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Discovery of Novel Ion Channel Modulators from *Physalia physalis*

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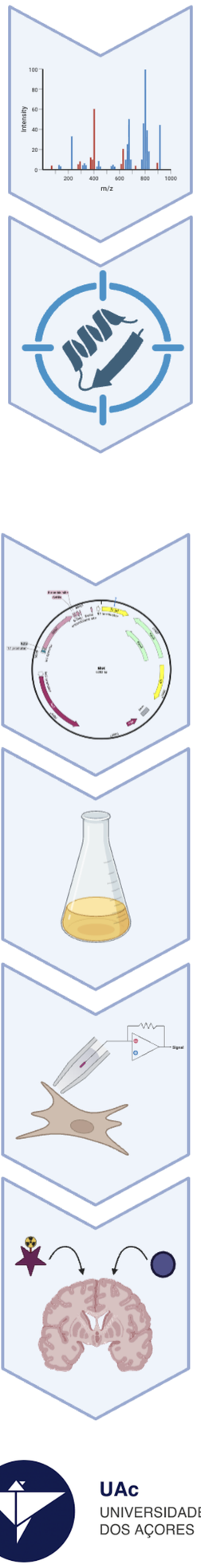
INTRODUCTION & AIM

Animal venoms are increasingly recognized as rich sources of bioactive molecules with therapeutic potential, particularly peptides targeting voltage-gated ion channels, which play critical roles in physiological processes and are implicated in a wide range of diseases. Among these, ShK-like peptides, originally discovered in sea anemone venom, have emerged as potent and selective modulators of Kv channels, highlighting the potential of venom-derived molecules in drug development. In this study, we report the discovery of 30 ShK-like domains in the venom of *Physalia physalis*, identified through proteomic analysis and predicted to selectively modulate specific Kv channel subtypes. **The aim of this work was to identify, characterize, and functionally validate one of these novel peptides, with a particular focus on their inhibitory activity against the Kv1.3 channel, a key therapeutic target involved in autoimmune disorders, pain, and neurological diseases.**

METHOD



Proteomic analysis

Venom was analyzed by LC-MS/MS (CIEX TripleTOF 6600), and spectra were searched against NCBI (Cnidaria-restricted) and a custom transcriptome-derived database (NCBI Accession SUB13325220).

Structure prediction and docking

3D structures of the ShK-like peptides were predicted using AlphaFold2 and subsequently docked against a panel of human Kv1 channels with ZDOCK.

Selection of the peptide based on the docking results

ShK26_D5

Vector construction

One of the most promising ShK variant was expressed in *E. coli* using the pET-40 vector, targeted to the periplasmic space in fusion with DsbC to promote correct disulfide bond formation, and carrying a His-tag.

Heterologous expression

Protein production was carried out in *E. coli* C41 using AI medium ZYM-5052 (2h at 37°C followed by 6h at 30°C, yield approx. 1 g/L pellet per cell culture) and purified by FPLC using a HisTrap and Desalting column.

Target validation

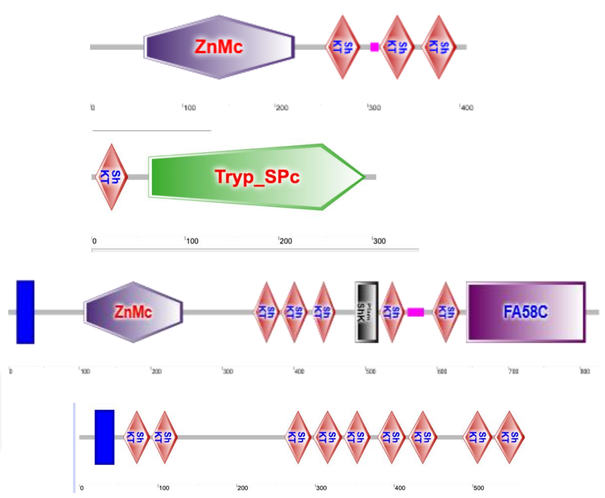
Functional characterization was performed using automated whole-cell patch-clamp electrophysiology (SynchroPatch 384i) on fibroblasts heterologously transfected with Kv1.3, and Quinidine as the reference compound. A peak pulse protocol, from a holding potential of -80 mV to +60 mV over 300 ms, was applied every 20 seconds to monitor the outward current.

Radioligand competition assay

Evaluation of the affinity of the compound (10 µM) for the Kv channel in the human cerebral cortex was determined in a competition assay with radiolabeled α-[125I]dendrotoxin (20nM and 50nM for non-specific binding).

