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# Insilico 3D Structure Prediction of Cell Membrane Associated Protein Ninjurin (Homosapiens)

[1] Sheetal Khatri, [1]Ashish Patel, [1]Jyotsana Choubey, [2] Shailendra Kumar Gupta and [1]M. K. Verma
[1]SUB-DIC Bioinformatics centre, NIT Raipur (C.G)
[2]Indian Institute of Toxicology Research Lucknow (U.P.)

**Abstract:** The protein Ninjurin is a cell membrane-associated protein that possesses homophilic adhesion properties. A comparative modeling of the ninjurin protein (Homo sapiens) is performed to understand the exact role of this protein and it's mechanism. Because of the very few structural information and poor templates similarities (for Ninjurin), the deviation of the model consisted in an iterative trial-and-error procedure using the comparative modeling program MODELLER9V3.The structure evaluation is done on the basis of DOPE (Discrete Optimized Protein Energy) Score that is a statistical potential used to assess homology models in protein structure prediction. The following structural validation programs Procheck, Prosa, Verify3D and WHATIF are also used for Model verification. The analysis of the final model reveals a scaffold of key residues that is believed to be essential in the folding mechanism and that coincides with the residues conserved throughout the ninjurin family.

**Key words:** Comparative modeling, DOPE score, ninjurin, neurite, organogenesis and WHATIF

## INTRODUCTION

Cell surface adhesion proteins play an important role in embryonic development, in organogenesis, and in tissue regeneration after injury (Miyamoto and Teramoto, 1995). Ninjurin was first identified as a molecule that is up regulated in Schwann cells and neurons after peripheral nerve injury. Subsequent analysis of ninjurin function revealed that it is a cell surface molecule that promotes cell aggregation and stimulates neurite outgrowth, suggesting that it may play an important role in nerve regeneration (Araki and Milbrandt, 1996).

In this study, our aim was to derive a model of the tertiary structure of the Ninjurin of Homo sapiens by using a comparative modeling approach. A drawback arises, however, from the fact that no homologues were found for this 152 residue long mature peptide. To overcome this problem our strategy was to consider an extensive iterative procedure that combines the following steps for the generation of models by comparative modeling, validation of a model by structure validation program. In MODELER9V3 the models are selected on the basis of GA341 (is a composite fold assessment score that combines a Z-score calculated with a statistical potential function, target-template sequence identity and a measure of structural compactness) (John and Sali, 2003; Melo *et al*., 2002) and the DOPE score (Discrete Optimized Protein Energy) (Shen and Sali, 2006; Chuang *et al*., 2003; Sali and Blundell, 2003).

## MATERIALS AND METHODS

**Selection of Templates and Input Preparation:** Selection of the secondary structure for the 152 residue long mature peptides of Ninjurin from Homo sapiens was performed through the JPRED server (http://www.combio.dundee.ac.uk/www-jpred) (Cuff *et al*., 1998). The result indicates two long helices between 41 and 69 and between76 and 103; two short helices between 109 and 114 and between120 and 137; and a shortest helices between 117and 118.Cystiene bridges are completely absent in ninjurin structure.

The comparative modeling program MODELER9v3 in conjunction with the following validation programs PROCHECK, PROSA and WHATIF were used throughout this study to derive a model for the ninjurin protein. The challenges we had to face were to derive a structure for this protein that does not have any known homologues in the PDB. Only protein structure sharing overall low similarities (<25%) were used in our modeling approach as template. Our basic idea was to select templates based on the following criteria: (i) the sequence must share sufficient similarity (at least 15% with respect to the 152 residue-long sequence of ninjurin); (ii) the secondary structure of the template must match the predicted secondary structure of the target (ninjurin) sequence. A template was rejected whenever one of the above criteria was not fulfilled.

