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# Voltage-gated ion channels and gating modifier toxins

William A. Catterall<sup>1\*</sup>, Sandrine Cestèle<sup>1,2</sup>, Vladimir Yarov-Yarovoy<sup>1</sup>, Frank H. Yu<sup>1</sup>, Keiichi Konoki<sup>1</sup>, Todd Scheuer<sup>1</sup>

<sup>1</sup> Department of Pharmacology University of Washington, Seattle, WA 98195-7280, US

<sup>2</sup> Canaux calciques, fonctions et pathologies INSERM : U607, CEA : DSV/IRTSV, Université Joseph Fourier - Grenoble I, 17, rue des martyrs 38054 Grenoble, FR

\* Correspondence should be addressed to: William Catterall <wcatt@u.washington.edu >

## Abstract

**Voltage-gated sodium, calcium, and potassium channels generate electrical signals required for action potential generation and conduction and are the molecular targets for a broad range of potent neurotoxins. These channels are built on a common structural motif containing six transmembrane segments and a pore loop. Their pores are formed by the S5/S6 segments and the pore loop between them and are gated by bending of the S6 segments at a hinge glycine or proline residue. The voltage sensor domain consists of the S1 to S4 segments, with positively charged residues in the S4 segment serving as gating charges. The diversity of toxin action on these channels is illustrated by sodium channels, which are the molecular targets for toxins that act at five or more distinct receptor sites on the channel protein. Both hydrophilic low molecular weight toxins and larger polypeptide toxins physically block the pore and prevent sodium conductance. Hydrophobic alkaloid toxins and related lipid-soluble toxins act at intramembrane sites and alter voltage-dependent gating of sodium channels via an allosteric mechanism. In contrast, polypeptide toxins alter channel gating by voltage-sensor trapping through binding to extracellular receptor sites, and this toxin interaction has now been modeled at the atomic level for a  $\beta$ -scorpion toxin. The voltage sensor trapping mechanism may be a common mode of action for polypeptide gating modifier toxins acting on all of the voltage gated ion channels.**

**MESH Keywords** Animals ; Humans ; Ion Channel Gating ; drug effects ; physiology ; Ion Channels ; drug effects ; Neurotoxins ; pharmacology ; Venoms ; pharmacology

**Author Keywords** Sodium channel ; neurotoxins ; tetrodotoxin ; saxitoxin ; scorpion toxins ; anemone toxins ; batrachotoxin ; veratridine ; conotoxins ; toxin receptor sites ; insect toxins

## The Voltage-Gated Ion Channels

Electrical signals control contraction of muscle, secretion of hormones, sensation of the environment, processing of information in the brain, and output from the brain to peripheral tissues. In excitable cells, electrical signals also have an important influence on intracellular metabolism and signal transduction, gene expression, protein synthesis and targeting, and protein degradation. In all of these contexts, electrical signals are conducted by members of the ion channel protein superfamily, a set of more than 140 structurally related pore-forming proteins (Yu and Catterall, 2004). In addition, members of this protein superfamily are crucial in maintaining ion homeostasis in the kidney and in many different cell types and participate in calcium signaling pathways in nonexcitable cells. Because of their importance in many aspects of cellular regulation and signal transduction, the voltage-gated ion channels are the molecular targets for a wide range of potent biological toxins, including the gating modifier toxins that alter the kinetics and voltage dependence of their activation and inactivation. This introductory chapter introduces the voltage-gated ion channels, gives an overview of the common features of their structure and function, presents the sodium channel as a primary example of neurotoxin action on ion channels, and describes the voltage-sensor trapping model for gating modifier toxin action, which may be a common mechanism of polypeptide toxin action on ion channels.

### Voltage-gated Sodium Channels

The founding member of the ion channel superfamily in terms of its discovery as a protein is the voltage-gated sodium channel. These channels are responsible for the rapid influx of sodium ions that underlies the rising phase of the action potential in nerve, muscle, and endocrine cells. Neurotoxin labeling, purification and functional reconstitution showed that sodium channels from mammalian brain contain voltage-sensing and pore-forming elements in a single protein complex of one principal  $\alpha$  subunit of 220 to 260 kDa and one or two auxiliary  $\beta$  subunits of approximately 33 to 36 kDa (Catterall, 1984, 2000a). The  $\alpha$  subunits of sodium channels are composed of four homologous domains that each contain six hydrophobic transmembrane segments (Fig. 1). A membrane-reentrant loop between the fifth and sixth (S5 and S6) transmembrane segments forms the narrow extracellular end of the pore while the S6 segments form the intracellular end (Fig. 1A, red). The pore is formed in the center of a pseudosymmetric array of the four domains, and a single  $\alpha$  subunit containing four domains is able to receive voltage signals and activate its intrinsic pore. The channel responds to voltage by virtue of its S4 segments (Fig. 1A, green), which contain repeated motifs of a positively charged amino acid residue followed by two hydrophobic residues, and move outward under the influence of the membrane electric field to initiate a conformational change that opens the pore. Nine voltage-gated sodium channel  $\alpha$  subunits, designated Na<sub>v</sub> 1.1 to Na<sub>v</sub> 1.9 have been functionally characterized. They comprise a single family of proteins with greater than 70% amino acid sequence identity in their transmembrane segments (Goldin et al., 2000).

Sodium channel auxiliary subunits, Na<sub>v</sub> β1 to Na<sub>v</sub> β4, interact with the different α subunits and alter their physiological properties and subcellular localization. These proteins have a single transmembrane segment, a large N-terminal extracellular domain that is homologous in structure to a variable chain (V-type) immunoglobulin-like fold, and a short C-terminal intracellular segment (Fig. 1B) (Isom et al., 1992; Isom et al., 1995; Morgan et al., 2000; Yu et al., 2003). The Na<sub>v</sub> β subunits interact with α subunits through their extracellular Ig-fold domains, modulate α subunit function, and enhance their cell surface expression (McCormick et al., 1999). Like other proteins with an extracellular Ig-fold, they also serve as cell adhesion molecules by interacting with extracellular matrix proteins, cell adhesion molecules, and cytoskeletal linker proteins (Ratcliffe et al., 2000; Srinivasan et al., 1998; Ratcliffe et al., 2001; Kazarinova-Noyes et al., 2001; Malhotra et al., 2002). The Na<sub>v</sub> β subunits are a recent evolutionary addition to the family of ion channel associated proteins, as they have only been identified in vertebrates.

