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Research Article

Docking of Homology Modeled Neurotoxin from *Acalyptophis peronii* onto the Crystal Structure of Acetyl Choline Binding Protein

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ABSTRACT

The present study focused on modelling the neurotoxin protein sequence from sea snake (*Acalyptophis peronii*) based on homology modelling technique and docking the predicted structure onto the crystal structure of acetyl choline binding protein (1YI5_F). The 2 neurotoxin models that were generated using the PDB templates 1IQ9_A and 1YI5_F had a similar gross topology, exhibiting a typical *three finger motif*. These predicted structures consisted of three loops (designated as loop I, II and III) which were clamped together by 4 disulphide bridges (cys^{3} – cys^{22}, cys^{17} – cys^{39}, cys^{41}-cys^{52}, cys^{53}- cys^{58}). Though both the models appeared to be reasonably good as revealed by the structure assessment reports, the model built using 1IQ9 template seems to be better since this has been predicted with greater accuracy. This is based on the fact that larger percentage of residues of the neurotoxin model predicted using 1IQ9 was present in the core region, with an overall G-factor value of 0.06 and also had the lowest energy.

Despite the fact that neurotoxin model generated using 1IQ9 was better, ZDOCK server was however, successful in docking the neurotoxin model predicted using 1YI5_F onto the acetyl choline binding receptor. The residues tyr^{185} and tyr^{192} of acetyl choline binding protein appeared to be in close proximity with the residues trp^{27}, ser^{28}, asp^{29}, his^{30}, gly^{32} and thr^{33} of loop II of the predicted neurotoxin structure.

INTRODUCTION

Venom informatics is a systematic bioinformatics approach in which classified, consolidated and cleaned venom data are stored into repositories and integrated with advanced bioinformatics tools for the analysis of structure and function of toxins. Various animals produce venom in specialized glands, for efficient hunting of prey and for defense against predators. Venomous animals are diverse and include, among others, species of jellyfish, cone snails, bees and wasps, spiders, scorpions, fish, snakes and even platypuses. They deliver venom through biting or stinging (Menez (1998)). Toxins are highly active molecules that target various cellular receptors and assist in prey digestion (Valentin and Lambeau (2000)). The probable ancestral function of venoms was enzymatic activity involved in prey digestion; however, in some animals including marine stingers, cone snails, spiders, scorpions and snakes, venom glands have evolved to produce potent toxins (Valentin and Lambeau (2000)).

Venom-toxins have wide pharmacological properties and exert their influence through interaction with a diverse range of targets which includes various cellular receptors, membranes

and ion channels (Valentin and Lambeau (2000); Hains *et al*., (1999); Kordis and Gubensek (2000)). Established methods for determining specific functions of venom-toxins are based on the experimental studies of naturally occurring peptides (Klenk *et al*., (2000)) site directed mutagenesis (Martinez *et al*., (1998)) or use of chemically modified variants (Hassani *et al*., (1999)). The pharmacological properties of venomtoxins are tested in animal models such as mouse, rat or insects. The experimentation is often supported by computational algorithms for sequence comparison (Possani *et al*., (2000); Tytgat *et al*., (1999)) or for modelling of venom-toxin three-dimensional (3D) structure (Ferrat *et al*., (2001); Juan *et al*., (1999)). Systematic functional study of even one individual toxin requires a significant experimental effort. Therefore, most research groups focus on determining functional properties of individual toxins or small groups of toxins. Bioinformatics analyses can improve the efficacy of research by assisting in selection of critical experiments. Bioinformatics approaches involve access to venom data across multiple databases, inspection for errors, analysis and classification of venom-toxin sequences and their structures and the design and use of predictive models for simulation of laboratory experiments.

Phylogenetic analysis has been used to determine diversification of conotoxins (Espiritu *et al*., (2001); Conticello *et al*., (2001)) and classification of scorpion (Tytgat *et al*., (1999); Heinrikson *et al*., (1977)) and snake toxins (Tsai *et al*., (2002); Okuda *et al*., (2001)). Multiple sequence alignment was used to identify related function of two novel defensins from scorpion venom (Legros *et al*., (1998)). The analysis of the 3D structures provides a complementary approach to site-directed mutagenesis for identification of functional residues in venom toxins (Carredano *et al*., (1998); Ward *et al*., (1998); Savarin *et al*., (1999); Renisio *et al*., (2000)). Further, the analysis of 3D structures of venom toxins have been used as molecular probes for acetylcholine (ACh) receptors (Lipkind and Fozzard (1997); Ferrat *et al*., (2001); Rowan (2001)).

The primary sequence of neurotoxin from sea snake (*Acalyptophis peronii*) is deposited by Pahari *et al*., (2007) into Genbank of NCBI. Since structural information of this protein through experimental means is not yet available, the present study has been undertaken with the following objectives:

A) To predict the 3D structure of neurotoxin from *Acalyptophis peronii* using computational tools.
B) To dock the predicted neurotoxin structure onto the crystal structure of acetyl choline binding receptor.

MATERIAL AND METHODS

An investigation was carried out at Department of Biotechnology, Indian Academy, Bangalore, for docking the homology modeled neurotoxin (AY742210; *Acalyptophis peronii*) onto the crystal structure of acetylcholine binding protein (PDB id: 1YI5_F; Bourne *et al*., (2005)). Sequence of neurotoxin (81 amino acids) from *Acalyptophis peronii* (AY742210) deposited in Genbank of NCBI (Pahari *et al*., 2007) was retrieved for the purpose of computational studies.

Prediction of signal peptide in neurotoxin

The signal peptide in neurotoxin of *Acalyptophis peronii*, was obtained from the SignalP tool developed by Jannick *et al.*, (2004). The method incorporates a prediction of cleavage sites and a signal peptide/non-signal peptide prediction based on a combination of several artificial neural networks and hidden Markov models. The output generated from the SignalP comprises different scores, C, S and Y. The *C-score* is the ``cleavage site'' score. For each position in the submitted sequence, a C-score is reported, which should only be significantly high at the cleavage site. The *S-mean* is the average of the S-score, ranging from the N-terminal amino acid to the amino acid assigned with the highest Y-max score, and the S-mean score is calculated for the length of the predicted signal peptide.

Selection of the PDB templates

Selection of the best PDB templates for the neurotoxin of *Acalyptophis peronii* was done by submitting the sequence information (devoid of

signal peptide) to the PSI-BLAST (Altschul, *et al.,* 1997) tool available at www.ncbi.nlm.nih.gov. All the parameters chosen at default and the degree of similarity is given in terms of a scoring parameter called the E-value.

Multiple sequence alignment

The selected PDB templates along with the neurotoxin from *Acalyptophis peronii* were submitted to CLUSTALW tool (Thompson *et al*., 1994) to know the presence of conserved amino acid residues in neurotoxin. We tried to calculate the best match for the selected sequences and lined them up to see the identities, similarities and differences.

Homology modelling of neurotoxin from *Acalyptophis peronii* was done using the Deep View (Swiss-PdbViewer) software developed by Guex and Peitsch (1997). The 3D structure of neurotoxin from *Acalyptophis peronii* was used for this purpose. The PDB file comprising of raw sequence of neurotoxin was threaded onto the PDB template and was later submitted to the SWISS-MODEL project (optimize) mode server (Schwede *et al*., 2003) for model building.

Loop refinement and structure validation

The initial 3D model (s) of neurotoxin was subjected to loop refinement based on the PROSA **(**Wiederstein and Sippl, 2007**)** and PROCHECK reports (Laskowski *et al*., 1993). PDB format of the generated neurotoxin model was uploaded and sent to the server for analysis. ProSA was done to calculate an overall quality score for the input structure and to check the stereochemistry of the protein structure. Ramachandran plot analysis was also done by PROCHECK program to display psi and phi backbone conformational angles for each residue in the protein.

