

# Structural and functional study of potassium channel inhibitor HsTX1

Mao-Feng Ger

## Introduction

Membrane proteins are thought to account for 30% of genes; thus there may be at least 10 000 membrane proteins encoded in the human genome. Ion channels are integral membrane proteins that allow movement of ions across membranes down their electro- chemical gradients. These channel proteins form water-filled, gated pores that are often highly selective for specific ions (such as sodium, calcium, potassium or chloride). Only when the channels are open, ions can flow in and out. The opening of ion channels depends on of membrane potential (voltage-gated channels), binding of signaling molecules such as neurotransmitters, ions or nucleotides (ligand-gated channels) or stretch of the membrane (mechanosensitive channels).

Potassium channels comprise a large family of ion channels. Their physiological functions are quite important, concerned with maintaining a negative voltage inside cells relative to outside. K channels have different families, according to their gating mechanism, i.e. the control of opening and closing of the channel.

All K channels share the same core structure. Voltage-gated potassium channel is made from 4 alpha polypeptides forming a central pore. Each polypeptide has 6 transmembrane regions. The beta chain is regulatory and can interact with the alpha subunit to regulate the gating kinetics and enhance the stability of the complex. Another class of potassium channels is in-ward rectifiers. The channel is made from 4 identical subunits. They have 2 membrane-spanning segments and 1 pore-lining segment.

Analysis of K channel sequences can understand the relationships between sequence motifs and various aspects of physiological function. In particular, it was shown that the selectivity of K channels for potassium ions is related to a conserved sequence motif TVGYG located in a re-entrant loop present in between two predicted transmembrane (TM)  $\alpha$ -helices. This sequence motif, conserved across all K channels, was proposed to correspond to the selectivity filter of the pore-forming region of the channel protein. K channels are made from 4 subunits and thus four copies of the filter motif come together to form the pore. More detailed molecular physiological

studies assigned functional roles (e.g. susceptibility to channel block; control of channel gating) to other regions of voltage-gated potassium channels. These studies enabled us to solve some basic questions through structural and computational methods.

In scorpion venom, we found several toxins that can bind ion channels with high affinity and thus be of pharmacological use. The main molecular targets of scorpion neurotoxins are the voltage-gated sodium channels and the voltage-gated potassium channels (and other K channels, including the calcium-activated and delayed-rectifier K channels). Excitable cells are where scorpion neurotoxins act mainly. That means nerve and muscle cells. Many scorpion toxins can block voltage-gated with varying selectivity of action. These peptides bind targets by the interactions of negatively charged residues in the channel with positively charged residues in the peptide toxins. Therefore, scorpion neurotoxins can be highly lethal by interrupting the ion channel mechanism, being comparable to those found in many snakes, and are less potent than only some bacterial toxins.

Recently, scientists have identified some toxins acting on K channels and made a formal classification. According to amino acid sequences comparison, those toxins can be classified into four distinct groups with 50-80% sequence identity within a group, and less than 50% between members of different groups. The first group contains charybdotoxin (ChTX) and Lq2 from *Leiurus quinquestriatus hebraeus* and iberiotoxin (IbTX) from *Buthus tamulus*. The second group contains the kaliotoxins (KTX) from *Androc-tonus* scorpions and the agitoxins (AgTX) from *L. quinquestriatus hebraeu*, with 80-90% sequence identity. The third group has noxiustoxin (NTX) from *Centruroides noxius*, margatoxin (MgTX) from *Centruroides margaritatus*, and TsKR from *Tityus serrulatus* with 60-80% sequence identity with each other. The fourth group was discovered recently and contains toxins with 35 amino acid residues and three or four disulfide bridges, all isolated from scorpions belonging to the *Scorpionidae* family, *Pandinus imperator* [Pi1, Pi2 and Pi3], and *Scorpio maurus* [maurotoxin (MTX)]. They display 50-70% sequence identity with each other, and less than 50% sequence identity with the other toxins.

HsTX1 has been identified in the venom of *Heterometrus spinnifer* (*Scorpionidae*), on the basis of its ability to block the rat Kv1.3 channels reversibly and efficiently.

