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IN-SILICO ANALYSIS FOR PREDICTING PROTEIN LIGAND INTERACTION FOR SNAKE VENOM PROTEIN

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ABSTRACT:

The ability to predict the conformations and energies involved in the binding of small molecules to proteins is quite crucial in designing potential drugs that can interact favourably with their target proteins. Docking is also used to predict protein-protein complexes, which are helpful in determining the quaternary structures of intrinsically multimeric proteins as well as to gain an understanding of the protein interaction networks. The objective of our research work is to predict protein ligand interaction for snake venom. Phylogenetic analysis was carried out using ClustalW2 software. ClustalW2 is a general purpose multiple sequence alignment program for DNA or proteins. Homology modelling was done using the SWISS-MODEL Workspace. Tropine was selected as ligand for performing docking. Docking was performed to find the inhibition activity of tropine against snake venom using Medock & Hex. Vibrational analysis was performed to check the energy changes and it was found that the energy of docking in case of Hex before and after vibrational analysis was same.

Keywords: Snake venom Protein, Protein ligand interaction, vibrational analysis of snake venom protein, homology modeling

INTRODUCTION:

The ability to predict the conformations and energies involved in the binding of small molecules to proteins is quite crucial in designing potential drugs that can interact favorably with their target proteins. Docking is also used to predict protein-protein complexes, which are helpful in determining the quaternary structures of intrinsically multimeric proteins as well as to gain an understanding of the protein interaction networks. The concept of protein flexibility forms the backbone of such analysis. The basic type of docking methods treats both the ligand and protein as rigid or fixed bodies, with constrained internal motions. Although, such methods speed up the calculation process, they might not stand true in case of highly flexible ligand or protein. This led to the development of modern docking methods which utilize ligand flexibility, and although, protein flexibility remains largely unsolved, a

simpler approximation used to introduce protein flexibility is by vibrational analysis. Thus, using these methods, normal vibrational modes of the proteins can be generated and normal mode analysis can be used to generate different protein conformations to which the ligand can be docked [1].

Snake bite is a serious medical, social and economic problem in many countries, especially in the tropical and subtropical countries including India. Snakes are cosmopolitan in distribution except in high altitudes, certain islands and Polar Regions. According to World Health Organisation (WHO), poisonous snakes are responsible for at least 5 million human fatalities annually [1,2]. The largest number of fatal snake bites occurs in South East Asia [3].

Nagarajan *et al.*,retrieved Short neurotoxin protein sequences of Cobra from Swiss prot database and performed multiple sequence alignment using ClustalX. Phylogenetic relationships were analyzed using Phylip software and sequences were modelled through Modeller 9v8. Tropine derived from Ashwagandha was investigated for its skeletal muscle relaxant property [3].

The focus of the present work is to predict protein ligand interaction for 7 different snake venom and to identify an inhibitor for snake venom protein by performing docking using bioinformatics tool and also vibrational analysis to the docked protein to identify the different modes to protein and further redock the ligand with different modes of protein obtained from vibrational analysis [4, 5].

Materials and Methods:

1. **Sequence retrieval:** From swissprot. url: http://epsasy.org/sprot/

Proteins and ligand used:

Naja pallid	Short neurotoxin1 (P01426)
Naja oxiana	Short neurotoxin1 (P01427)
Boulengerina christyi	Short neurotoxin1 (P34076)
Naja melanoleuca	Short neurotoxin1 (P01424)
Naja nivea	Short neurotoxin1 (P68419)
Naja samarensis	Short neurotoxin1 (P60774)
Naja haje haje	Short neurotoxin1 (P68418)
Ligand	Tropine

2. Phylogenetic analysis:

Phylogenetic analysis was carried out using ClustalW2 software. ClustalW2 is a general purpose multiple sequence alignment program for DNA or proteins. It attempts to calculate the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen and also has a feature of providing phylogram tree indicating phylogenetic relationship between proteins.

Retrieve all the seven neurotoxin sequences from Swiss-Prot: www.expasy.org/sprot in fasta format and paste in notepad leaving one line space between each sequence.