Protein – protein docking

Homology modeled neurotoxin from *Acalyptophis peronii* was docked onto the crystal structure of acetylcholine binding receptor protein (PDB: 1YI5_A) (Bourne *et* al., 2005) using the ZDOCK, a server for protein-protein docking. Since the PDB template 1YI5 consists of acetylcholine binding protein (AChBP) (chain A to E) complexed with neurotoxin (chain F to J), the coordinates for AChBP were separated for docking purpose. The PDB format files obtained for receptor (AChBP) and ligand (neurotoxin) were uploaded to the ZDOCK server. Depending upon the type of residues, they were either blocked from or forced into the binding site. The first three download links for the ZDOCK output file, ligand file and receptor file are downloaded and visualized through Deep view package

RESULTS

Signal peptide in neurotoxin

Based on the output generated by the SignalP tool, neurotoxin sequence from *Acalyptophis peronii* appeared to have a signal peptide. This was represented as most likely cleavage site present between position 21st and 22nd amino acid. While the probability for the presence of signal peptide suggested by the tool was 0.984, the probability of maximum cleavage site identified between position 21 and 22 was 0.640 (Fig 1). Subsequent computational analysis of the neurotoxin sequence was carried out by deleting the signal peptide (length 21 amino acid).

Selection of PDB templates and multiple sequence alignment

Analysis of PSI BLAST output at the end of 20th iteration revealed that, neurotoxin of *A. peronii* had very close homology with neurotoxins of other related organisms. Among several PDB structures that were recognized by the PSI BLAST tool, 1IQ9_A shared maximum sequence identity (70%) with the neurotoxin of *A. peronii* coupled with lowest E value of 5E – 13 (Table 1).

Remaining top 6 hits which include 1NTX_A, 1NOR_A, 1JE9_A, 1ONJ_A, 6EBX_A and 2ERA_A were chosen as the best template sequence set for multiple sequence alignment. Sequence identity of these selected subject sequences with the query sequence (i. e, neurotoxin from *A. peronii*) was more than 60 percent, with E value varying between 9E-13 to 3E-11 (Table 1).

The results of multiple sequence alignment clearly showed that majority of the template sequences, except 1YI5_F, had an alignment score between 65 and 71 with neurotoxin sequence of *A. peronii* (Fig 2). Alignment score between neurotoxin of *A. peronii* with 1YI5_F sequence was 35. Among various residues that were conserved in neurotoxin sequence of *A. peronii*, cysteine ($cys^{3, 17, 22, 39, 41, 52, 53, 58}$) which is involved in the formation of disulphide bridge, followed by tryptophan (trp^{27}), aspartate (asp^{29}) and arginine (arg^{31}) responsible for interaction with acetyl choline receptor, were found to be totally conserved with remaining template sequences (Fig 2). Further, on the basis of cladogram report, neurotoxin sequence of *A. peronii* was placed close to 1IQ9_A compared to other PDB template sequences (Fig 2).

Homology modeling of neurotoxin from *Acalyptophis peronii*

Using project optimization mode of SWISS-MODEL server, 2 models of neurotoxin (model_ 1 and model_2) were generated using the PDB templates, 1IQ9_A and 1YI5_F. Figure 3a and 3b illustrates the folding and arrangement of polypeptide chain in neurotoxin of *A. peronii*. The gross topologies of both the models were similar, exhibiting a characteristic "*three finger motif*". These predicted structures consisted of three loops (designated as loop I, II and III) which were clamped together by 4 disulphide bridges (cys^3 – cys^{22}, cys^{17} – cys^{39}, cys^{41}-cys^{52}, cys^{53}- cys^{58}). While loop I and II formed 2 stranded β- sheets [β1: residue 1-5 (loop I), β2: residue 13 – 17 (loop I), β3: residue 21-29 (loop II), β4: residue 32-40 (loop II)], loop III formed only 1 stranded β- sheet (β5: residue 49-53).

External validation

Validation of the predicted 3D neurotoxin structures (after loop refinement) by PROCHECK analysis showed that 82.4% of the residues (Fig 5 and Table 2a) of neurotoxin model_1 (using 1IQ9 as the template) and 72.9 % of the residues (Table 2b) of neurotoxin model_2 (using 1YI5_F as the template) were present in the core region of Ramachandran plot. However, Ramachandran plot analysis of the PDB templates (1IQ9 and 1YI5_F) showed that 87.8% of the residues of 1IQ9 and 78.9 % of 1YI5_F were present in the core region (Table 2a and 2b). The analysis also showed that only 2.0 % of the residues of neurotoxin model_1 (Fig 4 and Table 2a) and 0.0 % of residues of neurotoxin model_2 were located in the disallowed region of Ramachandran plot (Table 2b). While the overall G factor for neurotoxin model_1 was 0.06, it was -0.34 for the neurotoxin model_2 (Table 2a and 2b).

Superimposition of neurotoxin model of *Acalyptophis peronii* onto the PDB templates

Superimposition of neurotoxin models (1 and 2) onto the PDB templates using SWISS-PDB viewer, revealed that RMSD of Cα atoms was 0.81^{o}A for model_1 with 1IQ9 and 1.81^{o}A for model_2 with 1YI5_ Based on the illustration in figure 7 (B), it is noticed that loop I of neurotoxin model _2 was larger compared to corresponding loop in 1YI5_F. Although folding pattern of loop II and loop III segments of neurotoxin model_2 was similar to that of 1YI5_F, minor structural deviations could be noticed in these 2 loop segments. Backbone RMSD between neurotoxin models 1 and 2 upon superimposition was 2.51^{o}A (Fig 7). Although there was near perfect superimposition of loop II segment of the predicted neurotoxin structures, there was variation in the folding pattern of loop I and III.

Docking of neurotoxin of *Acalyptophis peronii* onto Acetyl choline binding protein

Attempt was made to dock both the homology modeled neurotoxin models (generated using 1IQ9 and 1YI5_F) onto acetyl choline binding protein using ZDOCK server. However, only neurotoxin model built using 1YI5_F got docked successfully onto the acetyl choline binding protein (Table 3 and Fig 6). Based on the intermolecular interactions revealed by PyMOL package, residues tyr^{185} and tyr^{192} of acetyl choline binding protein were in close proximity with the residues trp^{27}, ser^{28}, asp^{29}, his^{30}, gly^{32} and thr^{33} of loop II of neurotoxin (Fig 7).

Tables:

Table 1: Summary of the best PDB templates generated at the end of 20th iteration of PSI-BLAST analysis for neurotoxin (AY742210) from *Acalyptophis peronii*. Pairwise alignment between the query (AY742210) and the PDB template 1IQ9 is also shown at the bottom.

