Homology Modeling, Molecular Dynamics, Protein-Protein and Protein-Ligand Docking Studies of Inward Rectifier Human Potassium Channel

Wudayagiri Rajendra 1*, Tirumalasetty Munichandrababu, Baki Vijaya Bhaskar, Korla Praveen Kumar, Cherukupalle Bhuvaneswar
Bioinformatics Infrastructure facility, Department of Zoology, Division of Molecular Biology, Sri Venkateswara University, Tirupati- 517 502, A.P. India
Email: rajendra2k@yahoo.co.in

ABSTRACT
Inwardly rectifying potassium channels are potassium selective ion channels. In this study, the structure of inward-rectifying K⁺ channel was analyzed following the homology modeling utilizing the crystal structure of 1ORQ (voltage dependent K⁺ channel protein) as template from Aeropyrum pernix through MODELLER 9v1. Refined model of protein structure was obtained after energy minimization and molecular dynamics in a solvated water layer. The model was further assessed by PROCHECK, ERRAT programs and the results indicated that the proposed model is reliable for active site and docking analysis. The docking studies was carried out with inhibitors such as 3,4-diamino pyridine, 4-amino pyridine and amiloride to analyse the accurate conformation and orientation of protein with the inhibitors and through in silico analysis it was found that Amiloride is the best inhibitor for Potassium channel.

Keywords: Potassium channel, Homology Modeling, Inhibitor sensitivity, Molecular dynamics and Docking.

INTRODUCTION
Potassium (K⁺) plays a number of key roles in the cell such as osmeregulation, electrical neutralization, stomatal movements, and regulation of gas exchanges and transduction of various signals [1]. Several families of K⁺ transport systems were identified during 1990s, and the integration of various molecular, electrophysiological and reverse genetics approaches have already revealed the functions of some of these systems, including those of K⁺ channels belonging to the Shaker family [2]. K⁺ channels are structurally diverse group of proteins that facilitate the flow of K⁺ ions across cell membranes. They have diverse physiological functions depending on their type and their location. There are seven Kir channel subfamilies that can be classified into four functional groups: 1) Classical Kir channels (Kir2.x) are constitutively active, 2) G protein-gated Kir channels (Kir3.x) regulated by G protein-coupled receptors, 3) ATP-sensitive K⁺ channels (Kir6.x) tightly linked to cellular metabolism, and 4) K⁺ transport channels (Kir1.x, Kir4.x, Kir5.x, and Kir7.x) [3]. They are ubiquitous, being present virtually in all cell types. The channel-forming core is composed of two transmembrane (TM) helices separated by a re-entrant loop made up of a short pore (P) helix plus a more extended region of polypeptide that forms the selectivity filter (F) [4]. The glycine residue present in the inner helix hinge point in most of the K⁺ channels allows the inner helices to switch between a closed KcsA-like conformation and an opened MthK like conformation. All K⁺ channels share relatively same core topology and structure but differ in the number of P-loops and presence of transmembrane domains per monomer. Typical examples of these channels are shaker type 1P/6TM channels, the 1P/2TM channels, the ORK like 2P/8TM KCO channels and the TOK like 2P/8TM channels. Electrophysiological analyses have shown that various proteins e.g., kinases, phosphatases, syntaxins and G proteins are involved in the regulation of K⁺ channel activity.

Inwardly rectifying potassium channels (Kir, IRK) are potassium selective ion channels. To date, seven subfamilies have been identified in various mammalian cell types. These channels are termed inwardly rectifying - because they rectify current in the inward direction [5]. This means that under equal but opposite electrochemical potentials, these channels will pass more inward current than they do outward. In fact, the individual positive traces are difficult to discern. The current is created by the flow of K⁺ ions down their electrochemical gradient. However, the conductance of potassium ions is enhanced at more negative membrane potentials and is blocked when the cell is more depolarized. Under physiological conditions, these channels allow outward flow of potassium ions only when cells are 20 mV above the resting potential or lower. Thus in cells with a -60 mV resting potential, these channels would be inactivated at membrane potentials greater than -40 mV. Inward rectification of Kir channels is the result of high-affinity block by endogenous polyamines, namely spermine, which plugs the channel pore with more positive potentials. While the principal idea of polyamine block is well established already, the specific mechanisms are awaited.

