

# Structure-Guided Modeling and Binding Studies of GABA<sub>A</sub> Receptor Subunit Beta-3

B. Rajasekhara Reddy

Professor of ECE

K G Reddy College of Engineering and  
Technology, Moinabad, Ranga Reddy  
District, PIN: 501504, India.

L. Pratap Reddy, PhD.

Professor of ECE

JNTU College of Engineering  
JNTUH, Kukatpally  
Hyderabad – 500085, India.

Sravani Saragandla

Research Associate

Asterace Labs  
Windsor Plaza  
Nallakunta, Hyderabad-500044, India.

## ABSTRACT

The major inhibitory GABA systems are known to take part a vital role in epilepsy, associated with excessive neuronal circuitry excitation. This excitatory action reflects seizures which result from GABA inhibitory circuit dysfunction. GABA mediates its fast inhibition through GABA<sub>A</sub> receptors by activating it and then opens the chloride channels. This opening allows chloride ions to flow into the interior of the cell which inhibits the excitability. This inhibitory action devises anticonvulsive properties as a consequence. So GABA<sub>A</sub> receptors are primary targets in the pathophysiology of epilepsy. Enhancement in the the action of GABA<sub>A</sub> receptors is the basis of epileptic seizures reduction on which many antiseizure drugs act. Modulation of GABA<sub>A</sub> receptor can be shown by many synthetic and natural compounds. Valerenic acid, a plant origin compound shows modulatory effects on GABA<sub>A</sub> receptors. In our present study, the homology model of the GABA<sub>A</sub> receptor subunit beta-3 is build and docking studies are performed with Valerenic acid. Docking analysis is done to know the binding interactions and their binding affinity. The results revealed the interacting amino acids which are involved in binding of GABA<sub>A</sub> receptor subunit beta-3 with Valerenic acid are ASP35, GLN56, PHE55, TYR120, ARG111 and TYR 54.

## Keywords

GABA, GABA<sub>A</sub> receptor, epilepsy, antiseizure, Valerenic acid

## 1. INTRODUCTION

The  $\gamma$ -aminobutyric acid type A receptor (GABA<sub>A</sub>), a member of the Cys-loop ligand-gated ion channels, is the major mediator of GABAergic synaptic inhibition in the brain [1]. GABA-mediated inhibition through GABA<sub>A</sub> receptors is of major importance in the normal functioning of the nervous system and have also been the target of several clinically relevant anticonvulsant drugs [2, 3]. When GABA molecules or GABA-like compounds bind to the receptor and activate it, this channel temporarily opens and allows the passage of negatively charged molecules, such as chloride ions (Cl<sup>-</sup>), to pass from the cell's exterior to its interior. This ion flow decreases the cell's excitability. This cumulative neuronal inhibition caused by GABA binding to many neurons results anticonvulsive properties [4].

The GABA<sub>A</sub> receptors are composed of a pentameric structure, with five subunits arranged in a ring enclosing a central chloride ion channel. The five subunits arise from seven subunit families that contain multiple subtypes ( $\alpha_{1-6}$ ,  $\beta_{1-3}$ ,  $\gamma_{1-3}$ ,  $\delta$ ,  $\epsilon$ ,  $\pi$ ,  $\theta$  and  $\rho_{1-3}$ ) [1, 5]. The majority of GABA<sub>A</sub> receptors contain two  $\alpha$  subunits, two  $\beta$  subunits, and a  $\gamma$  or  $\delta$

