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Differences in binding energy for the interaction between tetrodotoxin and voltage-gated sodium channels may explain differences in channel block and whole animal resistance

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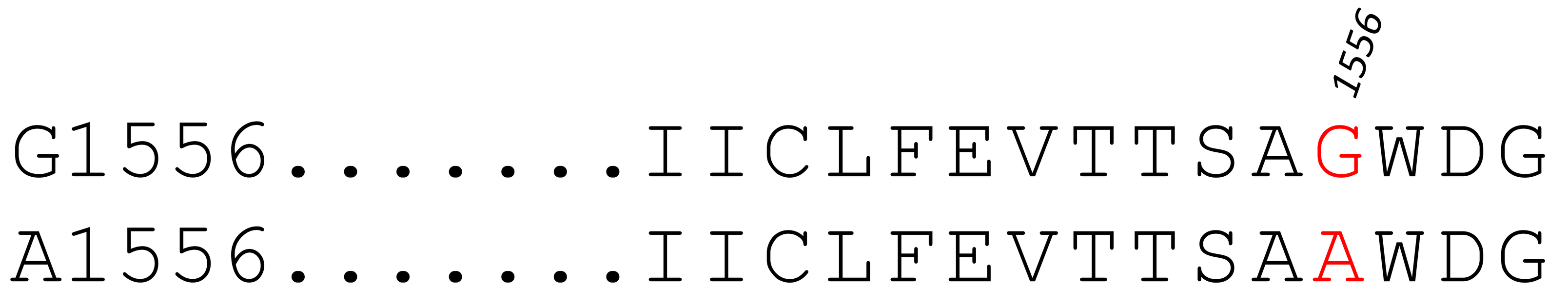
Introduction

Voltage-gated sodium ion (Na_v) channels are essential transmembrane proteins that initiate and propagate action potentials within excitable cells. In response to membrane depolarization, the channels open and conduct sodium ions into the cell.

Tetrodotoxin (TTX) is a potent toxin that specifically binds to Na_v channels. When TTX binds, it blocks the flow of sodium ions through the channel and prevents action potential generation.

Some populations of the garter snake *Thamnophis sirtalis* have evolved resistance to TTX through a few amino-acid changes in the TTX binding site of $Na_v1.4$. We know a single amino acid substitution (G1556A) changes the amount of TTX required to block $Na_v1.4$ channels. We tested if this change altered the structure of $Na_v1.4$ channels and changed the interaction between TTX and its binding site. Before this work, it was not clear how the G1556A substitution could cause a significant change in the shape of the TTX binding site of $Na_v1.4$. The work presented here demonstrates how the G1556A change might alter the interaction between TTX and$Na_v1.4$.

Figure 1 – Amino Acid Substitutions



Amino acid sequences for the identified TTX binding site in domain IV for two $Na_v1.4$ alleles. Highlighted amino acid are the single change within the sequences.