### **Voltage-gated Calcium Channels**

Voltage-gated calcium channels are the key signal transducers of electrical signaling, converting depolarization of the cell membrane to an influx of calcium ions that initiates contraction, secretion, neurotransmission, and other intracellular regulatory events (Catterall, 2000b). Skeletal muscle calcium channels first identified by drug labeling, purification, and functional reconstitution have a principal α1 subunit of 212 to 250 kDa, which is similar to the sodium channel α subunit (Takahashi et al., 1987; Tanabe et al., 1987). cDNA cloning and sequencing showed that the α1 subunit of calcium channels is analogous to the sodium channel α subunits in structural organization (Fig. 1A) and is approximately 25% identical in amino acid sequence in the transmembrane regions. The α1 subunit is associated with auxiliary α2δ, β, and γ subunits that are unrelated to the sodium channel auxiliary subunits (Fig. 1B). As for the sodium channel α subunit, the calcium channel α1 subunit is sufficient to form a voltage-gated calcium-selective pore by itself. Ten functional calcium channel α1 subunits are known in vertebrates, and they fall into three subfamilies that differ in function and regulation (Ertel et al., 2000). The Ca<sub>v</sub> 1 subfamily (Ca<sub>v</sub> 1.1 to Ca<sub>v</sub> 1.4) conduct L-type calcium currents that initiate contraction, endocrine secretion, and synaptic transmission at the specialized ribbon synapses involved in sensory input in the eye and ear (Hofmann et al., 1994; Striessnig, 1999). L-type calcium currents also are important regulators of gene expression and other intracellular processes. They are blocked by the classical calcium channel antagonists, including dihydropyridines, benzothiazepines, and phenylalkylamines. The Ca<sub>v</sub> 2 subfamily of calcium channels (Ca<sub>v</sub> 2.1 to Ca<sub>v</sub> 2.3) conduct N-, P/Q- and R-type calcium currents that initiate fast synaptic transmission at synapses in the central and peripheral nervous systems and are blocked specifically by peptide neurotoxins from spider and cone snail venoms (Snutch and Reiner, 1992; Dunlap et al., 1995; Catterall, 2000b; Olivera et al., 1994). The Ca<sub>v</sub> 3 subfamily of calcium channels (Ca<sub>v</sub> 3.1 to Ca<sub>v</sub> 3.3) conduct T-type calcium currents that are important for repetitive action potential firing of neurons in the brain and in the pacemaker cells of the sino-atrial node in the heart (Perez-Reyes, 2003). The protein-protein interactions as well as the functional and regulatory properties of different subfamilies of these ion channels are adapted to their distinct roles in electrical signaling and cellular signal transduction.

Ca<sub>v</sub> 1 and Ca<sub>v</sub> 2 channels have four distinct auxiliary subunits, Ca<sub>v</sub> α2, Ca<sub>v</sub> β, Ca<sub>v</sub> γ, and Ca<sub>v</sub> δ (Fig. 1B) (Takahashi et al., 1987; Catterall, 2000b), which each comprise a small protein family. The Ca<sub>v</sub> α2 and Ca<sub>v</sub> δ subunits are encoded by the same gene (Ellis et al., 1988), whose translation product is proteolytically cleaved and disulfide linked to yield the mature extracellular α2 subunit glycoprotein of 140 kDa and transmembrane disulfide-linked δ subunit glycoprotein of 27 kDa (De Jongh et al., 1990). Four Ca<sub>v</sub> α2δ genes are known (Arikkath and Campbell, 2003). The four Ca<sub>v</sub> β subunits are all intracellular proteins with a common pattern of alpha helical and unstructured segments (Ruth et al., 1989; Arikkath and Campbell, 2003). They have important regulatory effects on cell surface expression and they also modulate the gating of calcium channels, causing enhanced activation upon depolarization and altered rate and voltage dependence of inactivation (Arikkath and Campbell, 2003). Recent structural modeling and x-ray crystallography studies have revealed that these subunits contain conserved, interacting SH3 and guanylate kinase domains like the MAGUK family of scaffolding proteins (Van Petegem et al., 2004; Chen et al., 2004; Opatowsky et al., 2004; McGee et al., 2004; Takahashi et al., 2004), and therefore may interact with other intracellular proteins. Eight Ca<sub>v</sub> γ subunit genes encode glycoproteins with four transmembrane segments (Jay et al., 1990; Arikkath and Campbell, 2003). Although the Ca<sub>v</sub> γ1 subunit is associated specifically with skeletal muscle Ca<sub>v</sub> 1.1 channels, other Ca<sub>v</sub> γ subunits interact with other calcium channels, glutamate receptors and possibly with other membrane signaling proteins (Arikkath and Campbell, 2003). Thus, the γ subunits discovered as components of calcium channels apparently have a more widespread role in assembly and cell surface expression of other membrane signaling proteins.

### **Voltage-gated Potassium Channels**

Voltage-gated potassium channels are activated by depolarization, and the outward movement of potassium ions through them repolarizes the membrane potential to end action potentials, hyperpolarizes the membrane potential immediately following action potentials, and plays a key role in setting the resting membrane potential. In this way, potassium channels control electrical signaling in excitable cells and regulate ion flux and calcium transients in nonexcitable cells. The first voltage-gated potassium channels were cloned from *Drosophila* based on a mutation that causes the Shaker phenotype (Tempel et al., 1987; Papazian et al., 1987; Jan and Jan, 1997). They are composed of four transmembrane subunits that each is analogous to a single domain of the principal subunits of sodium or calcium channels (Fig. 1A). The voltage-gated potassium channels are remarkable for their diversity. They include 40 different channels

that are classified into 12 distinct subfamilies based on their amino acid sequence homology ( $K_v$  1 to  $K_v$  12) (Gutman et al., 2003). These  $\alpha$  subunits can assemble into homo- and hetero-tetramers, leading to a wide diversity of different channel complexes. The diversity of potassium channels allows neurons and other excitable cells to precisely tune their electrical signaling properties by expression of different combinations of potassium channel subunits.

$K_v$  1 channels are often associated with one of three intracellular  $K_v$   $\beta$  subunits ( $K_v$   $\beta$ 1–3) (Fig. 1B) (Scott et al., 1993; Rettig et al., 1994; Pongs et al., 1999), which interacts with the N-terminal T1 domain and forms a symmetric tetramer on the intracellular surface of the channels (Gulbis et al., 2000). The  $K_v$   $\beta$  subunits are superficially similar to the  $Ca_v$   $\beta$  subunits in their cytoplasmic location, but are not related in amino acid sequence or structure. The N-terminus of  $K_v$   $\beta$  subunits of vertebrates serves as an inactivation gate for  $K_v$  1  $\alpha$  subunits (Pongs et al., 1999), and is thought to enter the pore and block it during sustained channel opening (Zhou et al., 2001). This is a unique example of a direct physical role for an auxiliary subunit in channel gating, rather than modulating the gating process of its associated pore-forming  $\alpha$  subunit.  $K_v$  4 channels interact with the K channel interacting proteins KChIp1–4 (Fig. 1B), which are members of the neuronal calcium sensor family of calmodulin-like calcium regulatory proteins and have four EF hand motifs (An et al., 2000). The KChIps enhance expression of  $K_v$  4 channels and modify their functional properties by binding to a site in the intracellular T1 domain, similar to the interaction of  $K_v$   $\beta$  subunits with  $K_v$  1 channels. The  $K_v$  7,  $K_v$  10, and  $K_v$  11 channels associate with a different type of auxiliary subunit, the minK-like subunits. These five closely related proteins have a single transmembrane segment and small extracellular and intracellular domains (Takumi et al., 1988; Abbott et al., 2001b). Although these subunits are topologically similar to  $Na_v$   $\beta$  and  $Ca_v$   $\delta$  subunits, they do not have significant amino acid sequence similarity. The minK-like subunits are important regulators of  $K_v$  7 channel function (Sanguinetti et al., 1996; Barhanin et al., 1996), and mutations in one of these auxiliary subunits causes a form of familial long QT syndrome, which predisposes to dangerous cardiac arrhythmias (Abbott et al., 2001a). In addition, recent work indicates that these subunits also associate with  $K_v$  3 and  $K_v$  4 channels and are responsible for a form of inherited periodic paralysis (Abbott et al., 2001a; Zhang et al., 2001). In light of this work, it is possible that all  $K_v$  channels associate with minK-related subunits. If this hypothesis is true, the  $K_v$  channels would then resemble the  $Na_v$  and  $Ca_v$  channels in having an associated subunit with a single transmembrane segment and short intracellular and extracellular domains.