At first, several approaches were considered to search for templates, sharing global similarities with the ninjurin sequences. They are obtained from threading ninjurin

**Corresponding Author:** Sheetal Khatri, SUB-DIC Bioinformatics centre, NIT Raipur (C.G), India

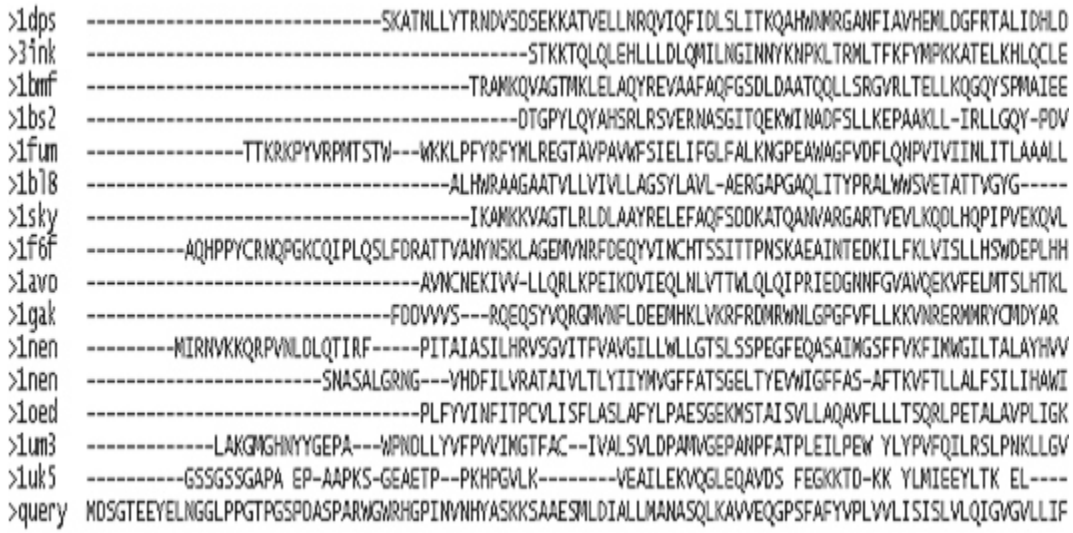



Fig. 1: Final alignment that enables the derivation for ninjurin (The identification code is taken from the RCSB protein data bank)

sequences onto known structure by using the 3D-PSSM server (Kelley *et al*., 2000), Despite the various databases used, very few good candidates were found. We finally selected one template structure from the 3D-PSSM server based on it's threading scores and the fulfillment of the above described requirements: the portion of the sequence of this selected template shares 15% global similarity with ninjurin, a good agreement is found between the observed and predicted secondary structures of the template and ninjurin.This template is chosen as first template in the final alignment (Fig. 1) and defines a general framework for the ninjurin fold.

A further step consisted in performing a multiple alignment of all the templates and target sequence using the program ClustalW with the PAM substitution matrix.
Modeling strategy

Five sets of models were generated by using the program MODELER9V3. In this program, the models are generated by satisfaction of spatial restraints. The restraints include distance and dihedral angles for the backbone and side chains. The values of DOPE score for five models are -12583.18945, -12849.71094, -12529.79883, -12625.21191 and -13016.87695. The 5$^{th}$ model has lowest DOPE score (-13016.87695) value so that this model is selected as a final model.

## RESULTS AND DISCUSSION

**Comparative Modeling:** The modeling approach can be summarized as follows. Firstly 3D models for Ninjurin is derived through MODELER9V3,out of this the model which shows the lowest DOPE score value is selected as a best model and this model is further validated by using PROCHECK (Laskowski, *et al*., 1993), PROSA (Wiederstein, 2004) and WHATIF (Vried, 1990).

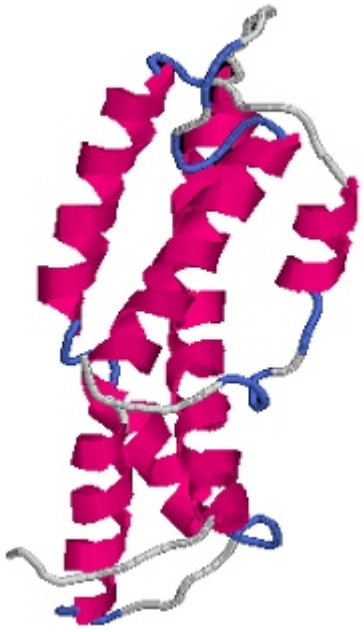


Fig. 2: Model derived for the Ninjurin

From the above methodology, it follows that the final alignment, presented in Fig. 1, is the result of many intermediate revised version. The model that satisfied all the validation criteria on the basis of WHATIF, PROSA and DOPE score is presented in Fig. 2. And Ramachandran plot shown in Fig. 3 which indicates that 88.2% of the residue having psi/phi angles falling in the most favored regions and 8.7% residues in the allowed region. The interaction energy per residue is also calculated by program PROSA. Fig. 4, displays the PROSA profile calculated for the Ninjurin model (shows