subunit [6] and that there is regional heterogeneity of the subunit composition of GABA<sub>A</sub> receptors in the mammalian brain [1,7]. The formation of functional GABA<sub>A</sub> receptors requires the coexpression of a least two subunit types,  $\alpha$  and  $\beta$  subunits, with both of these subunits contributing to the GABA binding site. GABA<sub>A</sub> receptors are activated by binding of agonist to recognition sites located at  $\alpha/\beta$  subunit interfaces [8, 9]. The functional and pharmacological properties of the GABA<sub>A</sub> receptors are determined in large part by their subunit composition [10]. These differential regulatory properties also underlie their role for fine tuning of neuronal circuits and genesis of network oscillations which represent a major facet of homeostatic synaptic plasticity and contribute to the excitation/inhibition (E/I) balance under physiological conditions and upon pathological challenges [11]. Imbalance between excitatory and inhibitory synaptic transmission in key brain areas are implicated in the pathophysiology of epilepsy, in which there is a decrease in the GABA mediated inhibition. [11]. Furthermore, these distinct receptor subtypes are preferentially expressed in specific regions and neuronal populations and they exhibit different sensitivities to modulators including neurosteroids, benzodiazepines, ethanol and barbiturates [12, 13]. By taking this diversity role of GABA<sub>A</sub> receptors, in our present study, Valerenic acid, a modulator of these receptors, is taken and performed docking studies with GABA receptor. To stimulate the structure-based design for new drugs that target restricted neuronal networks implicated in GABA related epilepsy medication and minimize side effects, it is vitally important to find the 3D structures of GABA<sub>A</sub> receptors subtypes. The present study initiates an attempt to find the 3D structures for the extracellular domain of beta-3 subunit of GABA<sub>A</sub> receptor and docking studies are carried out with Valerenic acid respectively. The results are further analyzed to know the interactions and binding mode of the receptor with Valerenic acid.

## 2. METHODOLOGY

### 2.1 Sequence Retrieval and Homology Modeling

The protein sequence of human GABA<sub>A</sub> receptor subunit beta-3 (GABRB3) is obtained from the SWISS-PROT database that has 473 amino acids. The extracellular domain sequence is retrieved and searched to find out the homologous protein structures to be used as a template by the BLAST program against Protein Data Bank database. The 3D homology model of GABRB3 is modeled using crystal structural coordinates of templates on the basis alignment of target and template. These procedures are performed by ICM MOLSOFT [14]. The

modeled structure is further optimized by energy minimization using Discovery Studio.

## 2.2 Model Evaluation

### 2.2.1 PROCHECK

A versatile protein structure analysis program [15] PROCHECK is used in validation of protein structure and models by verifying the parameters like Ramachandran plot quality, peptide bond planarity, Bad nonbonded interactions, main chain hydrogen bond energy, Calpha chirality and overall G factor and the side chain parameters like standard deviations of chi1 gauche minus, trans and plus, pooled standard deviations of chi1 with respect to refined structures [16].

### 2.2.2 ProSA

This program compares Z scores between target and template structure. The Z scores of model is a measure of compatibility between its sequence and structure. The model Z score should be comparable to the Z scores obtained from the template [17].

### 2.2.3 RMSD

Root Mean Squared Deviation (RMSD) is commonly used to represent the distance between two objects. In a structural sense, this value indicates the degree to which two three dimensional structures are similar. The lower the value, the more similar the structures are. The RMSD value [18] between the template 3RHW and our model structure is calculated using SPDBV program (Figure 39a0 & (b)).

## 2.3 Ligand Preparation

The structure of the Valerenic Acid is downloaded from pubchem and imported into Discovery Studio. Hydrogen bonds are added and the energy is minimized using CHARMM force field. The 3D structure is generated using catalyst.

## 2.4 Molecular Docking

The docking method used in this study is LigandFit in Discovery Studio software and the binding site of the proteins were identified by Eraser algorithm from the receptor site parameter of the tool. To perform docking process, a protocol called “Dockligands” (LigandFit) is selected among those listed under receptor-ligand interaction protocol cluster. The ligand compound is given as input in the parameter meant for “input ligands” and the protocol is run for each of the proteins selected for the study. The various conformations for ligand in this docking procedure were generated by Monte Carlo trials. The final energy refinement of the ligand pose (or) pose optimization in ligandfit occurs by Broyden-Fletcher Gold Farbshanno (BFGS) method. The determination of the ligand binding affinity is calculated using LigScore and PLP, JAIN and Dock score and the ligand binding energies were calculated based on the high Dock score of best conformation.

