Fishing for Function: From Predicted Binding to Functional Block by a Cone Snail Peptide

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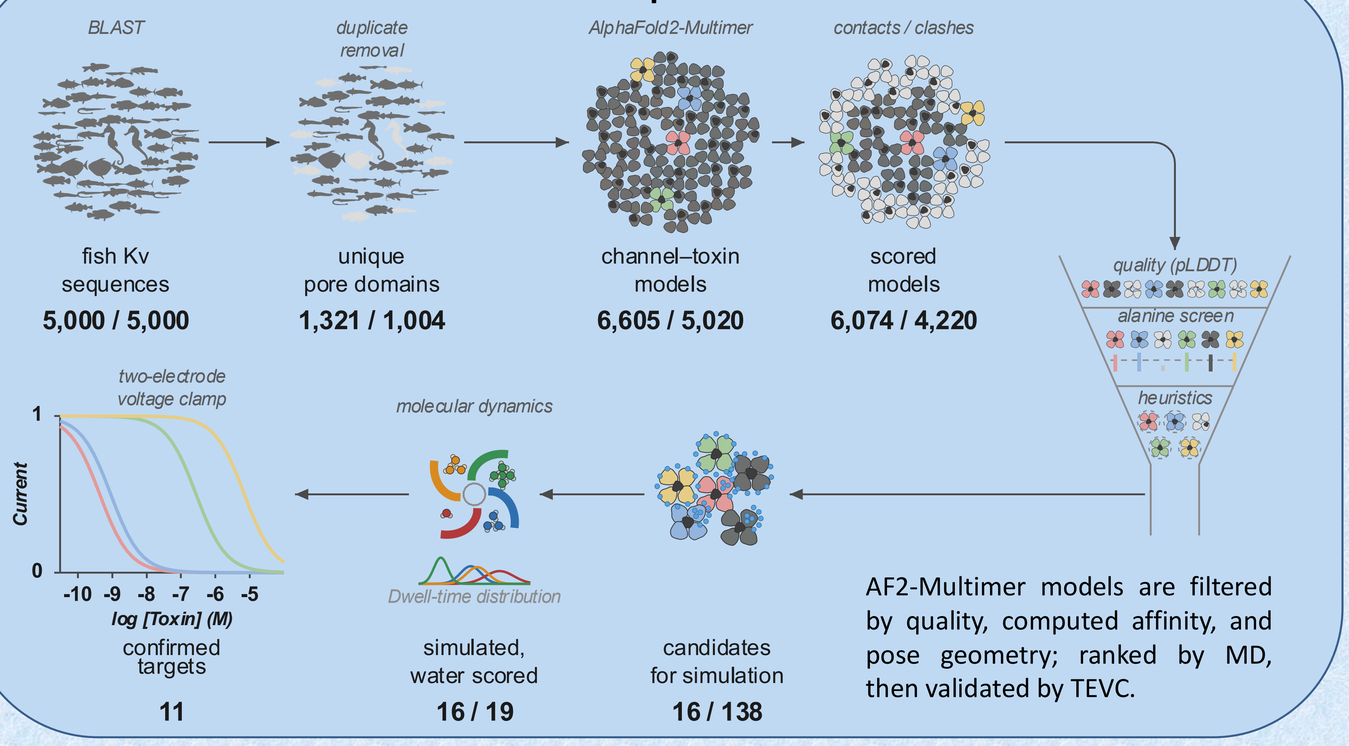
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Abstract

