

# Bioinformatics Analysis of BDNF-TrkB Interactions to Identify Nerve growth factor and Neurotrophin-3 as Agonist of TrkB Helpful in Cell Survival Signaling Pathways of Type 2 Diabetic Retinopathy

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**ABSTRACT:** Type 2 Diabetic Retinopathy is a communal cause of visual damage in the world and it is actually blinding complication of diabetes that damages the eye's retina. Type 2 Diabetic retinopathy is a complication of diabetes that distresses the blood vessels of the retina. Growth of new blood vessels, known as proliferative retinopathy, may lead to blindness through hemorrhage and scarring. A worsening of retinal blood vessels producing loss of blood vessels and leakage into the retina is known as maculopathy and leads to visual impairment and may progress to blindness. Brain-derived neurotrophic factor (BDNF) activates the receptor tropomyosin-related kinase B (TrkB) with high potency and specificity, promoting neuronal survival, differentiation, and synaptic function. In this research, NTF3, NGF interactions with TrkB has been done as NTF3 and NGF acts as agonist for TrkB, which may help in retinal cell survival which would in turn help in controlling Type 2 Diabetic Retinopathy.

**Keywords:** Type 2 Diabetic retinopathy, Brain-derived neurotrophic factor, Tropomyosin-related kinase B, Nerve growth factor, Neurotrophin-3

## INTRODUCTION

Type 2 Diabetic retinopathy (T2DR) remains a main cause of visual impairment and blindness in the worldwide with its timely detection and timely treatment capable of reducing the risk of visual loss [1]. Reasons for loss of vision are diabetic maculopathy and complications of proliferative diabetic retinopathy (PDR) such as vitreous hemorrhage, tractional retinal detachment, and neovascular glaucoma. By 2030 developing countries will face growth by 69% and industrialized countries by 20% of the number of patients with diabetes compared to 2010 [2,3,4]. Micro-angiopathy due to hyperglycemia in patients with diabetes mellitus outcomes in vascular leakage, which causes diabetic macular edema on one hand, and capillary occlusion on the other hand. Capillary occlusion then again causes retinal ischemia and increased levels of vascular endothelial growth factor (VEGF) which are responsible for the expansion of neovascularization and the proliferative stage of diabetic retinopathy [5]. Retina is a part of central nervous system. Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, was effective in guarding retinal neurons from hyperglycemia *in vitro*. BDNF promoted neuronal cell survival. Tropomyosin-related kinase B (TrkB) is a receptor protein involved in the development and maturation of the central and peripheral nervous systems. BDNF has a great affinity for TrkB and p75 increases the interaction between BDNF and TrkB [6].

BDNF is a member of the neurotrophin family of growth factors and is significant in the development, differentiation and maintenance of neurons [7]. Previous studies have discovered that BDNF is critical for photoreceptor cells and the repair of injury to the retina and the optic nerve. BDNF encourages survival in injured RGCs induced by axotomy or retinal ischemia, and also promotes regeneration of the nerve fiber [8, 9]. In addition, BDNF encourages the survival of retinal interneurons and is important for forming phenotypes and synaptic connections in the developing retina [10]. Tropomyosin-related kinase B (TrkB) is a receptor protein involved in the development and maturation of the central and peripheral nervous systems. BDNF has a great affinity for TrkB and p75 increases the interaction between BDNF and TrkB. Upon ligand-binding, TrkB undertakes homodimerization, autophosphorylation and activation. It then recruits and activates several downstream effectors to regulate gene expression and protect neurons. Members of the TrkB downstream signaling cascade, including ERK/MAPK and PI3K/PKB, have been reported to be responsive to BDNF [11, 12].

**Nerve growth factor (NGF)** is important for the development and maintenance of the nervous system. Extracellular ligand for the NTRK1 and NGFR receptors, triggers cellular signaling cascades through those receptor tyrosine kinase to regulate neuronal proliferation, differentiation and survival. NGF is initially in a 7S, 130-kDa complex of 3 proteins - Alpha-NGF, Beta-NGF, and Gamma-NGF (2:1:2 ratio) when expressed. This form of NGF is also referred to as proNGF (NGF precursor). The gamma subunit of this complex acts as a serine

protease, and cleaves the N-terminal of the beta subunit, thereby activating the protein into functional NGF. NGF is involved primarily in the growth, as well as the maintenance, proliferation, and survival of nerve cells (neurons). In fact, NGF is critical for the survival and maintenance of sympathetic and sensory neurons, as they go through apoptosis in its absence. However, numerous current studies suggest that NGF is also involved in pathways besides those regulating the life cycle of neurons [13].