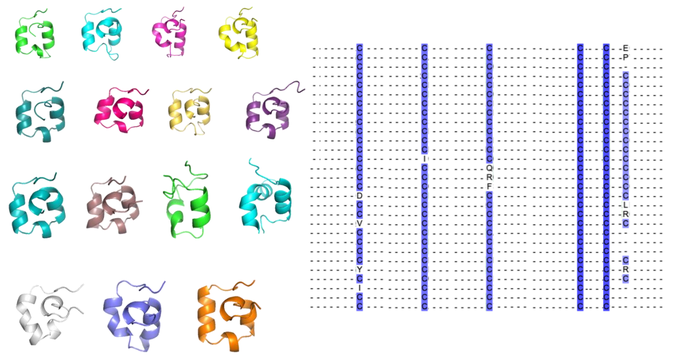
RESULTS & DISCUSSION

1. Proteomic analysis of *P. physalis* venom revealed the presence of multiple ShK-like domains.



Multiple ShK-like peptides tandemly arranged in a single coding sequence.

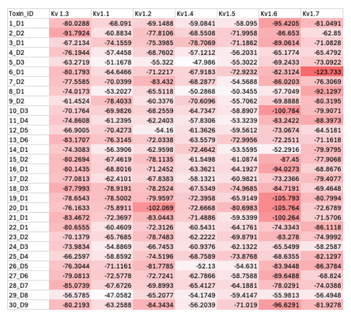
2. AI-based platform predicts the 3D structure of the peptides with highly conserved *Cys* residues.



X-C[1]-XXXXX-C[2]-XXXXX-C[3]-XXXXXXXX-C[4]-XXX-C[5]-XX-C[6]-X

- **Highly conserved** 2-3 **disulfide bridges** that stabilize the structure
- 33-47 residues

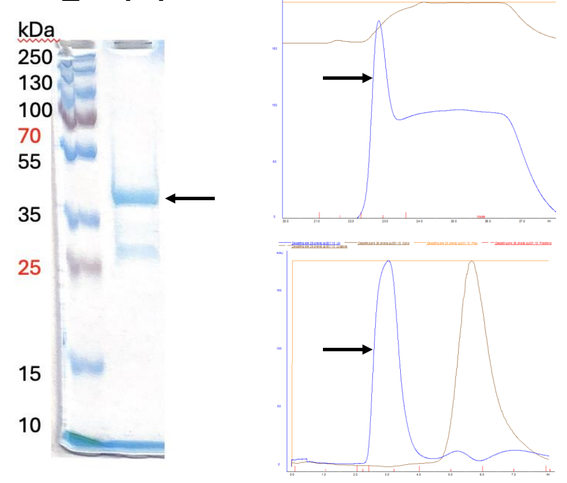
3. Docking against the panel of human Kv1 channels reveals peptides with Kv1.3 preference.



Toxin_ID	Kv 1.3	Kv1.1	Kv1.2	Kv1.4	Kv1.5	Kv1.6	Kv1.7
1_D1	-80.0288	-68.091	-69.1488	-59.0841	-58.095	-95.4205	-81.0491
2_D2	-91.7924	-60.8834	-77.8106	-68.5508	-71.9958	-86.653	-62.85
3_D1	-67.2134	-74.1559	-75.3985	-78.7069	-71.1862	-89.0614	-71.0828
4_D2	-76.1944	-57.4458	-68.7602	-57.1212	-56.2031	-65.1774	-65.4792
5_D3	-63.2719	-51.1678	-55.322	-47.986	-55.3022	-69.2433	-73.0922
6_D1	-80.1793	-64.6466	-71.2217	-67.9183	-72.9232	-82.3124	-123.733
7_D2	-77.5585	-70.0399	-83.432	-68.2877	-54.5688	-86.0203	-76.3069
8_D1	-74.0173	-53.2027	-65.5118	-50.2868	-59.3455	-57.7049	-92.1297
9_D2	-61.4524	-78.4033	-60.3376	-70.6096	-55.7062	-69.8848	-60.3195
10_D3	-70.1764	-69.9826	-68.2559	-64.7347	-58.8907	-100.784	-79.9071
11_D4	-74.8608	-61.2395	-62.2403	-57.8306	-53.3239	-83.2422	-88.3973
12_D5	-66.9005	-70.4273	-64.16	-61.3626	-59.5612	-73.0674	-64.5181
13_D6	-83.1707	-76.3145	-72.0338	-63.5579	-72.9956	-72.2511	-71.1618
14_D1	-74.3083	-56.3906	-62.9598	-72.4642	-53.5595	-52.2916	-79.9795
15_D2	-80.2694	-67.4619	-78.1135	-61.5498	-61.0874	-87.45	-77.9068
16_D1	-80.1435	-68.8016	-71.2452	-63.3621	-64.1927	-94.0273	-68.8676
17_D2	-77.0813	-62.4101	-67.8383	-58.1321	-60.9821	-73.2366	-79.4077
18_D3	-87.7993	-78.9191	-78.2524	-67.5349	-74.9685	-84.7191	-69.4648
19_D1	-78.6543	-78.5002	-79.9597	-72.3958	-65.9149	-105.793	-80.7994
20_D1	-76.1633	-75.8911	-102.069	-72.6668	-80.6983	-105.764	-72.6789
21_D1	-83.4672	-72.3697	-83.0443	-71.4886	-59.5399	-100.264	-71.5706
22_D1	-80.6555	-60.4609	-72.3126	-60.5431	-64.1761	-74.3343	-86.1118
23_D2	-70.1379	-65.7685	-78.7483	-62.2222	-69.8791	-83.278	-74.9992
24_D3	-73.9834	-54.8869	-66.7453	-60.9376	-62.1322	-65.5499	-58.2587
25_D4	-66.2597	-58.8592	-74.5196	-68.7589	-73.8768	-68.6355	-82.1297
26_D5	-76.3044	-71.1161	-81.7785	-52.13	-54.631	-83.9448	-86.3784
27_D6	-79.0813	-72.5778	-72.7241	-62.7866	-58.7588	-89.6488	-68.824
28_D7	-85.0739	-67.6726	-69.8993	-65.4127	-64.1881	-78.0291	-74.0386
29_D8	-66.5785	-47.0582	-65.2077	-54.1749	-59.4147	-55.9813	-66.4948
30_D9	-80.2193	-63.2588	-64.3434	-56.2039	-71.019	-96.6291	-81.9278