## Common Aspects of Ion Channel Structure and Function

The functions of the voltage-gated ion channel proteins can be divided into three complementary aspects: ion conductance, pore gating, and regulation. All members of the family share a common pore motif, with variations appropriate to determine their different ion selectivity. It is likely that this pore motif first evolved in the bacterial ion channels that resemble vertebrate inward rectifiers. The x-ray crystal structure of one of these channels showed that the narrow outer mouth of the pore is formed by the pore loops between the M1 and M2 segments that are analogous to the S5 and S6 transmembrane segments of the 6-TM family members while the length of the pore is formed by a tilted bundle, “inverted teepee” arrangement of the M2 segments (Doyle et al., 1998; Fig. 2). This structure suggests that the pore is closed at its intracellular end and discriminates ions at the narrow ion selectivity filter at its extracellular end. This model of pore formation fits well with a wealth of structure-function data on the voltage-gated ion channels and the cyclic nucleotide-gated channels. The closure of the pore at the intracellular end suggests that this is the site of pore gating. Support for this idea comes from recent work. The structure of a calcium-activated bacterial potassium channel with calcium bound shows that the crossing of the M2 segments at the intracellular end of the pore is opened by bending of the helix at a highly conserved hinge glycine residue, consistent with the hypothesis that this is the mechanism of pore opening in two-transmembrane-segment bacterial potassium channels (Fig. 2) (Jiang et al., 2002b; Jiang et al., 2002a) and this conclusion is further supported by mutagenesis studies of six-transmembrane bacterial sodium channels in which substitution of proline for the hinge glycine to favor bending in that position greatly stabilizes the activated state of the channel (Zhao et al., 2004). A similar pore structure is present in the voltage-gated potassium channel  $K_v$  1.2, which appears to have crystallized in an open state (Long et al., 2005; Fig. 2).

The opening and closing of the voltage-gated sodium, calcium, and potassium channels are primarily gated by changes in membrane potential (Armstrong, 1975), which cause movement of gating charges across the membrane and drive conformational changes that open and close the pore (Armstrong, 1981). Their voltage-sensing and voltage-dependent gating depends on the S1 to S4 segments of these channels, which can be viewed as an evolutionary addition to the pore-forming segments. The detailed mechanism of voltage-dependent gating remains unknown, but extensive structure-function studies, x-ray crystallography, and molecular modeling provide a progressively clearer picture. The positively charged S4 segments are thought to undergo outward and rotational movement through the protein structure during the gating process, as proposed in the ‘sliding helix’ and ‘helical screw’ models of gating (Catterall, 1986; Guy and Seetharamulu, 1986). This movement has been detected in fluorescent labeling experiments (Bezannilla, 2000), and the S4 voltage sensor is oriented across the membrane with its gating charges in an outward position in the structure of a  $K_v$  channel with its pore open (Long et al., 2005; Fig. 2). Remarkably, the S1–S4 voltage sensor domain interacts with the S5–S6 pore domain of the adjacent subunit or domain in the three-dimensional structure of voltage-gated channels (Long et al., 2005; Fig. 2). A recent structural model of the gating process at the atomic level gives a detailed view of the sliding helix movement of the S4 gating charges through a gating pore across the membrane upon depolarization and shows how the S4–S5 linker exerts a force on the pore-lining S6 segments to open the pore (Yarov-Yarovoy et al., 2006

). Altogether, these results nicely define the structure of the open channel with its activated voltage sensor and provide a detailed model for the closed state and the gating movements that activate the channel. These molecular movements are the targets for the action of gating modifier toxins.

## Neurotoxins and Sodium Channels

Sodium channels are unique in that they are the molecular targets for several groups of neurotoxins, which strongly alter channel function by binding to several different receptor sites (Catterall, 1980 ; Cestèle and Catterall, 2000). Due to their high affinity and specificity, neurotoxins provide powerful tools to study the structure and the function of sodium channels, affecting both permeation and gating properties. Six different neurotoxin receptor sites have been identified on voltage-gated sodium channels (Table 1 ). Early pharmacological studies led to the conclusion that neurotoxins act at four distinct receptor sites and have primary effects on either ion permeation or voltage-dependent gating (Table 1 ; Catterall, 1980 ; Martin-Eauclaire et al., 1992 ; Cestèle and Catterall, 2000). Subsequent studies provided evidence for site 5 (Catterall and Risk, 1981 ; Poli et al., 1986 ; Lombet et al., 1987 ) and site 6 (Fainzilber et al., 1994 ). The receptor sites at which neurotoxins affect gating were found to be allosterically coupled, suggesting that conformational changes induced by neurotoxin binding alter the equilibrium between the open and the closed/inactivated states and also alter conformation and toxin binding affinity at other neurotoxin receptor sites (Catterall, 1977 , 1980 ).

### Pore-blocking Toxins

Neurotoxin receptor site 1 on sodium channels is occupied by two different groups of toxins: the water soluble heterocyclic guanidines tetrodotoxin (TTX) and saxitoxin (STX) and the peptidic toxins  $\mu$ -conotoxins. TTX is isolated from the tissues of at least 40 species of puffer fish (Fuhrman, 1967 ), but it is also found in molluscs, crabs, octopus, fish and Central American frogs (Yamasuto et al., 1986 ; Mebs et al., 1989 ; Hwang et al., 1991 ). STX is produced by the marine dinoflagellate *Gonyaulax catenella* and can be found in bivalves such as clams and mussels that feed on the dinoflagellates (Schantz, 1986 ).  $\mu$ -Conotoxins are isolated from the venom of *Conus geographus* and related cone snails (Cruz et al., 1985 ; Sato et al., 1983 ). By binding to neurotoxin receptor site 1, these toxins block sodium conductance (Narahashi et al., 1964 ; Hille, 1968 , 1975 ; Narahashi, 1974 ; Ritchie and Rogart, 1977 ). The amino acid residues that form neurotoxin receptor site 1 are located in the pore loop and are thought to form the ion selectivity filter (Noda et al., 1989 ; Terlau et al., 1990 ; Heineman et al., 1992 ; Fig. 3 ). Surprisingly, selective pressure from the presence of STX in the natural environment or TTX in prey can select for mutations in the ion selectivity filter that cause resistance to these toxins in the softshell clam *Mya arenaria* and in garter snakes (Bricelj et al., 2005 ; Geffeney et al., 2005). Although binding experiments have shown that  $\mu$ -conotoxins inhibit TTX/STX binding on native sodium channels competitively (Moczydlowski et al., 1986 ; Ohizumi et al., 1986 ; Yanagawa et al., 1986 ), some of the mutations that affect TTX affinity do not alter  $\mu$ -conotoxin binding, suggesting that these two toxins share an overlapping but not identical receptor site (Dudley et al., 1995 ; Stefan et al., 1994 ; Chahine et al., 1995 , 1998 ). Moreover, recent studies of a toxin from the South American spider *Phoneutria nigriventer* showed that it blocks sodium currents in a state-dependent manner and competes for binding with  $\mu$ -conotoxins but not with TTX (Martin-Moutot et al., 2006 ). Localization of TTX/STX and  $\mu$ -conotoxins receptor sites has been instrumental in identifying the pore loop and in clarifying the regions of the channel structure involved in the ion selectivity filter, and one can anticipate that mapping the sites of interaction of *P. nigriventer* toxin will provide further insight into these overlapping toxin binding sites and their relationship to the ion selectivity filter.

### Gating Modifier Toxins Binding to Intramembrane Receptor Sites

#### *Neurotoxin receptor site 2*

Lipid-soluble grayanotoxins (found in rhododendron and other plants of the family Ericaceae ), the alkaloids veratridine (from the family of Liliaceae ), acotinine (from the plant *Acotinum napellus*) and batrachotoxin (from the skin of the Colombian frog *Phylllobates aurotaenia*) bind to receptor site 2. These toxins bind preferentially to the activated state of sodium channels and cause persistent activation at resting membrane potential via an allosteric mechanism that leads to block of sodium channel inactivation and shift of the voltage dependence of activation to more negative potentials (Ulbricht, 1969 ; Albuquerque et al., 1971 ; Catterall, 1977 , 1980 ; Khodorov et al., 1985 ).

