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Perspective

# The Paradox of Scorpion Toxin Interactions with Portable $Na_v$ Receptors

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Scorpion venom is rich in proteinaceous toxins that affect excitability by impeding ion channel gating. Channel blockers interact with the external region of the pore and obstruct ion conductance, whereas channel modifiers interact with the voltage sensor module hindering the activation or inactivation processes [1-3]. Scorpion toxin modifiers of voltage-gated sodium channels ($Na_v$s) are divided by their mode of action to alpha and beta classes [4], and further to distinct pharmacological groups by their binding features (Figure 1) [5-7]. Alpha toxins that prolong channel inactivation bind at the pharmacologically defined receptor site 3, whereas beta toxins that affect channel activation bind at receptor site 4 [4-12]. Although the study of the mode of action, binding features, three-dimensional structure and bioactive surface of these toxins encompasses more than four decades, it markedly accelerated once ways for toxin expression in heterologous systems (*Escherichia coli*, *Saccharomyces cerevisiae*) have been developed [9,13-15]. Of particular use was the efficient expression system of *Esherichia coli* which expedited the examination of the effects of any single or multiple amino acid substitutions on toxin binding and action. The considerable large quantities of pure recombinant toxins enabled also crystallization and determination of the structures of wild type and mutant derivatives. Undoubtedly, the simplification of toxin production and analysis accelerated the study of their bioactive surfaces, as shown for toxin representatives of all pharmacological groups [13,16-24]. These studies have shown that the bioactive surfaces are generally divided between two domains in both alpha and beta toxins (Figure 1 upper as an example), where one domain is associated with the molecule core and the other domain includes residues of the intertwining N and C tails. This division suggested that the 'mirror face' channel receptors might be composed as well of two distinct sites complementary to the toxin bioactive surfaces. Moreover, the ability of both alpha and beta toxins to modify channel gating has suggested that they interact, at least in part, with the voltage sensor module. Backed by this rationale and the available expression systems for both toxins (using *E. coli*) and $Na_v$s (expressed either in frog oocytes or cultured cell lines) a molecular study of the interaction between the toxins and the channels has been established.

Systematic mutagenesis at extracellular loops that connect trans-membrane segments, highlighted channel regions that dictate toxin selectivity toward mammalian versus insect $Na_v$s, as well as amino acid residues involved in channel sensitivity to the toxins [12,17,25-34]. Collectively, these findings enabled double-mutant cycle analyses and association/dissociation assays, which raised putative pairwise interactions between toxin and channel amino acid residues. Using the anti-mammalian toxin Lqh2 (from *Leiurus quinquestriatus hebraeus*) as a model of the alpha class, residues of the Core-domain have been suggested to interact with channel residues at the voltage sensing module of domain IV in the rat brain channel $Na_v1.2a$ (Figure 2), thus providing a partial view of receptor sites 3 [12,32,35-39,]. Since the movements of S4 voltage sensor at domain IV have been implicated in the inactivation process of the channel [40,41], this mutational analysis substantiated at the molecular level the specific effect of scorpion alpha toxins on channel inactivation. Similar analyses using Css4 (from *Centruroides sufussus sufussus*) as a representative of the beta class have suggested putative pairwise interactions of amino acids at the toxin core and the voltage sensing module at domain II of $Na_v1.2a$ (Figure 2) [12,36,37], rationalizing the specific effect of scorpion beta toxins on channel activation. Both studies raised the possibility of toxin interactions also with the Pore-module of the channel, although the supporting experimental evidence was less definitive [37,38].

Overall, this experimental approach offered an emerging view of receptor sites 3 and 4, but it also incited a confusing paradox that pertains to the dynamics of toxin binding and effects. Since one out of two channel regions that interact with the toxins is the cleft between segments S3 and S4 of the voltage sensor module [37-39], an evident question is how do the toxins sustain their grip over the channel during gating, when the S4 segments move outward and backward across the membrane? Assuming that the initial recognition and specificity of a toxin ligand is determined by complementary geometric shapes with the channel, does this interaction follow the 'lock and key' rules for protein-protein interactions, which were developed for enzymatic reactions [42], or is it a stepwise process that follows the principles of the 'induced fit theory' [43], where binding begins with recognition of complementary shapes and continues with molding of amino acid side-chains that strengthen the interaction? In assuming that the 'induced fit theory' provides a better description of the way toxins interact with $Na_v$s, the question of what happens to the complex upon membrane depolarization, when the S4 segments move toward their outward activated state, remains unanswered.

