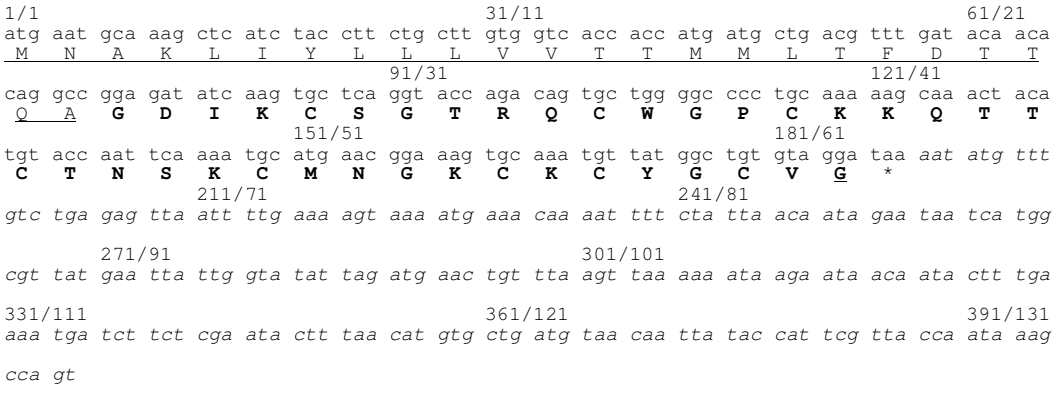
Structure, molecular modeling and function of the first potassium channel blocker, urotoxin, isolated from the venom of the Australian scorpion *Urodacus yaschenkoi*. Karen Luna-Ramírez, Adam Bartok, Rita Restano-Cassulini, Veronica Quintero-Hernández, Fredy I.V. Coronas, Janni Christensen, Christine E. Wright, Gyorgy Panyi and Lourival D. Possani. Molecular Pharmacology

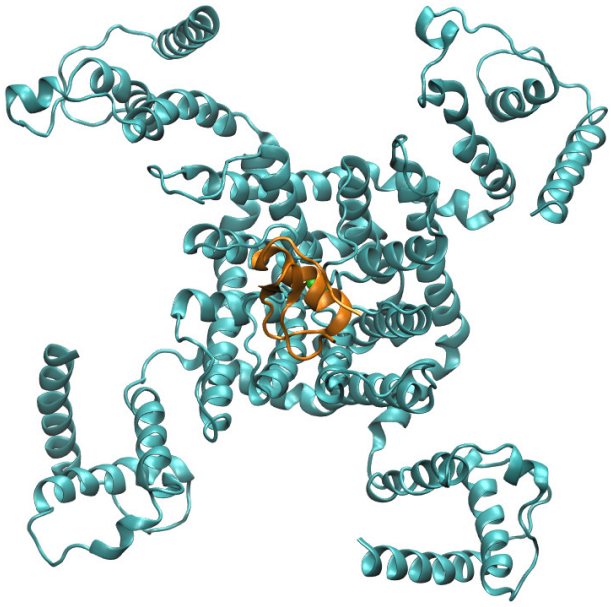
15. Supplemental Data



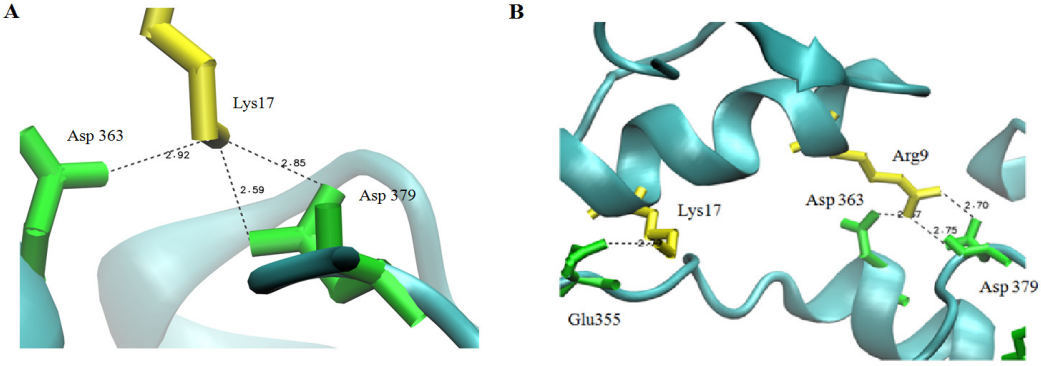
Supplemental Figure 1. Sequence of DNA encoding urotoxin and its translation.