## 3. RESULTS AND DISCUSSION

The extracellular domain (26-245) of human GABA<sub>A</sub> receptor subunit beta-3 is retrieved in FASTA format from the SWISS-PROT database (Accession No: P28472, Entry name: GBRB3\_HUMAN, Protein name: Gamma-aminobutyric acid receptor subunit beta-3). For modeling of GABA<sub>A</sub> receptor subunit beta-3, homology search for the best template is

carried out using PDB-BLAST and identified 3RHW from C.Elegans having 38% identity and 90% similarity with our target sequence. The 3D homology model of human GABRB3 is predicted using the crystal structure coordinates set of 3RHW on the basis of sequence structure alignment (Figure 1). The model building is done by ICM molsoft. The model structure (Figure 2) is further optimized by energy minimization using Discovery Studio by applying charmm forcefields.

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ID=95% pP=16.0      .#.#.....GYD#R#R.....GG.....#V.#.#.I.#.#.VDM.Y.#.#.#.N.DKRL.....C
sp_P28472_26_2451  1  ETVDKLLR-----GYDIRLRP-----FGGPFV--CVGNNDIASIMVSEVNMDTLTYFQQVWDRKLAIS-----F
3RHW_a             1  --SDSKI--LAHLFTSGVDVFRV--PPTDNGG---PVVSVNMLRLTISKIDVDMVEYSAQLLRESNIDKRLS--YGVKGD

G.P.#.L.....QH#PDT#P#N.K.#.H.#.#.N.#IR#H.DGTVLY.#RI...#.CAM.L..Y#D.Q.C.#.#)
sp_P28472_26_2451  63  GIPLNLTLDNRV--ADQLWVDTYFLNDKRSFVHGVTVENRMLRHPDGTLYGLRITTTAACHMDLRRYPLDEQNTLEIT)
3RHW_a             72  GQDFVLL----TVGHQIWRMFDITFFPNERQAYKHTLDKPEVILRLHNDGTLYVSRISLVLSCPMYLYYPMVQCCSIDL)

.SY.YTT.DIE#W.....#.G#.....LP.F.#.....V..TG#Y..L..#.LKR.#.#.....
sp_P28472_26_2451  142  ESYGYTDDIEFYNRGGDM---AVTGVRIE--LPQFSIVHRLSRNVVATGAVPRLSLSPRLKNTIGY-----
3RHW_a             149  ASYAYTKDIEYLNKHE--SELQLRVLSL---SSLPSQLNTSTTYCTVCTVNTVIGYSLCLATTQLKREFSYLLQLYIPSI

.....
sp_P28472_26_2451  207  .....
3RHW_a             225  CMLVIVSWSFWFORTAIPARVTLGVTLLMTAQSGAGINSQLPSPYIKALDVGWIGACMTFFICALLEFALVNHIANAGT)

.....
sp_P28472_26_2451  207  .....
3RHW_a             306  TENNDISHRVLLSRALFPVLFVFNILYWRGHRFGH
    
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Figure 1: Alignment of the template 3RHW and GABRB3

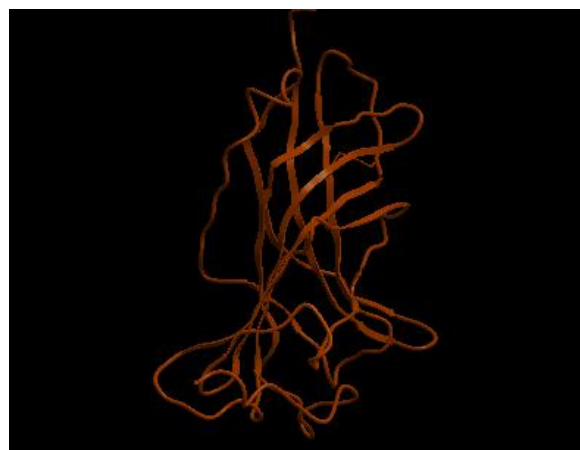


Figure 2: The 3D structure of human GABRB3 modelled using ICM MOLSOFT

The modeled protein is then analyzed for its structural validation by submitting to PROCHECK, ProSA and RMSD. Accuracy of the protein model generated was judged by validity report generated by PROCHECK. In the Ramachandran plot analysis, the residues are classified according to their regions in the quadrangle. The plot also shows that 84.0% of the residues are in most favored regions, 15.4% in additional allowed regions, 0.6 in generously allowed and with no regions in disallowed regions. The Ramachandran map for model is represented in Figure 3(a) & 3(b) and the plot statistics are shown in Table 1. Quality assessment of the model via ProSA revealed that the model matched with NMR region of the plot with Z score of -3.39 (Figure 4). ProSA analysis indicated that the model structure of GABRB3 obtained match with the known proteins whose structures have been already determined by NMR experiments. It signifies good quality of our model. The