E-value BETTER than threshold	Score (Bits)	E Value
pdb|1IQ9|A Chain A, Crystal Structure At 1.8 A Of Toxin A Fro...	68.9	5e-13
pdb|1NTX|A Chain A, Secondary Structure Determination For Alp...	68.1	9e-13
pdb|1NOR|A Chain A, Two-Dimensional 1h-Nmr Study Of The Spati...	67.3	1e-12
pdb|1JE9|A Chain A, Nmr Solution Structure Of Nt2	66.6	3e-12
pdb|1ONJ|A Chain A, Crystal Structure Of Atratoxin-B From Chi...	65.4	5e-12
pdb|6EBX|A Chain A, Structure Determination Of A Dimeric Form...	63.1	3e-11
pdb|2ERA|A Chain A, Recombinant Erabutoxin A, S8g Mutant	62.3	4e-11
pdb|1QKD|A Chain A, Erabutoxin >pdb|1QKD|B Chain B, Erabutoxi...	62.3	5e-11
pdb|1V6P|A Chain A, Crystal Structure Of Cobrotoxin >pdb|1V6P...	61.9	6e-11
pdb|3ERA|A Chain A, Recombinant Erabutoxin A (S8t Mutant) >pd...	61.9	6e-11
pdb|1G6M|A Chain A, Nmr Solution Structure Of Cbt2	61.6	8e-11
pdb|1NXB|A Chain A, Structure And Function Of Snake Venom Cur...	60.8	1e-10
pdb|1TFS|A Chain A, Nmr And Restrained Molecular Dynamics Stu...	60.8	1e-10
pdb|1QM7|A Chain A, X-Ray Structure Of A Three-Fingered Chime...	55.0	8e-09
pdb|1FAS|A Chain A, 1.9 Angstrom Resolution Structure Of Fasc...	53.1	3e-08
pdb|1DRS|A Chain A, Three-Dimensional Structure Of The Rgd-Co...	51.9	6e-08
pdb|1MAH|F Chain F, Fasciculin2-Mouse Acetylcholinesterase Co...	51.5	8e-08
pdb|2ABX|A Chain A, The Crystal Structure Of Alpha-Bungarotox...	45.4	6e-06
pdb|1HC9|A Chain A, A-Bungarotoxin Complexed With High Affini...	44.2	1e-05
pdb|1IK8|A Chain A, Nmr Structure Of Alpha-Bungarotoxin >pdb|...	43.8	2e-05
pdb|1W6B|A Chain A, Solution Nmr Structure Of A Long Neurotox...	43.5	2e-05
pdb|1HOY|A Chain A, Nmr Structure Of The Complex Between A-Bu...	43.1	3e-05
pdb|1JGK|A Chain A, Solution Structure Of Candoxin	41.5	7e-05
pdb|1TXA|A Chain A, Solution Nmr Structure Of Toxin B, A Long...	40.8	1e-04
pdb|1LXG|A Chain A, Solution Structure Of Alpha-Cobratoxin Co...	40.0	2e-04
pdb|1NTN|A Chain A, The Crystal Structure Of Neurotoxin-I Fro...	39.6	3e-04
Sequences with E-value WORSE than threshold		
pdb|1LSI|A Chain A, Lsiii (Nmr, 23 Structures)	32.3	0.046
pdb|1TGX|A Chain A, X-Ray Structure At 1.55 A Of Toxin Gamma,...	31.5	0.085
pdb|1UG4|A Chain A, Crystal Structure Of Cardiotoxin Vi From ...	30.7	0.15
pdb|2VLW|A Chain A, Crystal Structure Of The Muscarinic Toxin...	30.0	0.23
pdb|1CDT|A Chain A, Cardiotoxin V4II FROM NAJA MOSSAMBICA MOS...	30.0	0.26
pdb|1FF4|A Chain A, X-Ray Structure Of Muscarinic Toxin 2 At ...	30.0	0.27
pdb|2CCX|A Chain A, Determination Of The Nuclear Magnetic Res...	29.2	0.36
pdb|1CB9|A Chain A, Nmr Structure With Tightly Bound Water Mo...	29.2	0.43
pdb|2CDX|A Chain A, Structure Of Cobra Cardiotoxin Ctxi As De...	28.8	0.56
pdb|1I02|A Chain A, Nmr Structure Of Ctx A3 At Neutral Ph (20...	28.8	0.58
pdb|1RL5|A Chain A, Nmr Structure With Tightly Bound Water Mo...	28.4	0.66
pdb|1CHV|S Chain S, Elucidation Of The Solution Structure Of ...	28.4	0.79
pdb|1KXI|A Chain A, Structure Of Cytotoxin Homolog Precursor ...	28.4	0.80
pdb|1CRE|A Chain A, Cardiotoxin Ii From Taiwan Cobra Venom, N...	27.7	1.3
pdb|1KBS|A Chain A, Solution Structure Of Cardiotoxin Iv, Nmr...	27.3	1.7
pdb|1MR6|A Chain A, Solution Structure Of Gamma-Bungarotoxin:...	26.9	2.2
pdb|2JQP|A Chain A, Nmr Structure Determination Of Bungatoxin...	26.5	2.8
pdb|2H8U|A Chain A, Bucain, A Cardiotoxin From The Malayan Kr...	25.0	7.6

ALIGNMENTS

>pdb|1IQ9|A Chain A, Crystal Structure At 1.8 A Of Toxin A From *Naja Nigricollis* Venom

Score = 68.9 bits (167), Expect = 5e-13, Method: Composition-based stats.

Identities = 43/61 (70%), Positives = 47/61 (77%), Gaps = 1/61 (1%)

```
Query  1   MTCCNQQSSQPKTTTNCAGNS-CYKKTWSDHRGTIIERGCGCPQVKSGIKLECCHTNECN  59
           + C NQQSSQP TT  C G + CYKK W DHRGTIIERGCGCP VK GIKL CC T++CN
Sbjct  1   LECHNQQSSQPPTTKTCPGETNCYKKVWRDHRGTIIERGCGCPTVKPGIKLNCCTTDKCN  60
Query  60  N  60
           N
Sbjct  61  N  61
```

Table 2a: PROCHECK summary for **(A)** neurotoxin (*Acalyptophis peronii*) after loop refinement **(B)** PDB template: 1IQ9

A) Neurotoxin model (*Acalyptophis peronii*)

```
+----------<<< P R O C H E C K   S U M M A R Y >>>----------+
|                                                            |
| input_atom_only.pdb   2.5                  60 residues |
|                                                            |
*| Ramachandran plot:   82.4% core   15.7% allow    0.0% gener    2.0% disall |
|                                                            |
+| All Ramachandrans:    1 labelled residues (out of  58)              |
| Chi1-chi2 plots:      0 labelled residues (out of  36)             |
| Main-chain params:    6 better     0 inside      0 worse             |
| Side-chain params:    5 better     0 inside      0 worse             |
|                                                            |
+| Residue properties: Max.deviation:     4.0          Bad contacts:    2 |
+|                Bond len/angle:    4.2    Morris et al class:  1  1  2 |
|                                                            |
| G-factors          Dihedrals:  -0.06  Covalent:   0.22    Overall:   0.06 |
|                                                            |
| M/c bond lengths:100.0% within limits   0.0% highlighted              |
| M/c bond angles:  96.1% within limits   3.9% highlighted              |
+| Planar groups:    95.0% within limits   5.0% highlighted              |
|                                                            |
 +------------------------------------------------------------------------+
  + May be worth investigating further.  * Worth investigating further.
```

B) 1IQ9

```
 +----------<<< P R O C H E C K   S U M M A R Y >>>----------+
|                                                            |
| input_atom_only.pdb   2.5                  61 residues |
|                                                            |
*| Ramachandran plot:   87.8% core   10.2% allow    0.0% gener    2.0% disall |
|                                                            |
+| All Ramachandrans:    1 labelled residues (out of  59)              |
| Chi1-chi2 plots:      0 labelled residues (out of  39)             |
| Main-chain params:    6 better     0 inside      0 worse             |
| Side-chain params:    5 better     0 inside      0 worse             |
|                                                            |
+| Residue properties: Max.deviation:     4.0          Bad contacts:    0 |
+|                Bond len/angle:    3.9    Morris et al class:  1  2  2 |
|                                                            |
| G-factors          Dihedrals:   0.06  Covalent:   0.31    Overall:   0.17 |
|                                                            |
| M/c bond lengths:100.0% within limits   0.0% highlighted              |
| M/c bond angles:  95.9% within limits   4.1% highlighted              |
| Planar groups:   100.0% within limits   0.0% highlighted              |
|                                                            |
 +------------------------------------------------------------------------+
  + May be worth investigating further.  * Worth investigating further.
```

Note: *G-values are considered poor if they are less than -0.5 and are considered bad if they are less than -1.0

Table: 2b Procheck summary for homology modeled neurotoxin of *Acalyptophis peronii* (after loop refinement) generated using the PDB template: 1YI5_F

A) Neurotoxin model

```
 +----------<<< P R O C H E C K   S U M M A R Y >>>----------+
|                                                            |
| input_atom_only.pdb   2.5                  58 residues |
|                                                            |
*| Ramachandran plot:   72.9% core   25.0% allow    2.1% gener    0.0% disall |
|                                                            |
*| All Ramachandrans:    3 labelled residues (out of  55)              |
| Chi1-chi2 plots:      0 labelled residues (out of  33)             |
```

```
| Main-chain params:    6 better     0 inside      0 worse                 |
| Side-chain params:    5 better     0 inside      0 worse                 |
|                                                                          |
*| Residue properties: Max.deviation:     3.5             Bad contacts:    6 |
*|                  Bond len/angle:    9.1    Morris et al class:  2  1  3 |
+|     1 cis-peptides                                                       |
| G-factors          Dihedrals:  -0.33  Covalent:  -0.40    Overall:  -0.34 |
|                                                                          |
| M/c bond lengths: 96.4% within limits   3.6% highlighted                 |
*| M/c bond angles:  91.5% within limits   8.5% highlighted       2 off graph |
*| Planar groups:    75.0% within limits  25.0% highlighted       2 off graph |
|                                                                          |
 +--------------------------------------------------------------------------+
  + May be worth investigating further.  * Worth investigating further.
```