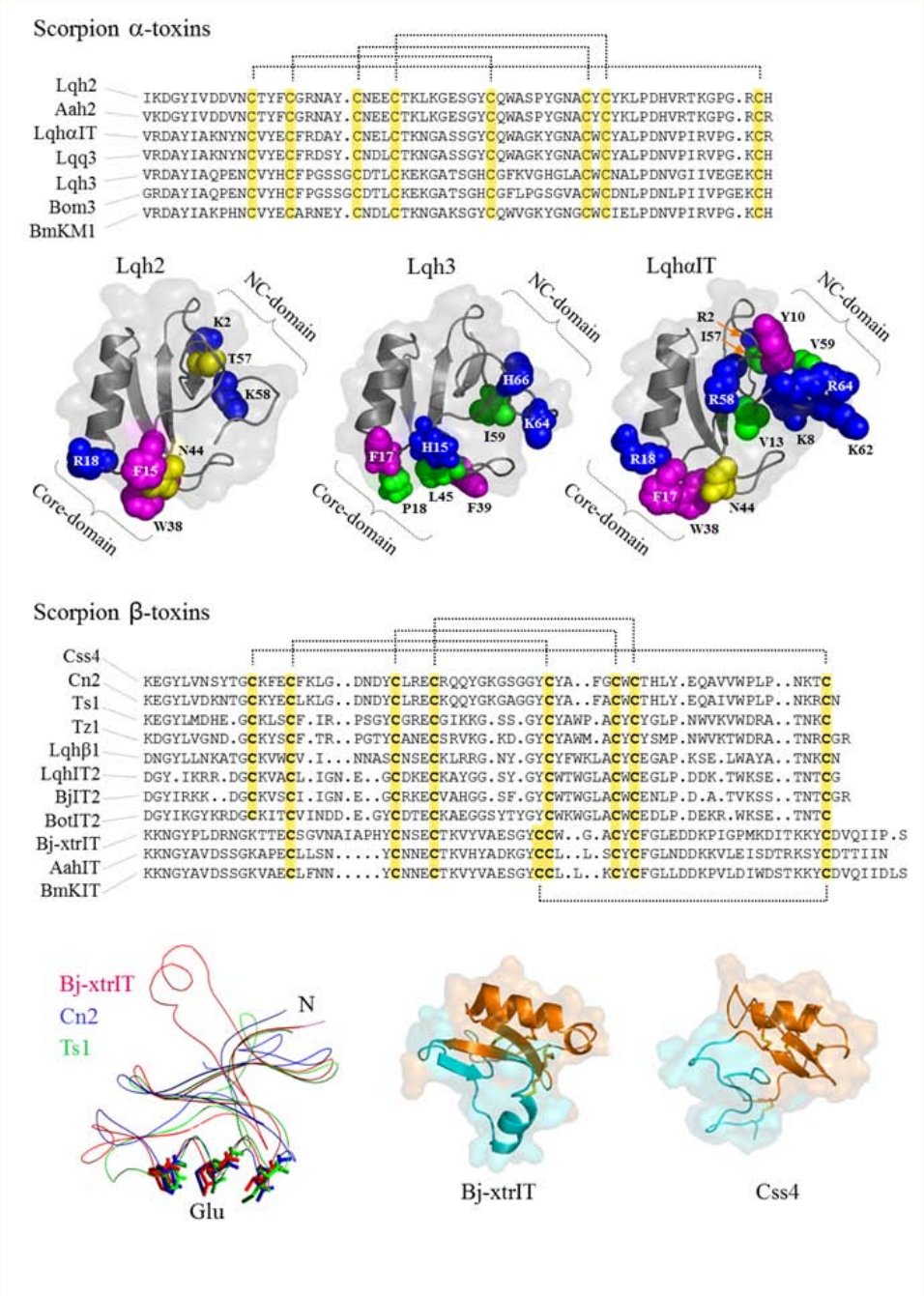


**Figure 1** Alignment of scorpion alpha and beta toxin representatives. Sequences are aligned by the conserved cysteines forming disulfide bonds. Dots indicate gaps for best alignment. Lqh, *Leiurus quinquestriatus hebraeus*; Aah, *Androctonus australis hector*; Lqq, *L. q. quinquestriatus*; Bom, *Buthus occitanus mardochei*; BmK, *Buthus martensii* Karsh; Css, *sufussus sufussus*; Cn, *Centruroides noxius*; Ts, *Tityus serrulatus*; Tz, *Tityus zulianus*; Bj, *Buthotus judaicus* (*Hotentota judaicus*); Bot, *Buthus occitanus tunetanus*. Upper: Lqh2 and Aah2 are classical anti-mammalian α-toxins; LqhαIT and Lqq3 are α-toxins highly effective on insects as well as on mammalian skeletal muscle channels; Lqh3, Bom3 and BmKM1 are α-like toxins (for further details on toxins and scorpion species [3,6]. Lqh3 and LqhαIT structures were determined (PDB codes 1FH3 and 2ASC, respectively). Lqh2 structure was modelled on the basis of the known structure of the almost identical Aah2 (PDB code 1AHO) using the SWISS-MODEL protein homology-modelling server (EXPASY). Ribbons indicate backbone structures. The molecular surface of the toxins is presented by a semi-transparent cover. Bioactive residues [28,29] are space-filled and colored according to their chemical nature (aliphatic, green; aromatic, magenta; polar, yellow; and positive, blue). The bioactive surface splits into a Core-domain and an NC-domain. Lower: Css4, Cn2, Ts1 and Tz1 are classical β-toxins; Lqhβ1 was found and characterized in the 'New World'; LqhIT2, BjIT2 and BotIT2 are depressant toxins; Bj-xtrIT, AahIT and BmKIT are excitatory toxins [46]. The structures of Bj-xtrIT, Cn2 and Ts1 were determined (PDB codes 1BCG, 1Cn2 and 1NPI, respectively). Note the common 'hot spot' at the 'pharmacophore' of the three toxins, which differ markedly in selectivity toward insects and mammals [22]. The structural models of Css4 (based on the NMR structure of Cn2; PDB code 1CN2) and Bj-xtrIT (PDB code 1BCG) are covered by semi-transparent molecular surfaces and are spatially aligned. The structural resemblance of the core in Lqh2 and Css4 as a result of common ancestry [15,25,48] is designated by the orange color. The models were prepared using PyMOL.

