## Phospholipid binding activity of the spider venom peptides ProTx-I and ProTx-II

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ProTx-I and ProTx-II are disulfide-rich, amphipathic peptides isolated from tarantula venom. They are able to inhibit the human voltage-gated sodium channel 1.7 ($hNa_V1.7$), a channel involved in pain sensation, and are thus promising lead molecules for the development of new analgesics. Both ProTx-I and ProTx-II inhibit the channel by binding to the membrane-embedded voltage sensor domain. However, the membrane-binding properties and its the importance in the inhibition of $hNa_V1.7$ by ProTx-I and ProTx-II remain poorly understood. In this study we examined the membrane-binding activities of ProTx-I and ProTx-II and analogues using unrestrained molecular dynamics (MD) simulations combined with surface plasmon resonance (SPR) and fluorescence spectroscopy experiments (Deplazes *et al.*, 2016; Henriques *et al.*, 2016).

Results from SPR experiments indicate that both ProTx-I and ProTx-II bind to neutral and anionic phospholipid bilayers with a preference for membranes that contain anionic lipids. The fluorescence spectroscopy experiments and MD simulations showed that the peptides do not partition into the hydrophobic core of the lipid bilayer but remain at the water-lipid interface when bound to the membrane. Results from MD simulations also revealed that both peptides exhibit a distinct lipid-interaction surface composed of a number of residues on or near the hydrophobic face of the peptides. Despite limited sequence identity (26%) between ProTx-I and ProTx-II the lipid-interaction surface of both peptides is dominated by Trp. This is consistent with the significantly reduced membrane-binding activity of [Trp/Tyr] mutants of ProTx-II. The combined data support a model whereby a hydrophobic patch on the peptide surface anchors the molecule at the cell surface.

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