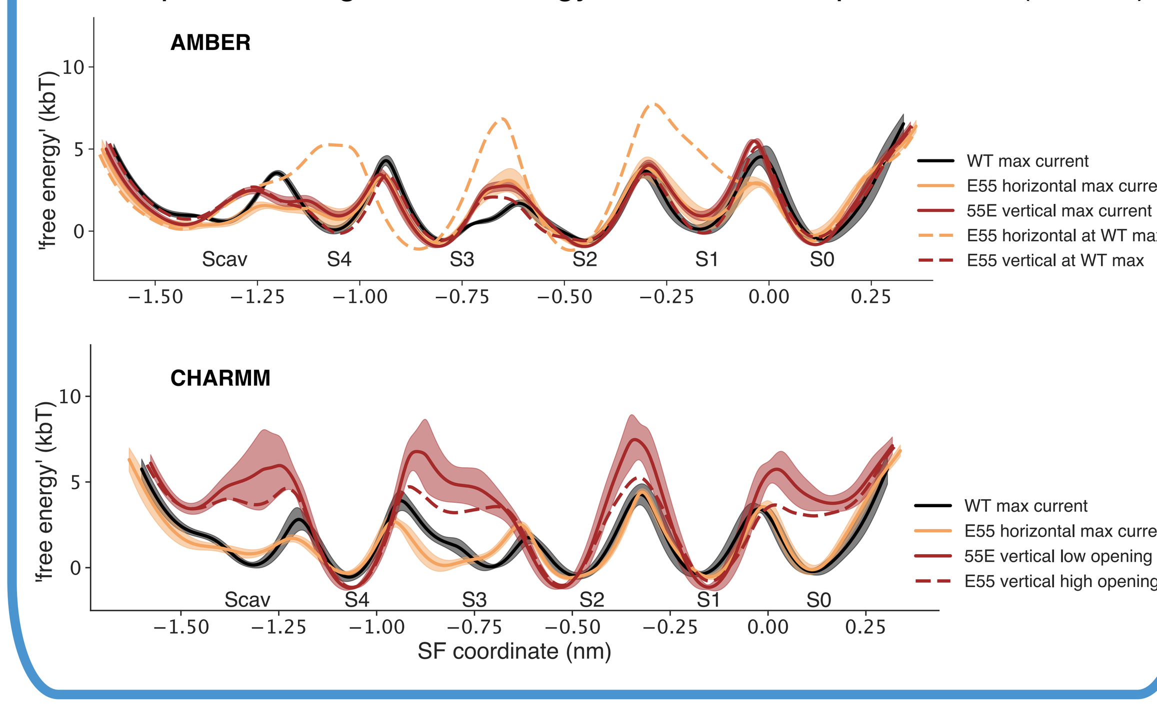
E55 rotameric state controls ion permeation in MD simulations