Photolabelling experiments using batrachotoxin derivatives have localized neurotoxin receptor site 2 to the S6 transmembrane region of domain I of the sodium channel  $\alpha$  subunit (Trainer et al., 1996 ). Site-directed mutagenesis has identified several molecular determinants of batrachotoxin binding and action in transmembrane segments IS6 (I433, N434 and L437 of  $\text{Na}_v$  1.4, Wang and Wang, 1998 ) and IVS6 (F1579 and N1584 in  $\text{Na}_v$  1.4, Wang et al., 1999 ; I1760 and F1764 (equivalent to F1579) in  $\text{Na}_v$  1.2, Linford et al., 1998 ) (Fig. 2 ). As for batrachotoxin, grayanotoxin binding affinity is also reduced by mutations of I433, N434, L437, I1575, F1579 on skeletal muscle  $\text{Na}_v$  1.4 channels and the mutations of the corresponding residues on cardiac sodium channels (Ishii et al., 1999 ). However, mutation of Y1586 in transmembrane segment IVS6, which does not alter batrachotoxin binding, completely eradicated grayanotoxin effect on  $\text{Na}_v$  1.4

channels (Kimura et al., 2000 ). These results indicate that the grayanotoxin receptor site shares overlapping but not identical molecular determinants with batrachotoxin in transmembrane segment IVS6 and has common molecular determinants in transmembrane segment IS6 (Table 1 ).

Involvement of IS6 and IVS6 transmembrane segments in the formation of receptor site 2 suggests that these toxins conform to a ' domain-interface' allosteric model of ligand binding and action (Fig. 2 ), as initially proposed for binding of calcium channel antagonists ( Catterall and Striessnig, 1992 ; Hockerman et al., 1997 ). It is proposed that the block of inactivation induced by this group of toxins is due to their interaction with the IVS6 transmembrane segment that is required for fast inactivation. Batrachotoxin binding to IVS6 segment may allow the toxin to alter the voltage-dependent movements of the adjacent IVS4 voltage-sensor and thereby affect both activation and coupling of activation to inactivation (Linford et al., 1998 ).

### ***Neurotoxin receptor site 5***

Like batrachotoxin and the other toxins acting at receptor site 2, the lipid-soluble toxins brevetoxin and ciguatoxin originating from the dinoflagellates *Karenia brevis* (formerly *Gymnodinium breve* or *Ptychodiscus brevis* ) and *Gambierdinus toxicus* , respectively, enhance sodium channel activity. They bind to neurotoxin receptor site 5 and cause a shift in activation to more negative membrane potentials and a block of inactivation (Catterall and Risk, 1981 ; Huang et al., 1984 ; Poli et al., 1986 , Benoit et al., 1986 ; Lombet et al., 1987 ). Transmembrane segments IS6 and IVS5 have both been identified to participate in the formation of neurotoxin receptor site 5 in photoaffinity labeling experiments (Trainer et al., 1991 , 1994 ; Fig. 3 ). Binding determinants for brevetoxin seem to be widely dispersed throughout these transmembrane segments (Konoki, Linford, Scheuer, and Catterall, unpublished). From a structural point of view, these results indicate that transmembrane segments IS6 and IVS5 are in close proximity in the native sodium channels and interact to form this receptor site (Trainer et al., 1991 , 1994 ), which is confirmed by the recent structure of  $K_v$  1.2 channels (Long et al., 2005 ).

### ***Allosteric modulation of sodium channel function***

The lipid soluble toxins that act at neurotoxin receptor sites 2 and 5 on sodium channel are allosteric modulators of channel function. They bind at sites that are distinct from the pore or the voltage sensors and favor the open state of the sodium channel through indirect, allosteric interactions. The initial proposal for this mechanism of toxin action came from studies of persistent activation of sodium channels by the lipid soluble toxins (Catterall, 1977 , 1980 ). These studies showed that the toxins act as full or partial agonists in causing persistent activation of sodium channels and that their effects can be quantitatively fit by an allosteric model, which assumes that they bind with high affinity to activated sodium channels and thereby shift a conformational equilibrium toward the activated state. Additional support for this mechanism of action comes from the pleiotropic effects of these toxins—negative shift of activation, block of inactivation, and in some cases altered ion permeation. We propose that these toxins alter the interactions of transmembrane segments within homologous domains as well as between adjacent homologous domains, resulting in alteration of voltage-dependent activation, its coupling to inactivation, and in some cases even ion permeation.

## **Polypeptide Gating Modifier Toxins Binding to Extracellular Receptor Sites**

### ***Neurotoxin receptor site 3***

Neurotoxin receptor site 3 of sodium channels is occupied by several groups of polypeptide toxins:  $\alpha$ -scorpion toxins, sea-anemone toxins and some spider toxins. These toxins slow or block sodium channel inactivation (Narahashi, 1974 ; Bergman et al., 1976 ; Catterall, 1979 , 1980 ; Catterall and Beress, 1978 ; Couraud et al., 1978 ; Schweiz et al., 1981 ; Lazdunski et al., 1986 ; Strichartz et al., 1987 ; Nicholson et al., 1994 , 1998 ). Alpha-scorpion toxins are a family of structurally and functionally related polypeptides neurotoxins, each containing 60 to 70 amino acid residues cross-linked by four disulfide bonds (Kopeyan et al., 1974 ; Rochat et al., 1979 ). They have complex phylogenetic and structure-function relationships, especially when toxins selective for mammals versus insects are considered ( Rodriguez de la Vega and Possani, 2005 ). In this review, we have used the traditional nomenclature for the two main classes of scorpion toxins that are active on mammals—the  $\alpha$ -scorpion toxins that slow inactivation and the  $\beta$ -scorpion toxins that enhance activation (Table 1 ; Dufton and Rochat, 1984 ).

Neurotoxin receptor site 3 was first identified on mammal sodium channels using  $\alpha$ -scorpion toxins (Catterall, 1976; Couraud et al., 1978 ). The binding affinity of this group of toxins for receptor site 3 is decreased by depolarization on rat brain sodium channels ( Catterall, 1977b ; Catterall and Beress, 1978 ). The voltage dependence of binding correlates closely with the voltage dependence of channel activation (Catterall, 1979 ). Therefore, the specific action of these toxins on inactivation implies that membrane potential affects the structure of receptor site 3 on rat brain sodium channels, that this region of the channel is important for coupling of activation and inactivation, and that toxin binding prevents the conformational change required for fast inactivation (Catterall, 1979 ).

