# KCa3.1 Channel: Computational Analysis of Three Known Toxin Inhibitors Towards New Extracellular Inhibitors

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## Introduction:

Ion channels are important drug targets that can be divided into two main classes: ligand-gated and voltage-gated ion channels. KCa3.1 is activated by $Ca^{2+}$ activated Calmodulin and insensitive to voltage. Not only is KCa3.1 important for immune regulation and the correct function of red blood cells, but it is also associated with certain types of cancer.[1]
Three toxins are known to bind to KCa3.1 from the extracellular side. They are called OSK1, Charybdotoxin (ChTx) and Maurotoxin (MTx). Based on the available NMR structures of these toxins and the KCa3.1 channel (PDB IDs: 1SCO, 2CRD, 1TXM and 6CNO), a computational analysis was conducted to model the toxin binding.[2]

## Sequence Alignment:

The alignment of the three toxins was performed using MOE. The residues were coloured according to the ClustalX scheme where the colour is based on occurrence and property of the amino acid. The most important conserved residue for the channel blockage corresponds to K28 in the alignment.[2]
In Figure 1 this residue is labelled as Lys27 (OSK1), Lys27 (ChTx) and Lys23 (MTx). The toxins have binding affinities ($K_d$) of 1 nM, 5 nM and 225 nM, respectively.[2,3]

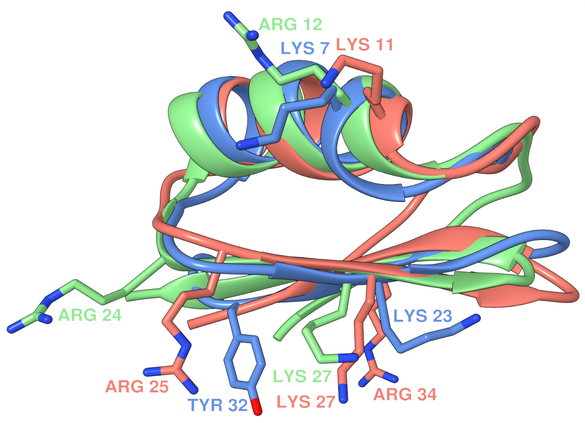



**Figure 1 and 2:** Above the models of the three toxins OSK1 (green), ChTx (orange) and MTx (blue) are displayed. The side chains that are important for binding to KCa3.1 are shown as sticks. Below the sequence alignment can be seen.


## Docking Study:

A flexible protein-peptide docking experiment was performed using the HADDOCK Webserver[4]. The docking poses were evaluated based on their HADDOCK score as well as manually inspected. The resulting binding pose can be seen in Figure 3.