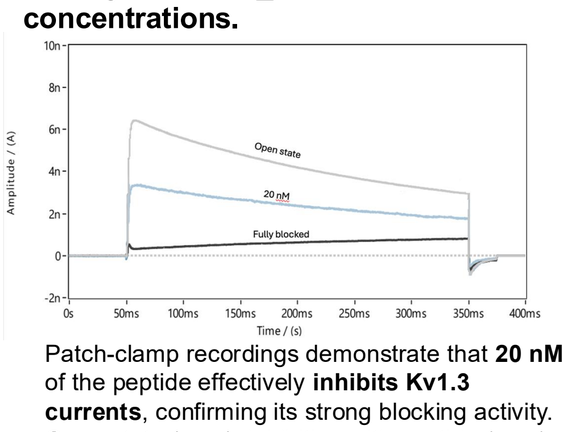
Peptide ShK26_D5 was selected for recombinant production based on its **low binding energy** and **direct binding to the pore** of the Kv1.3. Green structure represents the pore of the Kv1.3 channel while red represents ShK26_D5 peptide.

4. Expression and purification of the ShK26_D5 peptide.



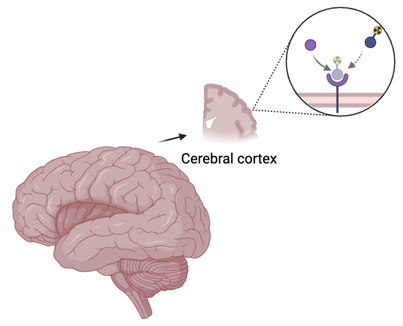
ShK26_D5 was expressed in a **soluble form**. Upper chromatogram: His-tag purification; lower chromatogram: desalting.

5. Whole-cell patch-clamp confirmed activity of ShK26_D5 at nanomolar concentrations.



Patch-clamp recordings demonstrate that **20 nM** of the peptide effectively **inhibits Kv1.3 currents**, confirming its strong blocking activity. Open state (grey), inhibition with peptide (blue), and fully blocked channel (black).

6. Competition assay on a cerebral cortex tissue with radiolabeled α-[125I]dendrotoxin



The recombinant ShK26_D5 peptide showed **low competition (4%) against Kv1.1, Kv1.2, and Kv1.6, even at 200x higher concentrations** than the reference compound, which minimizes the risk of neurological side effects.

CONCLUSION

- *Physalia physalis* venom **contains 30 ShK-like peptides with potential as selective Kv channel modulators**.
- The recombinant ShK26_D5 peptide showed **blocking activity on the Kv1.3** channel, with minimal competition against Kv1.1, Kv1.2, and Kv1.6, reducing the likelihood of off-target interactions associated with severe side effects, placing it **as a promising candidate for therapeutic applications in Kv1.3-mediated disorders.**

FUTURE WORK / REFERENCES

- Future studies should address functional validation in neuronal and immune cells and in a vertebrate model to evaluate therapeutic potential and safety.

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