Homology models of the *A.gambiae* para voltage-gated sodium channel were generated using Modeller software

* The sodium channel structure (PDB code 5X0M) from the cockroach *Periplaneta americana* provided the template for modelling the channel in the closed conformation
* The human voltage-gated sodium channel Nav1.4 sodium channel structure (PDB code 6AGF) provided the template for modelling the channel in the open, activated conformation
* Resistance-associated mutations were introduced using SwissPDBviewer software

Model figures are shown in ribbon and coloured pink (domain I), yellow (domain II), green (domain III) and blue (domain IV). Each position of interest (either the wild-type amino acid or the mutation) is shown as orange sticks with a transparent surface. Amino acids around that position are shown as black sticks

**R245 and R245K**

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| **R245** | |
| **R245 – closed-state** | **R245 – activated-state** |
| **R245K – closed-state** | **R245K – activated-state** |
| * This is a conservative mutation of a positively-charged arginine to a positively-charged lysine. It’s located on the DI S4-S5 linker (near the bend with the S5) and there is significant movement of this linker when the channel switches between closed and activated conformations. * One possible interaction this position might make is polar/charged amino acid(s) on intracellular loops of the protein. Unfortunately the DI-DII and DII-DIII loops weren’t resolved in the cryo-EM structure so we can’t identify specific interaction candidates. * A different interaction this position may make is with lipid molecules (e.g. with polar or negatively-charged head groups). Or one intriguing possibility is that at arginine at the 245 position partitions more readily into the hydrophobic interior of the lipid bilayer than a lysine (<https://pubs.acs.org/doi/10.1021/jp4068729>) | |

**V402 and V402L**

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| **V402** | |
| **V402 – closed-state** | **V402 – activated-state** |
| **V402L – closed-state** | **V402L – activated-state** |
| * The valine-to-leucine substitution on the DI S6 helix increases the length of the hydrophobic side chain at this 402 position. New interactions are therefore possible in the mutant * V402L might interact with the asparagine on the adjacent DII S6 helix. Movement of this asparagine is associated with the S6 helix movement that gates the channel. It’s possible that leucine sterically hinders S6 movement and so inhibits/slows down channel activation * Alternatively the leucine could interfere with movement of the DI and/or DIV S6 helices. There is an aromatic ring on the DI S6 and the DIV S6 (a tyrosine), which the longer leucine could intercalate between and interfere with movement (and possibly channel inactivation, which DIV movement is associated with) | |

**T791 and T791M**

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| **T791** | |
| **T791 – closed-state** | **T791 – activated-state** |
| **T791M – closed-state** | **T791M – activated-state** |
| * The T791M mutation introduces a large side-chain right into the centre of the DII voltage sensor. This would be expected to really perturb movement of this region. * DII voltage sensor movement is coupled with movement of the DII S4-S5 linker. Therefore a mutation that interferes with voltage sensor movement may delay formation of the pyrethroid binding site and confer resistance. | |

**L995 and L995F**

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| **L995** | |
| **L995 – closed-state** | **L995 – activated-state** |
| **L995F – closed-state** | **L995F – activated-state** |
| * L995 is found on the DII S6 helix. The side chain moves significantly when the channel transitions between closed and opened. The L995F mutation introduces a larger, more rigid aromatic ring at this position * L995 is orientated away from the pyrethroid binding site (unless you believe the dual binding site model of Dong and colleagues (which I don’t)) and so is not predicted to form a pyrethroid binding contact. What may happen is that the phenylalanine pushes against the DI S4-S5 linker and this changes position of the DII S6 helix, the other side of does form part of the pyrethroid binding site. Consequently, the pyrethroid binding site gets disturbed * The L995S substitution introduces a smaller side-chain. This mutation may have the opposite effect on the DI S4-S5 linker, in that it approaches closer instead of pushing it away. This would still alter the position of the DII S6 helix and disrupt the ligand binding site | |

**I1527 & I1527T**

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| **I1527T** | |
| **I1527 – closed-state** | **I1527 – activated-state** |
| **I1527T – closed-state** | **I1527T – activated-state** |
| * This position is located on the DIII S6 and is beside the selectivity filter. The I1527T introduces a smaller side-chain, which would change interactions with the hydrophobic pocket where its located. * Changing interactions involving the DIII S6 helix may effect movement of this segment (in some unpredictable way) and therefore modify channel gating * A more exciting possibility is that the channel selectivity or conductance gets altered. If the structure of the selectivity filter is changed then maybe its less conductive to Na+, which would be a novel form of resistance | |

**N1570 & N1570Y**

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| **N1570** | |
| **N1570 – closed-state** | **N1570 – activated-state** |
| **N1570Y – closed-state** | **N1570Y – activated-state** |
| * N1570 is located on the DIII-DIV linker, about 10 amino acids C-terminal to the ‘MFM’ fast inactivation particle. The N1570Y mutation replaces a polar hydrogen bond donating & receiving side chain with a larger aromatic ring. * In the closed state channel there are a number of nearby amino acids that the N1570 side chain can engage in hydrogen bonds with, including two at the cytoplasmic end of the DIV S6 helix. The DIII-DIV linker also forms extensive contacts with the C-terminal intracellular loop. The N1570Y mutation might disrupt these closed-state interactions. * Overall, I would expect altered channel inactivation, given the involvement of domain IV. It’s really odd, then, that Wang et al 2015 (Mol Pharmacol 87 421–9) found no effect on the N1570Y mutant channel properties. Maybe some mutant channels were already fast inactivated and so unavailable to be activated? How to test this, though… * The N1570 side chain isn’t interacting with anything in the activated state model, but that is because we are missing structure for most of the intracellular loops in this model * N1570 is too far to interact with the DII S6 helix so the explanation of Wang et al 2015 doesn’t work | |