B) 1YI5_F

```
 +----------<<< P R O C H E C K    S U M M A R Y >>>----------+
 |                                                           |
 | input_atom_only.pdb   2.5                    68 residues  |
 |                                                           |
*| Ramachandran plot:   78.9% core   19.3% allow    1.8% gener    0.0% disall |
 |                                                           |
*| All Ramachandrans:    8 labelled residues (out of  66)                 |
+| Chi1-chi2 plots:      1 labelled residues (out of  39)                 |
+| Main-chain params:    5 better     0 inside      1 worse                 |
+| Side-chain params:    2 better     0 inside      3 worse                 |
|                                                                          |
*| Residue properties: Max.deviation:     7.3             Bad contacts:    1 |
*|                  Bond len/angle:    5.1    Morris et al class:  1  3  3 |
+|     1 cis-peptides                                                       |
+| G-factors          Dihedrals:  -0.93  Covalent:   0.32    Overall:  -0.44 |
|                                                                          |
| M/c bond lengths: 99.4% within limits   0.6% highlighted                 |
| M/c bond angles:  97.8% within limits   2.2% highlighted                 |
| Planar groups:   100.0% within limits   0.0% highlighted                 |
|                                                                          |
 +--------------------------------------------------------------------------+
  + May be worth investigating further.  * Worth investigating further.
```

Note: *G-values are considered poor if they are less than -0.5 and are considered bad if they are less than -1.0

Table 3: Summary of the *ZDOCK output generated for AChBP – neurotoxin complex

---

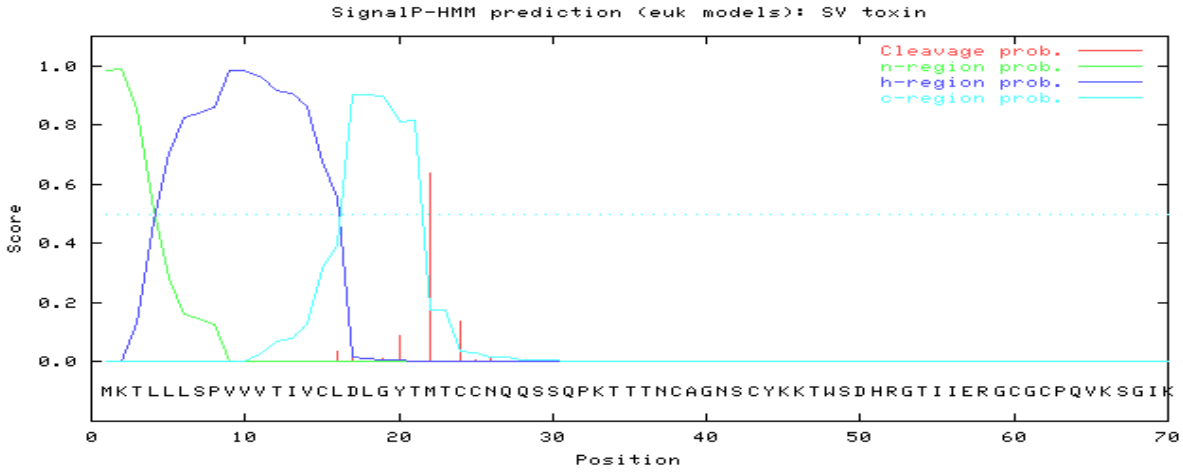
120 1.2
2.380392 2.500101 -0.120466
rec.job25031.bl.r.pdb 113.342 215.789 86.870
lig.job25031.bl.r.pdb 111.439 221.673 118.014

2.879793	1.721184	-0.153948	9	7	104	49.65
-2.617994	2.456751	-1.503343	11	6	105	44.82
-2.879793	1.732874	-0.951685	13	9	105	44.66
2.617994	1.721184	-0.153948	10	5	103	43.92
0.785398	0.379747	-1.713059	105	7	106	43.41
0.523599	1.335669	0.897240	119	13	102	43.39
-0.523599	1.325504	-1.671296	104	14	111	42.54
-1.832596	0.203748	0.590255	109	13	111	42.08
-2.617994	1.301460	-0.509251	105	4	101	41.78
-2.617994	2.180315	-1.357379	13	11	109	41.59
-3.141593	2.393226	1.857173	108	11	113	41.39
1.832596	1.875281	0.107086	3	5	100	41.31
-1.047198	1.335669	0.897240	4	8	102	40.91
0.261799	1.214854	-1.966664	101	5	104	40.88
0.261799	0.914445	2.687814	7	14	109	40.74
1.570796	2.587733	0.232235	12	9	107	40.45
2.617994	1.554776	0.100351	5	10	104	39.90
-2.356194	2.719179	-1.780644	7	10	107	39.52
-1.570796	0.379747	-1.713059	117	15	102	39.30
-3.141593	1.606104	-0.451151	9	11	107	39.21
-0.261799	2.979456	-1.009304	112	13	106	39.14
-0.523599	1.617114	-1.572037	105	15	112	38.98

-2.356194	1.510910	-0.740472	107	4	101	38.67
-1.832596	2.349581	-2.702517	109	11	107	38.59
1.047198	0.619654	-2.216909	103	7	108	38.50

***Note**: From a total of 2000 complexes that were generated, results of only top 25 complexes is shown in this table

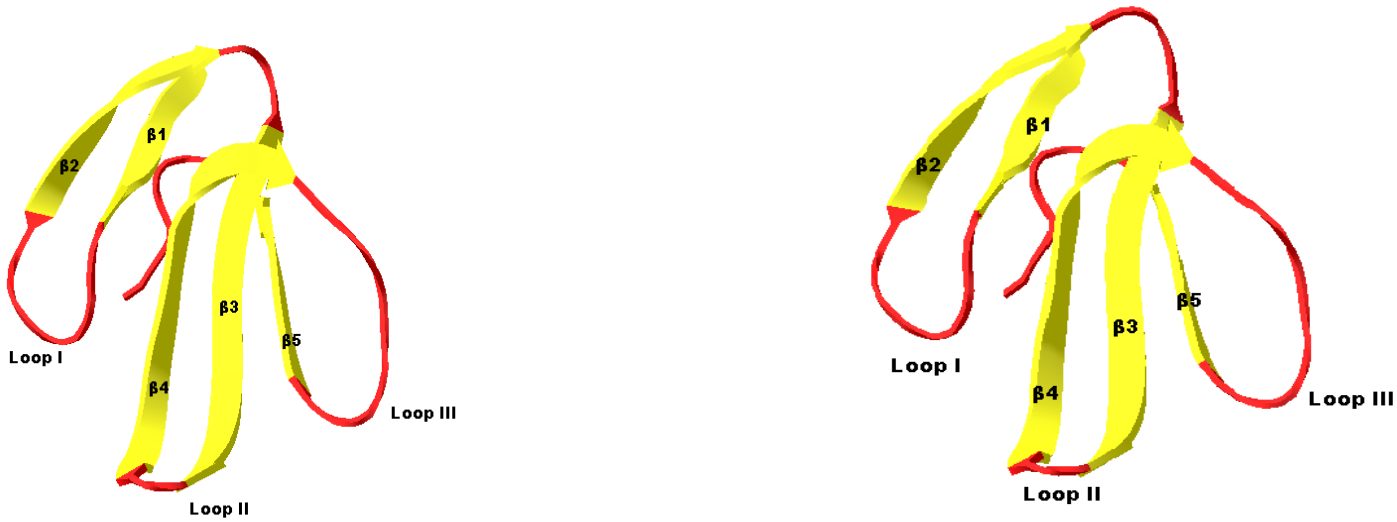
Figures: Most likely cleavage site between pos. 21 and 22: GYT-MT



