Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVIII

Computational Study of Gain of Function Point Mutations in a Potassium Channel

Agnese Roscioni
Modeling and Physiology laboratory, DiSVA

Introduction

Kv7.2/3 channels (Fig.1), encoded by members of the KCNQ gene family, are responsible for the generation of the M-current, a slowly activating and deactivating potassium conductance that plays a critical role in neuronal excitability. Over the years, in the context of the RIKEE project (www.rikee.org), Kv7.2 genetic defects have been linked to various neurological diseases, making this protein a prime candidate for drug development. In this regard, thorough functional and structural characterization of clinical mutants may open the door to the development of strategies to counteract protein dysfunction in either gain- (GoF) or loss-of-function (LoF) variants.

Here, we present the first steps in the investigation of the Kv7.2 channel by means of Molecular Dynamics (MD) simulations. We focus on the effect of different Force Fields (FFs) on the structure of the Selectivity Filter (SF) and on the impact of three Inner Gate (IG) mutations on the structure of the wild-type (WT) protein. We analyzed both closed and open channel configurations, starting from the cryo-EM WT structure (PDBID 7CR0) and a homology-based model, respectively. All of the examined mutations induced a widening of the gate of the closed configuration with respect to the WT protein, consistent with a GoF effect revealed by electrophysiological experiments, while they showed no effect on the open conformation.

We provide a preliminary atom-detailed structural characterization of different Kv7.2 variants that is of relevance for understanding the molecular mechanisms underlying KCNQ2-related pathologies, and that may lead to the development of personalized treatments.

Project Partners

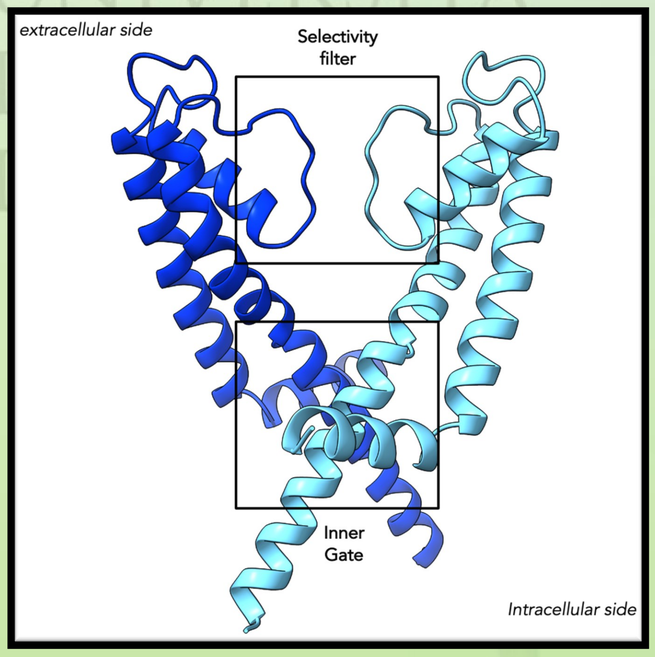

Fig.1 Cryo-em structure of the Kv7.2 in the closed configuration (PDBID 7CR0). Only chain A and C are represented for clarity.

Project workflow

KV7.2 WT and three different mutants

Objective 1

To investigate the effect of different Force Fields on the Selectivity Filter (SF) structure → MD equilibrium simulations with different Force Fields → Analysis of the SF structural properties

Objective 2

To characterize three GoF mutations → MD equilibrium simulations at different ion concentrations → Free Energy calculations to investigate ions permeation → Computational electrophysiology analysis

Objective 1 Preliminary results

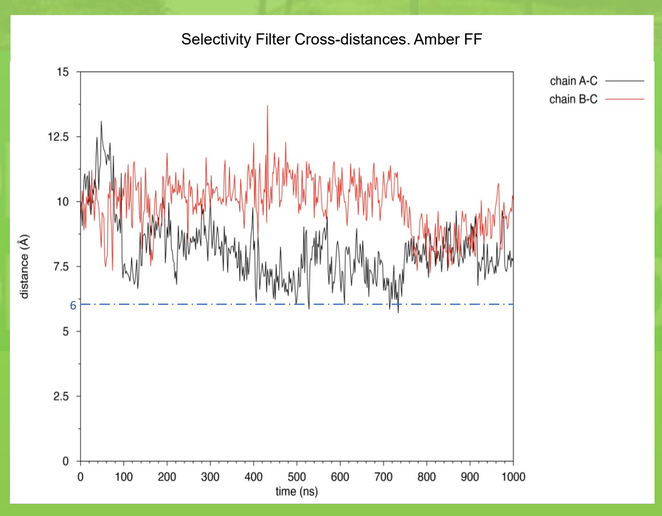

Fig. 3 Cross—distances of SF over 1 μs of production using Amber Force Field. In dashed blue line the collapse threshold