The DNA sequencing of urotoxin was obtained as described in Materials and Methods. The sequence of DNA encoding urotoxin and its translation are represented with the following individual components, corresponding to the open reading frame: signal peptide (underlined), mature peptide corresponding to urotoxin (amino acids in bold), amino acid involved in post-translational modification (amidation): G62 (Bold and underlined), stop codon (asterisk), 3'-UTR (italics). The nucleotide sequence of urotoxin reported in this paper has been submitted to GenBank under the accession number: KC818423.

Structure, molecular modeling and function of the first potassium channel blocker, urotoxin, isolated from the venom of the Australian scorpion *Urodacus yaschenkoi*. Karen Luna-Ramírez, Adam Bartok, Rita Restano-Cassulini, Veronica Quintero-Hernández, Fredy I.V. Coronas, Janni Christensen, Christine E. Wright, Gyorgy Panyi and Lourival D. Possani. Molecular Pharmacology

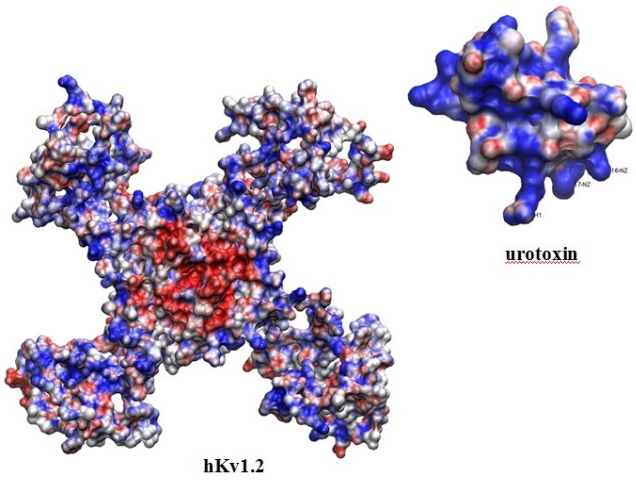


Supplemental Figure 2. Bound state of urotoxin with hKv 1.2. Position of the toxin when forming one of the most recurring bound states.

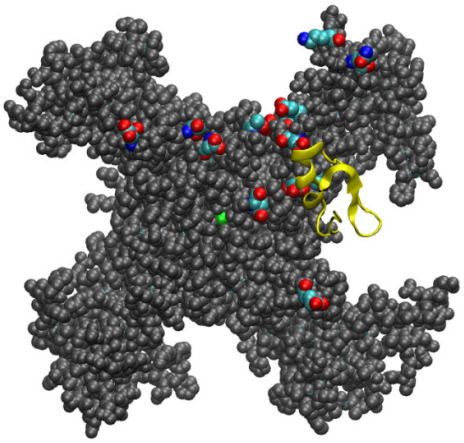


Supplemental Figure 3. Bound states of urotoxin and hKv1.2. (A) Lys17 is forming an H-network with Asp**363** and Asp**379**. (B) Lys17-Glu**355** are forming an H-bond occluding the ion conduction and, at the same time on the other side of urotoxin's α-helix, Arg9 forms an H-network with Asp**363** and Asp**379**.

Structure, molecular modeling and function of the first potassium channel blocker, urotoxin, isolated from the venom of the Australian scorpion *Urodacus yaschenkoi*. Karen Luna-Ramírez, Adam Bartok, Rita Restano-Cassulini, Veronica Quintero-Hernández, Fredy I.V. Coronas, Janni Christensen, Christine E. Wright, Gyorgy Panyi and Lourival D. Possani. Molecular Pharmacology