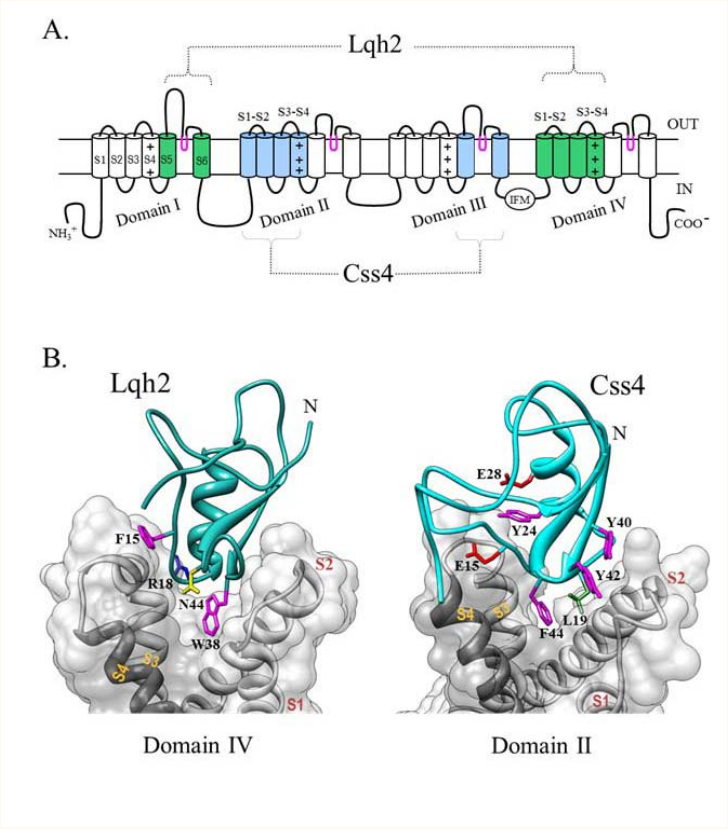


**Figure 2** Interaction of alpha and beta toxins with the voltage sensor module of $Na_v1.2a$. A, Scheme of a sodium channel pore-forming α-subunit and regions assigned to receptor sites 3 (green) and 4 (blue). DI, DII, DIII and DIV assemble in the membrane around the channel pore (magenta). The S1-S2 and S3-S4 linkers at the gating module of DIV and S5-S6 at the pore module of DI form receptor site-3 of scorpion α-toxins [40]. The equivalent regions in DII and DIII form receptor site-4 of scorpion β-toxins [38,39], Face of interaction between Lqh2 α-toxin and Css4 β-toxins with the rat brain channel $rNa_v1.2a$ at the voltage sensor module. The toxins (in blue) intrude at a cleft between S1-S2 and S3-S4 of domains IV (Lqh2) and II (Css4). Both toxins are believed to also interact with the external linkers of the juxtaposed pore domain (DI for Lqh2 and DIII for Css4), which are not shown. Bioactive toxin residues are indicated. N indicates N-terminus.

Strong depolarizations might detach the toxin from its binding site [44-48], a scenario that not necessarily occurs under weak to moderate physiological changes in membrane potential. We may assume that the toxin accommodates to the conformational alterations by interaction with a different subset of channel residues at the voltage sensor module, or sustain its hold over the channel and avoid fall-off due to its interaction with the less labile Pore-module [49]. In any event, the toxin-channel interaction involves transient conformational intermediates of the channel, and therefore it seems at present that a comprehensive clarification of the way scorpion toxins interact with $Na_v$s is more challenging than anticipated and likely requires determination of the structures of the channel-toxin intermediary complexes.

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