**Neurotrophin-3** is a protein that in humans is encoded by the **NTF3 gene**. The protein encoded by this gene, NT-3, is a neurotrophic factor in the NGF (Nerve Growth Factor) family of neurotrophins. It is a protein growth factor which has activity on certain neurons of the peripheral and central nervous system; it helps to maintaining the survival and differentiation of existing neurons, and inspires the growth and differentiation of new neurons and synapses. NT-3 was the third neurotrophic factor to be categorized, after nerve growth factor (NGF) and BDNF (Brain Derived Neurotrophic Factor) [14]. NT-3 is unique in the number of neurons it can possibly stimulate, given its ability to activate two of the receptor tyrosine kinase neurotrophin receptors [15].

## Materials and Methods

### 1. Datasets

TRKB, NGF and NTF3 protein sequences in the FASTA format were retrieved from the UniProtKB database [16]. For protein-protein docking analysis using bioinformatics studies, 7,8-dihydroxyflavone structure is retrieved from PubChem database [17]. Brain Derived Neurotrophic Factor (BDNF) protein structure retrieved from protein databank (<https://www.rcsb.org/pdb/home/home.do>) and ProBias (<http://probis.cmm.ki.si/>)

### 2. Protein Modeling

Protein structure prediction for TRKB, NGF and NTF3 was accomplished by homology modeling method Swiss Model server (<https://swissmodel.expasy.org/>).