Simulated currents in MthK V55E

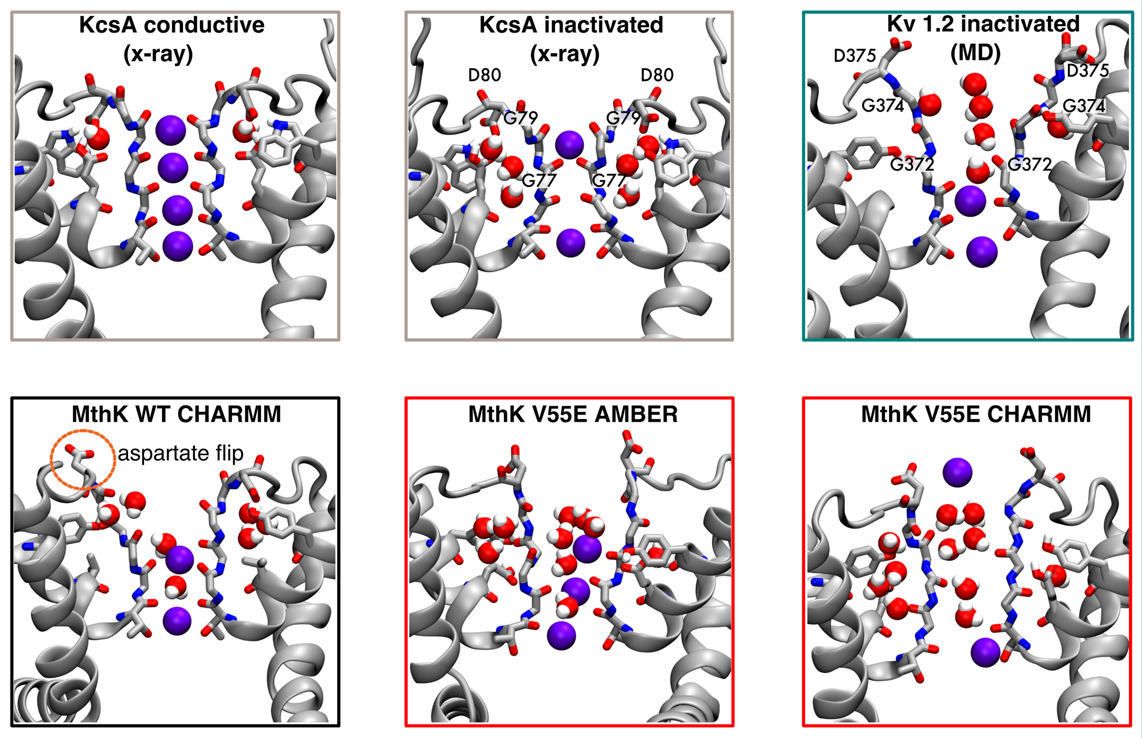


Vertical orientation of E55 (red) leads to low currents (top), which is a consequence of higher free energy barriers for ion permeation (bottom).



Selectivity filter gating in MthK WT and MthK V55E

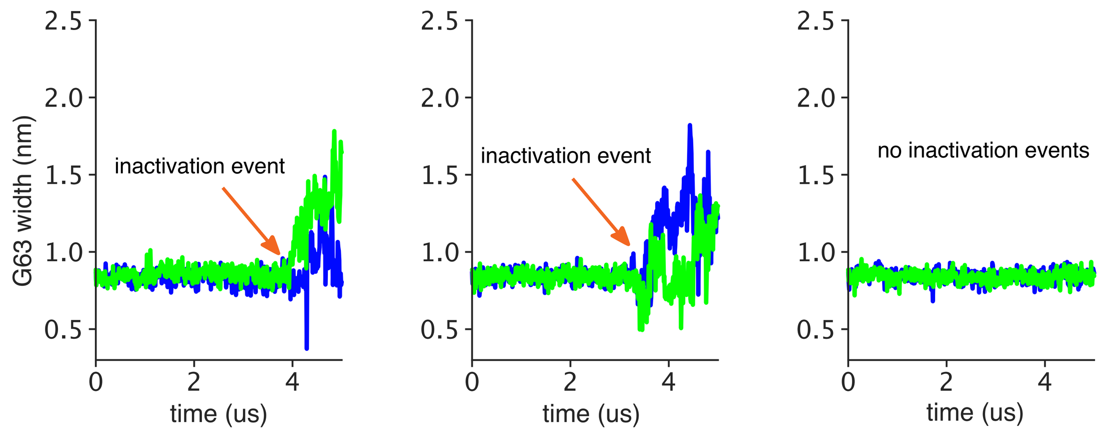
Selectivity filter conformations



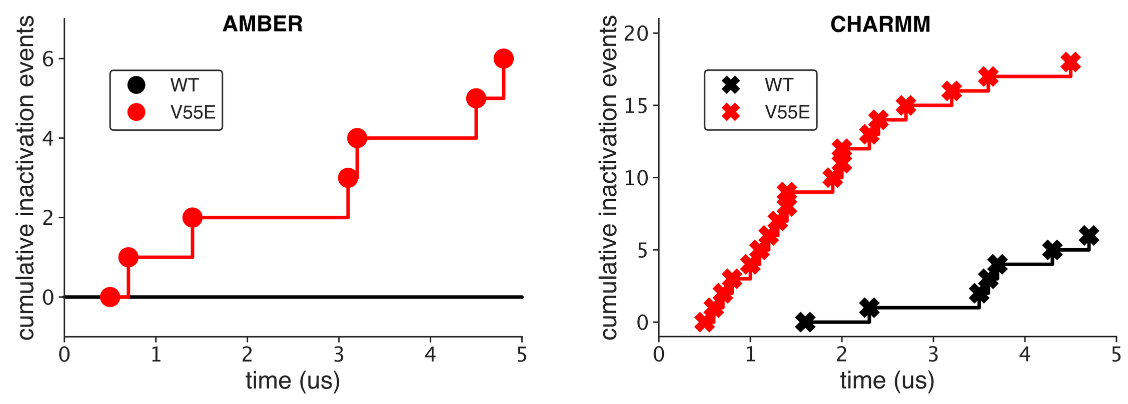
Widening of the selectivity filter, especially at the level of the 'second glycine' (G63 in MthK) has been postulated as the selectivity filter gating mechanism in voltage gated channels (e.g. Kv1.2) [5,6]. We observe similar transitions in MD simulations of MthK WT and MthK V55E.