Localization of receptor site 3 was first investigated by photoaffinity labeling, cleavage with proteases or cyanogens bromide, and antibody mapping with sequence-specific antibodies and by block of binding with sequence-specific antibodies (Tejedor et al., 1988 , Thomsen et al., 1989 ). These results suggested that the extracellular loops between transmembrane segments S5 and S6 in domains I and

IV of the  $\alpha$ -subunit are involved in binding of  $\alpha$ -scorpion toxins. Subsequent site-directed mutagenesis studies further identified individual amino acid residues in the extracellular loop connecting the S3 and S4 segments in domain IV, which appear to be essential for both binding and action of these toxins (Rogers et al., 1996). Since basic amino acid residues of the toxins are known to be implicated in the interaction with the channels (Habersetzer-Rochat and Sampieri, 1976; El Ayeb et al., 1986; Kharrat et al., 1989; Gallagher and Blumenthal, 1994; Khera et al., 1994), it is of interest that E1613 was identified as a major determinant for both  $\alpha$ -scorpion toxin and sea-anemone toxin binding (Table 1; Rogers et al., 1996). Likewise, the mutation of the homologous residue in cardiac (D1612) and skeletal muscle (D1428) sodium channels alters the binding of sea-anemone toxins (Benziger et al., 1998). Extensive mutagenesis in the loop S3–S4 of domain IV of rat brain sodium channels indicated that interaction of  $\alpha$ -scorpion toxin and sea-anemone toxin required overlapping but distinct molecular determinants on the channel.  $\alpha$ -Scorpion toxin affinity is increased by mutations K1617A and P1622A; sea-anemone toxin affinity is increased by mutations L1614A, K1617A and S1621A and decreased by mutations E1616A, V1620A and L1624A (Table 1; Rogers et al., 1996). Thus, as suggested previously by binding experiments (Catterall and Beress, 1978; Couraud et al., 1978),  $\alpha$ -scorpion toxins and sea-anemone toxins share overlapping receptor sites (Rogers et al., 1996).

Location of neurotoxin receptor site 3 just on the extracellular side of transmembrane segment IVS4 suggested a molecular mechanism for toxin action (Rogers et al., 1996). The S4 segments of sodium channels serve as voltage sensors, moving outward under the influence of the electric field when the membrane is depolarized. Therefore,  $\alpha$ -scorpion toxins and sea-anemone toxins are proposed to slow sodium channel inactivation by preventing the outward movement of the IVS4 transmembrane segment, thereby preventing conformational changes that are necessary for fast inactivation (Rogers et al., 1996). Prolonged depolarization forces the outward movement of the IVS4 segment and causes voltage-dependent dissociation of the bound  $\alpha$ -scorpion toxins (Catterall, 1977b). This mechanism of action is supported by recent evidence that  $\alpha$ -scorpion toxins prevent a component of outward gating charge movement that is associated with inactivation of the channel (Sheets et al., 1999). This voltage-sensor trapping model of toxin action has wide applicability to peptide gating modifier toxins (see below).

#### ***Neurotoxin receptor site 4***

Neurotoxin receptor site 4 is occupied by another group of scorpion toxins: the  $\beta$ -scorpion toxins. They are composed of 60–65 amino acid residues crosslinked by four disulfide bridges (Rochat et al., 1979; Dufton and Rochat, 1984).  $\beta$ -scorpion toxins induce both a shift in the voltage dependence of sodium channel activation in the hyperpolarizing direction and a reduction of the peak sodium current amplitude (Cahalan, 1975; Jover et al., 1980; Jaimovich et al., 1982; Meves et al., 1982; Wang and Strichartz, 1983; Vijverberg et al., 1984; Jonas et al., 1986). Recently, Magi toxin 5 from the spider *Macrothele gigas* has also been shown to bind to neurotoxin receptor site 4, the first spider toxin to have this activity (Corzo et al., 2003).

In order to localize receptor site 4, chimeric sodium channels were used. Analysis of the activity of  $\beta$ -scorpion toxins on chimeric sodium channels, having a combination of domains from cardiac and skeletal muscle or brain sodium channels indicated that domain II is required for binding (Marcotte et al., 1997) and that specific amino acid residues in the extracellular loops connecting the S1–S2 and S3–S4 segments are primarily responsible (Cestèle et al., 1998; Fig. 3). In fact, the single residue chimeric mutation G845N strongly decreased the affinity of the toxin for its receptor site on rat brain sodium channels and markedly altered the electrophysiological effects of  $\beta$ -scorpion toxins. No negative shift in the voltage-dependence of activation was observed for this mutant. Thus, the change of G845 to N is responsible for most of the difference of affinity between the brain and cardiac isoforms and also for the absence of effect of  $\beta$ -scorpion toxin on voltage-dependent activation of the cardiac sodium channel (Cestèle et al., 1998).

These results provide new insight into the mode of action of  $\beta$ -scorpion toxins on sodium channels. The voltage dependence of activation of the brain sodium channels is modified by  $\beta$ -scorpion toxin only after a strong depolarizing prepulse. Therefore, the interaction of the toxin with its receptor site must be dependent on the activated conformational state of the toxin receptor site. Evidently, binding of  $\beta$ -scorpion toxins to this high affinity conformation is disturbed by the mutation G845N. The proposed mechanism of action of  $\beta$ -scorpion toxins on sodium channels therefore involves interaction with the extracellular end of the IIS4 segment in activated sodium channels through binding to the S3–S4 loop. Since the IIS4 segment moves outward during depolarization, it is proposed that the toxin binds to newly accessible residues in the IIS3–S4 loop and the extracellular end of the IIS4 segment during the conditioning prepulse. By binding to the extracellular end of the IIS4 segment,  $\beta$ -scorpion toxins are proposed to trap and stabilize the IIS4 segment in its outward, activated position, thereby enhancing channel activation in response to subsequent depolarization and negatively shifting the voltage dependence of activation (Cestèle et al., 1998).

$\beta$ -scorpion toxins are macromolecular ligands and, like  $\alpha$ -scorpion toxins (Tejedor et al., 1988; Thomsen et al., 1989), may be expected to have additional points of contact with the extracellular domains of sodium channels in addition to their binding site in the IIS3–S4 linker. The first evidence for this has recently come from studies of the specificity of interaction of the  $\beta$ -scorpion toxin Tz1 from *Tityus zulianus* (Leipold et al., 2006), in which three amino acid residues in the C-terminal pore loop in domain III was shown to determine the specificity for  $\beta$ -scorpion toxin action on different sodium channel subtypes. Because the voltage sensor domain (segments S1 to S4) of one  $K_v$  1.2 channel subunit interact with the pore domain (S5 and S6 segments and the P loop) of the adjacent subunit in

clockwise rotation, this novel interaction is consistent with the probable structure of the sodium channel in which the S1 to S4 segments of domain II should interact with the S5 to S6 segments and P loop of domain III.

### ***Neurotoxin receptor site 6***

$\delta$ -conotoxins bind at neurotoxin receptor site 6 and slow sodium channel inactivation (Fainzilber et al., 1994), as indicated in Table 1. Recent structure-function studies indicate that  $\delta$ -conotoxins interact with a set of amino acid residues in the IVS4 segment, near the neurotoxin receptor site 3 (Leipold et al., 2005). Their binding and action is synergistic with  $\alpha$ -scorpion toxins, probably because both of these toxins trap the IVS4 voltage sensor in a similar conformation (see the voltage-sensor trapping model below). These results suggest that the  $\delta$ -conotoxins may slow inactivation by the same molecular mechanism as  $\alpha$ -scorpion toxins, sea-anemone toxins, and spider toxins.

## **Voltage-Sensor Trapping—A Common Mechanism of Action of Polypeptide Gating Modifier Toxins**

### **The voltage-sensor trapping mechanism**

Studies on sodium channels indicate that polypeptide neurotoxins from scorpions and sea anemones have evolved to use a novel voltage-sensor trapping mechanism to alter channel gating (Rogers et al., 1996; Cestèle et al., 1998, 2001). The voltage sensors responsible for sodium channel activation and its coupling to inactivation are transmembrane segments, inaccessible to binding of hydrophilic polypeptide toxins. To alter gating, these toxins have evolved to bind to the S3–S4 loop at the extracellular ends of the S4 segments of sodium channels and impede their transmembrane movements that are required in the gating process. Thus,  $\alpha$ -scorpion toxins and sea-anemone toxins specifically block inactivation by holding the transmembrane segment in domain IV in its inward position, while  $\beta$ -scorpion toxins specifically enhance activation of sodium channels by holding the S4 segment in domain II in its outward position. The  $\delta$ -atrachotoxins from spider venoms are likely to act by a similar voltage-sensor trapping mechanism. All of these toxins directly alter the movements of a voltage sensor and therefore have quite specific effects on channel function. Evidently, animals from at least two different phyla—coelenterates and arthropods—have independently evolved toxins that act on sodium channels through this mechanism.