HsTX1 is a 34-residue peptide with four disulfide bridges and belongs to the fourth group. The disulfide pairing was determined by sequencing heterodimers produced by mild enzymic hydrolysis. HsTX1 shares 53% and 59% sequence identity with *Pandinus imperator* toxin1 (Pi1) and maurotoxin. We went through the determination of the 3D solution structure of HsTX1, to have a detailed structure of a four-disulfide-bridged potassium channel blocker. This structure was then docked on

a model of Kv1.3. From our complex models, an original configuration is proposed for the function of HsTX1.

## Analysis

### (1) Sequence analysis

HsTX1 shares 53-59% identical with those four-disulfide-bridged scorpion toxins (Pi1, MTX) and Compared to those three-disulfide-bridged scorpion toxins, the identities are only 32-47 %. Thus, according to chain length, disulfide bridge number and sequence similarity, HsTX1, MTX and Pi1 constitute a new structural class of scorpion K channel inhibitors. Indeed, they are from the venom of Scorpionidae.

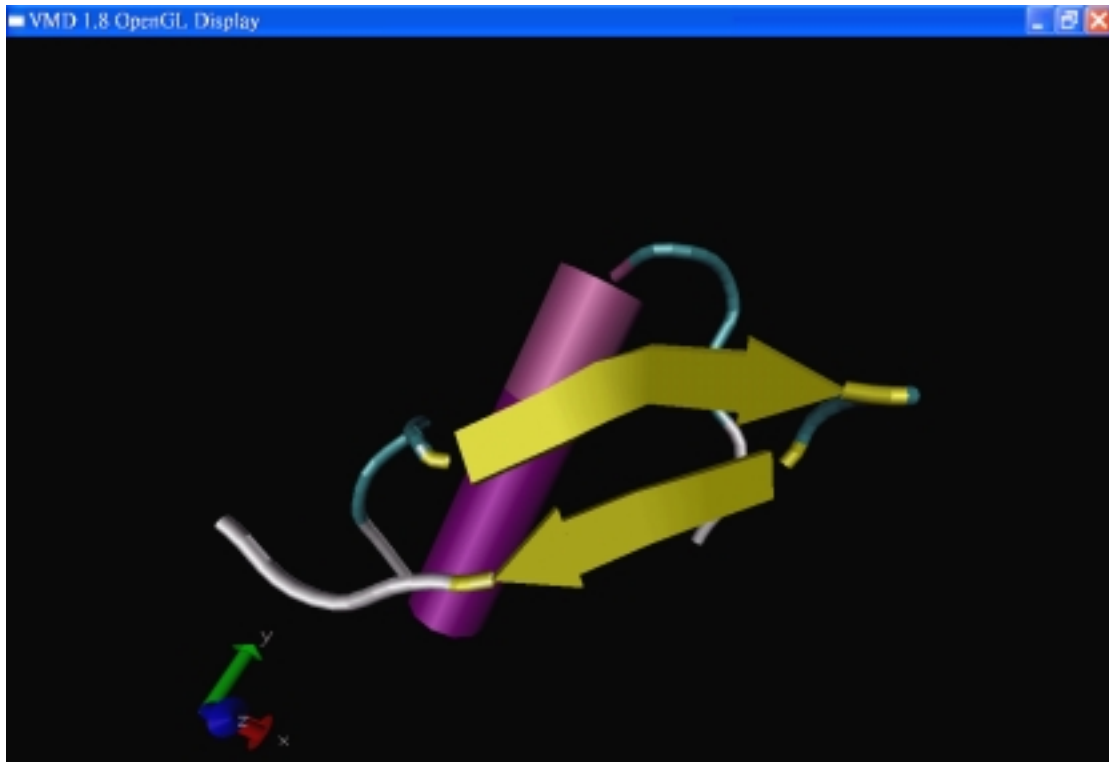
	1	10	20	30	40	Similarity (%)
<b>HsTX1</b>	-----ASCRT	<b>PKDCADPC</b> CRK	ETG <b>CPYG</b> -KC	MNR <b>KCKCNRC</b> *		100
<b>maurotoxin</b>	-----VSCTG	<b>SKDCYAPC</b> RK	QTG <b>CPN</b> -AKC	IN <b>KSCKCYGC</b> *		59
<b>Pi1</b>	---L-VKCRG	<b>TSDCGRPC</b> QQ	QTG <b>CPNS</b> -KC	IN <b>RMCKCYGC</b> *		53
<b>Cl1TX 1</b>	-ITINVKCTS	<b>PQQCLRP</b> CKD	RFGQHAGG <b>KC</b>	ING <b>KCKCYP</b> -		41
<b>MgTX</b>	-TIINVKCTS	<b>PKQCLPP</b> CKA	QFGQSAG <b>AKC</b>	MNG <b>KCKCYPH</b>		47
<b>NTX</b>	-TIINVKCTS	<b>PKQCSKP</b> CKE	LYGSSAG <b>AKC</b>	MNG <b>KCKCYNN</b> *		47
<b>KTX</b>	GVEINVKCSG	<b>SPQCLKP</b> CKD	AGMRF-G- <b>KC</b>	MNR <b>KCHCTPK</b>		38
<b>AgTX1</b>	GVPINVKCTG	<b>SPQCLKP</b> CKD	AGMRF-G- <b>KC</b>	ING <b>KCHCTPK</b>		32
<b>AgTX2</b>	GVPINVSC <b>TG</b>	<b>SPQCIKP</b> CKD	AGMRF-G- <b>KC</b>	MNR <b>KCHCTPK</b>		41
<b>AgTX3</b>	GVPINV <b>PCTG</b>	<b>SPQCIKP</b> CKD	AGMRF-G- <b>KC</b>	MNR <b>KCHCTPK</b>		38
<b>ChTX</b>	-ZFTNV <b>SCTT</b>	<b>SKECWSV</b> CQR	LHNTSRG- <b>KC</b>	MN <b>KKCRCYS</b> -		41
<b>IbTX</b>	-ZFTDV <b>DCSV</b>	<b>SKECWSV</b> CKD	LFGVDRG- <b>KC</b>	MG <b>KKCRCYQ</b> -		35
<b>LQ2</b>	-ZFT <b>QESCTA</b>	<b>SNQCWSI</b> CKR	LHNTNRG- <b>KC</b>	MN <b>KKCRCYS</b> -		35