Selectivity filter transitions in unbiased MD simulations

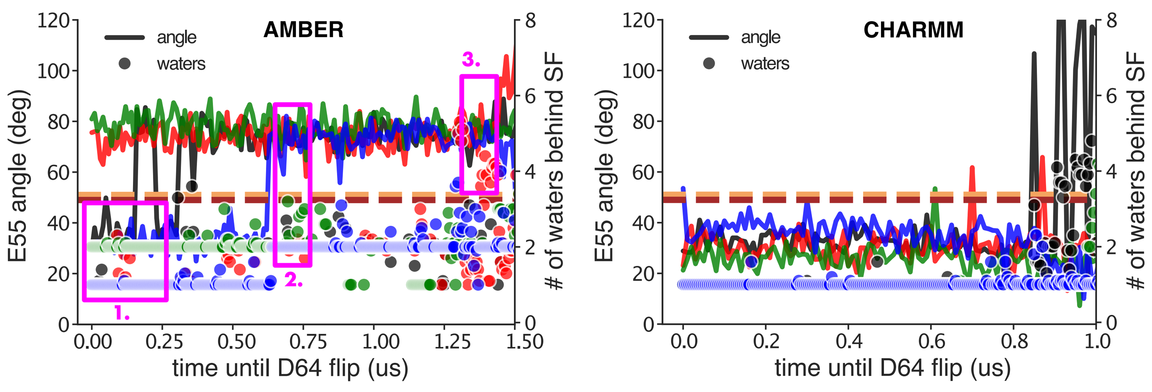
Examples of individual inactivation events in MD simulations. As the channel is tetrameric, each curve refers to a distance between oppositely oriented subunits.

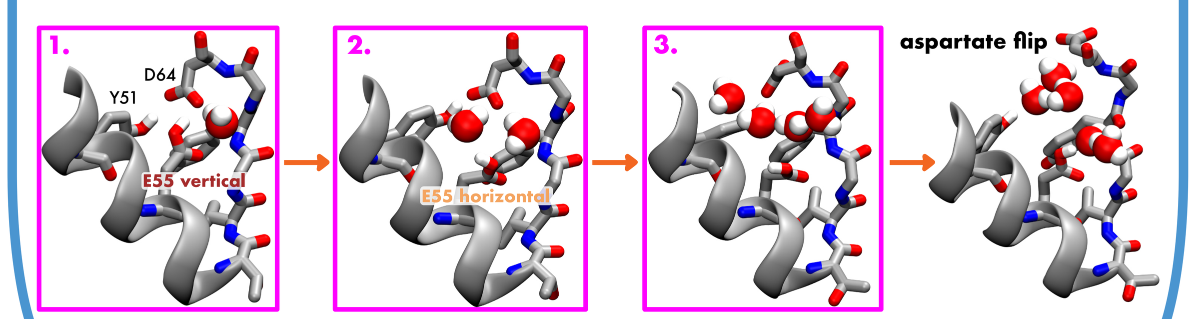


Based on 20 independent, 5 us long simulations per system/force field, we counted number of inactivation events occurring in MD simulations of MthK WT and MthK V55E



Flipping of an aspartate behind the SF leads to the SF widening. This flip is a consequence of the E55 horizontal orientation that causes water flux behind the SF and destabilization of the D64-Y51 hydrogen bond.





Conclusions

- Experiments and MD simulations are in good agreement, showing the reduced stability of the conductive state in MthK V55E, as compared to MthK WT, due to the higher propensity of entering the inactivated conformation.
- E55 transitions between two rotameric states affect ion permeation rates in MD simulations, by increasing the free energy barriers.
- Inactivation of both MthK WT and MthK V55E seems to be structurally more related to inactivation in voltage gated channels (widening of the SF at the top part) than in the Kcsa channel (narrowing of the filter in the middle part).
- Inactivation in MthK V55E is enhanced due to the 'horizontal' orientation of E55, that attracts water molecules from the extracellular space, which in turn destabilizes the critical hydrogen bond between D64 and Y51, leading to an 'aspartate flip'.
- A single mutation in the pore-helix of potassium channels can dramatically alter the gating behavior at the selectivity filter
- Further work is required to understand diverging trajectories of inactivation in Kcsa, MthK and voltage gated potassium channels