Template identification is carried out using Protein Blast ([https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE\\_TYPE=BlastSearch&LINK\\_LOC=blasthome](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome)). The validation for structural models were selected on the basis of QMEAN4 global scores, Z-score, SAVES server and Ramachandran plot RAM-PAGE and PROCHECK.

### 3. Energy Minimization of BDNF Protein And Protein Models

Swiss-PdbViewer software ([https://spdbv.vital-it.ch/energy\\_tut.html](https://spdbv.vital-it.ch/energy_tut.html)) was used to evaluate the energy of BDNF proteins and protein models.

### 4. Biological Active Site Selection

The Active site of receptor protein and its residues are predicted using online server as Sitehound, Coach and cofactor.

### 5. Protein Docking Studies

The protein-protein docking of TRKB receptor model and BDNF proteins and its derivatives was calculated using offline software HEX and online server PatchDock and FireDock for advance molecular docking. Autodock software was used for TRKB receptor and 7,8-dihydroxyflavone ligand for docking study.

### 6. Visualization of H-Bond

UCSF Chimera software is used for visualization of docked structure and Hydrogen bonds analysis formed between docked molecules.

## RESULTS

### 1. Retrieval and ligand preparation

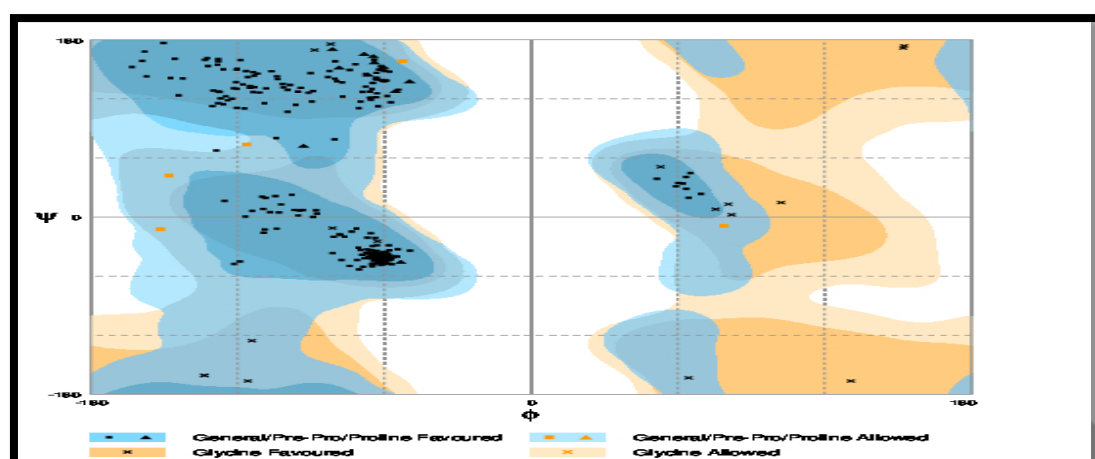
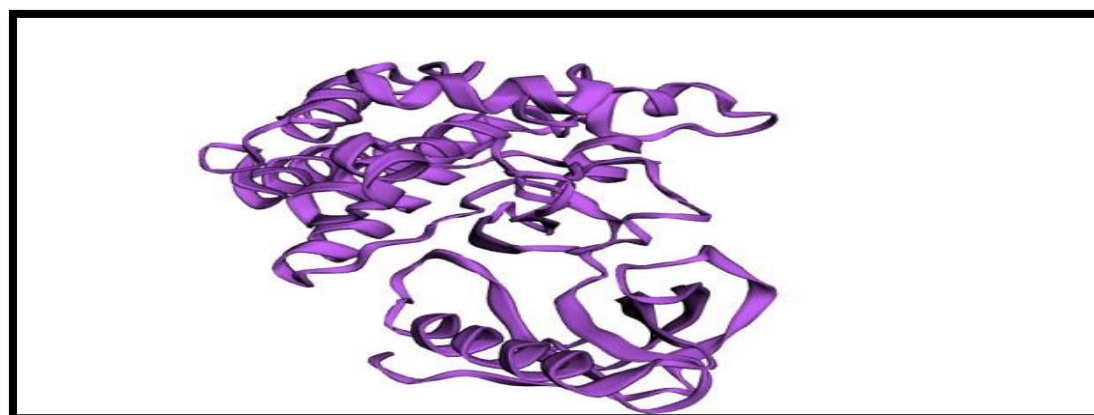
Trkb(Q16620), NGF(P01138) and NTF3(P20783) sequences were retrieved from UniProtKB database. BDNF proteins structures i.e. 1B8M and 1BND tertiary structure retrieved and optimized from Protein Data Bank and ProBias database. 7,8-dihydroxyflavone structure is retrieved from PubChem and converted using Openbabel software for docking preparation.