overall general similarities and subtle differences among predicted model of GABRB3 and the 3D structure of template 3RHW can be seen from the backbone superposition (Figure 5). As evident from superposition, general folding topology of the structure is similar; however, some structural differences appear between the predicted model and template. These differences are mainly due to insertion and deletions in different loop regions. The RMSD (Root Mean Square Deviation) between predicted model and template is 0.56 Å. The low RMSD between the target and template reflects the presence of strong homology (The lower the value, the more similar the structures are).

The 3D structure of Valeric Acid, which is generated by catalyst after energy minimization using CHARMM forcefield, is shown in Figure 6.

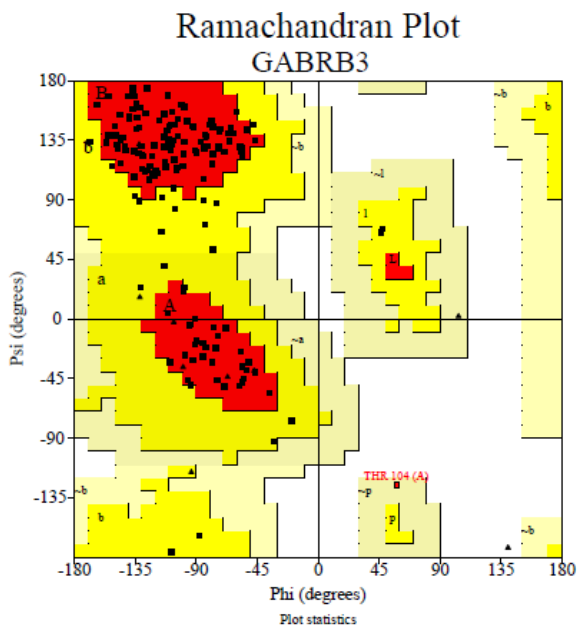


Figure 3(a): Ramachandran Map of GABRB3 from *Homo sapiens*

The Protein-Ligand interaction plays a significant role in structural based drug designing. In the present work, to study