Open the URL:http://www.ebi.ac.uk/clustalw which contains Graphical User Interphase(GUI) of the ClustalW software.Copy the sequence form notepad and paste them in textbox provided in ClustalW page. Default Parameters are used for the analysis.Click on the “submit” button.Result including alignment and tree appear in the window after the page refreshes. The alignment may be seen in colours by selecting the option “show colours”.

3. Homology modeling:

Homology modeling was done using workspace. The SWISS-MODEL Workspace is a web-based integrated service dedicated to protein structure homology modelling. It assists and guides in building protein homology models at different levels of complexity.Retrieve all the 7 snake neurotoxin sequences from Swiss-Prot: www.expasy.org/sprot and carry out homology modelling using SWISS MODEL:

http://swissmodel.expasy.org/. Template identification is done for each sequence using the template identification tool provided in SWISS MODEL. Carry out the modelling using the same server in alignment mode. Follow the online instructions to carry out the task and get the results.

In order to facilitate the use of alignments in different formats, the submission is implemented as a three step procedure:

Prepare a multiple sequence alignment--We used CLUSTAL W to carry out multiple sequence alignment of the query protein sequences with the homologous target sequences obtained using the "template Idendification" tool in SWISS-MODEL.The multiple sequence alignment was carried out using the site http://www.ebi.ac.uk/Tools/msa/clustalw2/.

Submit your alignment to the Workspace Alignment Mode--Possible formats are: FASTA, MSF, CLUSTALW, PFAM and SELEX.One may either upload the file or cut & paste.One should not forget to specify the correct alignment format.We submitted the input to the SWISS-MODEL>Alignment Mode in CLUSTAL W format.

Select Target and Template--The alignment (as it was interpreted by the server) was displayed in the bottom part of the page.The script will try to make a good guess for the correct names based on your submission.The sequence name of the target sequence was selected.(e.g. THN_DENCL).The sequence of the template structure was selected (e.g. 1crnA). You don't need to use PDB IDs; one may use any name one likes.The template structure was specified to which this sequence belongs. This template MUST be part of the ExPDB template library. One should not forget to specify the correct CHAIN ID. Note that PDB's chain IDs are normally in capital letters.

Validation of model: Validation for generated models was done using Verify 3D: http://nihserver.mbi.ucla.edu/verify_3D/

4. Selection of ligand:

Tropine is a small sized molecule with a molecular weight of 141.2108(g/mol). It has one hydrogen bond donor and two hydrogen bond acceptors with no rotatable bonds. The compound Tropine has the LogP value of 0.477. Thereby it satisfies all the criteria of Lipinski's rule of five.

5. Docking using Medock:

The most important feature of MEDock((Maximum Entropy based Docking)) in this regard is the use of a novel optimization algorithm that exploits the maximum entropy property of the Gaussian distribution.Generate PDBQ format for ligand (tropine) by Dundee's PRODRG server (http://davapc1.bioch.dundee.ac.uk/programs/prodrg/).PRODRG server will convert coordinates for small molecules in PDB format to the following topology formats: GROMOS, GROMACS, WHAT IF, REFMAC5, CNS, O, SHELX, HEX and MOL2.Upload the protein(snake venom) file in PDB format and ligand(tropine) file in PDBQ format to Medock server(http://medock.ee.ncku.edu.tw/).Specify the docking setting such as docking runs, generation limit, grid spacing, local search, population size and click on the proceed button.Click on report to get the final report of the task. Perform docking for seven different neurotoxins of Naja spp using tropine as ligand. Note down the docked energy for docking of different neurotoxins from Naja spp.

6. Docking using Hex:

Upload a pair of protein and ligand structures in PDB format in Hex server.Provide an e-

mail address for notification of the status of their jobs.Default parameters are used for carrying out the jobs.To be able to analyze the docking, the e-values were obtained using the Hex software. Install the free Hex version6.0 software available online suitable to the existing operating system. In the Hex window, go to File>Open>Receptor. The receptor and ligand molecules must be previously saved in the hex/examples directory of Hex. This done by opening the pdb files of receptor and ligand in SWISS Pdb Viewer and saving them in project mode using "magic fit" tool.The ligand file must be opened, File>Open>Ligand.The process of docking is started by using the option Controls>docking. There are five stages in the process which take fifteen minutes to be completed. Once the five stages are completed it gives a docked structure indicating the corresponding e-values. The more negative the e-value, the more efficient is the docking process.

