Poring over why pufferfish do not poison themselves
(by Assoc Prof Soong Tuck Wah, Department of Physiology)

About a year ago, in a discussion with Dr Venkatesh Byrappa of the Institute of Molecular and Cell Biology (IMCB), he was musing over a long-standing question that has intrigued the pufferfish research community which was, "Why is the pufferfish not poisoned by tetrodotoxin (TTX) that it produces?" Combining IMCB's expertise in comparative genomics and our experience in voltage-gated ion channels and the whole-cell patch clamp technique, we decided to have a go at the question.

The IMCB team quickly generated the alignments of all the 9 known voltage-gated sodium (Na_v) channels, through their easy access to the most up-to-date pufferfish and zebra fish genomic databases. They also compared them to mammalian and invertebrate Na_v channel genes to provide a very comprehensive overview of their similarities and differences.

One major difficulty, however, was to identify which one of the many substitutions that were naturally found across genes of different species should be picked to test our hypothesis: that a mutation(s) in the Na_v channel gene confers resistance to TTX.

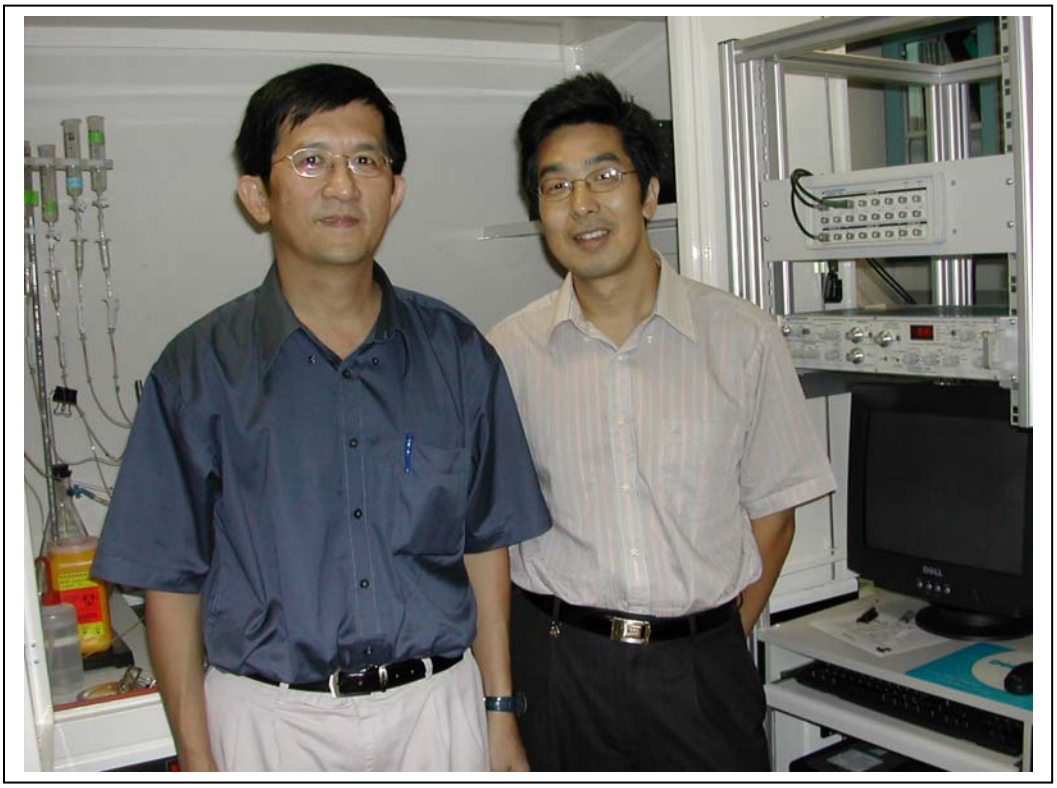
Our group's working knowledge and understanding of the mechanistic function of Na_v channels helped both teams to decide on a single critical residue of the skeletal muscle $Na_v1.4$ gene to test for TTX resistance. This residue resides on the outer vestibule of the P-loop that lines the pore of the channel. Binding of TTX in this region should occlude the pore to block the function of the channel.

This project was also coincidentally well timed as my postdoctoral fellow, Dr Lu Songqing, had previously worked in the laboratory of Prof Stefen Heinemann on the blockade of $Na_v1.4$ channels by α-scorpion toxin. Dr Lu was therefore able to very quickly introduce the mutations found on domain IS5-S6 region of the channels in pufferfish into the rat $Na_v1.4$ cDNA obtained from Prof Heinemann. Over 4 months, Dr Lu performed whole-cell electrophysiology to determine the dose-dependent inhibition of the rat $Na_v1.4$ and mutant channels by TTX. He found at least a 2000-fold increase in TTX resistance in the mutant Na_v channels as a result of a single base change in the channel transcripts.

While many have pored over the question, Occam's razor came in useful again as the answer to a simple question is also elegantly simple: a mutation in the pore region of the Na_v channel gives rise to resistance to TTX. The simple answer belied the huge amount of work performed computationally, at the bench and also at the patch clamp set-up. Above all, this was a collaborative effort that brought together two institutions, each contributing their own expertise to solving an interesting scientific question.

Reference:

Venkatesh B*, Lu SQ, Dandona N, See SL, Brenner S, Soong TW*
Genetic basis of tetrodotoxin resistance in pufferfishes.
Curr Biol. 2005 Nov 22;15(22):2069-72. * Corresponding authors



Left: A/P Soong Tuck Wah
Right: Dr Lu Songqing