### 2. Protein modeling and validation of models

The sequence homology approach to generate Tertiary structure of desire protein using sequence alignment to identify the homology template structure for different components such as conserved portion, loop portion and side chains from the database, and threads them to predict and generate tertiary structure. Templates selected using Protein blast tool based on E-value and percentage ident for TRKB, NGF and NTF3.

The models were evaluated and selected on the basis of QMEAN4 and QMEAN6 global scores, Z-score and Ramachandran plot. The QMEAN4 global score was obtained by using QMEAN server while the Ramachandran plot was obtained using PROCHECK and RAM-PAGE. Ramachandran plot was very much useful in evaluating backbone conformation as well as in checking non GLY residues at disallowed regions.

PROTEIN MODELING USING SWISSMODEL SERVER QUERY SEQUENCE	TEMPLATE ID	GMEAN4	QMEAN6	RAMACHANDRAN PLOT
TRKB	4AT5_A	-0.10	-0.14	98.3%
NGF	1WWW_B	-0.75	-0.95	98.2%
NTF3	1BND_B	-0.89	-1.22	98.1%

**TRKB MODEL:**

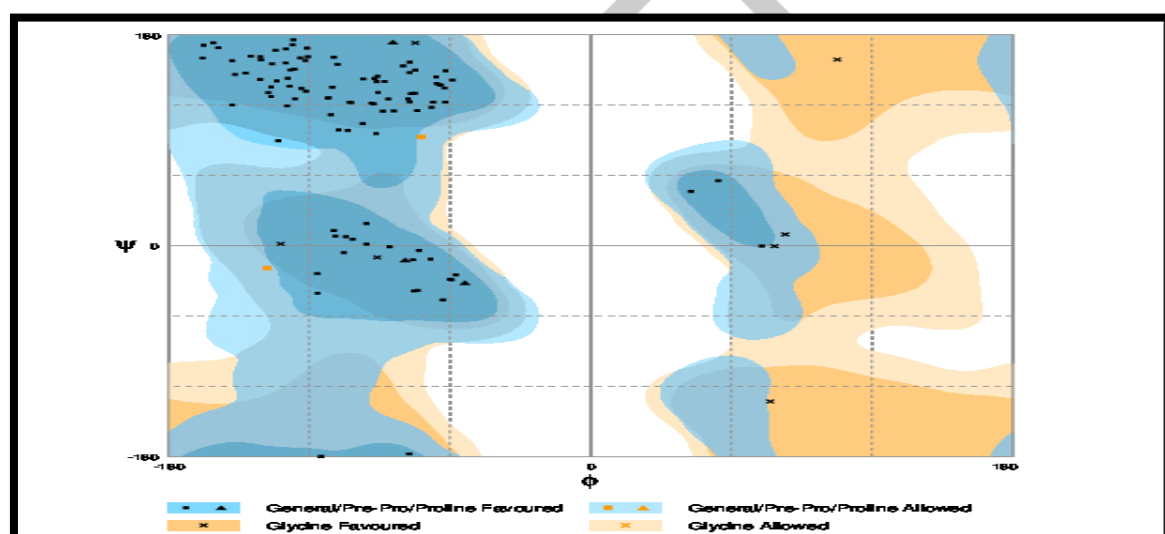
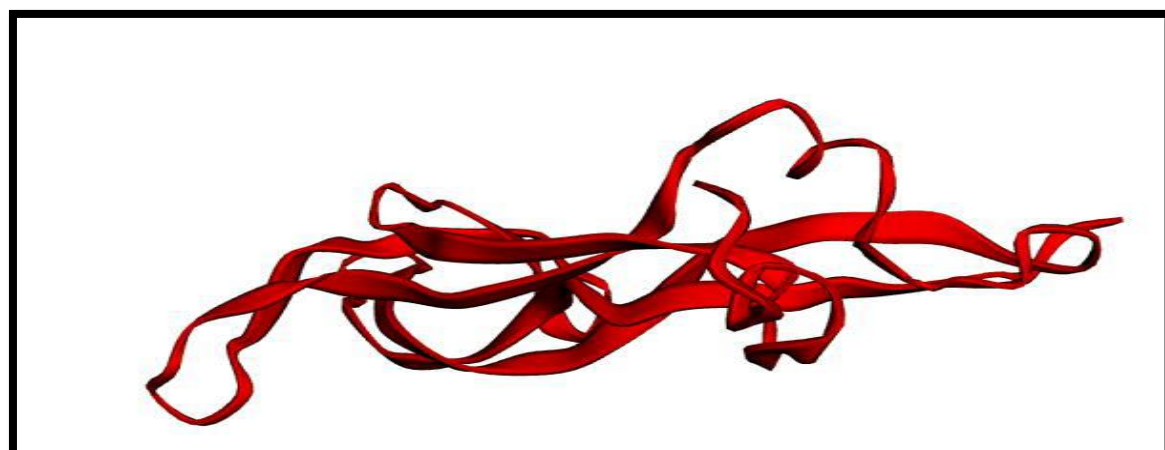
## Evaluation of residues

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Residue [A 578 :SER] ( -52.48, 158.34) in Allowed region
Residue [A 675 :ARG] ( 78.91, -8.94) in Allowed region
Residue [A 676 :ASP] (-148.02, 42.08) in Allowed region
Residue [A 702 :TYR] (-116.26, 73.81) in Allowed region
Residue [A 817 :TYR] (-151.53, -12.30) in Allowed region
Number of residues in favoured region (~98.0% expected) : 289 ( 98.3%)
Number of residues in allowed region (~2.0% expected) : 5 ( 1.7%)
Number of residues in outlier region : 0 ( 0.0%)