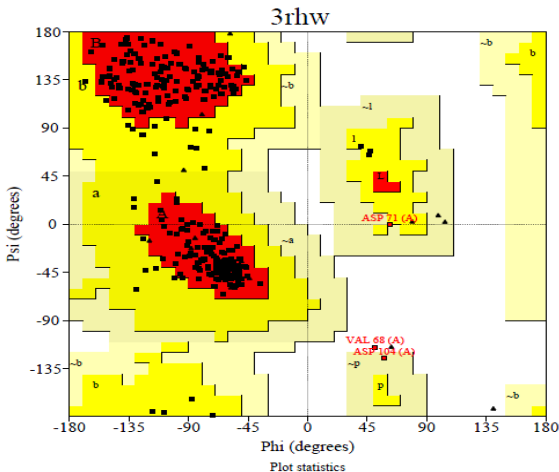


Figure 3(b): Ramachandran Map of 3RHW from *C. Elegans*

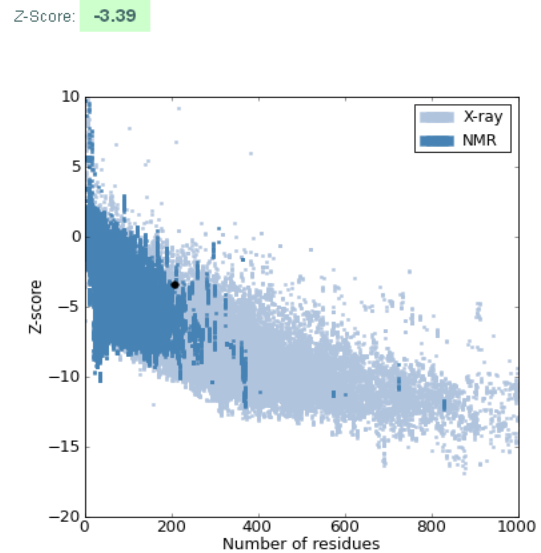


Figure 4: The plan of Z-Score shows spots of Z score values of proteins determined by NMR (represented in dark blue color) and by X- ray (represented in light blue color) using ProSA program. The black dot represents Z-Scores of our model.

the binding modes of the Valeric acid in the active site of gabrb3 Molecular docking study was performed by ligand fit program. The binding modes are calculated using intermolecular flexible docking simulations of LigandFit program. As a result, ten different conformations were generated, but only top ranked docked complex score compound is taken for binding affinity analysis. The different score values include Ligscore1, Ligscore2, PLP1, PLP2, JAIN, PMF and Dockscore as represented in Table 2. Ligscores indicates the protein ligand affinity energy; higher PLP score indicates stronger receptor-ligand binding, high PMF score indicates a stronger receptor-ligand binding affinity and DockScore is used to estimate the ligand-binding energies. Candidate ligand poses are evaluated and prioritized according to the DockScore functions.

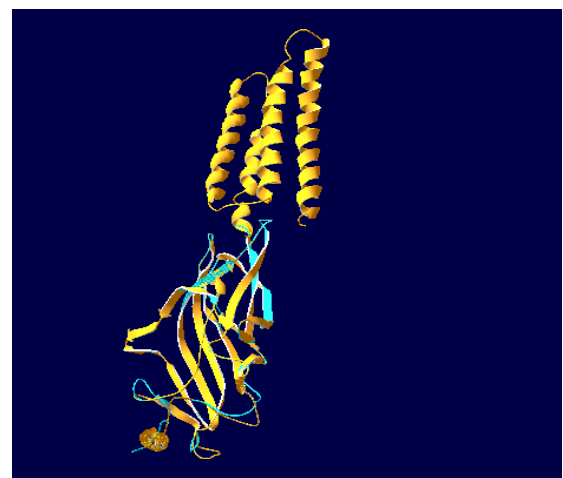


Figure 5: Superimposed structure of Modeled GABRB3 (in blue colour) and 3RHW(ingold colour)

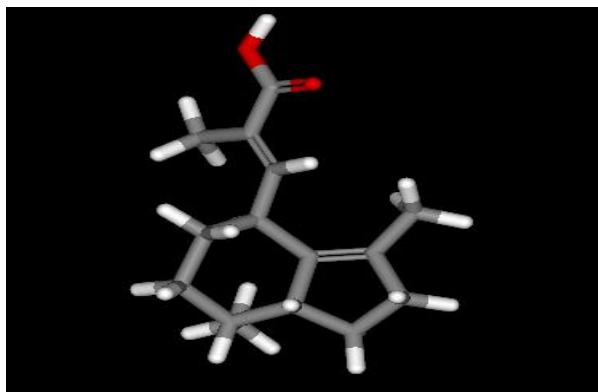


Figure 6: structure of Valerianic acid

Two important parameters have been considered for selecting potential compounds among the given input. They are

(i) Hydrogen bond details of the top-ranked pose and (ii) prediction of binding energy between the docked ligand and the protein using the mentioned scores as obtained for the analysis (Table 2).

From the ten conformations, the compound with the highest dockscore is taken for interaction analysis of the hydrogen bonding. By enlarging this interaction analysis the hydrogen bond interaction is contributed as major parameter. The Hydrogen bonding interaction of the valerianic acid with GABRB3 (Figure 2) are analyzed. Results are analyzed using Hbond Monitor of Discovery studio.2.1 involved in hydrogen bond formation with amino acids. The results reveal that docked complex with a dock score of 47.665 with two hydrogen bonds and it is the best conformation. The binding mode of the compound has been shown in Figure 7.

