Combining experiment and simulations to characterize the interactions of a sea-anemone toxin with the analgesic target acid-sensing ion channel 3

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Acid-sensing ion channel 3 (ASIC3) is a proton-gated, sodium-selective channel present in peripheral sensory neurons. ASIC3 plays an important role in inflammatory and ischemic pain, making it a promising drug target for the development of novel analgesics. The sea-anemone toxin APETx2 is a potent and selective inhibitor of ASIC3 and is effective in animal models of both neuropathic and inflammatory pain. This study combines experimental and *in silico* approaches to characterize the pharmacophore of APETx2 and identify the main toxin-channel interactions with a view to facilitating rational design of new molecules with increased selectivity and affinity for ASIC3.

Using alanine-scanning mutagenesis we identified a set of residues representing the core APETx2 pharmacophore that drives interaction of toxin with ASIC3. A second set of mutants showed reduced activity but it was unclear if this change in activity was a direct result of lost toxin-channel interactions due to the mutation or a consequence of structural changes induced in neighbouring residues. We employed molecular dynamics (MD) simulations to investigate the structural effect of the mutations. The results suggest that the change in activity is likely a result of changes induced by the mutation in the backbone structure and/or sidechain flexibility of key pharmacophore residues. These MD simulations enabled us to conclude that this second set of residues is not part of the APETx2 pharmacophore.

To identify the binding pocket and predict specific toxin-channel interactions between APETx2 and ASIC3 the experimental data on key pharmacophore residues was used for restraints-based docking of APETx2 to a homology model of ASIC3. In addition, MD simulations were carried out to model the spontaneous binding of APETx2 to ASIC3. Testing the activity of APETx2 on mutants of ASIC3 will be used to validate the predicted toxin-channel interactions.