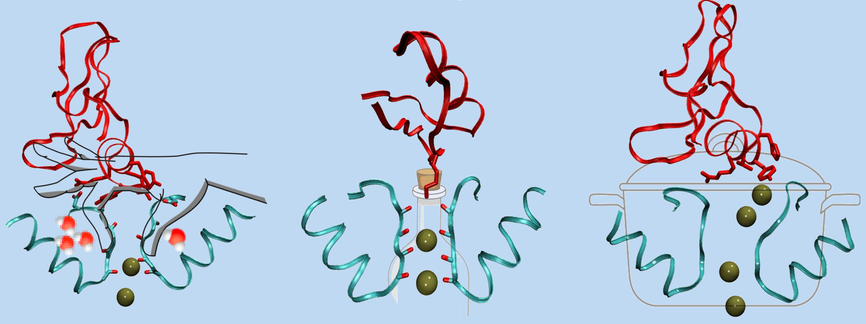
Pore-modulating peptide toxins block K^+ channels by perturbing water dynamics around the selectivity filter (SF). The cone-snail toxin Conkunitzin-S1 (Cs1), the first identified toxin of this class, was initially characterized on the Drosophila Shaker channel, but its natural targets remained elusive. Initial screening with conventional ML-based methods yielded numerous false positives. We therefore asked whether molecular dynamics, informed by the toxin's mode of action, could pinpoint the true targets. To test this, we combined AlphaFold2-Multimer structure prediction with automated molecular dynamics screening and an Equivariant Transformer (ET3) trained to classify the water network surrounding the SF. Screening over 2,300 unique fish K^+ channel sequences for toxin-induced water asymmetry identified eleven previously unknown high-affinity Cs1 targets, validated electrophysiologically (EC_{50} 0.9–8.7 nM). Crucially, resistant channels bound Cs1 in the same pose and remained stably associated throughout, showing that neither structural compatibility nor binding stability predicts peptide activity. Instead, sensitivity depended on the channel's intrinsic water dynamics: a single residue, Shaker 449, determines whether the asymmetry Cs1 induces becomes sustained and structured. Our computational framework showcases the power of integrating structure prediction, machine learning, and mechanism-aware molecular dynamics to decode toxin-channel interactions, opening new possibilities for in silico ecological research and drug interaction studies.

The Pipeline



AF2-Multimer models are filtered by quality, computed affinity, and pose geometry; ranked by MD, then validated by TEVC.