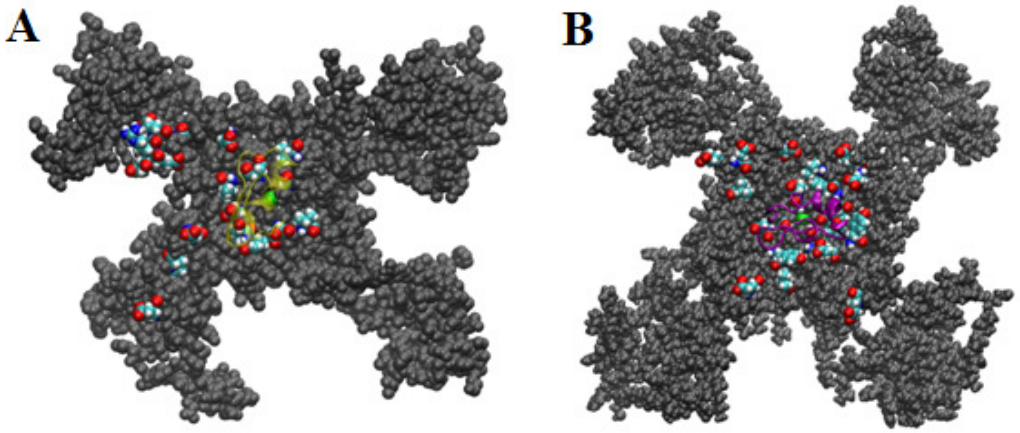


Supplemental Figure 4. Electrostatic surfaces of the human Kv1.2 channel and urotoxin. The negatively charged pore region of hKv1.2 (red) and the positively charged surface of urotoxin (blue) facilitates their interaction.