Kir channels are found in multiple cell types, including macrophages, cardiac and kidney cells, leukocytes, neurons and endothelial cells. Their roles in cellular physiology vary among cell types. In cardiac myocytes, Kir channels close upon depolarization, slowing membrane repolarization and helping to maintain a more prolonged action.
Potassium channels are involved in regulation of the resting membrane potential. This type of inward-rectifier channel is distinct from delayed rectifier K⁺ channels, which help to repolarize nerve and muscle cells after action potentials; and potassium leak channels, which provide much of the basis for the resting membrane potential. In endothelial cells, Kir channels are involved in regulation of nitric oxide synthase. In the kidneys, Kir export surplus potassium into collecting tubules for removal in the urine, or alternatively may be involved in the reuptake of potassium back into the body [6]. In view of its physiological significance, the present study is taken up with a specific aim to construct the structure and also to study the sensitivity of this ion-channel for selective inhibitors using \textit{insilico} analysis.

MATERIALS AND METHODS

SEQUENCE ALIGNMENT
Sequence alignment of K⁺-Channel was performed using different web-based tools. Prediction of the location of the TM helices in Potassium channel employed a number of methods, namely, TopPred2 (http://www.biokemi.su.se/server/toppred2) [7]; TMAP (http://www.embl-heidelberg.de/tmap/emapinfo.html) [8]; DAS (http://www.biokemi.su.se/;server/DAS) [9]; PHDhtm (http://www.embl-heidelberg.de/predictprotein) [10]; TMHMM (http://www.cbs.dtu.dk/services/TMHMM1.0) [11]; and TMPred (http://www.isrec.isb-sib.ch/software/TMPRED_form.html) [12].

HOMOLOGY MODELING
The initial model of Potassium channel protein from \textit{Human} was built by using homology-modeling methods and the MODELLER software [13] a program for comparative protein structure modeling optimally satisfying spatial restraints derived from the alignment and expressed as probability density functions (pdfs) for the features restrained. The pdfs restrain Cα-Cα distances, main-chain N-O distances, main-chain and side-chain dihedral angles. The three-dimensional (3D) model of a protein is obtained by optimization of the molecular pdf such that the model violates the input restraints as little as possible. The molecular pdf is derived as a combination of pdfs restraining individual spatial features of the whole molecule. The query sequence from \textit{Human} was searched to find out the related protein structure to be used as a template by the BLAST (Basic Local Alignment Search Tool) [14,15] program against PDB (Protein Databank). Sequence that showed maximum identity with high score and less e-value were aligned (Figure 1), and was used as a reference structure to built a 3D model for Potassium channel. The sequence of Potassium channel protein (Accession Number: CAC88112) was obtained from NCBI. The co-ordinates for the structurally conserved regions (SCRs) for Potassium channel were assigned from the template using multiple sequence alignment, based on the Needleman-Wunsch algorithm [16].

MOLECULAR DYNAMICS SIMULATIONS
The structure having the least modeller objective function, obtained from the modeller was improved by molecular dynamics and equilibration methods using NAMD2.5 (Nanoscale Molecular Dynamics) software [17] using CHARMM27 (Chemistry at Harvard Macromolecular Mechanics) force field for lipids and proteins [18,19] along with the TIP3P model for water. The energy of the structure was minimized with 1,00,000 steps in 2,50,000 runs and 500 ps of molecular evolution.

- **Figure 1**: Sequence alignment of 1ORQ vs. Potassium channel.
dynamics. A cut off of 12Å (switching function starting at 10Å) for Van der Waals interactions was assumed. Spherical periodic boundary conditions were included in this study. An integration time step of 2 fs was used, permitting a multiple time-stepping algorithm [20, 21] was employed in which interactions involving covalent bonds were computed for every time step. Short-range non-bonded interactions were computed for every two-time step and long-range electrostatic forces were computed for every four-time steps. The pair list of the nonbonded interaction was recalculated for every ten-time steps with a pair list distance of 13.5Å. The short-range non-bonded interactions were defined as Van der Waals and electrostatic interactions between particles within 12Å. A smoothing function was employed for the Van der Waals interactions at a distance of 10Å. The equilibrated system was simulated for 1 ps with a 500 kcal/mol/Å² restraint on the protein backbone under 1 atm constant pressure and 310 K constant temperature, constant pressure and Number of Particles (NPT). The Langevin damping coefficient was set to 5 ps unless otherwise stated. Finally, the structure having the least RMSD of Cα trace generated during the molecular dynamics was used for further studies. In this step, the quality of the initial model was improved.