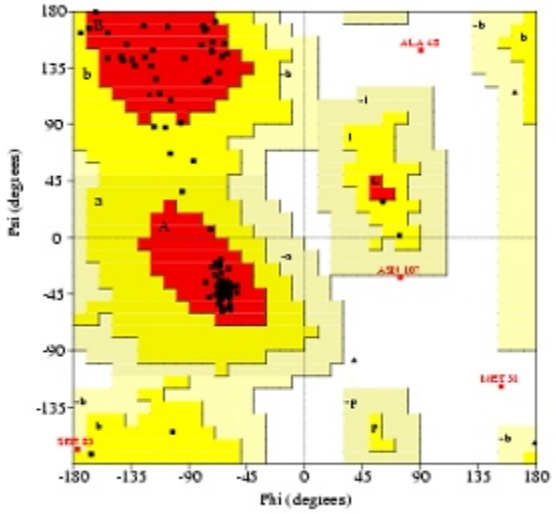



Fig. 3: Ramachandran plot of the psi/phi distribution of the Ninjurin model as obtained by PROCHECK: 88.2% residues are in most favored region and 8.7% are in additional allowed regions.

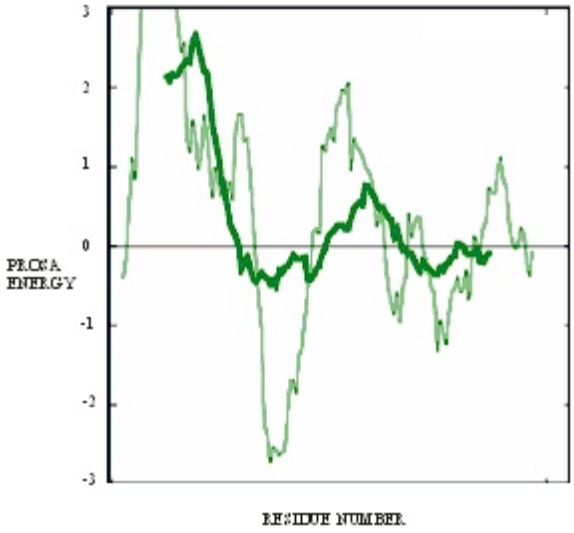



Fig. 4: Prosa energy plot for the Ninjurin.

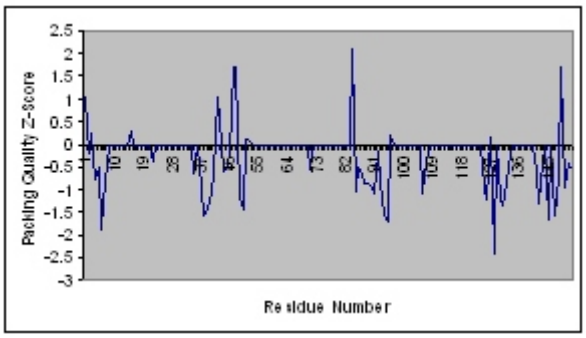



Fig. 5: WHATIF quality control values calculated for the Ninjurin

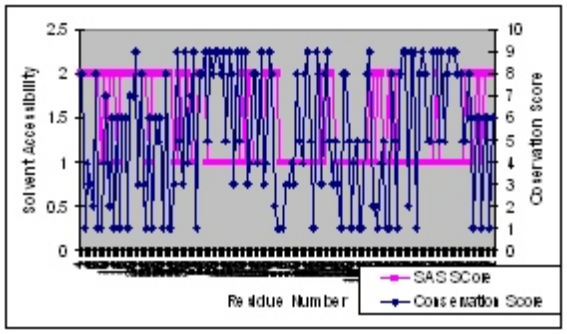



Fig. 6: Analysis of buried residue in modeled Ninjurin and residue conservation. Solvent accessibility was calculated using SAS program. Residue conservation score (1, low conservation; 9, high conservation) were calculated from Consurf server