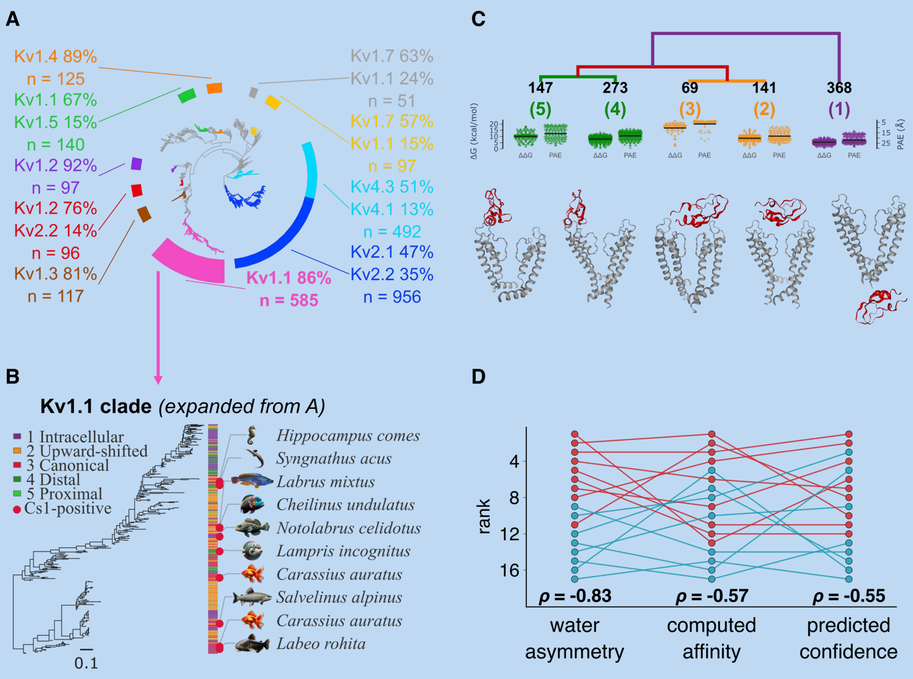
K^+ Pore-Blockers : Modes of action



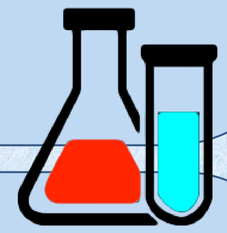
Voltage-gated potassium channels (K_vs) mediate the flow of potassium ions in and out of cells, playing a crucial role in cell signaling and regulation. These channels are targeted by multiple animal toxins which block ion conduction, leading to toxic outcomes. Classical scorpion K_v blockers physically occlude the ion permeation pathway by inserting a charged Lysine residue into the pore, akin to a “Cork” or a “Plug”. Recently , we described two alternative modes of action for K_v channel blockers [1,2]. The first is the “Pinch” mechanism, in which the toxin modifies the permeation of water molecules into protein cavities surrounding the channel pore, creating highly asymmetric water distributions at these regions and trigger a local collapse of the channel pore, analogous to slow inactivation. The second is the “Molecular Lid” block mechanism, in which K^+ ions are diverted and trapped in ectopic binding sites formed at the channel-toxin interface.

1. Saikia, C. *et al.* A Molecular Lid Mechanism of K+ Channel Blocker Action Revealed by a Cone Peptide. *J. Mol. Biol.* 166957 (2021) doi:10.1016/j.jmb.2021.166957.
2. Karbat, I. *et al.* Pore-modulating toxins exploit inherent slow inactivation to block K^+ channels. *Proc. Natl. Acad. Sci.* **116**, 18700–18709 (2019).

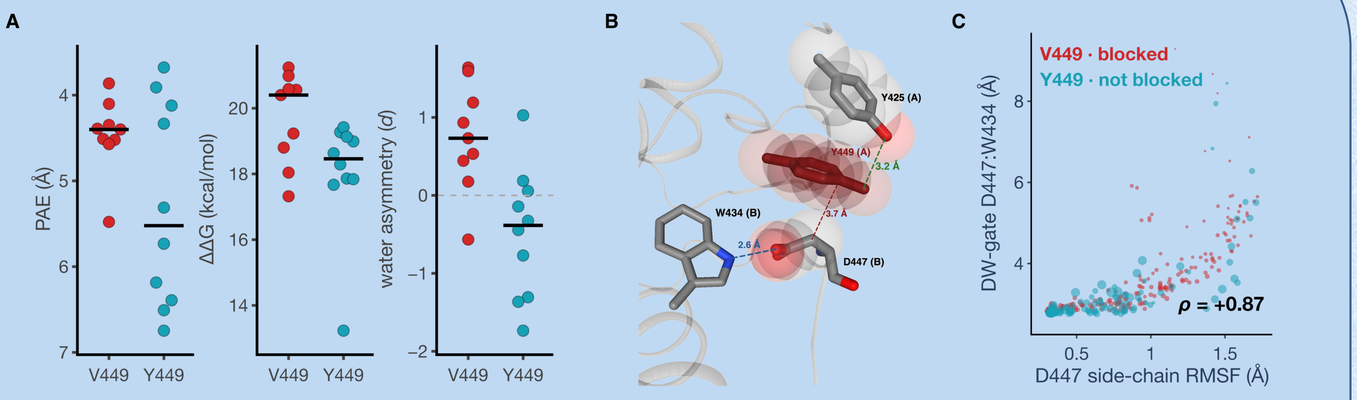
Results



All confirmed targets map exclusively to the $Kv_{1.1}$ clade (585 sequences; **A, B**) and universally adopt the tightest predicted binding pose (canonical, 69 models, 1.0 ± 0.4 Å; **C**). Among complexes adopting this pose, static affinity and interface confidence are blind to functional outcomes, falsely ranking inert binders alongside active targets (ρ = −0.57 and −0.55; **D**). Dynamic ET3 - assigned water asymmetry resolves this computational blind spot, successfully tracking actual the measured toxicity (ρ = −0.83).