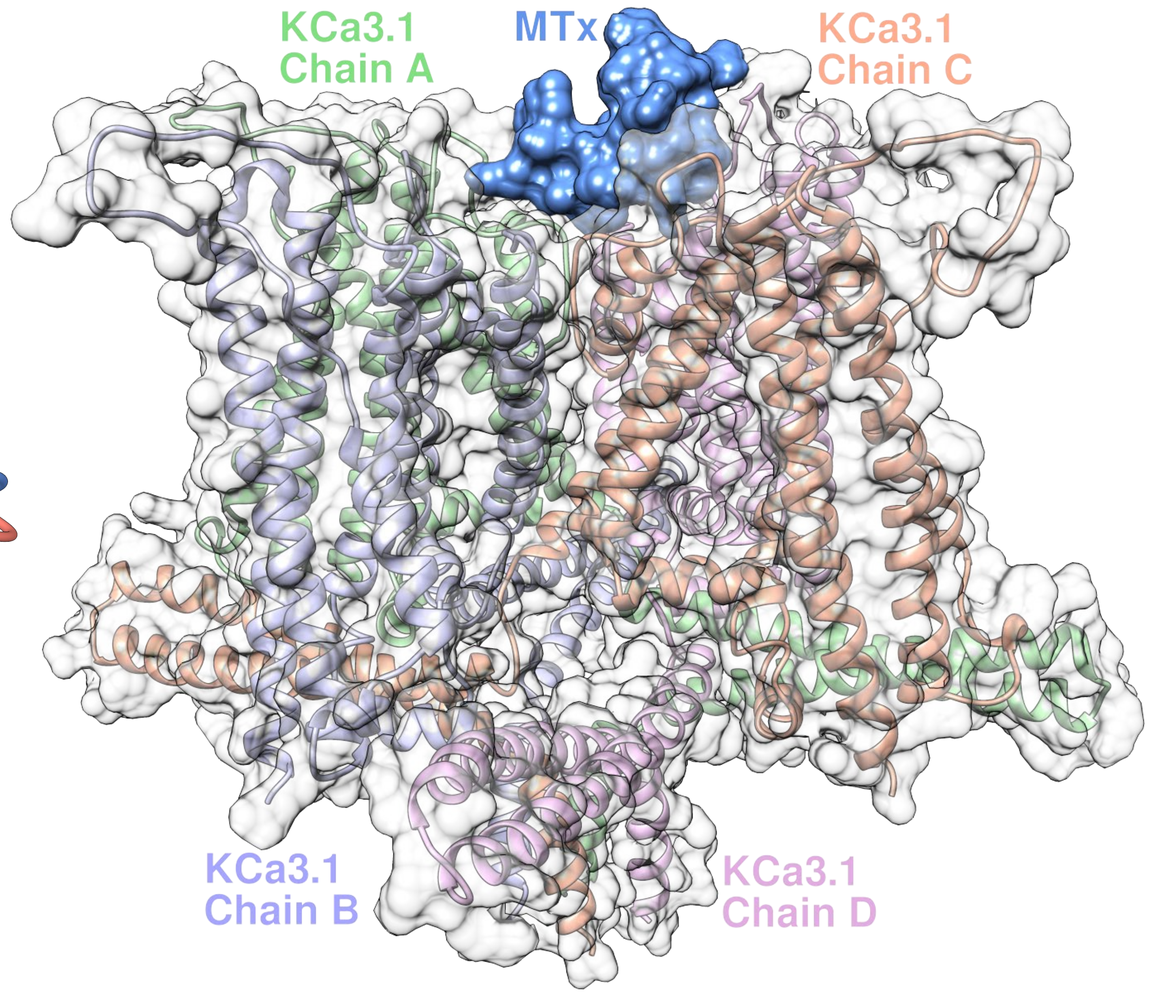
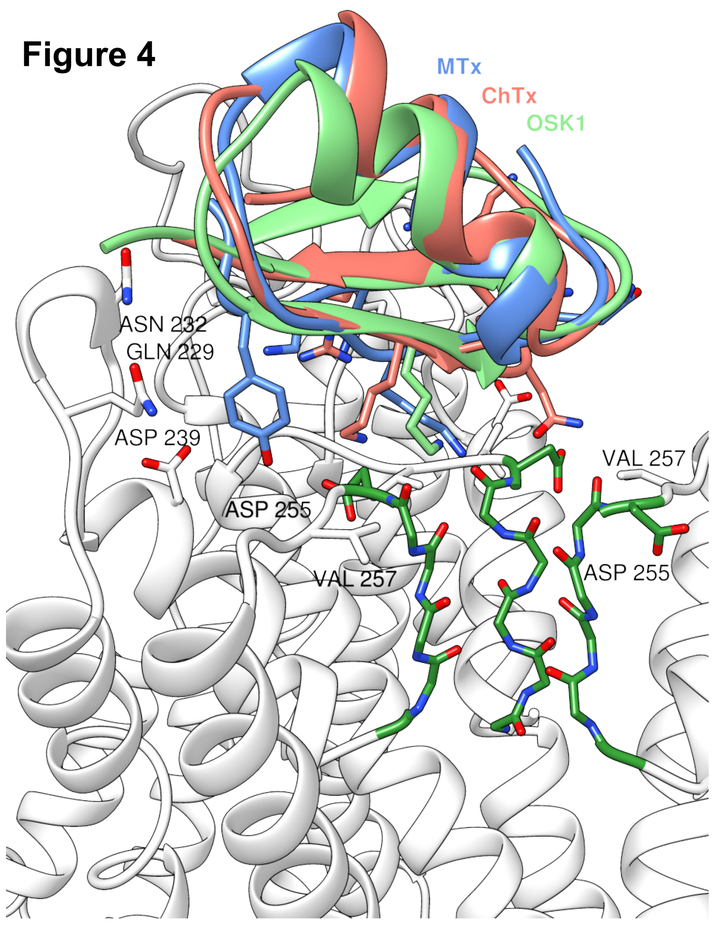



**Figure 3:** Overview of the result of the docking of MTx (blue) to KCa3.1. The channel is a homotetramer and the four chains are shown as ribbons. This result reinforces the importance of the Lys23 residue as it is sticking into the pore thus impairing ions to enter the selectivity pore.

## Molecular Dynamics Simulations:

The docking result of MTx in KCa3.1 was used as the starting point for the simulations. Due to their structural similarity, the other two toxins were structurally aligned to MTx, and these positions were used as starting points. The dimensions of the rectangular box are 154x154x270 Å. The receptor and the toxins were parametrized using AMBER99SB-ILDN.
Water and ions ($K^+$ and $Cl^-$) were added to the system. All preparations and the simulations were done using Gromacs 2020.6 and were mostly run on the GPU. A peak performance of around 20 ns/day was reached.
The table below summarizes the results of the hydrogen bond analysis conducted with VMD. The residues of KCa3.1 that are important for the binding of the toxins are listed there as well.

