

# Sequence Analysis and Homology Modeling of NF2 Protein

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## ABSTRACT

Diabetes is diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. In India more than 62 million individuals currently diagnosed with the diabetes. Diabetes is resulting from insulin deficiency or pancreatic cells become insulin resistant. Pancreatic cell ( $\beta$ -cell) death by apoptosis is one of main reason which results in diabetic condition in patients. Neurofibromatosis 2 is involved in  $\beta$ -cell death. Neurofibromatosis 2 (NF2/Merlin) is a tumor suppressor protein, which belongs to the ezrin–radixin–moesin family of actin-binding proteins and regulates the Hippo signaling pathway in mammals and also involved in the regulation of cell proliferation and apoptosis. Merlin regulates the Hippo signaling pathway by controlling the Hippo kinases cassettes MST1/2 and LATS1/2. Therefore, targeting  $\beta$ -cell apoptosis and dysfunction can be a therapeutic approach for the treatment of diabetes. Hence our present investigation focus mainly to understand the detailed molecular features of NF2 by its protein sequence annotation by implementing tools and techniques of Bioinformatics.

**Keywords** : Insulin deficiency, Hippo signaling pathway,  $\beta$ -cell apoptosis, NF2 protein, Merlin, Neurofibromatosis.

## I. INTRODUCTION

Diabetes is a complex metabolic disease resulting from dysregulation of glucose homeostasis and hyperglycemia. If remain untreated diabetes leads to multiple abnormalities such as retinopathy, nephropathy, neuropathy, cardiovascular disease, and impaired wound healing. In India with more than 62 million individuals currently diagnosed with the diabetes. In 2000, India topped the world with the 31.7 million diabetic people. Diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India. Type 1 diabetes (T1D) is mainly due to an autoimmune destruction of the pancreatic  $\beta$  cells and in type 2 diabetes (T2D) pancreatic cells become resistant to insulin. Type 1 diabetes often develops

suddenly and characterized by symptoms such as polydipsia, polyuria, enuresis, lack of energy, extreme tiredness, polyphagia, sudden weight loss, slow-healing wounds, recurrent infections and blurred vision. The loss of pancreatic  $\beta$ -cells, mainly because of increased  $\beta$ -cell apoptosis is a central feature of both type 1 diabetes and type 2 diabetes. Merlin (NF2) regulates the Hippo signaling pathway by controlling the Hippo kinases cassettes MST1/2 and LATS1/2. Two different mechanisms are responsible for pancreatic  $\beta$ -cell apoptosis by Merlin one through direct activation of the MST–Sav complex and subsequent MST1/2-induced LATS1/2 phosphorylation and activation or other being direct recruitment of LATS1/2 to the membrane. Merlin (NF2) functions as scaffold for MST-induced LATS

phosphorylation without altering MST1/2 auto-phosphorylation.

## II. METHODS

### A. Retrieval of protein sequence information of NF2

Sequence of NF2 protein was retrieved from Uniprot database. The retrieved sequence was stored in FASTA format with its accession number [Barker (2001)]

### B. Analysis of Physicochemical properties

For physicochemical characterization of NF2 theoretical isoelectric pI, molecular weight, total number of positive and negative residues, extension coefficient, half life, instability index, aliphatic index, Grand average were computed using protparam server.[William L (2009)]

### C. Secondary structure prediction of NF2

The secondary structure of NF2 was predicted by using online secondary structure prediction tool SOPMA, which provides the information of Alpha helix, beta sheets, extended strands and random coils. [Pirovano (2010)]

### D. Domain analysis of NF2

Domain analysis of NF2 protein was performed by using Pfam tool.

### E. Binding site prediction of NF2

Binding site for NF2 was predicted using CASTp server. [Binkowski TB (2003)]

### F. Prediction of Tertiary structure of NF2

The Tertiary structure prediction of NF2 was performed by using SWISS Model tool by selecting the template with maximum homology and with optimized parameters. The predicted tertiary structure was stored in pdb format for visualization.

### G. Visualization of tertiary structure of NF2

The visualization of predicted tertiary structure of NF2 was performed by using structure visualization tool Rasmol. [Waltz SE (1996)]

### H. Structure validation of predicted tertiary structure

The quality and accuracy of the NF2 protein structure was evaluated using Ramachandran Map calculation computed with PDBsum generate server.