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## NGF MODEL



## Evaluation of residues

Residue [B 193 :ASP] ( -72.09, 92.72) in Allowed region

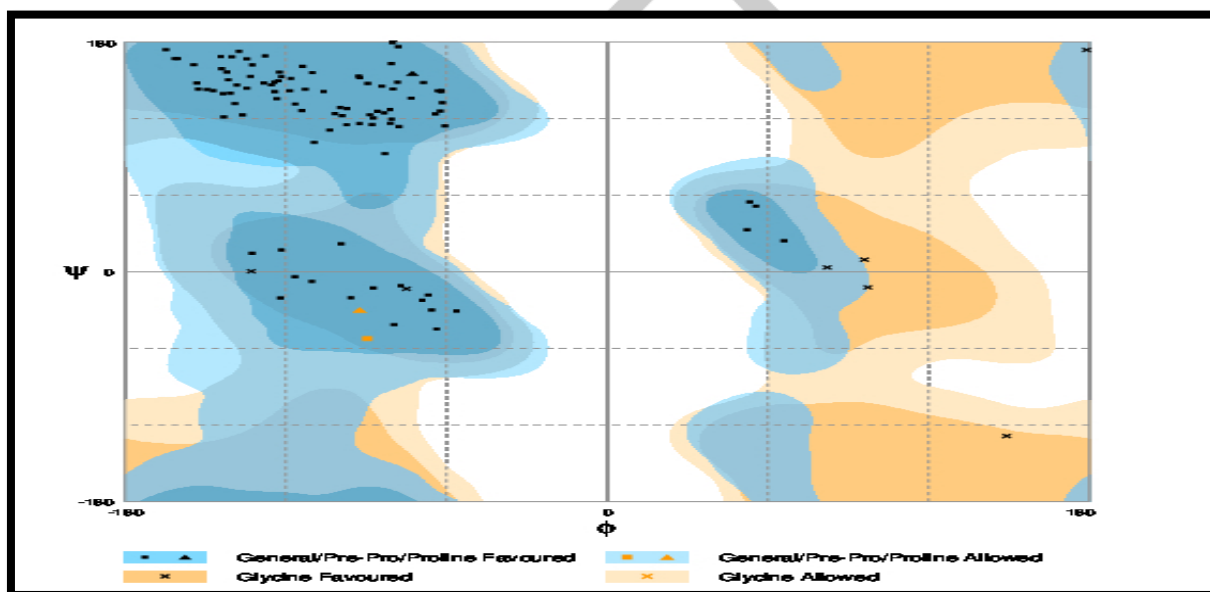
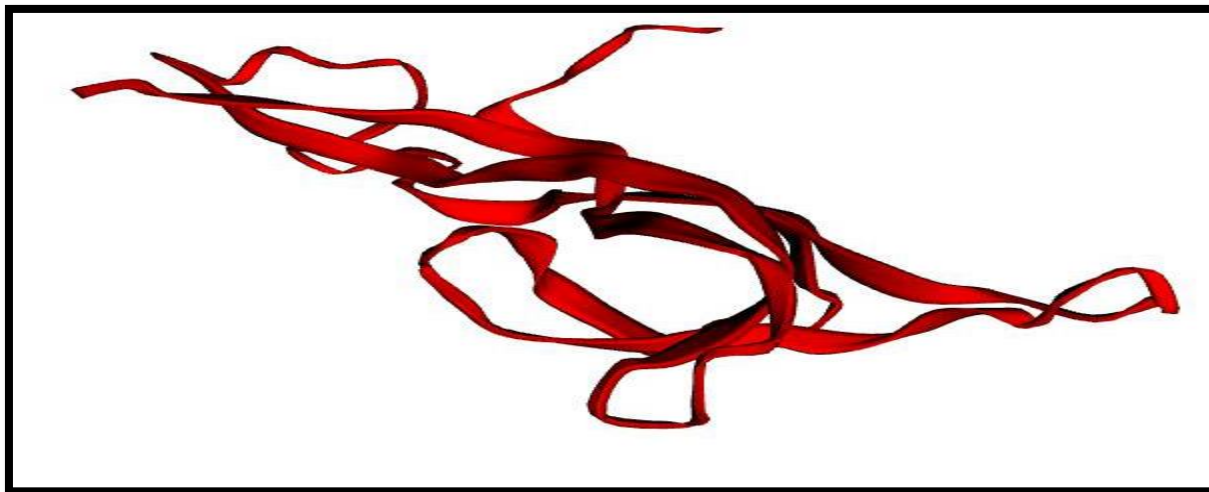
Residue [B 227 :THR] (-137.88, -19.11) in Allowed region

Number of residues in favoured region (~98.0% expected) : 111 ( 98.2%)

Number of residues in allowed region (~2.0% expected) : 2 ( 1.8%)

Number of residues in outlier region : 0 ( 0.0%)