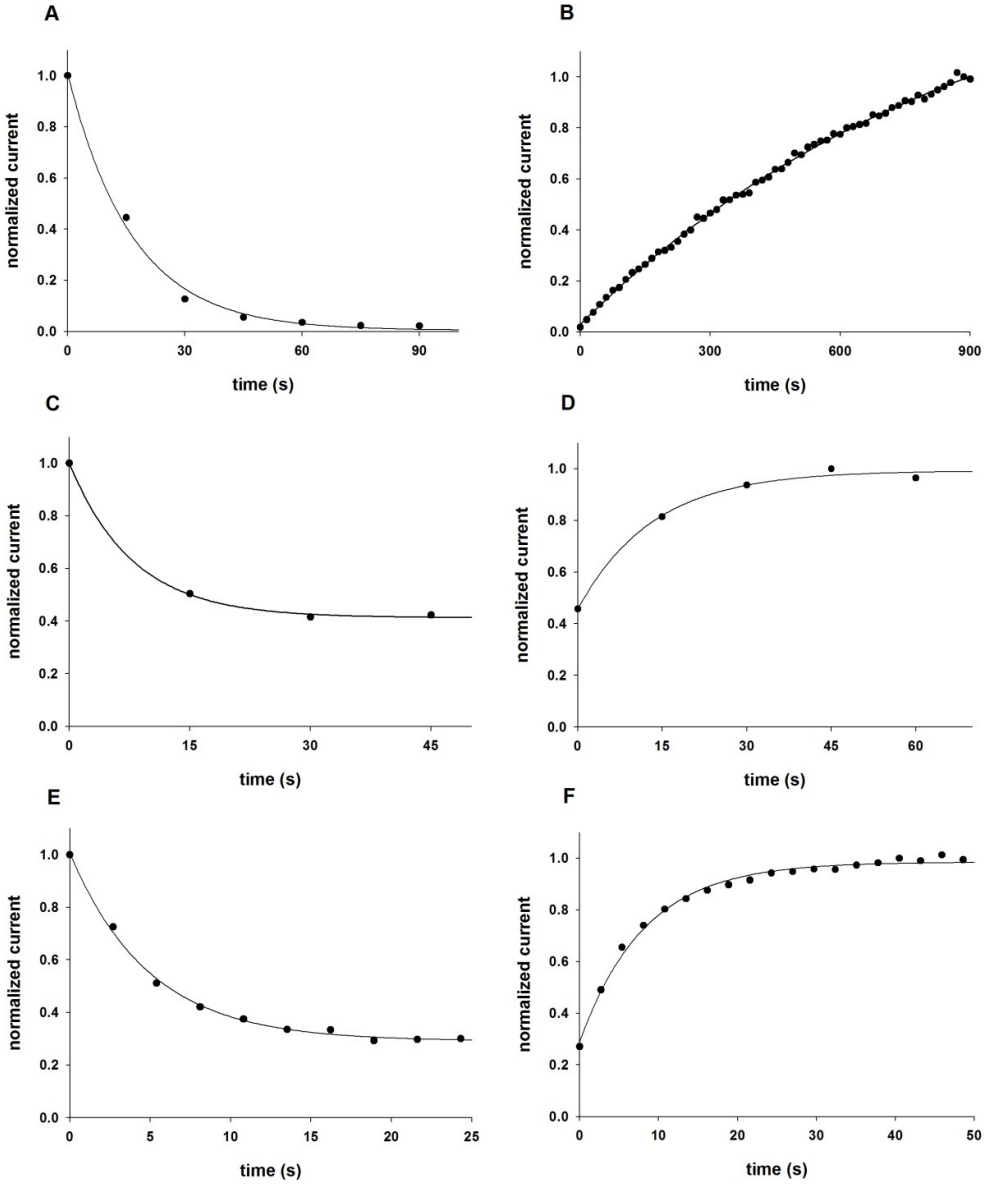


Supplemental Figure 5. Average position of urotoxin with respect to hKv1.1 during the MD simulation. Urotoxin in yellow is positioned close to the turret region (or far away from the pore, compare to the urotoxin-Kv1.2 complex in Fig. 9). The channel is in grey. Highlighted (in color) are the most active residues forming H-bonds and salt bridges during the 500 ns simulation.

Structure, molecular modeling and function of the first potassium channel blocker, urotoxin, isolated from the venom of the Australian scorpion *Urodacus yaschenkoi*. Karen Luna-Ramírez, Adam Bartok, Rita Restano-Cassulini, Veronica Quintero-Hernández, Fredy I.V. Coronas, Janni Christensen, Christine E. Wright, Gyorgy Panyi and Lourival D. Possani. Molecular Pharmacology



Supplemental Figure 6. MD with a mutant-urotoxin. **(A)** K25A-urotoxin with hKv1.2 channel. Average position of the mutant toxin throughout the simulation. The image shows how the toxin (in light yellow) is positioned above the pore suggesting the binding is stronger without K25. Highlighted in color are the most active residues forming H-bonds and salt bridges during the whole simulation. (**B**) K25A-urotoxin with hKv1.1 channel. Average position of mutant toxin (in purple) throughout the simulation. The toxin is above the pore and the residues highlighted in color are the most active in forming H-bonds and salt bridges.



Supplemental Figure 7. Peak currents of hKv1.2 **(A,B),** KCa3.1 **(C,D) and** hKv1.1 **(E,F)** were recorded during the wash-in (**A,C,E**) and wash-out (**B,D,F**) procedure. To determine the time constant of the association a single exponential function was fitted to the data points: $A(t) = B \times \exp(-t/T_{ON}) + C$, where $A(t)$ indicates the amplitude of the current at time t, C is the peak current at equilibrium block and $B = A(t = 0) - C$. The time constant (T_{ON}) yielded 18.6 ± 1.2 s at 10 nM urotoxin for hKv1.2, (N = 6) **(A)**, 7.9 ± 3.7 s, (n = 3) at 30 nM urotoxin for hKv1.3 and 5.7 ± 0.8 s, (N = 4) at 1 μM

urotoxin for hKv1.1. The wash-out kinetics was also fitted using a single exponential function: $A(t) = B \times (1 - \exp(-t/T_{OFF})) + C$, where $B = A(t = \infty) - C$, A(t) indicates the amplitude of the measured current at time t, C is the peak current at equilibrium block. The resulting time constants (T_{OFF}) were 958.6 ± 68.1 s (n = 4), 14.4 ± 0.6 s (n = 3) and 8.1 ± 0.5 s (n = 4) for hKv1.2, hKCa3.2 and hKv1.1, respectively.