Figure: The sequences shown are Heterometrus spinnifer toxin 1 (HsTX1), MTX, Pi1, Centruroides limpidus limpidus toxin 1 (Cl1TX1), MgTX, noxiustoxin (NTX), KTX, AgTX1,2 and 3, ChTX, iberiotoxin (IbTX) and Leiurus quinquestriatus toxin 2 (Lq2). Cys residues are indicated in bold type. Percentages of sequence identity are given relative to HsTX1.

In HsTX1, the five residues proposed to be characteristic of the inhibitory activity against voltage-gated potassium channel are Y (position 26), K (position 29), M (position 31), N (position 32) and R (position 39).

### (2) Backbone structure

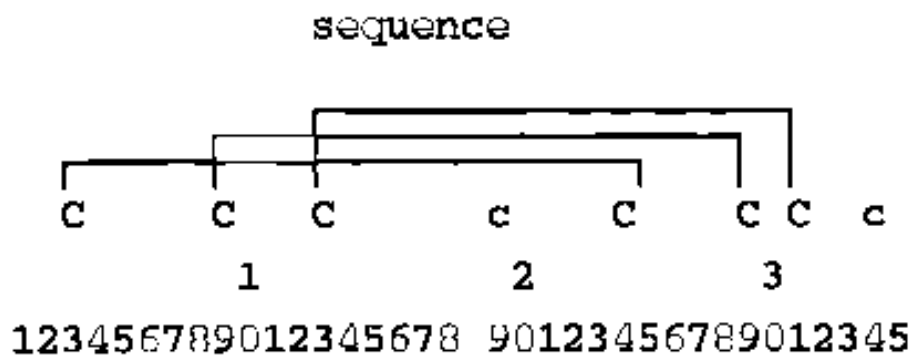
The backbone structure is well defined and we can explore it through VMD.