## NTF3 MODEL



## Evaluation of residues

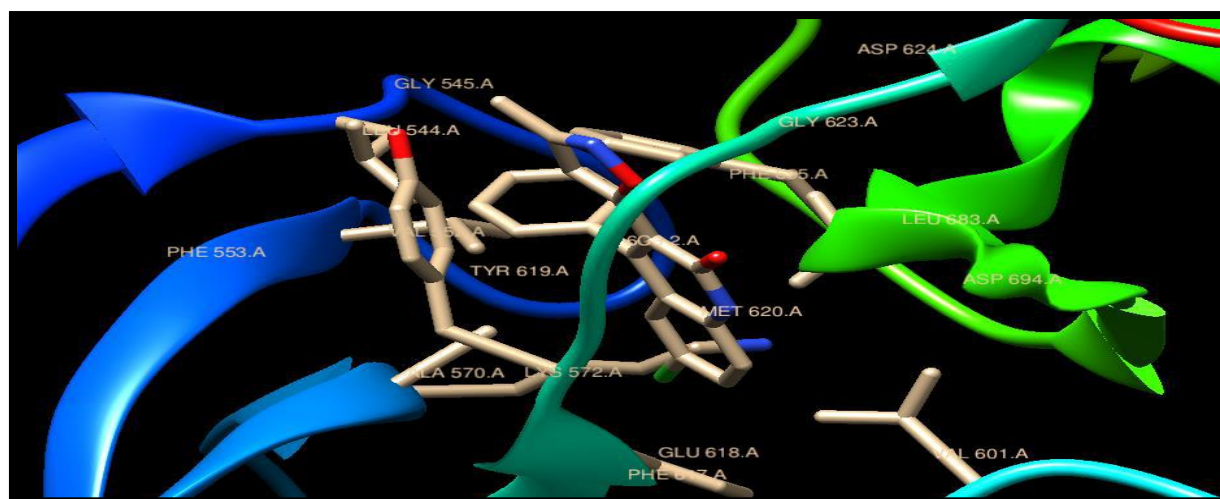
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Residue [B 184 :SER] ( -89.53, -52.32) in Allowed region
Residue [B 200 :PRO] ( -92.34, -29.94) in Allowed region
Number of residues in favoured region    (~98.0% expected) : 104 ( 98.1%)
Number of residues in allowed region     ( ~2.0% expected) :   2 (  1.9%)
Number of residues in outlier region      :   0 (  0.0%)
  
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### 3. Energy Minimization and Biological Active Site Prediction

SwissPdbViewer software used to overhaul distorted geometries through protein structures to achieved energy minimization. Energy minimization was performed to improve the quality of BDNF protein and Protein models of TRKB, NGF and NTF. TRKB receptor protein biological active site predicted using Sitehound, Coach and Cofactor active site prediction online tool. Active sites of a protein are a key element in the binding interaction as they targeted by macromolecules or ligand in attempts to alter related biological processes.