Signal peptide probability: 0.984; Signal anchor probability: 0.003;
Max cleavage site probability: 0.640 between pos. 21 and 22

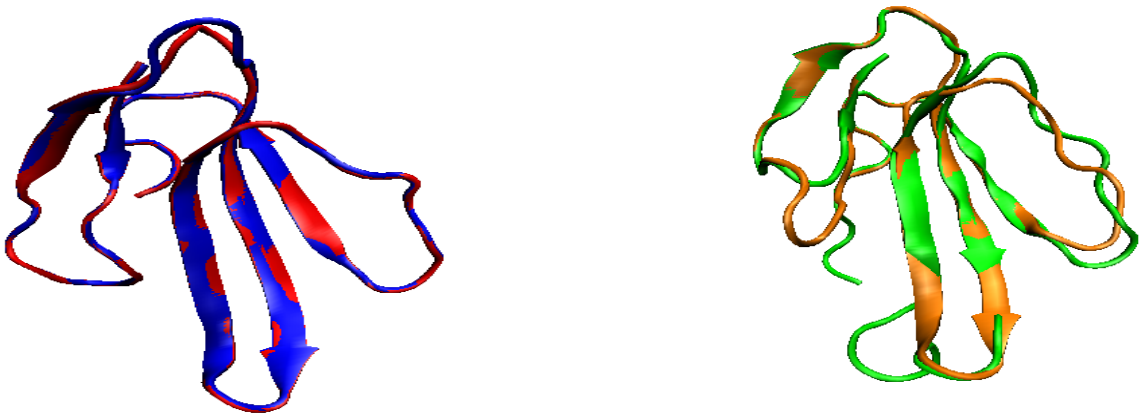
Figure 1: Analysis of neurotoxin from *Acalyptophis peronii* (AY742210) for the presence of signal peptide by SIGNALP tool (www.expasy.org)

Figure 2: Mutiple sequence alignment of neurotoxin (AY742210) from *Acalyptophis peronii* with other selected PDB templates using CLUSTAL W tool (www.ebi.ac.uk)



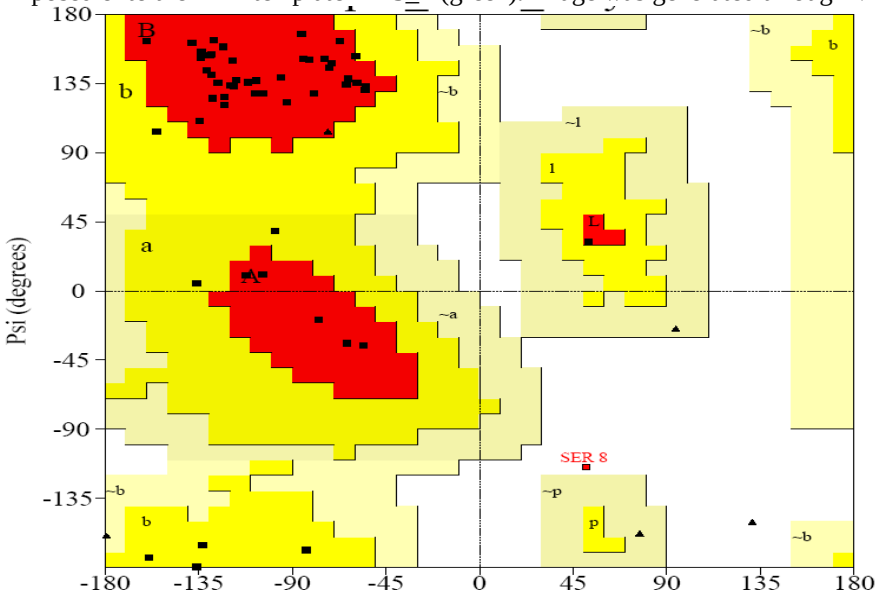
(A) Homology modelled neurotoxin from *Acalyptophis peronii* (B) X-ray structure of neurotoxin from *Naja nigricollis.*

Figure 3a: Comaprsion of homology modelled neurotoxin (AY742210) from *Acalyptophis peronii* with X-ray structure of neurotoxin from *Naja nigricollis* (PDB: 1IQ9)



(A) Neurotoxin model_1 **(B)** Neurotoxin model_2

Figure 3b: Superimposition of neurotoxin models (model_1 and model_2) onto the PDB templates. **(A)** While neurotoxin model_1 (red) is superimposed onto the PDB template 1IQ9 (blue), **(B)** neurotoxin model_2 (orange) is superimposed onto the PDB template 1YI5_F (green). Image was generated through VMD package.



Plot statistics

Residues in most favoured regions [A,B,L]	42	82.4%
Residues in additional allowed regions [a,b,l,p]	8	15.7%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.0%
Residues in disallowed regions	1	2.0%
Number of non-glycine and non-proline residues	51	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	5	
Number of proline residues	2	
Total number of residues	60	

Figure 4: Ramachandran plot analysis for the loop refined neurotoxin model_1 generated using 1IQ9

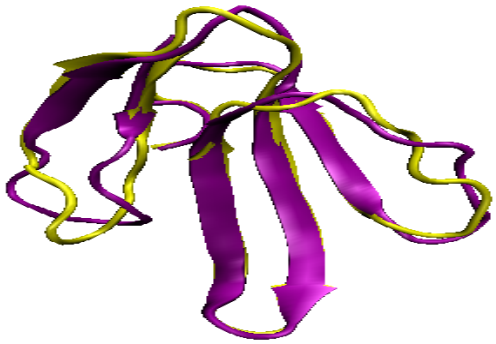


Figure 5: superimposition of neurotoxin models (model_1 and model_2). The neurotoxin model_1 colored is purple, whereas the neurotoxin model_2 is colored yellow. Backbone RMSD between the two models is 2.51^{o}A.

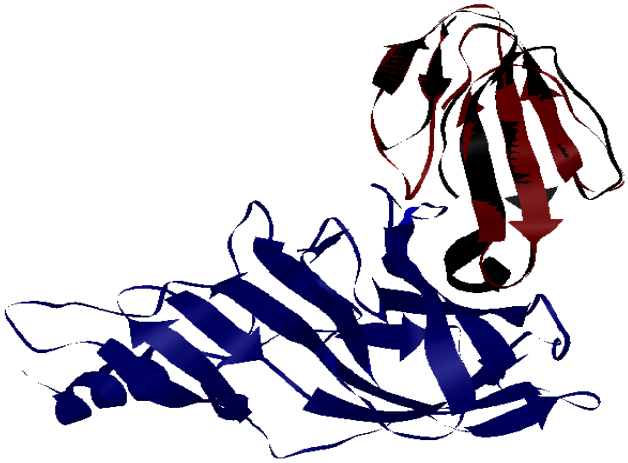


Figure 6: Neurotoxin model_2 (colured red) of *Acalyptophis peronii* docked onto acetyl choline binding receptor (blue) is superimposed onto the PDB template 1YI5_A_F (neurotoxin (colored black) complexed with acetyl choline binding receptor (colored blue)). Neurotoxin model_2 was generated using F chain of PDB template 1YI5_F. Image was generated using Chimera package.

Figure 7: Intermolecular interactions between neurotoxin (*Acalyptophis peronii*) and acetyl choline binding protein (AChBP). The residues tyr^{185} and tyr^{192} of AchBP are in close proximity with the residues asp^{29}, his^{30}, arg3, gly32 and thr^{33} of neurotoxin. Image was generated using PyMOL package.

DISCUSSION

The growing number of known protein sequences exceeds the number of protein structures determined experimentally by NMR and X-ray crystallography (Baker and Sali, 2001). Therefore, computational assignment of three – dimensional structures to newly determined protein sequences is very much

essential to keep in pace with the rate at which the experimental structures are being determined (Fischer *et al*., (2001)). In the present study, tertiary structure of neurotoxin of sea snake (*Acalyptophis peronii*) deposited into the NCBI databank by Pahari *et al*., (2007) has been predicted by homology modelling technique and the same was docked onto the acetyl choline binding receptor protein (PDB ID:1YI5_A; Bourne *et al*., (2005)).