In fact, this voltage-sensor trapping mechanism may be the molecular basis for the actions of all polypeptide toxins that alter the voltage dependence of ion channel gating. For example, hanatoxins from tarantula bind to the S3–S4 loop of potassium channels and inhibit channel activation by shifting the voltage dependence of activation to more positive membrane potentials (Swartz et al., 1997), and agatoxins from spiders bind to the IVS3–S4 loop of calcium channels and shift their voltage dependence of activation to more positive membrane potential (Bourinet et al., 1999; Li-Smerin et al., 1999). It is highly likely that they also act by trapping these voltage sensors in their inward, resting positions. We predict that additional polypeptide neurotoxins that alter the voltage dependence of ion channel gating will also be found to bind to S3–S4 loops and act by a ‘voltage-sensor trapping’ mechanism.

### **Interaction sites for voltage-sensor trapping**

Several types of gating-modifier toxins that inhibit activation or inactivation bind to their target ion channel through interactions with acidic and hydrophobic amino acid residues in S3–S4 loops, which include a key glutamate that is conserved at the extracellular end of the S3 segment (Rogers et al., 1996; Cestèle et al., 1998; Swartz and MacKinnon, 1997; Bourinet et al., 1999; Winterfield and Swartz, 2000; Li-Smerin and Swartz, 1998, 2000).

Scheme 1 illustrates the alignment of the S3–S4 loops in domain IV of Na<sub>v</sub> 1.2 channels containing the  $\alpha$ -scorpion toxin and sea anemone toxin receptor sites, in domain IV of Ca<sub>v</sub> 2.1 channels containing the  $\omega$ -agatoxin receptor site, and in the K<sub>v</sub> 2.1 channel containing the hanatoxin and grammotoxin receptor sites. This alignment reveals a common toxin-binding motif at the extracellular end of the S3 segment composed of 2 hydrophobic residues and 1 acidic residue in the sequence  $\Phi\Phi\text{XXE}$  (Scheme 1, shaded residues).

In contrast, our studies of the receptor site for  $\beta$ -scorpion toxins have revealed a different interaction motif. Six amino acid residues in loops IIS1–S2 and IIS3–S4 have been identified, whose mutations would reduce the affinity for the  $\beta$ -scorpion toxin CssIV by 13,650-fold if their effects were additive (Cestèle et al., 1998, 2006). The cumulative effects of these mutations would increase the K<sub>D</sub> for CssIV from 0.2 nM to 2.7  $\mu$ M, indicating that these amino acid residues are essential for high-affinity binding of Css IV. Because we have made conservative amino acid substitutions in order to retain protein expression and function, it is likely that the substituted amino acid residues at these critical positions retain a substantial contribution to the residual low-affinity binding of CssIV and therefore that this is a lower limit of their contribution to high affinity binding. The three amino acid residues involved in the formation of the  $\beta$ -scorpion toxin receptor site on IIS3–S4 linker of Na<sub>v</sub> 1.2 channels are quite different in sequence context from those forming the interaction site for  $\alpha$ -scorpion toxins and the toxins that inhibit calcium and potassium channels (Scheme 1). Thus,  $\beta$ -scorpion toxins are the first group of gating modifier toxins to interact with a different binding motif in the S3–S4 linker, and they are unique in enhancing activation of their target channel. We speculate that this novel mode of interaction is essential for the unique effect of  $\beta$ -scorpion toxins to trap the voltage sensor in its activated conformation.

## Structural basis for voltage-sensor trapping

In order to gain insight into the three-dimensional structure of the  $\beta$ -scorpion toxin/sodium channel complex, we modeled the three-dimensional structure of the toxin-receptor complex with the Rosetta algorithm. This modeling method is informatics-based, using the structures in the Protein Databank to predict the folding of each segment of the protein of interest and then assembling the most favored structure based on all known protein structures (Rohl et al., 2004a, b). The Rosetta method has been the most accurate in predicting the structures of folded domains in the CASP trials of modeling (Bradley et al., 2005; Chivian et al., 2005). Moreover, we have recently developed a membrane mode of the Rosetta algorithm that successfully predicts the transmembrane folds of several membrane proteins (Yarov-Yarovoy et al., 2006a, b). We used the three-dimensional structures of related toxins and ion channels to construct a model of the C<sub>ss</sub> IV toxin-sodium channel complex, based on the x-ray crystallographic structure of *Centruroides sculpturatus* variant II, a structurally related  $\beta$ -scorpion toxin (Cook et al., 2002), and the x-ray crystallographic structure of the related voltage sensor of the bacterial voltage-gated potassium channel, K<sub>v</sub> AP (Jiang et al., 2003). This K<sub>v</sub> AP structure was derived from expression and crystallization of the S1 to S4 segments of that channel, and it is likely that this structure represents the activated state of the voltage sensor. We used the Rosetta-Dock algorithm to fit the toxin into the voltage sensor structure, after a manual adjustment of  $-10^\circ$  in the  $\psi$  torsion angle of G822 in the K<sub>v</sub> AP voltage sensor to increase toxin access. The resulting structural model predicts that the C<sub>ss</sub>IV toxin fits tightly into the crevice between the S1–S2 and S3–S4 helical hairpins of the sodium channel IIS1–S4 structure (Fig. 4), a position that would fix movements of the S1–S2 and S3–S4 helical hairpins relative to each other.

Our structural model reveals close interactions between amino acid residues in the toxin and sodium channel that have been shown by mutagenesis studies to be important for toxin binding and action. Residues E837 and L840 in the IIS3–S4 loop make productive binding interactions with amino acid residues in the C<sub>ss</sub> IV toxin (Fig. 4). E837 forms a salt bridge with R27 and has a secondary charge- $\pi$  interaction with Y24. L840 also interacts with Y24 and secondarily with E28 (Fig. 4). Rotating the model  $60^\circ$  reveals E779 in the IIS1–S2 loop, which makes a productive charge- $\pi$  interaction with F44 of C<sub>ss</sub> IV and also interacts with L19 (Cestèle et al., 2006). Although these interactions between two pairs of E and L residues initially seemed surprising, interactions between E and L are very common in protein-protein interfaces of known structure (Glaser et al., 2001) and therefore must be quite favorable for binding interactions. All six of these predicted interactions are consistent with mutagenesis results, as we have found important effects of mutations of E779, E837, and L840 on C<sub>ss</sub> IV binding and action on the sodium channel in this work and Cohen et al. have reported important effects of mutations of L19, Y24, R27, E28, and F44 for binding and toxicity of C<sub>ss</sub> IV (Cohen et al., 2005). The close fit between the structural model and the results of mutagenesis studies strongly supports the accuracy of the structure prediction.

Our previous results also demonstrated a crucial role for G845 in the sodium channel. Mutation of this residue to N decreased toxin-binding affinity by 13-fold and completely prevented voltage sensor trapping, even when high concentrations of toxin were tested (Cestèle et al., 1998). Our model reveals the molecular basis for this strong effect (Fig. 4). Even though G845 does not make a productive interaction with C<sub>ss</sub>IV itself, substitution of the much larger N residue creates a steric conflict with the side chains of N7 and F14 of C<sub>ss</sub>IV, which would reduce toxin binding affinity and potentially prevent voltage sensor trapping. Thus, this correlation also supports the accuracy of our structural model.