7. Vibrational analysis:

The site used for this provides online servers for algorithms such as normal mode calculation, structural refinement, solvation and mutation and transition path calculation.Calculate the first major vibrational mode of the assigned protein using the web server NOMAD-REF: http://lorentz.immstr.pasteur.fr/ .Under "Normal Model Calculation", go to, "Submit a Job (PDB)" and follow the online instructions to upload the protein pdb file. Follow the instructions to obtain the results. Re-dock the proteins obtained from vibrational calculations with ligand Tropine using HEX server.

Results:

1. Sequences retrieved from Swiss-Prot:

- >sp|P01426|NXS1_NAJPA Short neurotoxin 1 OS=***Naja pallida*** PE=1 SV=1 LECHNQQSSQPPTTKTCPGETNCYKKV WRDHRGTIIERGCGCPTVKPGIKLNCC TTDKCNN
- >sp|P01427|NXS1_NAJOX Short neurotoxin 1 OS=***Naja oxiana*** PE=1 SV=1 LECHNQQSSQPPTTKTCSGETNCYKK WWSDHRGTIIERGCGCPKVKPGVNLN CCRTDRCNN
- >sp|P34076|NXS1_BOUCH Short neurotoxin 1 OS=***Boulengerina christyi*** PE=1 SV=1 MECHNQQSSQPPTTTHCSGGETNCYE KRWHDHRGTIIERGCGCPTVKPGVKL NCCTTDKCNN
- >sp|P01424|NXS1_NAJME Short neurotoxin 1 OS=***Naja melanoleuca*** PE=1 SV=1 MECHNQQSSQPPTTKTCPGETNCYKK QWSDHRGTIIERGCGCPSVKKGVKINC CTTDRCNN
- >sp|P68419|NXS1_NAJNI Short neurotoxin 1 OS=***Naja nivea*** PE=1 SV=1 LECHNQQSSQPPTTKTCPGETNCYKKR WRDHRGSITERGCGCPSVKKGIEINCC TTDKCNN
- >sp|P29180|TXW6_NAJNA Weak neurotoxin 6 OS=***Naja naja*** PE=1 SV=1 LTCLICPEKYCNKVHTCLNGEKICFKR YSERKLLGKRYIRGCADTCPVRKPREI VQCCSTDKCNH
- >sp|P60774|NXS1_NAJSA Short neurotoxin 1 OS=***Naja samarensis*** PE=1 SV=1 LECHNQQSSQAPTTKTCSGETNCYKK WWSDHRGTIIERGCGCPKVKPGVKLN CCTTDRCNN
- >sp|P68418|NXS1_NAJHH Short neurotoxin 1 OS=***Naja haje haje*** PE=1 SV=1 LECHNQQSSQPPTTKTCPGETNCYKKR WRDHRGSITERGCGCPSVKKGIEINCC TTDKCNN

2. **Resultsfor Phylogenetic analysis:** Phylogenetic analysis was performed using CLUSTALW and the results were as shown below