Figure 7 shows the amino acid residues involved in hydrogen bond interactions with protein GABRB3 and the ligand Valerianic acid. The interacting amino acids are ASP35, GLN56, PHE55, TYR120, ARG111 and TYR 54. The hydrogen bonds are formed between oxygen atom of ligand molecule interacting with hydrogen of GLN56 amino acid (O2:L...HE22:GLN56) and hydrogen atom of ligand interacting with oxygen of ASP35 (H39:L... OD1:ASP35). The other interactions are O1 of ligand molecule interacting with OD2 of ASP35 (O1:L...OD2:ASP35) and PHE55, TYR120, TYR54 and ARG111 show non-bonded interactions respectively.

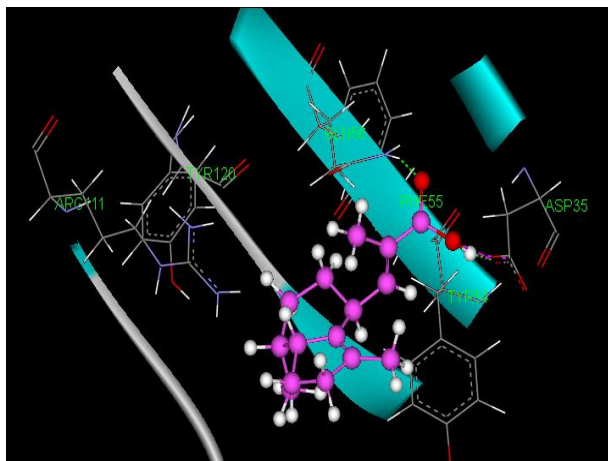


Figure 7: Docking of valerianic acid with GABRB3:  
Hydrogen bond interactions

## 4. CONCLUSION

The present study demonstrates that based on the selected template 3RHW, the 3D structural model of GABA<sub>A</sub> receptor subunit beta-3 is predicted and optimally minimized. The refined structure is further validated by PROCHECK, ProSA and RMSD. In Ramachandran plot analysis, it is observed that maximum residues (84%) lie in the most favored and no residues in disallowed region. Further docking studies are carried out with the valerianic acid to study the binding mechanism between GABA<sub>A</sub> receptor subunit beta-3 and valerianic acid. Based on the docking score, out of ten conformations of the receptor ligand complex, the complex structure having the highest dockscore is taken for interaction analysis. The results show that the interacting amino acids are ASP35, GLN56, PHE55, TYR120, ARG111, and TYR54 and hydrogen bonding forming with amino acids GLN56 and ASP35. This study would be useful in both understanding the activity mode of valerianic acid the most favorable binding mode of the top – ranking complex will be useful in designing new derivatives as human GABRB3 modulators.

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**Table 1: Percentage of residues in the core region of the Ramachandran plot for the model built and template.**

Structure	Core	Allowed	Generous	Disallowed
GABRB3	84.0	15.5	0.6	0.0
3RHW	88.6	10.4	0.7	0.3

**Table 2: Summary of docking information of the ten conformations of the docked poses of the Valerianic acid with GABRB3.**

Compound	Protein	Lig Score 1	Lig Score 2	PLP1	PLP2	JAIN	PMF	Dock score	Pose number
Valerianic Acid	GABRB3	1.63	2.72	29.62	30.26	-0.91	52.26	47.665	1
		1.74	2.78	38.92	39.5	0.01	45.77	45.346	2
		1.81	2.47	30.3	34.78	-0.22	36.34	44.883	3
		1.81	2.47	30.3	34.78	-0.22	36.34	44.883	4
		2.35	3.2	27.6	28.37	-0.65	55.24	41.855	5
		1.78	2.8	27.14	28.38	-1.22	53.5	40.661	6
		1.28	1.98	19.49	26.23	-0.87	45.9	40.515	7
		2.35	2.87	25.58	28.12	-0.82	55	39.059	8
		1.67	3.34	39.91	38.18	-0.25	48.54	38.261	9
		1.67	3.34	39.91	38.18	-0.25	48.54	38.261	10