Figure 2: Calculated RMSD graph of molecular dynamics simulations using NAMD software. Time (Ps) was taken in X-axis and RMSD in Y-axis

STRUCTURE VALIDATION
The final refined model obtained was analyzed by Ramachandran’s map using PROCHECK (programs to check the stereo chemical quality of protein structures) [22] and environment profile using ERRAT graph [23] (structure evaluation server). This model was used to identify the active site and for docking of the sweeteners, modulators and inhibitors with the receptor.

ACTIVE SITE IDENTIFICATION
The binding pockets of potassium channel from human were identified using CASTP (Computed Atlas of Surface Topography of Proteins) [24] a program for identifying and characterizing protein active sites, binding sites, and functional residues located on protein surfaces and voids buried in the interior of proteins by measuring concave surface regions on three-dimensional structures of proteins. It also measures the area and volume of pocket or void by solvent accessible surface model (Richards’ surface) and by molecular surface model (Connolly’s surface). It can also be used to study surface features and functional regions of proteins.

OTHER COMPUTATIONAL DETAILS
Structural diagrams were prepared using OPENEYE (OpenEye Scientific Software, Santa Fe, NM) and SPDBV software.

PROTEIN-PROTEIN DOCKING
The program HEX was employed to conduct the docking of the model built with itself to form a dimer. The basic approach to the docking problem is to model each molecule using 3D parametric functions, which encode surface shape, electrostatic charge and potential distributions. The surface shape representation uses a novel 3D surface skin model of protein topology, and a novel soft molecular mechanics. Energy minimization procedure is used to refine the candidate docking solutions. Unlike conventional 3D fast Fourier transform (FFT) docking approaches; HEX uses spherical polar Fourier correlations to accelerate the docking between 10 and 100 times faster than FFT docking algorithm [25]. The docking procedure proceeds in several steps. The four main steps of this algorithm are first; the ligand molecule is (optionally) oriented along the negative z-axis to face the receptor, if knowledge of the
ligand-binding surface is available. Second, a low resolution (L=5) spherical harmonic surface is calculated for the receptor. The surface is discretised by projecting it onto an icosahedral tessellation of the sphere. At each triangular facet of the surface, a normal vector is calculated and a 15Å radius sphere is centered on each outward normal, tangential to the surface. This smoothes the surface with spheres. In the third step, the surface spheres are culled by iteratively identifying and striking out that sphere which has the greatest volume overlap with its neighbors. This procedure is repeated until no overlap volume exceeds 5Å. This yields a fairly even distribution of the surviving spheres over the surface of the receptor. Finally, each surviving sphere (normal vector) was used to define a local intermolecular axis for docking, with the initial ligand/axis orientation being transferred onto the outward normal, and a local coordinate origin for the receptor being defined at an equal distance along the inward normal. Here, we used the following parameter set: correlation type = shape, post-processing = none, steric scan = 16 (maximum), final search = 25 (maximum), the others were default set.

PROTEIN-LIGAND DOCKING

The ligands, including all hydrogen atoms, were built and optimized with Chemskech software suite [26]. Extremely Fast Rigid Exhaustive Docking (FRED) version 2.1 was used for docking studies (OpenEye Scientific Software, Santa Fe, NM). It is an implementation of multi-conformer docking, meaning that a conformational search of the ligand is first carried out, and all relevant low-energy conformations were then rigidly placed in the binding site. This two-step process allows only the remaining six rotational and translational degrees of freedom for the rigid conformer to be considered. The FRED process uses a series of shape-based filters, and the default scoring function is based on Gaussian shape fitting.