The results of PSI BLAST suggested that 1IQ9 was the best template that got aligned with the neurotoxin of *Acalyptophis peronii*, with sequence identity of 70 %. The results also suggest that there are currently more than 100 neurotoxins with high sequence homology being isolated and characterized. The results of multiple sequence alignment illustrates that active site residues comprising trp^{27} asp^{29} and arg^{31} has been conserved in the neurotoxin sequence of *Acalyptophis peronii* as well as other neurotoxin sequences of PDB templates considered in this study. This is in conformation with the observations made by Bourne *et al*., (2005); Bernard *et al*., (2002); Golovanov, *et al*., (1993).

SWISS-MODEL server was successful in generating 2 neurotoxin models using the PDB templates 1IQ9_A and 1YI5_F, whose gross topologies were similar, exhibiting a characteristic "*three finger motif*". Both the neurotoxin models had three adjacent loops (loop I, II and III) forming a large β-pleated sheet with five anti-parallel β-strands. These loops could be seen as 3 fingers emerging from a small globular core where four disulfide bonds are embedded. These observations are in agreement with the studies carried out by Betzel *et al*., (1991); Bernard *et al*., 2003 and Bourne *et al*., (2005), who have noticed similar type of folding pattern for neurotoxins isolated from various snake species.

Superimposition of the loop refined neurotoxin models onto the respective PDB templates revealed, RMSD of Cα atoms between neurotoxin model_1 and 1IQ9 was lesser compared to RMSD of Cα atoms between neurotoxin model_2 and 1YI5_F. Based on the backbone RMSD between both the predicted neurotoxin models (2.51^{o}A), it is possible to safely conclude that these models adopted a similar topology (i.e. "*three finger motif*"), except minor structural variation. While loop I of neurotoxin model_2 appeared to be larger compared to the corresponding loop in 1YI5_F, loop II and loop III segments of neurotoxin model_2 differed slightly from that of 1YI5_F. This is mainly due to the variation observed in neurotoxin sequences of *Acalyptophis peronii* and *Naja mossambica mossambica* (1YI5_F).

Validation of refined neurotoxin models (1 and 2) using various tools suggest that model_1 appeared to be better than model_2. This is because nearly 82.0 % of the residues of model_1 (predicted using template 1IQ9) were present in the core region of Ramachandran plot, in contrast to nearly 73 % of the residues of the model_2 (predicted using 1YI5_F) being present in the same region. Ramachandran plot analysis for neurotoxin model_1 as well as the template 1IQ9 reveals that spot distributions of amino acid is very similar to the standard X-ray structure 1IQ9. Other sterochemical parameters (such as % residues in A, B, L regions, omega angle, bad contacts / 100 residues etc) calculated by PROCHECK tool was well within the blue band, suggesting the overall quality of the model was reasonably good.

Further, overall G factor for neurotoxin model_1 was better (0.06) compared to that of neurotoxin model_2 (-0.34). However, the G-values calculated by PROCHECK for both the neurotoxin models was well above the acceptable threshold of -0.5, indicating the model prediction to be sensibly good. This is in confirmation with the observation of Lee and Briggs who had used similar kind of tools for validating the 3D structure of *E.coli* leucyl-tRNA synthetase predicted using X-ray structure of *Thermus thermophilus* leucyl-tRNA synthetase.

Despite the strong resemblance of the neurotoxin model_1 to the template 1IQ9 and both the neurotoxin models (1 and 2) adopting the typical "*three finger motif*", ZDOCK server was able to successfully dock only the neurotoxin model 2, generated using 1YI5_F onto the crystal structure of acetyl choline

binding protein (AChBP). This could be mainly due to the easy availability of the surface residues of neurotoxin model_2 and AChBP to enter into favorable interaction. The fact that residues tyr^{185} and tyr^{192} of acetyl choline binding protein are involved in interaction with the residues trp^{27}, ser^{28}, asp^{29}, his^{30}, gly^{32} and thr^{33} of neurotoxin model_2 of *Acalyptophis peronii*, complies with the observation made by Bourne *et al*., (2005), who noticed similar kind of interaction between loop II of α- cobratoxin and AChBP.

Based on the results of above study, it is possible to conclude that predicted 3D structure of neurotoxin of sea snake (*Acalyptophis peronii*) adopts a typical "*three finger*" fold, which is common among other type of neurotoxins. Interaction of neurotoxin with AChBP has shed some light on the possibility of considering the neurotoxin as a potential (synthetic) biopesticide. The study also offers a strong basis for undertaking molecular dynamics simulations of neurotoxin –AChBP complex, which can reveal protein conformational changes in presence of solvent environment, which is more realistic.

REFERENCES:

1. Abola, EE; Bernstein, F. C., Bryant, SH., Koetzle, T.F., and Weng, J., *Data Commission of the International Union of Crystallography*, (Cambridge) 1987, 107-132.
2. Ali, S. A., Stoeva, S., J, G. Grossmann *et al*., Purification, characterization, and primary structure of four depressant insect selective neurotoxin analogs from scorpion (*Buthus sindicus*) venom *Arch. Biochem. Biophys*., **391,** (2001), 197–206
3. Altschul S F, Madden T L, Schäffer A A, Zhang J, Zhang Z *et al,* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, *Nucleic Acids Res*., **25** (1997) 3389-3402.
4. Baker & Sali, Protein structure prediction and structural genomics, *Science* **294** (2001) 93-96.
5. Betzel C, Lange G, Pal G P, Wilson K S, Maelicke A *et al,* The refined crystal structure of α-cobratoxin from *Naja naja siamensis* at 2.4 ^{o}A resolution, *J Biol Chem*., **266** (1991) 21530 – 21536.
6. Bernard G, Marjorie B, Renee M, Marie-Helene Le Du, Denis S *et al,* Motions and structural variability within toxins: Implications for their use as scaffolds for protein engineering, *Protein Science* **12** (2002) 266-277.
7. Bourne Y, Talley T T, Hansen S B, Taylor P & Marchot P, Crystal structure of a Cbtx-AChBP complex reveals essential interactions between snake α-neurotoxins and nicotinic receptors, *EMBO J*., **24** (2005) 1512–1522
8. Bowersox S S & Luther R, Pharmaco therapeutic potential of omegaconotoxin MVIIA (SNX-111), an N-type neuronal calcium channel blocker found in the venom of *Conus magus*, *Toxicon*, **36** (1998) 1651–1658.
9. Bowman W C, Pharmacology of neuromuscular function, 2nd.London: Butterworth & Co. Ltd, (1990) 36–126.
10. Braud S, Bon C & Wisner A, Snake venom proteins acting on hemostasis *Biochimie*, 82 (2000) 851–859.
11. Brejc K, van Dijk WJ, Klaassen RM *et al*, Crystal structure of an acetylcholine-binding protein reveals the ligand-binding domain of nicotinic receptors. *Nature*, **411** (2001) 269–276.
12. Carredano E, Westerlund B, Persson B *et al*, The three-dimensional structures of two toxins from snake venom throw light on the anticoagulant and neurotoxic sites of phospholipase A2, *Toxicon,* **36** (1998) 75–92
13. Cervenansky C, Dajas F, Harvey A L & Karlsson E, Fasciculins, anticholinesterase toxins from mamba venoms, in *Snake toxins: biochemistry and pharmacology*, edited by Harvey AL, (Pergamon Press, New York) 1991, 303–321.
14. Chang C C, The action of snake venoms on nerve and muscle. In *Snake venoms, Handbook of experimental pharmacology*, Vol. 52, edited by Lee C Y, (Springer-Verlag, Berlin) 1979, 309 - 376