There are one helix and two sheets in this molecule. In the helix (residue 6-16), residue 6-9 is  $3_{10}$  helix and residue 10-16 is alpha-helix.

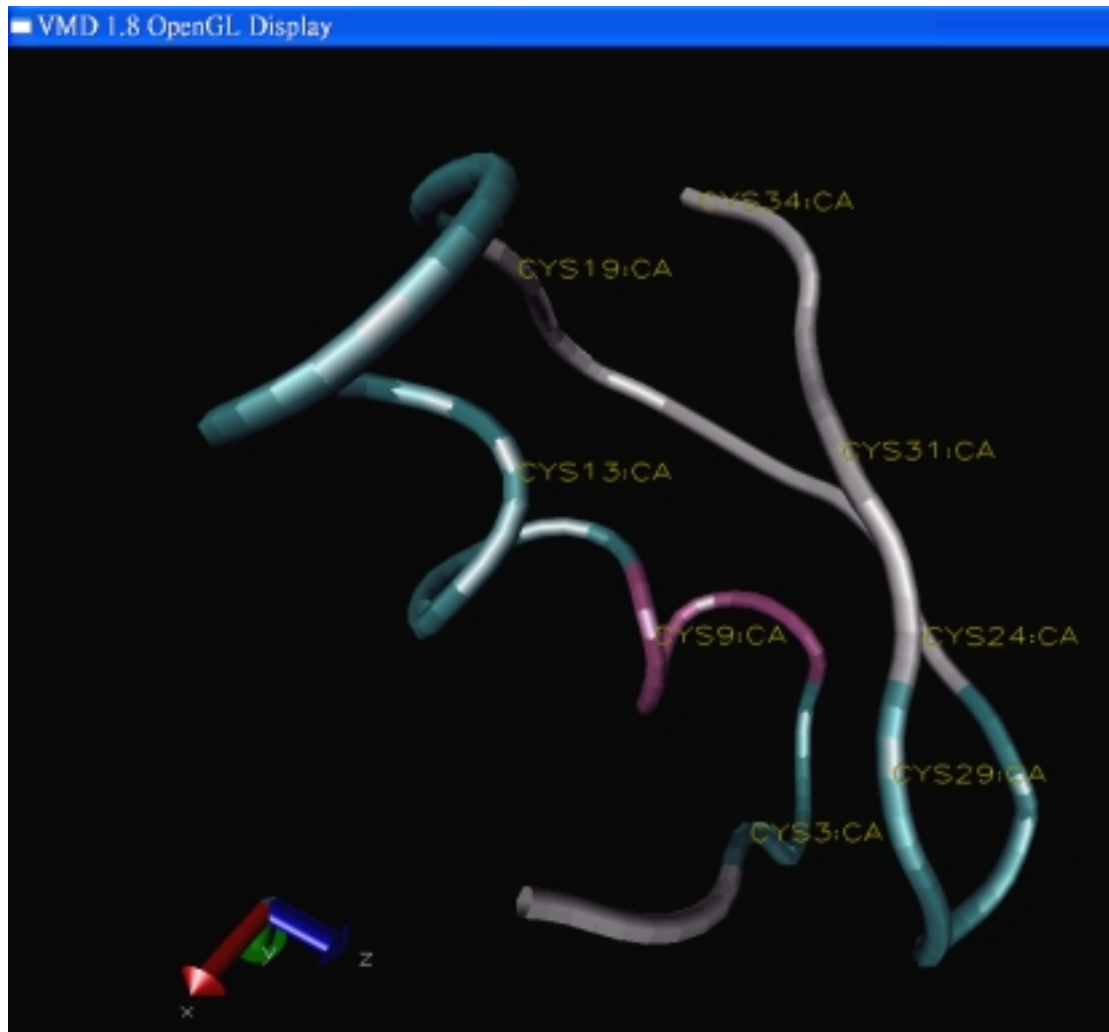
### (3) Disulfide bridge pairing determination

There are eight cysteines (3,9,13,19,24,29,31, and 34) in HsTX1. In other scorpion toxins, three disulphide bridges are 3-24, 13-31 and 9-29. HsTX1 has an extra one, 19-34.