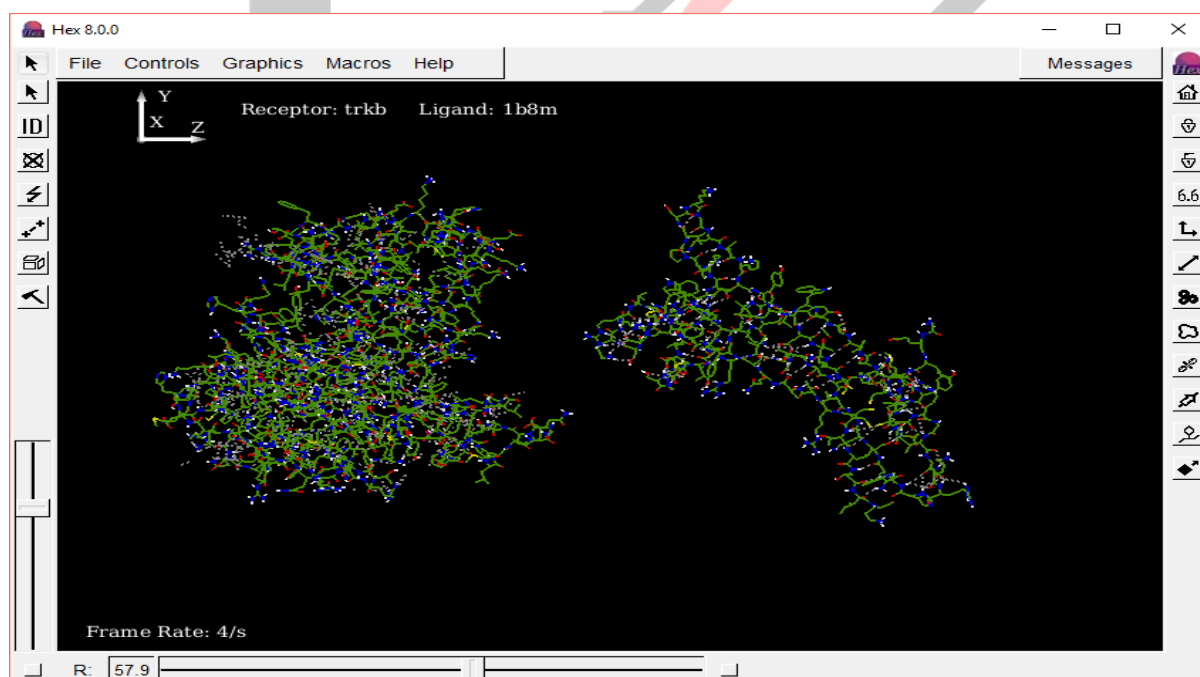
## ACTIVE SITE OF TRKB

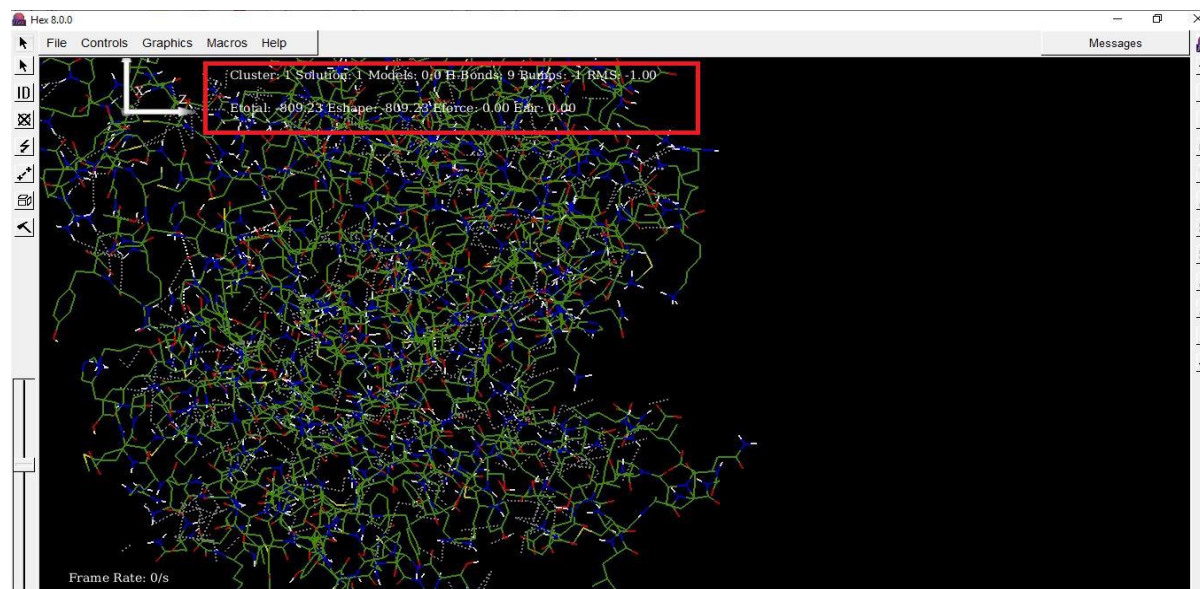
### 4. Protein Docking Studies

Protein-Protein docking studies to deal with the conformational changes between unbound and bound structures, as well as the inaccuracies of the interacting modeled structures and the interaction of macromolecules structure complex. The protein-protein docking carried out between receptor protein model i.e. TRKB and BDNF proteins and its derivatives NGF and NTF using HEX software, PatchDock and FireDock server.

Protein-ligand docking studies to predict and analyze the position and orientation of a 7,8-dihydroxyflavone ligand when it is bound to a protein TRKB protein receptor using AutoDock 4.0 software.