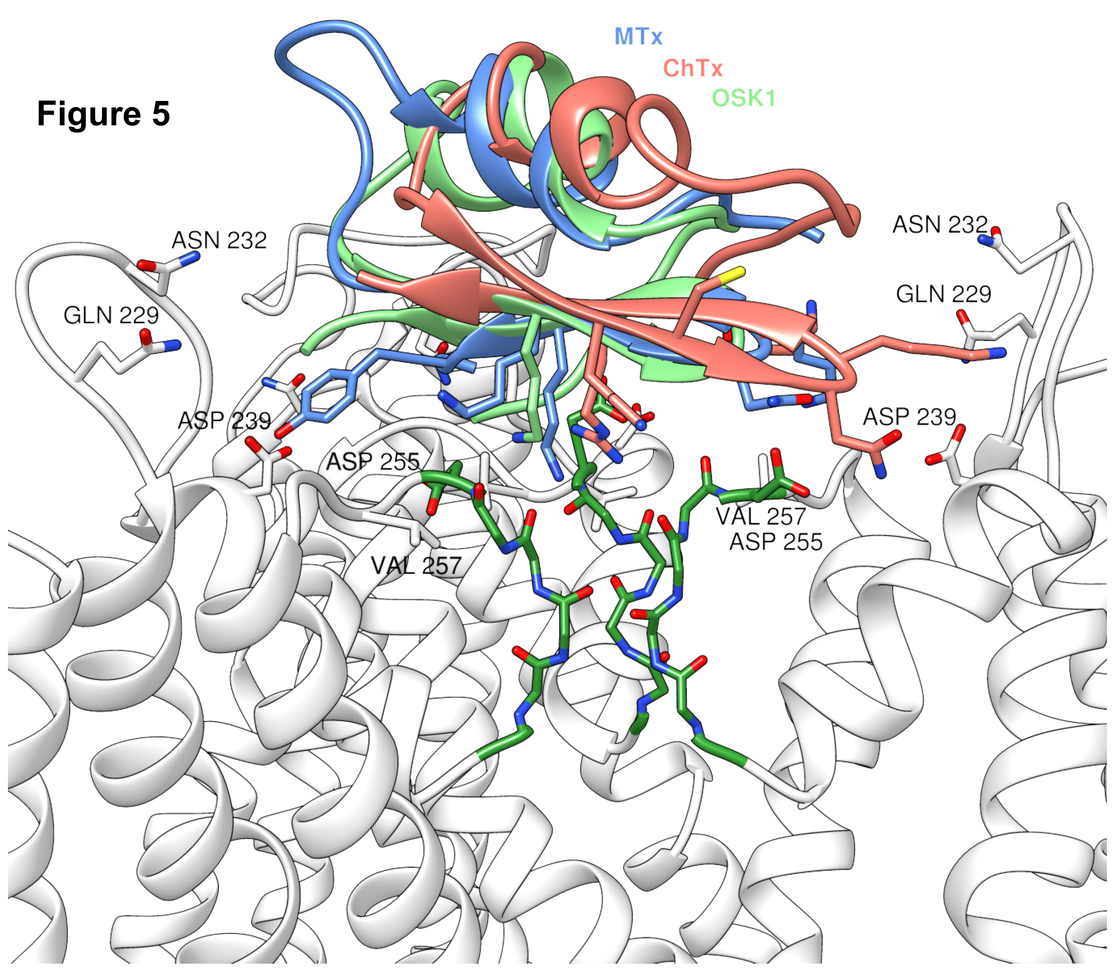
**Figure 4**




| Receptor | Identified residues |
|---|---|
| KCa3.1 | Gln229, Asn232, Asp239, Tyr253, Gly254, Asp255, Val257 |

**Figure 4 and 5:** In both figures important residues of the toxins as well as the receptor are depicted as sticks, in order to visualize the interactions.
The three toxins have the following colors: OSK1 is green, ChTx is orange and MTx is blue. The amino acids forming the selectivity filter of KCa3.1 are colored dark green.
In figure 4 the starting poses of the three simulations are displayed.
Figure 5 shows the last frame of the currently on-going simulations.
The positions of the toxins do not change significantly, thereby confirming the validity of the docking results.

**Figure 5**




## Conclusion and Outlook:

In this work the importance of the pore blocking lysine of the three toxins was confirmed. Moreover, common residues of the receptor responsible for this binding were identified. Further studies are needed to validate these results, i.e., the simulations are currently extended, or replicas will be made. This knowledge will be used to define constraints for a virtual screening campaign to identify small molecules that bind to the outer pore of the channel. Additionally, these results are useful for the design of small peptides that have a higher specificity for KCa3.1 than the here examined toxins. Therefore, a binding mode comparison of these toxins to other potassium ion channels is needed to identify interactions that enhance the selectivity.

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References:
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