**E1597 & E1597G**

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| **E1597** | |
| **E1597 – closed-state** | **E1597 – activated-state** |
| **E1597G – closed-state** | **E1597G – activated-state** |
| * E1597 is located on the short ‘S0’ helix of the domain IV voltage sensor * This side chain isn’t orientated to interact with much, although one possible hydrogen-bond interaction partner is an asparagine at the bottom of the S1 helix. The E1597G would eliminate this interaction, although it’s not clear that there would be any major structural consequences of this * One interesting possibility is that E1597G introduces the flexible glycine residue that might affect the secondary structure of this S0 helix and so disturb function of the voltage sensor. * Again, I would predict some altered channel inactivation would be the functional effect of this mutation | |

**K1603T**

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| **K1603** | |
| **K1603 – closed-state** | **K1603 – activated-state** |
| **K1603T – closed-state** | **K1603T – activated-state** |
| * K1603 is the first amino acid of the domain IV S1 helix. The K1603T mutation eliminates the positive charge and reduces the length of the side chain. * The side chain is orientated towards the lipid bilayer and so might be able to interact with a negatively-charged or polar lipid head group. Notably the residue preceding it is also a lysine, so maybe together they could chelate a negatively-charged head group (the is an interaction the mutation could knock out)? * Alternatively K1603 might be able to form a hydrogen bond with an asparagine on the S0 helix. Actually, the K1603T might also be able to form some hydrogen bond … that asparagine would be a good one to mutate to test the interaction hypothesis. * Overall, movement of the DIV voltage sensor might be perturbed by the mutation, with a knock-on effect on channel inactivation | |

**A1746S**

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| **A1746** | |
| **A1746 – closed-state** | **A1746 – activated-state** |
| **A1746S – closed-state** | **A1746S – activated-state** |
| * A1746 is located on the top of the DIV S5 helix. It is very snuggly positioned in a pocket lined with hydrophobic amino acids * The A1746S mutation (slightly) increases the size of the side chain and might push against its neighbours, perturbing the structure in that region of the channel. It is not clear what the functional effect of that would be, although the 1746 residue is in contact with the DIV pore-pointing helix of the selectivity filter, so speculatively there may be altered channel conductance and/or selectivity? | |

**V1853I**

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| **V1853** | |
| **V1853 – closed-state** | **Not in activated-state model (this region missing)** |
| **V1853I – closed-state** | **Not in activated-state model (this region missing)** |
| * V1853 is at the cytoplasmic end of the DIV S6 helix. The V1853I mutation (slightly) increases the length of this hydrophobic side chain * One possible effect of this mutation is that it interferes with a hydrogen bond between a threonine on the DIV S4-S5 linker and a lysine on the C-terminal domain. Disrupting this interaction might result in the S4-S5 linker adopting the activated conformation more readily. * Again, I would predict altered channel inactivation to be a functional outcome because of the unique role of domain IV in channel inactivation. | |

**I1868T**

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| **I1868T** | |
| **I1868 – closed-state** | **Not in activated-state model (this region missing)** |
| **I1868T – closed-state** | **Not in activated-state model (this region missing)** |
| * Located in the C-terminal domain. Mutation from the large hydrophobic isoleucine to the small polar threonine could disrupt the hydrophobic interactions around this residue. * One effect of the mutation might be to release the DIII-DIV linker from its interaction with the C-terminal domain, allowing it to bind to the fast inactivation receptor site. Could many channels be transitioning from closed to fast-inactivated, thus conferring resistance? | |

**P1874S / P1874L**

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| **P1874** | |
| **P1874 – closed-state** | **Not in activated-state model (this region missing)** |
| **P1874L – closed-state** | **Not in activated-state model (this region missing)** |
| * This 1874 proline is performing its role as a helix-breaker at the end of the first helix of the C-terminal domain. Replacing this proline could change the secondary structure in this region. * Notably this 1874 position is very close to an interaction point of the DIII-DIV linker so disrupting this interaction might release the fast inactivation particle to do its thing? | |

**A1934V**

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| **A1934** | |
| **A1934 – closed-state** | **Not in activated-state model (this region missing)** |
| **A1934V – closed-state** | **Not in activated-state model (this region missing)** |
| * A1934 is located on a helix on the C-terminal domain. It really isn’t positioned to interact with anything of note. * A1934V is a conservative mutation so it’s not clear what functional effect (if any) would result from this substitution. | |

**I1940T**

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| **I1940** | |
| **I1940 – closed-state** | **Not in activated-state model (this region missing)** |
| **I1940T – closed-state** | **Not in activated-state model (this region missing)** |
| * I1940 is at the end of one of the helices on the C-terminal domain. The I1940T mutation replaces a large hydrophobic amino acid with a small polar one. * Unlike the other mutations in the C-terminal domain, this mutation might actually promote interactions with the domain III-IV linker. The threonine could form a hydrogen bond with an aspartate or glutamine on this linker. It’s not clear what the functional might be here, although delayed channel fast inactivation might be a possibility (as the DIII-DIV might not be released if there’s an extra stabilising interaction with the C-terminal domain). | |