## III. RESULTS AND DISCUSSION

### A. Retrieval of protein sequence information of NF2

Protein sequence of NF2 protein was retrieved from Uniprot database. The retrieved sequence was stored in FASTA format with its accession number. The length of the sequence was found to be 595 amino acids as shown in figure 1.

**Accession no.-** P35240

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>sp|P35240|MERL_HUMAN Merlin OS=Homo sapiens OX=9606 GN=NF2 PE=1 SV=1
MAGAIASRMSFSSLKRKQPKTFTVRIVTMDAEMEF
NCEMKWKGGKDLFDLVCRTLGLRETWFFGLQYTIK
DTVAWLKMDKKVLDHDVSKEEPVTFHFLAKFYF
ENAEELVQEITQHLLFQVKKQILDEKIYCPPEAS
VLLASYAVQAKYGDYDPSVHKGFLAQEELLPKR
VINLYQMTPEMWEERITAWYAEHRGRARDEAE
MEYLKIAQDLEMYGVNYFAIRNKKGTLELLGVDA
LGLHIYDPENRLTPKISFPWNEIRNISYSKDEFTIKP
LDKKIDVFKFNSSKLRVNLKILQLCIGNHDLFMRRR
KADSLEVQQMKAQAREEKARKQMERQLAREKQ
MREEAERTRDELERRLLQMKEEATMANEALMRSE
ETADLLAEKAQITEEEAKLLAQKAAEAQEMQRIK
ATAIRTEEEKRLMEQKVLEAEVLALKMAEESERRA
KEADQLKQDLQEAREARAKQKLEIATKPTYYP
MNPAPLPPDIPSFNLIGDSLDFKDTDMKRLSM
EIEKEKVEYMEKSKHLQEQNLKTEIEALKLKERE
TALDILHNENSDRGGSSKHNTIKKLTLSAKSRVAF
FEEL
```

Figure 1: Showing Protein sequence of human NF2.

### B. Analysis of Physicochemical properties

The analysis of physicochemical properties of NF2 was done by using protein prediction tool i.e.

protparam which gives a detailed information of protein. Computed parameters of protein using ExPASy's Protparam tool was represented in table I. The computed pI value of NF2 protein is less than 7 (pI<7). Total numbers of negatively charged residue (Asp+Glu) were greater than positively charged residue (Arg +Lys). In atomic composition number of Hydrogen (H) atoms was dominated. Instability index of protein was greater than 40(>40).

TABLE I  
SHOWING PHYSICOCHEMICAL PROPERTIES OF NF2

Properties	Values
No. of amino acid	595
Molecular weight	69690.16
Theoretical pI	6.11
Total no. of negative charged residue (Asp+Glu)	107
Total no .of positively charged residue(Arg +Lys)	101
Atomic composition	
1)Carbon(C)	3084
2)Hydrogen(H)	4962
3)Nitrogen(N)	852
4)Oxygen(O)	926
5)Sulfur(S)	28
Total no .of atoms	9852
Extinction coefficient No.	55600
Half life	30hrs
Instability index	Unstable(53.26)
Aliphatic index	81.08

**C. Secondary structure prediction of NF2**

Secondary structure prediction of NF2 was performed by using SOPMA and all the secondary structural elements like alpha helix, beta sheets, random coils and extended strands were predicted as shown in table II. Secondary structure of NF2 protein was dominated by alpha helix (Hh) followed by random coils (Cc), extended strands (Ee) and beta turn (Tt).

TABLE II  
SHOWING SECONDARY STRUCTURE INFORMATION OF NF2

Structural component	Residues	Percentage
Alpha helix (Hh)	350	58.82%
310 helix (Gg)	0	0.00%
Pi helix (Ii)	0	0.00%
Beta bridge (Bb)	0	0.00%
Extended strand(Ee)	70	11.76%
Beta turn (Tt)	22	3.70%
Bend region(Ss)	0	0.00%
Random coil (Cc)	153	25.71%
Ambiguous	0	0.0%