The helix is linked to the sheet by two disulfide bridges 9-29 and 13-31. The 3-24 bridge links the N-terminus to the sheet. The 19-34 bridge links the loop connecting alpha and beta structures to the C-terminus.

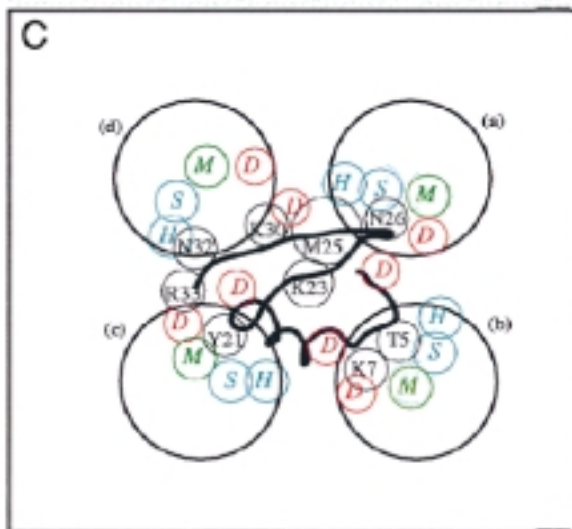
The eight cysteines in HsTX1 are shown through VMD.



#### (4) Interaction of HsTX1 with Kv1.3 channel

The affinity of HsTX1 towards the Kv1.3 channels has been proved. This binding is reversibly and efficiently.

HsTX1 was docked on a model of Kv1.3 deduced from the crystal structure of the *Streptomyces lividans* potassium channel. The magnitude of the electrostatic and van der Waals energies between HsTX1 and the channel suggests a nice complementary of the two structures. The interaction between HsTX1 and Kv1.3 is mediated by two salt bridges linking positively charged residues of the toxin to negatively charged residues of the channel and six additional hydrogen bonds.