In this structure, the interactive face of the  $\beta$ -scorpion toxin is inserted between the S1–S2 loop and the S3–S4 loop, and the amino acid residues known to be important for binding on the toxin and on the channel interact directly in the binding interface. The crevice formed by the S1–S2 and the S3–S4 helical hairpins of the voltage sensor accommodates all of the interactive surface of the toxin that has been defined by mutagenesis studies, arguing against any other significant interaction sites in the resting state. This mode of interaction allows the toxin to control the relative movement of these two extracellular loops and thereby potentially influence the gating movements of the S4 voltage sensor. Based on this structural model, we propose that movement of the IIS4 segment under the influence of the electric field allows new interactions between the channel and the C<sub>ss</sub>IV toxin. This strengthened toxin-receptor interaction would then stabilize the voltage sensor in its activated conformation and enhance subsequent activation of the sodium channel by negatively shifting its voltage dependence.

## Conclusion

Voltage-gated Na<sup>+</sup>, Ca<sup>2+</sup>, and K<sup>+</sup> channels are encoded by 60 genes in the human genome, and these channel proteins are the founding members of the larger superfamily of voltage-gated-like ion channels numbering 143 in total (Yu and Catterall, 2004). These channels have a common pore motif and a common mechanism for pore opening and voltage-dependent gating. Animals and plants from many phyla have targeted these channels with potent neurotoxins, which paralyze prey by altering electrical signaling. The voltage-gated Na<sup>+</sup> channel illustrates this diversity of toxin action, with six receptor sites at which toxins from many different phyla interact and modify channel function. Studies of neurotoxins and Na<sup>+</sup> channels have revealed three broad classes of toxin action (Catterall, 1980; Cestèle and Catterall, 2000). Pore-blocking toxins bind in the transmembrane pore and physically occlude it, thereby preventing ion conductance. Lipid-soluble gating-modifier toxins acting at intramembrane receptor sites bind preferentially to specific conformations of the channel and stabilize unique functional states via an allosteric mechanism, thereby altering activation, inactivation, and even ion selectivity

concurrently. Polypeptide gating-modifier toxins acting at extracellularly exposed receptor sites including the S3–S4 linker and modify the voltage-driven gating movements of the S4 transmembrane segments via a voltage-sensor trapping mechanism, thereby enhancing or inhibiting voltage-dependent activation or inactivation. It is likely that these mechanisms defined for Na<sup>+</sup> channels will be relevant for gating modifier toxins acting on other members of the voltage-gated ion channel family.

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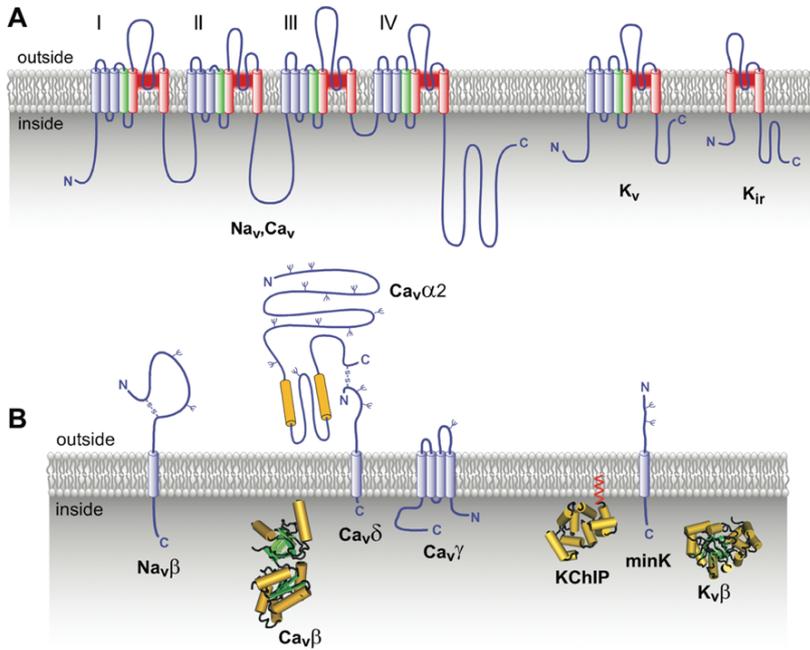
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### Figure 1

#### The voltage-gated ion channels

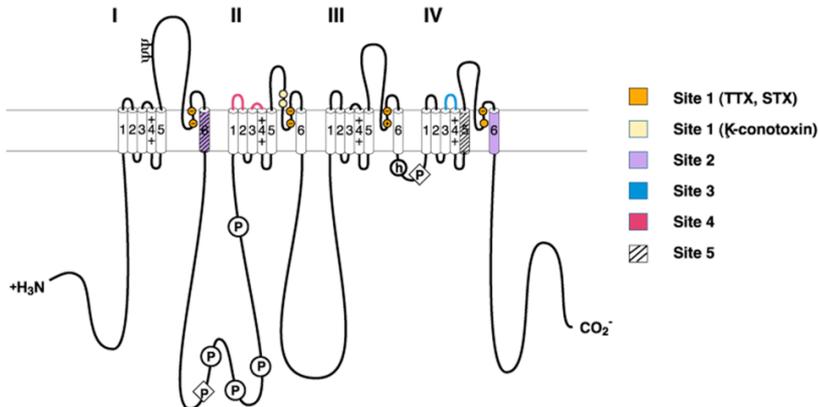
A. The different members of the ion channel protein family structurally related to the voltage-gated ion channels are illustrated as transmembrane folding diagrams in which cylinders represent probable transmembrane alpha helices. Red, S5-S6 pore-forming segments; green, S4 voltage sensor; and blue, S1-S3 transmembrane segments. B. Auxiliary subunits of the voltage-gated ion channels. The auxiliary subunits of  $\text{Na}_v$ ,  $\text{Ca}_v$ , and  $\text{K}_v$  channels are illustrated, with cylinders representing predicted alpha helices of the transmembrane subunits. N-linked carbohydrate chains are indicated by  $\Psi$ . The intracellular auxiliary subunits are illustrated by their predicted three-dimensional structures.



### Figure 2

#### Neurotoxin receptor sites on voltage-gated sodium channels

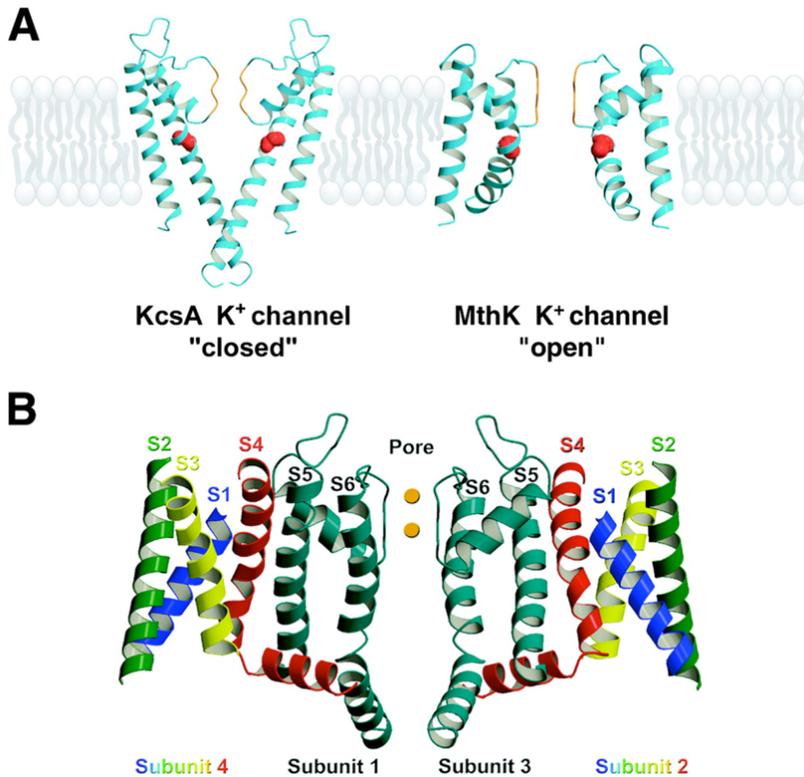
Locations of the neurotoxin receptor sites on mammalian sodium channels are illustrated by colors, as indicated in the Figure.