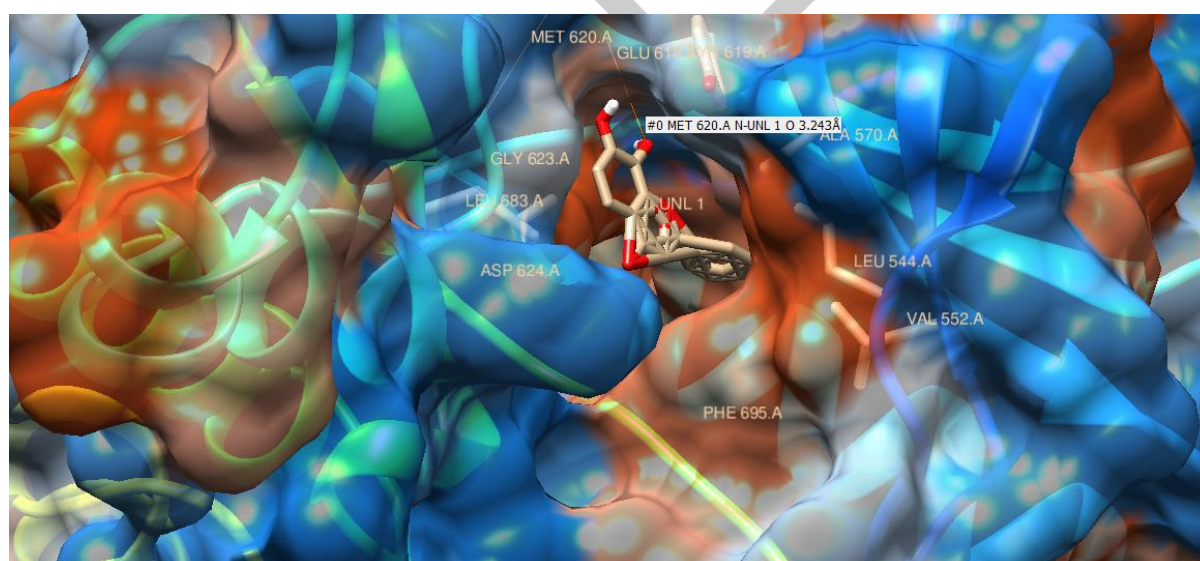
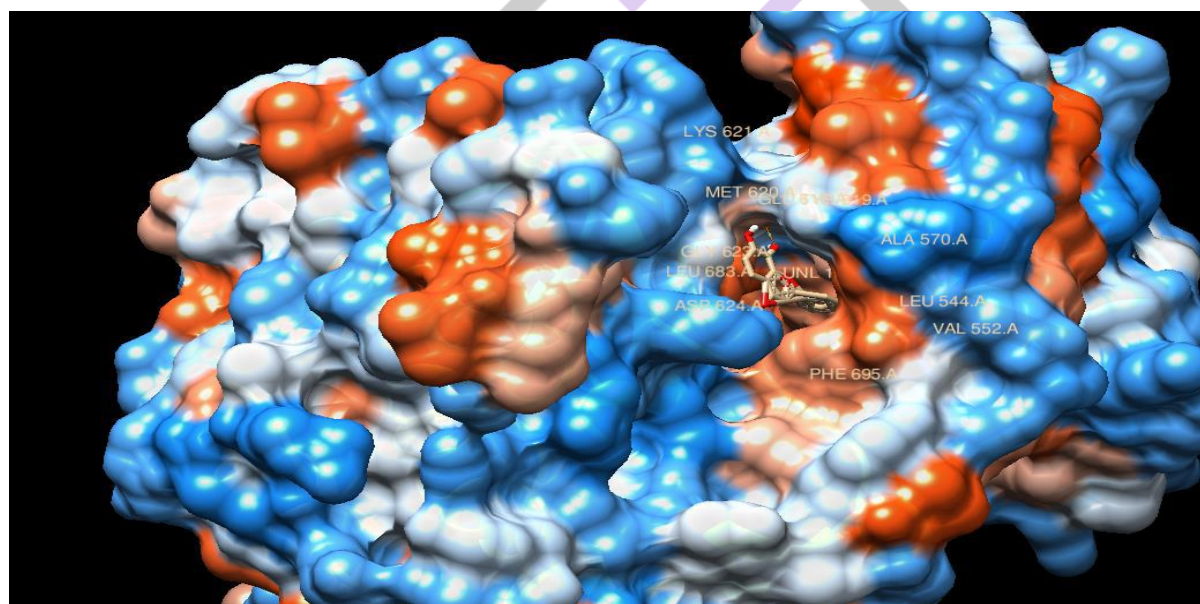
SOFTWARE/SE RVER	TRKB-1BND	TRKB-1B8M	TRKB-NGF	TRKB-NTF3	TRKB-7,8- dihydroxyflavone
HEX	-912.15	-809.23	-580.24	-742.44	-
PATCHDOCK	-57.92	-65.5	-76.93	-65.69	-
AND FIREDOCK					
AUTODOCK	-	-	-	-	-6.41





### VISUALIZATION OF PROTEIN COMPLEX

UCSF Chimera software is used to visualize the protein complex and the H- bond formed between the complex structure.





## CONCLUSION

NTF3 and NGF docked with TrkB act as agonist for neural protection against progression of diabetic retinopathy. NTF3 has better result as compared to NGF while both has much better result as compared to 7,8DHF. Therefore, it is new finding and can help in protection of retinal neurons. This *in silico* study helps in finding new factors which help in protection of retinal neuron clearly more study and *in vitro* study can be done in this field to establish NTF3 and NGF as factors for neural survival signal which can be of medicinal importance to decrease the effect of diabetic retinopathy and protecting neural cell. Thus, this research and study conclude that NTF3 and NGF can protect retinal cell like BDNF and establish it by means of *in silico* analysis of docking methods.

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20. <https://swissmodel.expasy.org/>
21. [https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE\\_TYPE=BlastSearch&LINK\\_LOC=blasthome](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome)
22. [https://spdbv.vital-it.ch/energy\\_tut.html](https://spdbv.vital-it.ch/energy_tut.html)