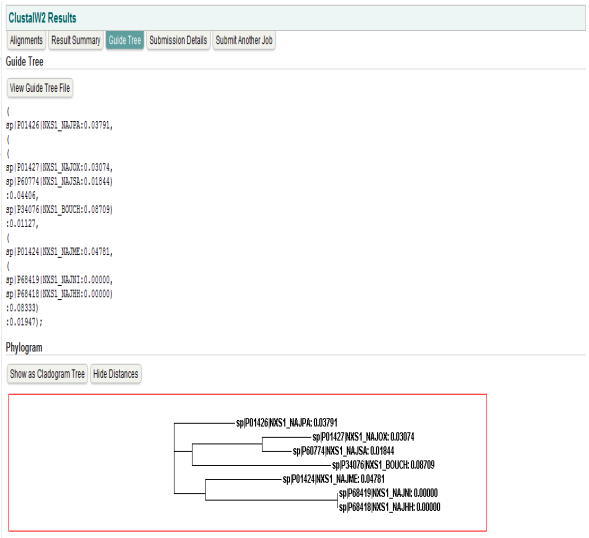
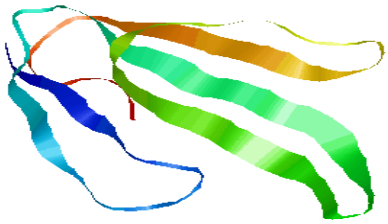


Figure 1: Phogenetic analysis.

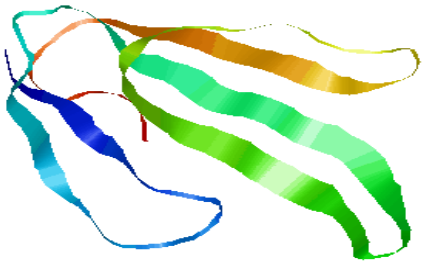
3. **Results for homology modeling:** Homology modeling was performed using SWISSPROT and the different structure were obtained and are as follows

Naja haje haje:

Naja melanoleuca:



Naja nivea:

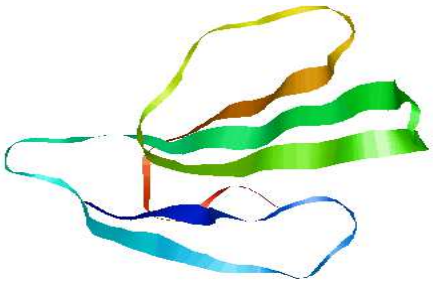


Naja oxiana:

Naja pallida:

Naja samarensis:

Boulegrina christyi:

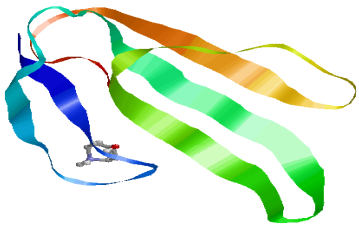


Result of MEDock: These were the structures obtained from MEDock server. These were the structures observed by using RasMol software. Energy values were obtained and are tabulated as below

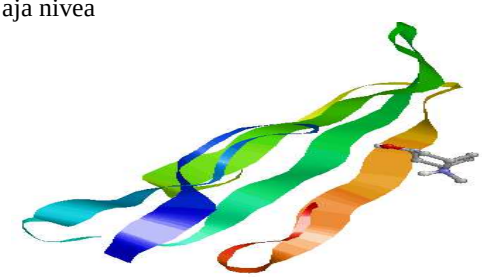
SL NO	ID	NAME	e-values
1	P34076	Boulengerina christyi	-115.21
2	P01424	Naja melanoleuca	-103.62
3	P01427	Naja oxiana	-106.53
4	P01426	Naja pallida	-111.73
5	P68419	Naja nivea	-119.96
6	P60774	Naja samarensis	-92
7	P68418	Naja haje haje	-117.55