Structure, molecular modeling and function of the first potassium channel blocker, urotoxin, isolated from the venom of the Australian scorpion *Urodacus yaschenkoi*. Karen Luna-Ramírez, Adam Bartok, Rita Restano-Cassulini, Veronica Quintero-Hernández, Fredy I.V. Coronas, Janni Christensen, Christine E. Wright, Gyorgy Panyi and Lourival D. Possani. Molecular Pharmacology

Supplemental Table 1. Residues forming **H-bond** during the 500 ns brute force simulation for the urotoxin-**hKv1.2** channel complex. Chains A, B, C and D belong to the channel while urotoxin is channel F. Atoms NHx or ODx form the H-bonds. Bold letters indicate residues in the pore region of the channel.

Farg9NH2_Dglu353OE1	Farg9NH2_Dser356OG	Fgln10NZ_Basp379OD1	Flys16NZ_B**asp363**OD1	Flys16NZ_Dgln357OD1
Farg9NH2_Dglu353OE2		Fgln10NZ_Basp379OD2	Flys16NZ_B**asp363**OD2	
	Flys16NZ_Dser356OG			Flys32NZ_Bglu357OE1
Flys16NZ_Dglu353OE1		Flys16NZ_Basp379OD2	Flys17NZ_B**asp363**OD1	
Flys16NZ_Dglu353OE2			Flys17NZ_B**asp363**OD2	Fthr20OG1_Bgln357NE2
		Flys4NZ_Dasp379OE1		Fthr20OG1_Bgln357NE2
Flys25NZ_Dglu353OE1		Flys4NZ_Dasp379OE2		
Flys25NZ_Dglu353OE2				
		Flys16NZ_Basp379OD1		
		Flys17NZ_Dasp379OD1		
		Flys17NZ_Dasp379OD2		

Supplemental Table 2. Residues forming **salt bridges** during the 500 ns brute force simulation for the **hKv1.2**-urotoxin channel complex. Chains A, B, C and D belong to the channel while urotoxin is channel F. Bold letters indicate residues in the pore region of the channel.

Asp352A_lys16F	**asp363**B_arg9F	asp379A_arg9F	glu353A_lys16F	glu355A_arg9F
	asp363B_lys16F		glu353A_lys32F	glu355A_lys4F
	asp363B_lys17F	asp379B_arg9F		glu355A_lys16F
		asp379B_lys4F	glu353D_arg9F	glu355A_lys17F
	asp363C_lys16F	asp379B_lys16F	glu353D_lys16F	glu355A_lys25
	asp363C_lys17F	asp379B_lys17F	glu353D_lys25F	
		asp379B_lys30F		glu355B_lys16F
	asp363D_lys17F			glu355B_lys17F
		asp379D_arg9F		glu355B_lys32F
		asp379D_lys4F		
		asp379D_lys17F		glu355D_arg9F

Supplemental Table 3. Residues forming **H-bond** during the 500 ns brute force simulation for the urotoxin-**hKv1.1** channel complex. Chains A, B, C and D belong to the channel while urotoxin is channel F. Atoms (NHx or ODx) form the H-bonds. Red residues indicate residues in the selectivity filter region of the channel.

C377AspOD1_F25LysNZ	B351GluOE1_F28AsnND2	B353GluOE1_F30LysNZ	C375TyrO_F9ArgNH2	D379Tyr_F9ArgNH1
C377AspOD2_F25LysNZ	B351GluOE2_F28AsnND2	B353GluOE2_F30LysNZ		
B377AspOD1_F25LysNZ				
B377AspOD2_F25LysNZ				
D377AspOD1_F9ArgNH1				
D377AspOD2_F9ArgNH1				

Supplemental Table 4. Residues forming **salt bridges** during the 500 ns brute force simulation for the **urotoxin-hKv1.1** channel complex. Chains A, B, C and D belong to the channel while urotoxin is channel F. Bold letters indicate residues in the pore region of the channel.

B377Asp_F25Lys	BGlu353_F30Lys	B351GLu_F30Lys	**A361Asp**_F9Arg
C377Asp_F25Lys			
	B353Glu_F32Lys	C351Glu_F9Arg	**C361Asp**_F25Lys
A377Asp_F4Lys			
B377Asp_F4Lys	A353Glu_F16Lys	C351Glu_F25Lys	
B377Asp_F30Lys	C353Glu_F9Arg		
D377Asp_F9Arg			