

Supplemental material

Table S1. Oligonucleotides used for Osu1 gene synthesis					
Name	Sequence (5' – 3')				
Osul -Upl	GAGGGATCCATCGAGGGACGCCGCCTGGCGCTTCCGCCTGGCGCCGT GTGCAACGGTCATAAGAGTG				
Osu1 -Lw2	CCAAAAGAACGGGCAGCTACACTTATATTTCGCGCCAAAGCACTGAC AATCACTCTTATGACCGTTGCAC				
Osu1 -Up3	GCTGCCCGTTCTTTTGGCGTTTCCGCAAAAGTGCAGAATGTCACTGCA AGAAAGGTTGGGCGTGGACC				
Osu1 -Lw4	CTCCTGCAGCTATTAGTCACTCCATTGGTAGCGATTATGACAGCTACG TTTCTTAATGGCGGTCCACGCCCAACC				

Table S2. Animal venoms tested on hK_v 1.5.

Scorpions	Spiders	Others
Androctonus crassicauda	Aphonopelma serratum	Paragaleodes pallidus
Centruroides tecomanus	Brachypelma albiceps	Ambrysus lunatus
Centruroides suffusus suffusus	Brachypelma auratum	
Centruroides noxius	Brachypelma smithi	
Centruroides limpidus	Dolomedes gertschi	
Centruroides gracilis	Drassodes sp.	
Hadrurus gertschi	Dysdera sp.	
Hadrurus sp	Eresus sp.	
	Gnaphosa taurica	
	Lachesana sp.	
	Misumena vatia	
	Oculicosa supermirabilis	
	Palpimanus sp.	
	Phidippus octopunctatus	
	Phyxioshema sp.	
	Pisaura mirabilis	
	Sparassus sp.	
	Steatoda grossa	
	Thomisus onustus	
	Tibellus oblongus	

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Table S3. Comparative table that show statistical parameters of different Osu1 models obtained from 3D prediction software

	I-Tasser	Modeller	Swiss-model	Robetta
Method	Threading	Homology	Homology	Ab-initio
	Cys10-Cys26	Cys10-Cys26	Cys10-Cys26	Cys10-Cys42
Cys-Cys bonds	Cys17-Cys56	Cys17-Cys56	Cys17-Cys56	Cys17-Cys28
	Cys19-Cys42	Cys19-Cys42	Cys19-Cys42	Cys19-Cys56
	Cys28-Cys40	Cys28-Cys40	Cys28-Cys40	Cys26-Cys40
RMSD	5.3	3.8	4.4	11.3
Ramachandran plot MFR (%)	36.4	90.9	63.3	89.1
Ramachandran plot AAR (%)	52.7	5.5	34.7	9.1
Ramachandran plot GAR (%)	9.1	1.8	2	0
Ramachandran plot DR (%)	1.8	1.8	0	1.8
Secondary structure	Antiparallel	Antiparallel and helix	Antiparallel and helix	Antiparallel and helix

MFR=Most favored regions AAR=Additional allowed regions

GAR=Generously allowed regions **DR**= Disallowed regions



Figure S1. Reverse-phase separation of alkylated fractions. The alkylated native Osu1 was enzymatically cleaved by Lys-C. The chromatographic separation was performed using an analytical C₁₈ column and a gradient of aqueous acetonitrile containing 0.1% TFA (solution B), starting after 5 min from 0 to 60% CH₃CN during 60 min at a flow rate of 1 mL/min. Mass spectra in panels A, B and C correspond to HPLC fractions to retention times 22.4, 31.9 and 32.5 min (a, b and c), respectively. Mass spectrum in panel D corresponds to the full alkylated Osu1.





Figure S2. Endoproteolytic cleavage of rOsu1 by Factor Xa. Lane 1, Molecular weight markers; Lane
2, rOsu1 control sample; Lanes 3-6, cleavage for 9 h. Lane 8, rOsu1 control sample; Lanes 9-12, cleavage
for 16 h. The Fxa to rOsu1 ratio is shown. 15% SDS-PAGE.





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Figure S3. Comparative reverse-phase separation of rOsu1 and native Osu1. Superimposed in this chromatogram is a profile of rOsu1 (solid line) and native Osu1 (dashed line). Chromatographic separation was performed in a RP-HPLC using an analytical C₁₈ column and a gradient of aqueous acetonitrile containing 0.1% TFA (solution B), starting after 5 min from 20 to 60% CH₃CN during 40 min at a flow rate of 1 mL/min.

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Figure S4. Recombinant *Osu1* effect is not reversible on $hK_v 1.5$. Panel A shows the current traces recorded under control conditions (black) and in the presence of recombinant Osu1 (3 μ M; orange) as a response to +50 mV depolarization. Panel B displays the peak current amplitudes during +50 mV depolarization pulse. Black horizontal bars represent that the peak currents were measured under control conditions; orange bar indicates the presence of Osu1 in the external solution and green bar refers to perfusion with high potassium external solution (HK; 150 mM K⁺) to test the perfusion system on this given cell.

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- 72 Figura S5. Three-dimensional models of Osu1 obtained from different modeling programs. A) I-
- 73 Tasser. B) Modeller. C) Swiss-model. D) Robetta.





Figure S6. Osu1 models generate by the server Modeller. A) Distribution of 10,000 models generated
 by Modeller. B) Distribution of density population of 10,000 models generated by Modeller. C)
 Ramachandran graphics for the best model according to the percentage of residues in the favorable regions.