Boulengerina christyi

Naja oxiana



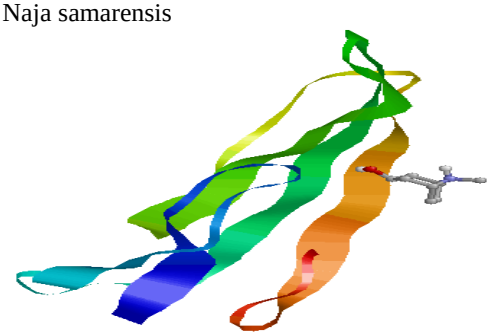
Naja nivea



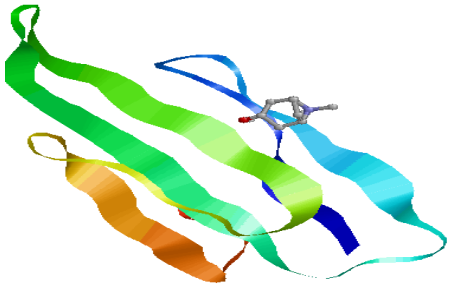
Naja melanoleuca

Naja pallid

Naja samarensis



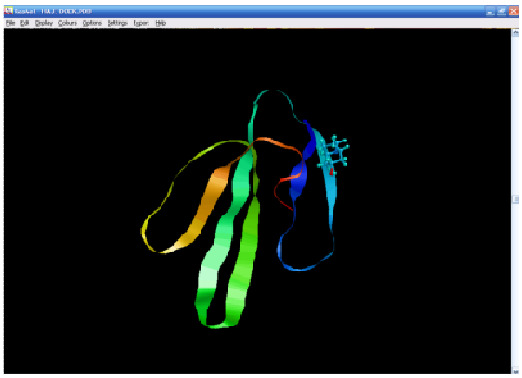
Naja haje haje



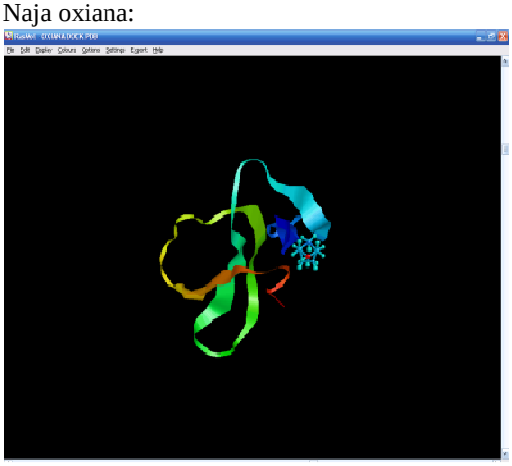
Hex Docking Results: Energy values were obtained and are tabulated as below

When viewed in visualization tool like RASMOL, the docking between receptors of proteins and the ligand can be clearly observed as shown below:

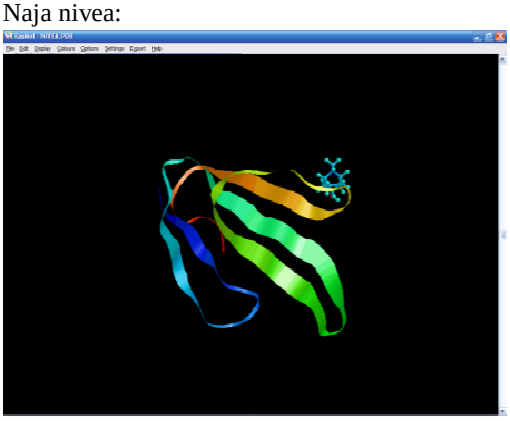
Naja haje haje



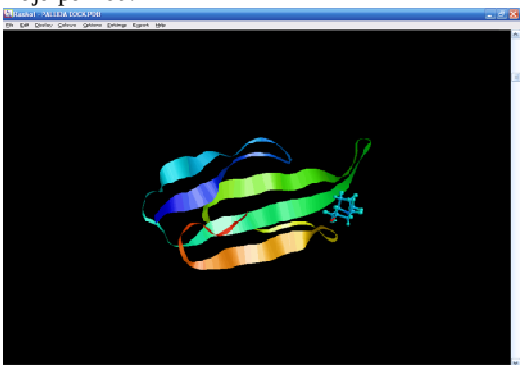
Naja oxiana:



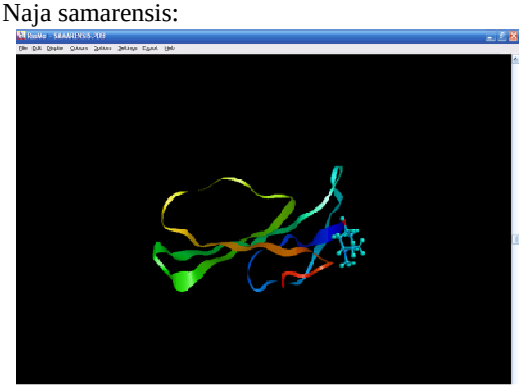
Naja nivea:



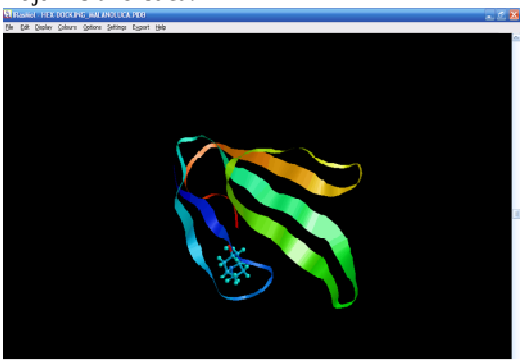
Naja pallida:



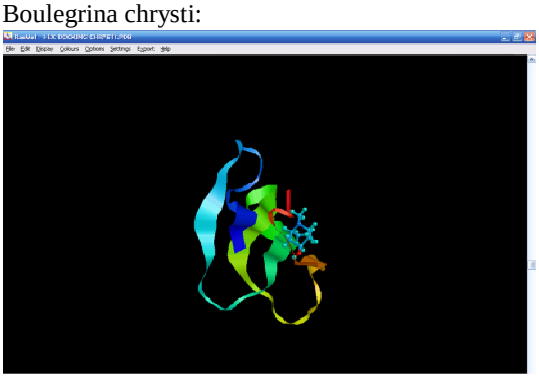
Naja samarensis:



Naja melanoleuca:



Boulegrina chrysti:



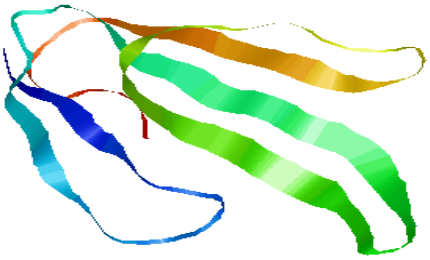
Results of docking using <u>Hex Server</u>:

4.9 Results for vibratinal analysis:

Different modes obtained for vibrational tool for Naja nivea:

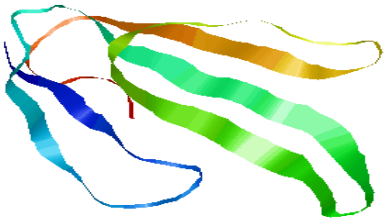
1. **Nivea**

Mode1:

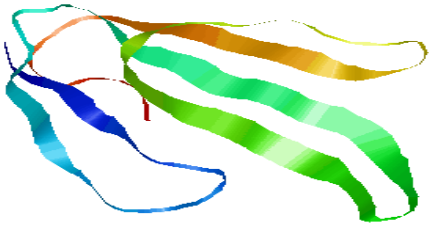


Mode 2:

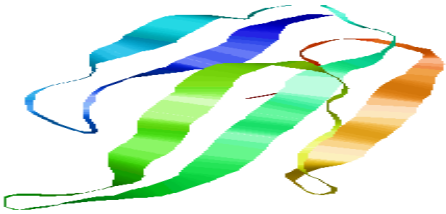
Mode3:



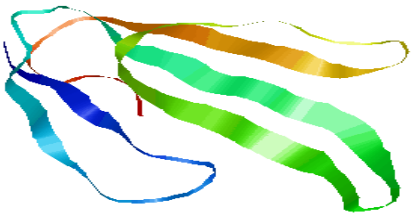
Mode 4:



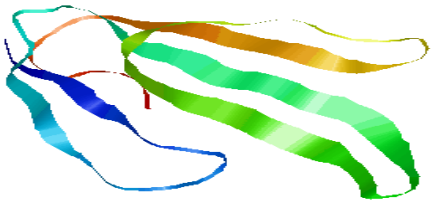
Mode 5:



Mode 6:



Mode7:



Like these different modes were obtained for rest of the 6 proteins, rest all had 7 different modes.