15. Cheng Y, Meng Q, Wang W & Wang J, Structure–function relationship of three neurotoxins from the venom of *Naja kaouthia*: A comparison between the NMR derived structure of NT2 with its homologues, NT1 and NT3', *Biochim. Biophys. Acta*, **1594** (2002) 353–363.
16. Chiappinelli V A, κ-neurotoxins and α-neurotoxins: effects on neuronal nicotinic acetylcholine receptors. in *Snake toxins*, edited by Harvey A L, (Pergamon Press, New York) 1991, 223– 258
17. Chiappinelli V A, Weaver W A, McLane K E, Conti-Fine B M, Fiordalisi J J *et al*, Binding of native kappa-neurotoxins and site-directed mutants to nicotinic acetylcholine receptors, *Toxicon*, **34** (1996) 1243–1256.
18. Chippaux J P, The treatment of snake bites: analysis of requirements and assessments of therapeutic efficacy in tropical Africa. In *Perspectives in molecular toxinology*, edited by Menez A, (John Wiley & Sons, Chichester, England) (2002) 457–473.
19. Conticello S G, Gilad Y, Avidan N *et al*, Mechanisms for evolving hypervariability: The case of conopeptides, *Mol. Biol. Evol*., **18** (2001) 120–131.
20. Corringer P J, Le Novère N & Changeux J P, Nicotinic receptors at the amino-acid level, *Annu Rev Pharmacol Toxicol*., **40** (2000) 431–458.
21. Devaux C, Clot-Faybesse O, Juin M *et al*, Monoclonal antibodies neutralizing the toxin II from *Androctonus australis* hector scorpion venom: Usefulness of a synthetic, non-toxic analog, *Vaccine*, **15** (1997) 187–194
22. Dufton M J & Hider R C, Classification of phospholipases A2 according to sequence. Evolutionary and pharmacological implications *Eur. J. Biochem.*, **137** (1983) 545–551
23. Eddy S R, *Current Opinions in Structural Biology* **6** (1996) 361-365
24. Endo T & Tamiya N, Structure-function relationships of postsynaptic neurotoxins from snake venoms, In *Snake toxins*, edited by Harvey A L, (Pergamon Press, New York) 1991, 165–222.
25. Espiritu D J, Watkins M, Dia-Monje V *et al*, Venomous cone snails: molecular phylogeny and the generation of toxin diversity, *Toxicon*, **39** (2001) 1899– 1916.
26. Ferrat G, Bernard C, Fremont V *et al*, Structural basis for alpha-K toxin specificity for Kþ channels revealed through the solution 1H NMR structures of two noxiustoxin-iberiotoxin chimeras, *Biochemistry*, **40** (2001) 10998–11006.
27. Fischer D, Baker D & Moult J, We need both computer models and experiments, *Nature*, **409** (2001) 558.
28. Fiser A & Sali A, ModLoop: automated modeling of loops in protein structures, *Bioinformatics*, **19** (2003) 2500-2501.
29. Fiser and Sali, http://www.guitar.rockfeller.edu
30. Froy O, Zilberberg N, Chejanovsky N *et al*, Scorpion neurotoxins: structure/ function relationships and application in agriculture, *Pest Manage. Sci*., **56** (2000) 472–474.
31. Golovanov A P, Lomize A L, Arseniev A S, Utkin Y N & Tsetlin V I, Two-dimensional 1H-NMR study of the spatial structure of neurotoxin II from *Naja naja* oxiana, *Eur.J.Biochem*. **213** (1993) 1213-1223.
32. Gribskov M, McLachlan A D & Eisenberg D, *Proc Nat Acad Sci USA* **84** (1987) 4355 - 4358.
33. Gribskov M, *Methods Mol Biol* **25** (1994) 247-266.
34. Grutter T & Changeux J P, Nicotinic receptors in wonderland, *Trends Biochem. Sci*. **26** (2001) 459–463.
35. Gordon D, Savarin P, Gurevitz M & Zinn-Justin S, Functional anatomy of scorpion toxins affecting sodium channels, *J. Toxicol. Toxin Rev*., **17** (1998) 131–159.
36. Guatimosim S C, Kalapothakis E, Diniz, C R & Chavez-Olortegui C, Induction of neutralizing antibodies against *Tityus serrulatus* toxins by immunization with a recombinant nontoxic protein, *Toxicon*., **38** (2000) 113–121.
37. Guex N & Peitsch M C, SWISS-MODEL and the Swiss-PdbViewer: an environment

for comparative protein modeling, *Electrophoresis* **18** (1997) 2714-23.

38. Hains P G, Ramsland P A & Broady K W, Modeling of acanthoxin A1, a PLA2 enzyme from the venom of the common death adder (*Acanthophis antarcticus*), *Proteins* **35** (1999) 80–88.
39. Harvey A, Strategies for discovering drugs from previously unexplored natural products, *Drug Discov. Today*, **5** (2000) 294–300.
40. Harvey A L, Bradley K N, Cochran S A, *et al*, What can toxins tell us for drug discovery?', *Toxicon*, **36** (1998) 1635–1640.
41. Hassani O, Mansuelle P, Cestele S *et al*, Role of lysine and tryptophan residues in the biological activity of toxin VII (Ts gamma) from the scorpion *Tityus serrulatus'*, *Eur. J. Biochem*., **260 (**1999) 76–86.
42. Heinrikson R L, Krueger E T & Keim P S, Amino acid sequence of phospholipase A2-alpha from the venom of *Crotalus adamanteus*. A new classification of phospholipases A2 based upon structural determinants, *J. Biol. Chem.*, **252** (1977) 4913–4921.
43. Higgins D, Thompson J, Gibson T, Thompson J D, Higgins D G *et al*, CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22** (1994) 4673-4680.
44. Hoffmann J J Vijgen M, Smeets, R. E. & Melman P G, Haemostatic effects in vivo after snakebite by the red-necked keelback (*Rhabdophis subminiatus*) , *Blood Coagul. Fibrinolysis*, **3** (1992) 461–464.
45. Hsueh-Fen Juan, Chin-Chun Hung, Kung-Tsung Wang & Shyh-Horng Chiou, Comparison of Three Classes of Snake Neurotoxins by homology modeling and computer simulation graphics, *Biochemical and Biophysical Research Communications* **257** (1999) 500–510.
46. Hunt K J, Valentine M D, Sobotka A K *et al*, A controlled trial of immunotherapy in insect hypersensitivity, *N. Engl. J. Med*., **299** (1978) 157–161.
47. Jannick D B, Henrik Nielsen, Gunnar von Heijne & Søren Brunak, Improved prediction of signal peptides: SignalP 3.0., *J. Mol. Biol*., **340** (2004) 783-795
48. Joyce Phui Yee Siew, Asif M. Khan, Paul T. J. Tan, Judice L. Y. Koh, Seng Hong Seah *et al*, Systematic analysis of snake neurotoxins functional classification using a data warehousing approach., *Bioinfromatics* **20** (2004) 3466 -3480.
49. Juan H F, Hung C C, Wang K T and Chiou S H, Comparison of three classes of snake neurotoxins by homology modeling and computer simulation graphics', *Biochem. Biophys. Res. Commun*., **257** (1999) 500–510.
50. Karlin A, Emerging structure of the nicotinic acetylcholine receptors, *Nature Rev Neurosci*, **3** (2002) 102–114.
51. Karlsson E, Chemistry of protein toxins in snake venoms. In: Lee CY, editor. Snake venoms, Handbook of experimental pharmacology, Vol. 52. (Springer-Verlag, Berlin) 1979, 159– 212
52. Kini R M, Molecular moulds with multiple missions: functional sites in three-finger toxins, *Clin. Exp. Pharmacol Physiol*., **9** (2002) 815–822.
53. Klenk K C, Tenenholz T C, Matteson D R *et al*, Structural and functional differences of two toxins from the scorpion, *Pandinus imperator Proteins* **38** (2000) 441–449.
54. Kleywegt G J & Jones T A, Phi/psi-chology: Ramachandran revisited, *Structure* **4** (1996) 1395 – 1400.
55. Kordis D & Gubensek, F, Adaptive evolution of animal multigene families, *Gene*, **216** (2000) 43–52.
56. Krogh A, Brown M, Mian I S, Sjolander K & Haussler D, *J Mol Biology* **235** (1994) 1501- 1531.
57. Laskowski R A, MacArthur M W, Moss D S & Thornton J M, PROCHECK: A program to check the stereochemical quality of protein structures, *J. Appl.* Cryst., **26** (1993) 283-291.