### Figure 3

#### Structures of potassium channels

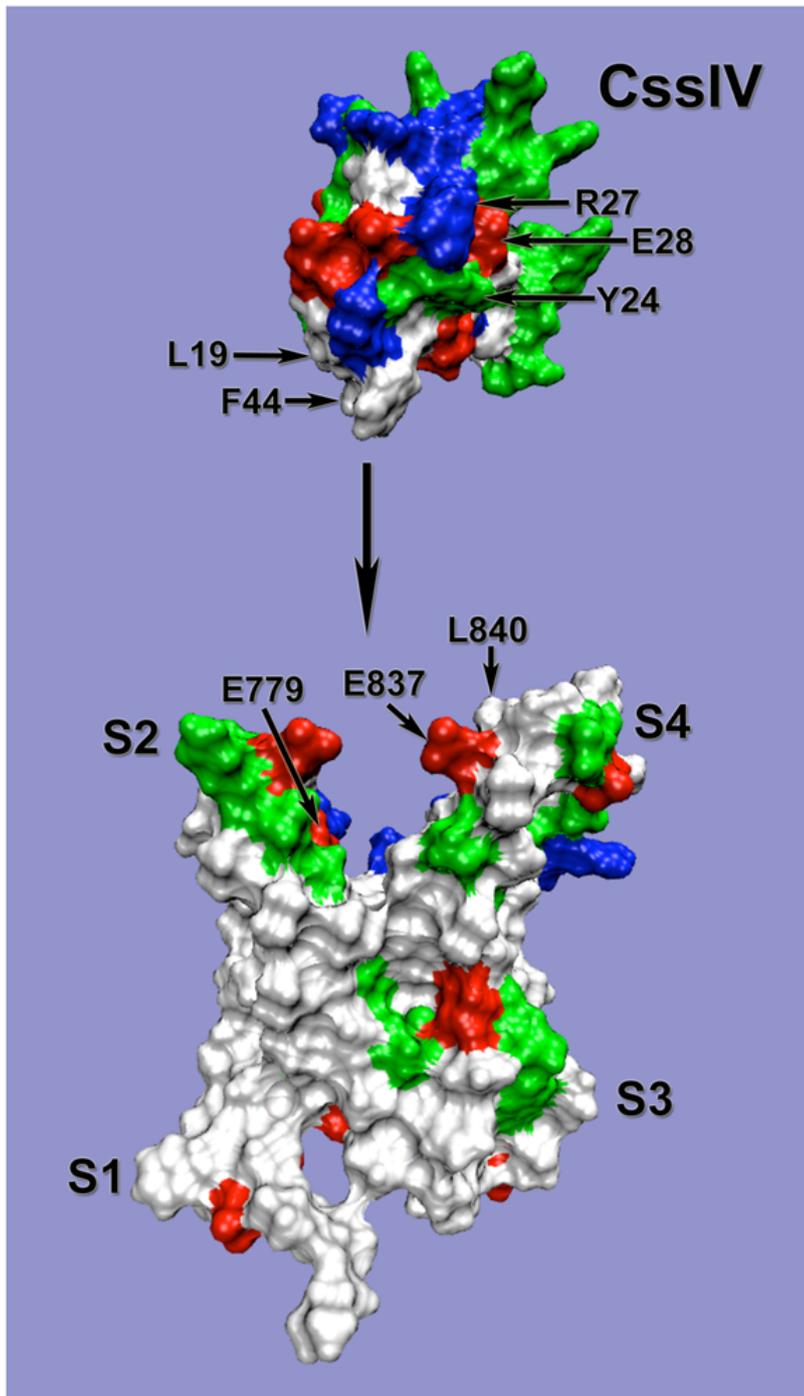
A. Open and closed conformations of the potassium channel pore structures. Two subunits of the bacterial  $K^+$  channels of KcsA (Doyle et al., 1998), representing a "closed" conformation, and MthK (Jiang et al., 2002b), representing an "open" conformation, are shown in the figure. The selectivity filter is yellow, and the outer helix (analogous to S5) is depicted adjacent to the lipid bilayer. The inner helix (analogous to S6) is marked with a space-filling model of the conserved glycine residue (red), which is thought to be critical for the bending of the S6 helix in the open conformation (Zhao et al., 2004). B. Cross-section through the three-dimensional structure of a tetrameric  $K_v$  1.2 channel (Long et al., 2005a,b). Center, the pore regions S5-P-S6 of two subunits, designated 1 and 3, in blue-green. Left, S1 through S4 of subunit 4, whose S5-P-S6 segments project in front of the plane of the figure and form the near side of the pore. Right, S1 through S4 segments of subunit 2, whose S5-P-S6 segments project behind the plane of the figure and form the far side of the pore. As illustrated, the voltage-sensing domain composed of the S1 to S4 segments is positioned to interact with the S5-P-S6 segments of the adjacent subunit located clockwise (as seen from the extracellular side) in the symmetrical tetrameric array.



**Figure 4**

Structural model of  $\beta$ -scorpion toxin binding to  $\text{Na}_v 1.2$

Docking model of the  $\beta$ -scorpion CssIV toxin binding to the voltage-sensing segments of domain II of  $\text{Na}_v 1.2$ , generated as described in the text and in Cestèle et al. (2006). A molecular surface representation of the toxin-receptor complex is illustrated. To demonstrate overall shape complementation, the model for the  $\beta$ -scorpion toxin CssIV was separated from the sodium channel model. Side chains of selected residues are labeled with corresponding residue numbers. Side chains are colored as white for hydrophobic residues, blue for positively charged residues, red for negatively charged residues, and green for polar but uncharged residues.

**Scheme 1**

$\text{Na}_v 1.2$ Domain II	S3	<b>IVSLSLMELGLANVEG</b>	S4
$\text{Na}_v 1.2$ Domain IV	S3	VVILSIVGMFLAELIEKYFV---SPT	S4
$\text{K}_v 2.1$	S3	AILPYYVTIFLTESNKSVLQFQNVRR	S4
$\text{Ca}_v 2.1$ Domain IV	S3	TVLGSITDILVTEFGNNFIN----LS	S4

**Table 1**  
Neurotoxin Receptor Sites on Voltage-gated Sodium Channels

<b>Receptor Site</b>	<b>Neurotoxin</b>	<b>Functional Effect</b>
Site 1	Tetrodotoxin Saxitoxin $\mu$ -Conotoxin	Pore block
Site 2	Batrachotoxin Veratridine Grayanotoxin Aconitine	Persistent activation, enhanced activation, & block of inactivation
Site 3	$\alpha$ -Scorpion toxins Sea anemone toxins Atrachotoxins	Slowed inactivation
Site 4	$\beta$ -Scorpion toxins	Enhanced activation
Site 5	Brevetoxins Ciguatoxin	Enhanced activation & block of inactivation
Site 6	$\delta$ -Conotoxins	Slowed inactivation