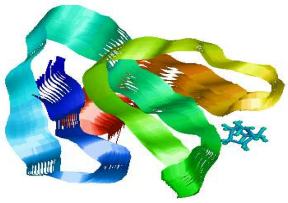
Results of Re-docking using Hex Server:

Species Name	Mode	e-values
1.Naja Pallida	Mode1	-111.73
	Mode2	
	Mode3	
	Mode4	
	Mode5	
	Mode6	
	Mode7	
2. Boulengrina chrysti	Mode1	-115.21
	Mode2	
	Mode3	
	Mode4	
	Mode5	
	Mode6	
	Mode7	
3. Naja haje haje	Mode1	
	Mode2	
	Mode3	
	Mode4	

	Mode5	-117.55
	Mode6	
	Mode7	
4. Naja oxiana	Mode1	
	Mode2	
	Mode3	
	Mode4	
	Mode5	-106.53
	Mode6	
	Mode7	
5.Naja samarensis	Mode1	
	Mode2	
	Mode3	
	Mode4	
	Mode5	-92
	Mode6	
	Mode7	
6. Naja malanoleuca	Mode1	
	Mode2	
	Mode3	
	Mode4	
	Mode5	-103.62
	Mode6	
	Mode7	
7. Naja nivea	Mode1	
	Mode2	
	Mode3	
	Mode4	-119.96
	Mode5	
	Mode6	
	Mode7	

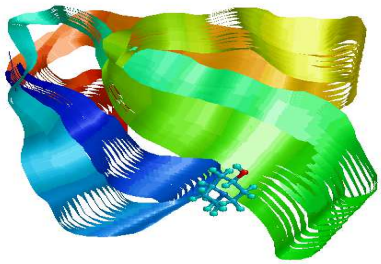
Nivea_redock after vibrational analysis:

Mode1:

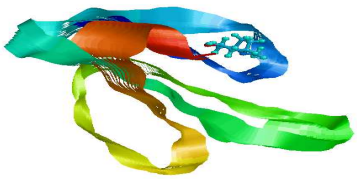


+

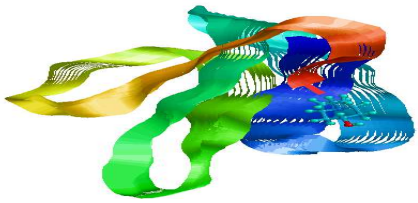
Mode2:



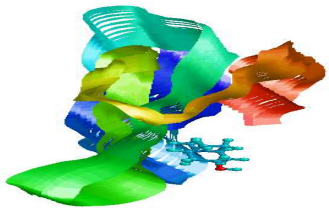
Mode3



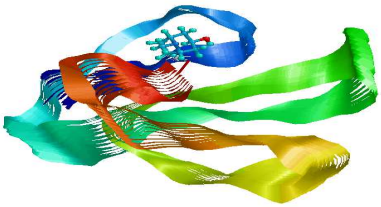
Mode4



Mode5



Mode6



Mode7

Similarly redock was performed for rest of the six different proteins with 7 different modes for each.

DISCUSSION:

The Phylogenetic Analysis was carried out using CLUSTAL W tool. This showed the evolutionary relationships between different species of *Naja* which were selected for the study. The relationship established by phylogenetic trees often describe species evolutionary history and, hence, its phylogeny-the historical relationship among the lineages or organisms. Study of protein evolution often involves the comparison of homologs, sequences that have common origins but may or may not have common activity. These homologous sequences are inherited from a common ancestor that possesses similar structure, although the ancestor may be difficult to determine because it has been modified through descendent.

Homology modeling was performed for the seven different species of *Naja* using the alignment mode of SWISS Model. The template identification was performed for all the sequences. *Naja nigricolis* was found to be the common template for all the sequences. Further modeling of all the seven proteins was carried out using this template. Homology modeling relies on the identification of one or more known protein structures likely to resemble the structure of the query sequence, and on the production of an alignment that maps residues in the query sequence to residues in the template sequence. It has been shown that protein structures are more conserved than protein sequences amongst homologues, but sequences falling below a 20% sequence identity can have very different structure. The models developed were first checked for pass or fail using structure analysisjh and validation tool which performs different methods like what check, verify 3D, prove and errat. Average of 75% residues should have 3D-1D score greater than 0.2. 3D-1D scores for all the models were found to be greater than 0.2. The range of the percentage of residues was 75.81- 100%. The lowest percentage was obtained for *Naja malanoleuca* and the highest percentage was for *Naja nivea.* Verify3D plots were considered for the validation of models which are the residue number v/s 3D-1D averaged score plots.