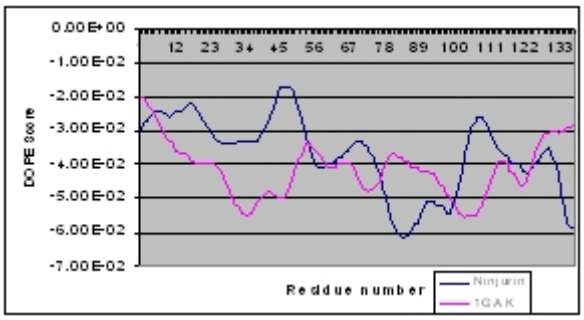
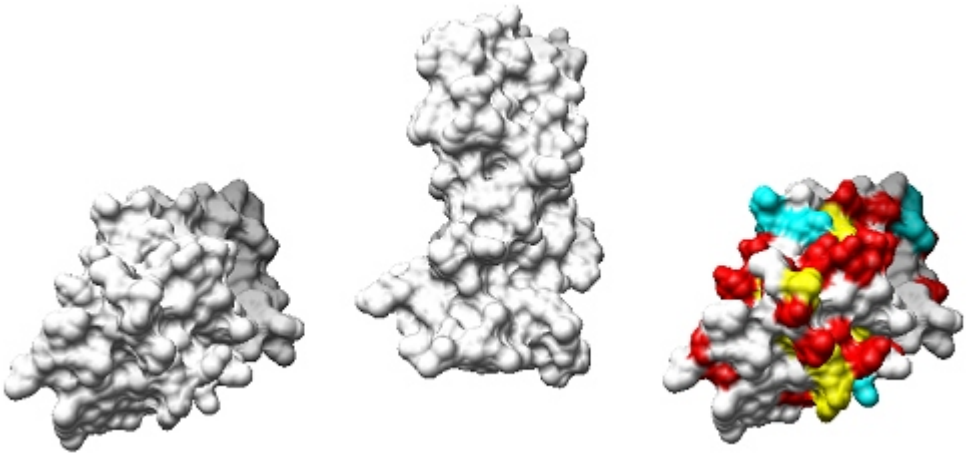



Fig. 7: Analysis of DOPE Score profile of Ninjurin and 1GAK (template)

that the model which shows lowest Prosa score [as compared to template] is the best model). A final test is the packing quality (threshold value -3) of each residue as assessed by the WHATIF program. Fig.5, present the profile obtained with respect to the residues and all residues show satisfactory packing values.

**Analysis of Model:** We have performed solvent accessibility calculation on the model using SAS program (Rost and Sander, 1994) and residue conservation score (1, low conservation; 9, high conservation) were calculated from Consurf server (Glaser *et al.*, 2003). Fascinatingly it can be noted from Fig.6 that lower solvent accessibility is associated with higher residue conservation score among aligned Ninjurin. The buried residues (ILE-36, 84,137; ALA42, 48, 55,122,138; SER-50, 87; MET-51; VAL-67, 126,130,131) are highly conserved among Ninjurin accessions. Further analysis of the alignment using the Conseq server shows a scaffold of residues that are expected to be essential for the function of the Ninjurin, its mechanism and overall stability of the system.

In Fig.7 the DOPE score has been represented for Ninjurin and 1GAK in which at position ASN37-ASP53



(a) (b) (c)

Fig 8: Surface representation of the Ninjurin: two front views (a) and (c) and one side view (b). Polar, negatively and positively charged atoms are shown in red, yellow and cyan respectively

(Loop Region), TYR8-ILE36, LEU97-ASP105 and PRO109-GLY124 (Helical Region) shows higher DOPE score value in Ninjurin as compared to the template (1GAK) and that region was further refine by the loop refinement method.

In Fig. 8 the surface of the Ninjurin model has been represented with negative and positive charges displayed in cyan and yellow, respectively and the polar atoms are shown in red. First it is noteworthy that the protein is rather flat as seen in Fig. 8b; second the two surface of each face exhibit different distribution of charges. A large whitish grey surface beside cyan colored areas predominates in the helices part of the protein.

## CONCLUSION

In the process of modeling of Ninjurin, we had to face a major concern that is Absence of homologous structures from structural databases; we were able to identify useful templates that share low sequence similarity with Ninjurin.

Interestingly one of the models (the model which has lowest DOPE score) derived from comparative modeling through MODELER9V3 was validated and displayed several meaningful features: secondary structure, charge distribution, conserved residues engaged in non-bonded interaction.

Analysis of the model of Ninjurin proposed in this paper suggested further experimental investigation and simulations. These are mainly site directed mutagenesis and docking. The validated model of Ninjurin protein shows higher DOPE Score At several regions (Residues) due to the presence of loop and that region will be further refined by the loop refinement method to get a more stable protein model.

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