**D. Domain analysis of NF2**

The analysis of Domain of NF2 protein was performed by using Pfam tool. Domains of NF2 are as shown in figure 2. This image shows arrangement of domains that found on protein sequence and ERN indicates Ezrin/radixin/moesin family.



Figure 2: Domain of NF2.

**E. Binding site prediction of NF2**

Binding site for NF2 was predicted using CASTp server. It predicts the 7 active pockets as shown in Figure 3.

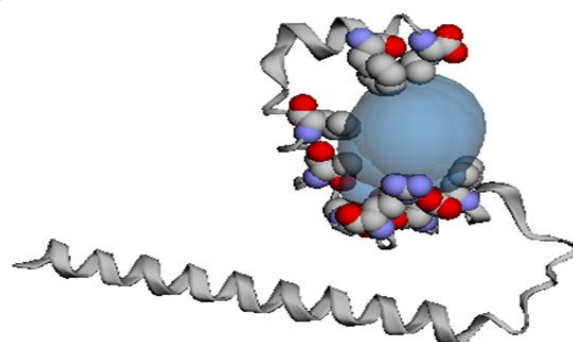


Figure 3 : Binding site of NF2.

**F. Prediction Tertiary structure of NF2**

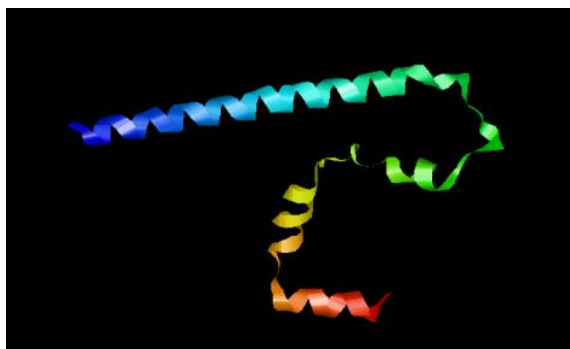
The Tertiary structure of NF2 was obtained by using SWISS Model tool by selecting the template with maximum homology and with optimized parameters. The obtained structure was stored in pdb format for visualization. The details of template selected for structure prediction was as shown on table III. Oligostate of predicted tertiary structure of NF2 protein was Monomer in nature.

TABLE III  
SHOWING TEMPLATE INFORMATION OF NF2  
FOR HOMOLOGY MODELLING

Name	Title	Identity	Oligostate
4zrj.1.B	Merlin	97.78	Monomer
2i1k.1.A	Moesin	45.55	Monomer
4o9b.2. B	Stromal interaction molecule 1	24.10%	Monomer
3jax.1.A	Myosin 2 heavy chain	22.94	Monomer

**G. Visualization of tertiary structure of NF2**

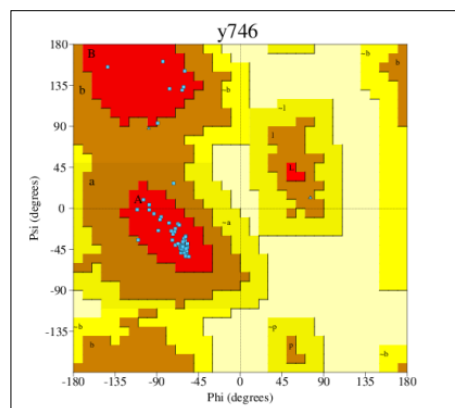
The predicted Tertiary structure of NF2 was visualized by using structure visualization tool Rasmol. Visualization was done to understand structural features of NF2. The predicted tertiary structure of NF2 was represented in Figure 4.



**Figure 4 :** Visualization of 3D structure of NF2 protein.

**H. Structure validation of predicted tertiary structure**

The quality and accuracy of the NF2 protein structure was evaluated using Ramachandran Map calculation computed with PDBsum generate server. Ramachandran Map of NF2 protein was as shown in figure 5. It indicates out of 80 residues 76 residues (95%) were present in most favored regions and 4 residues (5%) in additional allowed region.



**Figure 5 :** Ramachandran map of NF2 protein.

**IV. CONCLUSION**

Diabetes results from either insulin deficiency or insulin resistant pancreatic cell. Merlin is highly conserved protein in mammals and plays a key role in organ size control and development through the regulation of cell proliferation and apoptosis. Merlin is a principal inducer of apoptosis in the heart by activating MST1/Hippo signaling. The instability index value for NF2 was found to be above 40 it indicates that the protein may be unstable. Very high aliphatic index (81.08) of protein indicates this protein may be stable for a wide temperature range. The predicted structure conformed well to the stereochemistry indicating good quality. Hence our preliminary investigation mainly leads to understand the basic primary, secondary and tertiary structure of NF2 using various in-silico tools and techniques of bioinformatics. This preliminary work and further

investigations on pancreatic cell death can lead to a novel therapeutic approach for treatment of diabetes.

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