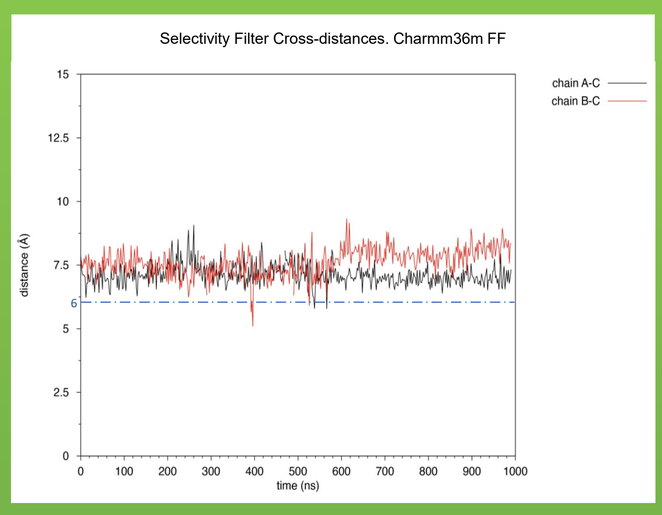

Fig. 4 Cross—distances of SF over 1 μs of production using Charmm36m Force Field. In dashed blue line the collapse threshold

Recently, MD investigations of ion channels revealed a different structural behavior of the SF when different FFs are used. Furini and Domene tested different potassium channels with Amber and Chamm36m FFs and the NAMD code, observing a collapse of the filter when the Charmm36m FF was used.

To further investigate this problem, we performed two equilibrium MD simulations, one with each force field (Fig.3 Amber FF, Fig.4 Charmm36m FF) of the wild type Kv7.2 using GROMACS code.
Surprisingly, the SF does not collapse in any of the two considered cases. Moreover, it seems to be more stable with the Charmm36m FF.

More replicas of the WT channel will be simulated and the behaviour of the SF in the mutants will be also tested.

Objective 2 Preliminary results

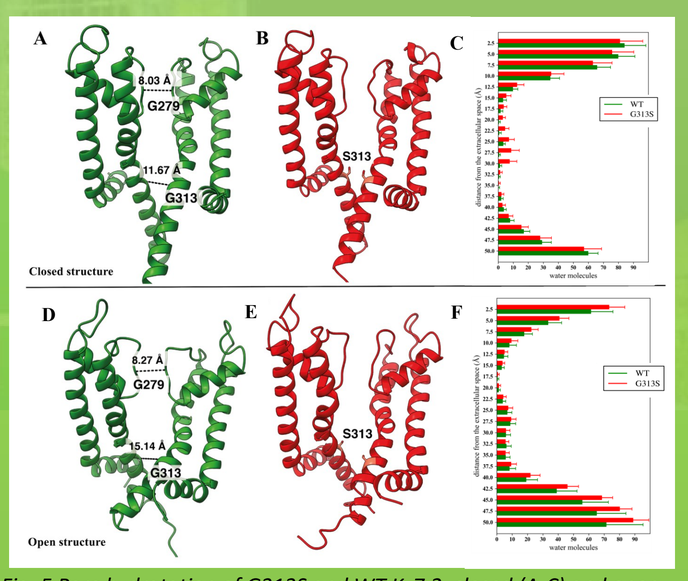

Fig. 5 Pore hydratation of G313S and WT Kv7.2, closed (A-C) and open conformations (D-F)

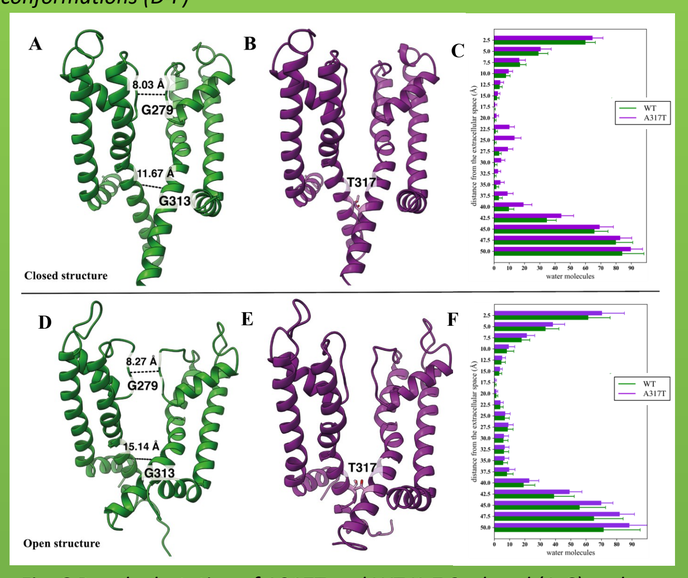

Fig. 6 Pore hydratation of A317T and WT Kv7.2, closed (A-C) and open conformations (D-F)

The extent of pore hydratation is correlated to pore conductivity.

Fig.5 and 6 show the hydration degree along the pore for the closed (A-C) and open (D-F) conformations of the G313S and A317T mutant, respectively. Water molecules are averaged over six independent replicas of a 500ns trajectory.

As it is possible to observe in panels C, in both mutants the closed structure present a higher hydratation rate in the central region of the pore, with respect to the wild type.

On the contrary, panels F show that there are no significant changes in hydratation between mutants and wild type.

These data are consistent with experimental results showing GoF effects of these two mutations.

The analysis will be repeated for the third mutant.