Figure 2 – TTX docking within the pores of two $Na_v1.4$ alleles

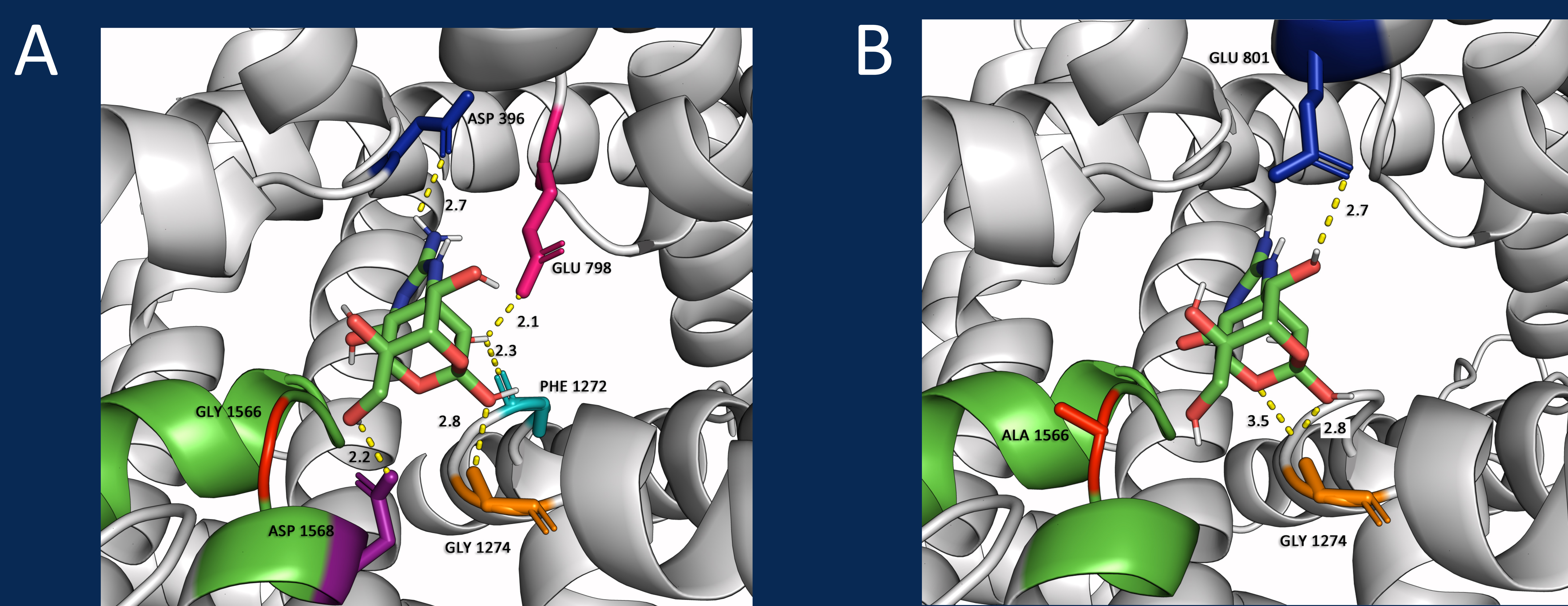


Figure A – TTX docked within the G1556 Channel.

Figure B – TTX docked within the A1556 channel.

Amino acid substitution G1556A shows a resulting cascade effect on how TTX forms bonds with atoms in the channel pore. Highlighted amino acids in the channel proteins show the specific interactions TTX has with atoms in the channel pore. The substitution G1556A is shown in red. Measurements are shown in angstroms.

Table 1- Channel Allele Comparisons

Alleles	Binding Energy	Polar Contacts
T. sirtalis G1556 $Na_v1.4$	-6.68 kcal/mol ± 0.41	5
T. sirtalis A1556 $Na_v1.4$	-6.58 kcal/mol ± 0.37	3

Table 1 shows a comparison between the two channel allele models of the measured mean binding energy (± standard deviation) for modes 1-5 as well as the number of polar contacts for mode 1.

Methods

Homology models were constructed using Swiss-Model. The resulting models with the largest QMEAN score were selected.

Auto dock and Autodock Vina were used to model ligand bonding and calculate mean binding energy.

PyMol 2.5 was used to display TTX binding sites, selected side chains, polar contacts, and associated measurements shown in angstroms.

Results

The G1556A substitution reduced the mean binding energy by -0.1 kcal/mole for the top 5 modes of TTX binding. (Table 1). When we compared the number of polar contacts for the top binding energy mode (mode 1) between the two channel alleles, we measured that the number of polar contacts were reduced by two for the A1556 allele. (Figure 2 & Table 1).

Conclusions

The measured differences between the two channel allele models, for mean binding energy (modes 1-5) and the number of polar contacts (mode 1), suggest that the G1556A amino-acid substitution may alter pore structure enough to affect TTX binding. The effect of this change in protein structure could scale up to affect channel block and snake resistance to TTX.

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