58. Labesse G, MulBlast 1.0: a multiple alignment of BLAST output to boost protein sequence similarity analysis, *CABIOS* **12** (1996) 463-467.
59. Lee C Y, Chemistry and pharmacology of polypeptide toxins in snake venoms, *Annu Rev Pharmacol*., **12** (1972) 265–286.
60. Lee K L & Briggs J M, *Proteins: Structure, Function and Bioinformatics*, **54** (2004) 693-704.
61. Legros C, Ceard B, Bougis P E & Martin-Eauclaire M F, Evidence for a new class of scorpion toxins active against Kþ channels, *FEBS Lett*., **431** (1998) 375– 380.
62. Lipkind G M and Fozzard H A, A model of scorpion toxin binding to voltagegated Kþ channels, *J. Membr. Biol*., **158** (1997) 187–96.
63. Martinez F, Munoz-Garay C, Gurrola G. *et al*, Site directed mutants of Noxiustoxin reveal specific interactions with potassium channels, *FEBS Lett*., **429** (1998) 381–384.
64. McIntosh J M, Gardner S, Luo S, *et al*, *Conus* peptides: Novel probes for nicotinic acetylcholine receptor structure and function, *Eur. J. Pharmacol.*, **393** (2000) 205–208.
65. Menez A, Functional architectures of animal toxins: a clue to drug design? *Toxicon*, **36** (1998) 1557–1572.
66. Menez A, Servent D & Gasparini S, The binding sites of animal toxins involve two components: a clue for selectivity, evolution and design of proteins? In *Perspectives in molecular toxinology*, edited by Menez A, (John Wiley & Sons, Chichester, England) 2002, 175–200.
67. Miyazawa A, Fujiyoshi Y & Unwin N, Structure and gating of the acetylcholine receptor pore, *Nature* **423** (2003) 949–955.
68. Naguib M, Flood P, McArdle J J & Brenner H R, Advances in neurobiology of the neuromuscular junction: implications for the anesthesiologist, *Anesthesiology*, **96** (2002) 202–231.
69. Nirthanan S, Gopalakrishnakone P, Gwee M C E, Khoo H E & Kini R M, Non-conventional toxins from Elapid venoms, *Toxicon*., **41** (2003) 397–407.
70. Okuda D, Nozaki C, Sekiya F & Morita T, Comparative biochemistry of disintegrins isolated from snake venom: Consideration of the taxonomy and geographical distribution of snakes in the genus Echis, *J. Biochem.*, (Tokyo) **129** (2001) 615–620.
71. Olof Emanuelsson, Søren Brunak, Gunnar von Heijne & Henrik Nielsen, Locating proteins in the cell using TargetP, SignalP, and related tools, *Nature Protocols*, **2** (2007) 953–971.
72. Pahari S, Bickford D, Fry B G & Kini R M, Expression pattern of three-finger toxin and phospholipase A2 genes in the venom glands of two sea snakes, *Lapemis curtus* and *Acalyptophis peronii*: comparison of evolution of these toxins in land snakes, sea kraits and sea snakes, *BMC Evol. Biol*., **7** (2007) 175.
73. Pollard B J, Components of the neuromuscular junction. In *Applied neuromuscular pharmacology*, edited by Pollard B J, (Oxford University Press New York) 1994, 17–43.
74. Possani L D, Merino E, Corona M *et al*, Peptides and genes coding for scorpion toxins that affect ion-channels, *Biochimie*, **82** (2000) 861–868.
75. Rehm H, Molecular aspects of neuronal voltage-dependent Kþ channels, *Eur. J. Biochem*., **202** (1991) 701–713.
76. Renisio J G, Romi-Lebrun R, Blanc E *et al*, Solution structure of BmKTX, a Kþ blocker toxin from the Chinese scorpion *Buthus martensi*, *Proteins*, **38** (2000) 70–78.
77. Rogers D F, Scorpion venoms: taking the sting out of lung disease, *Thorax* **51** (1996) 546–548.
78. Rowan E G, What does β-bungarotoxin do at the neuromuscular junction? *Toxicon*, **39** (2001) 107–118.
79. Savarin P, Romi-Lebrun R & Zinn- Justin S, Structural and functional consequences of the presence of a fourth disulfide bridge in the scorpion short toxins: Solution structure of the potassium channel inhibitor HsTX, *Protein Sci*., **8** (1999) 2672–2685.
80. Schwede T**,** Kopp J, Guex N & Peitsch M C, SWISS-MODEL: an automated protein

homology-modeling server, *Nucleic Acids Research*, **31**(2003) 3381-3385.

81. Servent D & Menez A, Snake neurotoxins that interact with nicotinic acetylcholine receptors, In *Handbook of neurotoxicology*, Vol. 1, edited by Massaro E J, (Humana Press, Totowa, New Jersey) 2001, 385–425.
82. Sixma TK & Smit AB, The acetylcholine binding protein: A secreted glial protein that provides a high resolution model for the extracellular domain of pentameric ligand-gated ion channels, *Annu Rev Biophys Biomol Struct.*, **32** (2003) 311–334.
83. Smit A B, Syed N I, Schaap D, *et al*, A glia-derived acetylcholine binding protein that modulates synaptic transmission, *Nature* **411** (2001) 261–268.
84. Tsai I H, Hsu H Y & Wang Y M, A novel phospholipase A (2) from the venom glands of *Bungarus candidus*: Cloning and sequence-comparison, *Toxicon* **40** (2002) 1363–1367.
85. Tsetlin, Snake venom alpha-neurotoxins and other 'threefinger' proteins, *Eur J Biochem*., **264** (1999) 281–286.
86. Tytgat J, Chandy K G, Garcia, M. L. *et al*, A unified nomenclature for short chain peptides isolated from scorpion venoms: alpha-KTx molecular subfamilies, *Trends Pharmacol. Sci.*, **20** (1999) 444–447.
87. Valentin E & Lambeau G, What can venom phospholipase A (2) tell us about the functional diversity of mammalian secreted phospholipases A (2)? *Biochimie.*, **82** (2000) 815–831.
88. Wang C Y, Tan Z Y, Chen B *et al*, Antihyperalgesia effect of BmK IT2, a depressant insect-selective scorpion toxin in rat by peripheral administration', *Brain Res. Bull*., **53** (2000) 335–338.
89. Ward R J, de Azevedo, W F Jr & Arni R. K, At the interface: Crystal structures of phospholipases A2, *Toxicon*, **3** (1998) 1623–1633.
90. Warrell D A, Clinical features of envenoming from snake bites, In *Envenomings and their treatments*, edited by Bon C & Goyffon M (Foundation Marcel Merieux, Lyon) 1996, 63–76.
91. **Wiederstein M & Sippl M J,** ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins, *Nucleic Acids Res.*, **35** (2007) W407-W410.
92. Yang C C, Structure-function relationship of phospholipase A2 from snake venoms, *J Toxicol Toxin Rev*., **13** (1994) 125–177.
93. Yasuda O, Morimoto S, Jiang B *et al*, FS2, a mamba venom toxin, is a specific blocker of the L-type calcium channels, *Artery*, **21** (1994) 287–302.
94. Zenouaki I, Kharrat R, Sabatier J M *et al*, In vivo protection against *Androctonus australis* hector scorpion toxin and venom by immunization with a synthetic analog of toxin II, *Vaccine*, **15** (1997) 187–194.
95. Zieler H, Keister D B, Dvorak J A & Ribeiro J M, A snake venom phospholipase A(2) blocks malaria parasite development in the mosquito midgut by inhibiting ookinete association with the midgut surface, *J. Exp. Biol.*, **204** (2001) 4157–4167.
96. Zlotkin E, Fishman Y & Elazar M, AaIT: From neurotoxin to insecticide, *Biochimie*, **82** (2000) 869–881.