Hydropathcity plots were obtained for different proteins that characterize its hydrophobic character which may be useful in predicting membrane spanning domains potential antigenic sites and regions that are likely to exposed on prortein surface.

Kyte-Doolittle is widely applied scale for delineating hydrophobic character of of a protein. Regions with values above 0 were hydrophobic in character

Hopp-Woods scale was designed for predicting potentially antigenic regions of polypeptide values greater than 0 were hydrophilic and thus klikely to be exposed on the surface of the folded protein.

The ligand, Tropine is a small sized molecule with a molecular weight of 141.2108(g/mol). It has one hydrogen bond donor and two hydrogen bond acceptors with no rotatable bonds. The compound Tropine has the LogP value of 0.477. Thereby it satisfies all the criteria of Lipinski's rule of five.

Molecular Docking was performed using two different servers, Hex and Medock and the performances were verified for further study. The first tool used was Medock which exploits maximum entropy property of Gaussian distribution. Short neurotoxin of Naja samarensis showed higher affinity towards the compound tropine. It has the binding energy value of -6.52896Kj/mol. Hence tropine coild act as better skeletal muscle relaxant. Lower binding energy

indicates less entropy. Hence more stable the system will be.

Second docking tool used was Hex. Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. *Hex* can also calculate protein ligand docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes. Hex program uses *spherical polar Fourier* (SPF) correlations to accelerate the calculations, and it is still one of the few docking programs which have built-in graphics to view the results. Also, it is the first protein docking program to be able to use modern graphics processor units (GPUs) to accelerate the calculation.

The Hex software gives corresponding e-values for each docking. More negative the e-value more efficient is the docking. Short neurotoxin of Naja nivea showed higher affinity towards the compound tropine. It gave an e-value of -119.96 which is the most negative among docking for all the other proteins.

These models could be used for further analysis by some more user friendly visualizing and manipulating tool like SPDBV and RASMOL.

Vibrational analysis was used to find vibrational modes of the receptor and a docking conducted on the most structurally different form with the ligand. This needs to be compared with the previous dockings which only took into account the flexibility of the ligand. The flexibility concept is important in concern to the proper bind and biological relevance. By performing the vibrational studies we were able to find out more conformational possibilities and best fit by the ligand to receptor molecule. The docking energy for Naja nivea before and after the vibrational analysis was found to be the same, i.e 119.96. So was the case for all the other proteins. Hence it was observed that there is no change in the energy value for the docking of the proteins, obtained from the vibrational analysis, with the ligand. This shows that the conformational change achieved by vibrational tool are not associated with the ligand binding site.

CONCLUSION:

The total of seven snake venom neurotoxins were chosen and analysed for phylogenetic relationship. For all the seven neurotoxins models were built using Swiss Model server and all models were validated using verify3D.Docking was carried out using Hex Server and Medock Server for all the seven proteins with the ligand Tropine. The hydrophilllic and hydrophobic regions of the snake venom protein were obtained from the hydropathicity plot.Using MEDock server, the short neurotoxin of Naja samarensis (P60774) species showed the higher affinity towards the compound tropine. It has the binding energy value of -6.52896(KJ/mol). Hence tropine could act as a better skeletal muscle relaxant.Using Hex server, Short neurotoxin of Naja nivea showed higher affinity towards the compound tropine with the highest negative e-value of -119.96.7 modes of structures were obtained for each protein by Vibrational analysis, done using protein vibration calculating tool. Redocking by Hex Server for all the protein structures of vibrational studies gave same e-values. This shows that the conformational changes are not associated with the binding site of ligand.

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