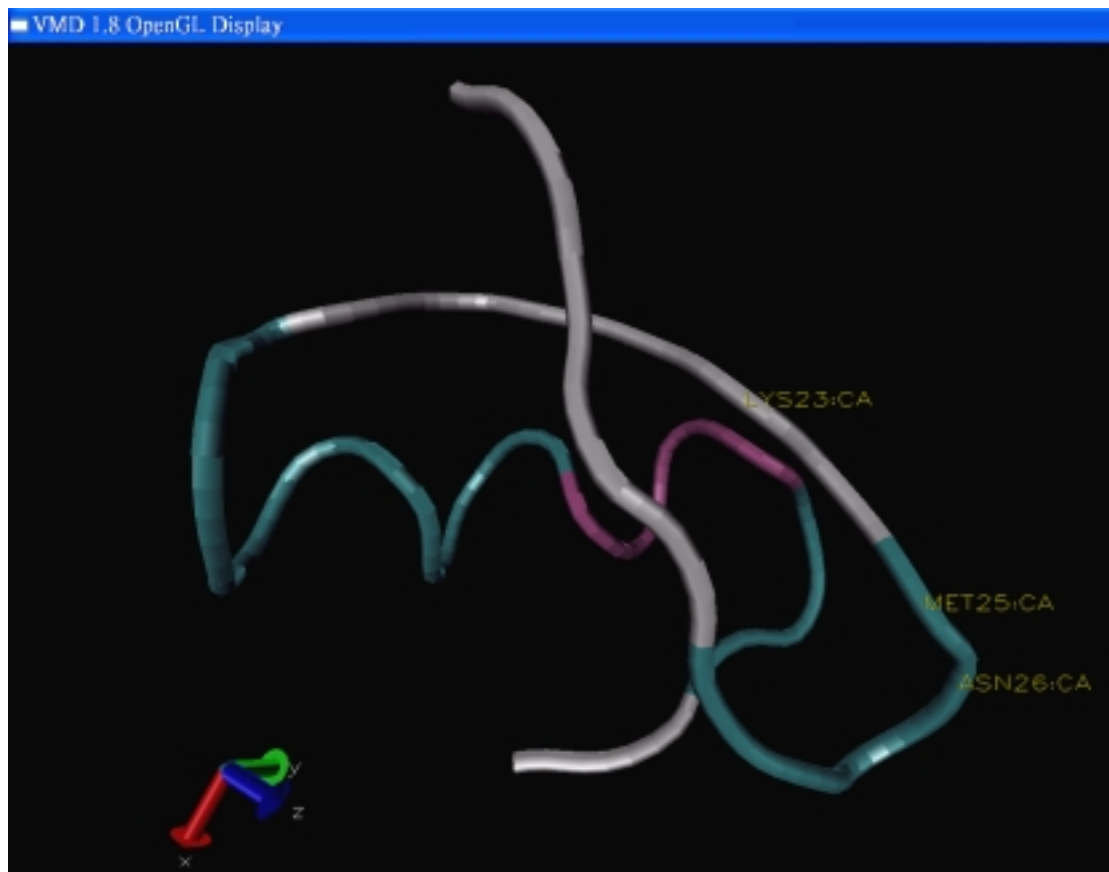


## Discussion

The 3D structure of HsTX1 is similar to those of three-disulfide-bridged toxin which can block the voltage-gated potassium channels. The extra disulfide bridge does not change the protein folding.

The similar folding of HsTX1 to other three-disulfide-bridged toxins might help to highlight some important amino acid residues for the mechanism of toxins blocking potassium channels.

The triplet Lys23, Met/Ile25 and Asn26 found in all the voltage-gated potassium channel inhibitor functional site is probably critical for the binding to its biological target.



HsTX1 can be a new tool to investigate voltage-gated potassium channels. We need to go further to define its 3D structure and its selectivity towards different subtypes of voltage-gated potassium channels.

Multiple toxins may be present in the venom of a single species of scorpion, which serves to ensure that the venom will be capable of producing potent synergistic actions when discharged into a victim. The bioactivity of the neurotoxins present in scorpion venoms also displays a high degree of host specificity: thus, the venom from

a single species of scorpion may contain a toxin preferentially targeting only insects, another only crustaceans and yet another only mammals.

## Reference

- Aiyar, J., Withka, J. M., Rizzi, J. P., Singleton, D. H., Andrews, G. C., Lin, W., Boyd, J., Hanson, D. C., Simon, M., Dethlefs, B., Lee, C. L. et al. (1995) *Neuron* 15, 1169-1181
- Gwee MC, Nirthanan S, Khoo HE, Gopalakrishnakone P, Kini RM, Cheah LS. Autonomic effects of some scorpion venoms and toxins. *Clin Exp Pharmacol Physiol.* 2002 Sep;29(9):795-801
- Kharrat R, Mabrouk K, Crest M, Darbon H, Oughuideni R, Martin-Eauclaire MF, Jacquet G, El Ayeb M, Van Rietschoten J, Rochat H, Sabatier JM. 1996. Chemical synthesis and characterization of maurotoxin, a short scorpion toxin with four disulfide bridges that acts on K<sup>+</sup> channels. *Eur J Biochem* 242:491–498.
- Lebrun B, Romi-Lebrun R, Martin-Eauclaire MF, Yasuda A, Ishiguro M, Oyama Y, Pongs O, Nakajima T. A four-disulphide-bridged toxin, with high affinity towards voltage-gated K<sup>+</sup> channels, isolated from *Heterometrus spinnifer* (Scorpionidae) venom. *Biochem J.* 1997 Nov 15;328 ( Pt 1):321-7
- Romi-Lebrun R, Lebrun B, Martin-Eauclaire MF, Ishiguro M, Escoubas P, Wu FQ, Hisada M, Pongs O, Nakajima T. Purification, characterization, and synthesis of three novel toxins from the Chinese scorpion *Buthus martensi*, which act on K<sup>+</sup> channels. *Biochemistry.* 1997 Nov 4;36(44):13473-82.
- Savarin P, Romi-Lebrun R, Zinn-Justin S, Lebrun B, Nakajima T, Gilquin B, Menez A. Structural and functional consequences of the presence of a fourth disulfide bridge in the scorpion short toxins: solution structure of the potassium channel inhibitor HsTX1. *Protein Sci.* 1999 Dec;8(12):2672-85.