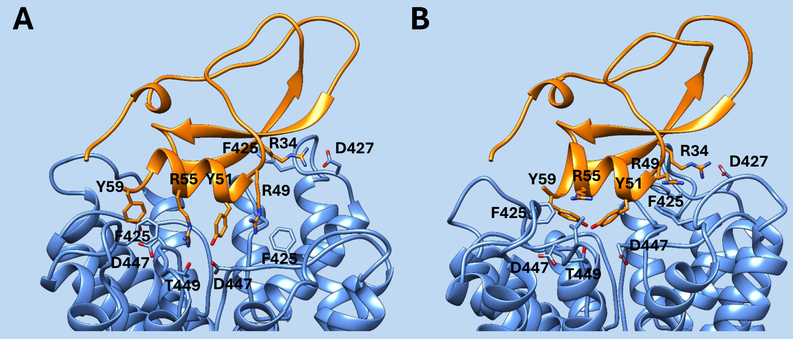


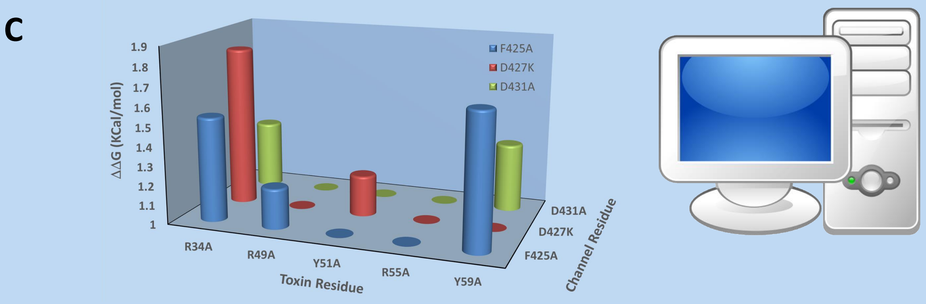
The Mechanism



A single substitution cleanly sorts the functional outcomes: Cs1 blocks channels with valine but not tyrosine at position 449. Both variants form a similar interface with the toxin, and static ML metrics rank them equally (A). The functional divergence traces to the channel’s intrinsic mechanics. To establish asymmetric water flow, Cs1 must trap the D447-W434 gate open. A tyrosine at 449 prevents this by packing against F/Y425; the resulting rigid ring restrains the adjacent subunit's D447, firmly stabilizing the closed state (B). Gate opening strictly depends on this baseline D447 mobility (C). Lacking this aromatic lock, V449 leaves D447 highly mobile, providing the flexibility the toxin requires to trap the gate open.

AlphaFold multimer model of Cs1-Shaker





AlphaFold (B) Vs Experimental (A) complex between Conkunitzin-S1 and dmK_v1.1. Experimental restraints derived from double-mutant cycle analysis and used for model validation are displayed in (**C**).

Conclusion

Distinguishing mere binding from a functional consequence remains a fundamental challenge in computational protein-peptide interaction screening. By anchoring a simulated dynamic rate to a macroscopic functional measurement, we established a generalizable strategy to separate active targets from inert binders.

- **Prospective Discovery**: Yielded 11 novel targets, all confirmed in vitro (EC_{50} 0.9–8.7 nM).
- **The Static Blind Spot:** Affinity filters failed; unresponsive channels held the toxin in the identical canonical pose throughout 200 ns simulations, forming perfectly stable but functionally inert complexes.
- **The Dynamic Solution**: The rate of asymmetric water exchange behind the filter successfully resolved the cohorts, tracking the macroscopic slow-inactivation time constant across a position 449 mutant series.

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