RESULTS AND DISCUSSION

SEQUENCE ALIGNMENT

An optimal sequence alignment is essential to the success of homology modeling. The sequence identity between Potassium channel protein and 1ORQ is 39% and the percentage of similarity is 14 in the M1 M2 and P-loop regions, making this element of the alignment relatively straightforward. However, for the M1 and M2 helices the sequence identity is rather low. The residues from 33-102 are removed from the homology model, which thus corresponds to the central pore-forming domain of the channel. The loop between the end of M1 and M2 helices constitute 49 residues and the loop between the end of M1 and the start of the P-helix contains 22 residues. Within the P-helix the LWW motif of 1ORQ is replaced by MYW. It is possible that this conserved glycine may confer some degree of flexibility upon M2, which in turn may be related to channel gating [27]. Further more, TM helices were also predicted from different programs. It can be seen that although the same core regions are identified by most of the algorithms, there are significant differences in the lengths of the TM helices. Predictions of TM helices by four different programs are given in Table 2. In general TM helices predicted by TMHMM were longer than those predicted by other three methods.

HOMOLOGY MODELING OF POTASSIUM CHANNEL PROTEIN

A high level of sequence identity should guarantee more accurate alignment between the target sequence and template structure. In the results of BLAST search against PDB, only three reference proteins, including crystal structure of a Mammalian shaker Kv1.2 K⁺ beta subunit complex from *Rattus norvegicus* (PDB :2A79) , crystal structure of Kvap-33h1 Fv complex from *Aeropyrum pernix* (PDB:2A0L) , X-Ray structure of a voltage-dependent K⁺ channel from *Aeropyrum pernix* (PDB:1ORQ) have a high level of sequence identity and the identity of these three reference proteins with the Potassium channel protein are 25%, 39%, and 39% respectively (Table 1).

Table 1: Data for the closest homologue Potassium channel with known 3D structures obtained with the Blast server against PDB

<table>
<thead>
<tr>
<th>PDB Code</th>
<th>Protein</th>
<th>Chain</th>
<th>Ref</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1ORQ</td>
<td>X-Ray Structure of a Voltage-Dependent Potassium channel from <em>Aeropyrum pernix</em></td>
<td>C</td>
<td>[28]</td>
<td>39%</td>
</tr>
<tr>
<td>2A0L</td>
<td>Crystal structure of kvap-33h1 Fv complex from <em>Aeropyrum Pernex</em></td>
<td>A,B</td>
<td>[29]</td>
<td>39%</td>
</tr>
<tr>
<td>2A79</td>
<td>Mammalian shaker kv 1.2 Potassium channel beta-sub unit complex from <em>Rattus norvegicus</em></td>
<td>B</td>
<td>[30]</td>
<td>25%</td>
</tr>
</tbody>
</table>
Structurally conserved regions (SCRs) for the model and the template were determined by superimposition of the two structures and multiple sequence alignment (Figure 1). Reference protein 1ORQ was chosen for modeling Potassium channel protein of Human in our subsequent studies. Coordinates from the reference protein (1ORQ) to the SCRs, structurally variable regions (SVRs), N and C-termini were assigned to the target sequence based on the satisfaction of spatial restraints. All side chains of the model protein were set by rotamers. Thus we know that residues 1-219 do not locate near the active site. In our study, residues 1-219 are removed from the model because no homologous region occurs in 1ORQ and these residues do not locate near the active site. Thus the model is made up of residues 220-380. This model was refined by molecular dynamics method and the final stable structure of the Potassium channel protein obtained is shown in Figure 3. The final structure was further checked by ERRAT graph and the results have been shown in Figure 3. The overall quality score in the ERRAT graph (Figure 4) corresponded to acceptable protein environments. From Figure 4 it appears that all residues are reasonable, which makes us to believe that the structure of Potassium channel protein is reliable.

Figure 3: The final 3D structure of Potassium channel protein. The structure is obtained by energy minimization and equilibration over the last 25000 runs with 50 pico seconds of molecular dynamics simulation.
VALIDITY OF THE HOMOLOGY MODEL

Validation of the model built was carried out using Ramachandran plot calculations computed with the PROCHECK program. The $\phi$ and $\varphi$ distributions of the Ramachandran plots of non-glycine, non-proline residues are summarized in Figure 5 and Table 3. The RMSD (Root Mean Square Deviation) for covalent bonds relative to the standard dictionary was -4.54 Å and for the covalent angles was −0.13 Å. Altogether, 99.3% of the residues was in favored and allowed regions. The overall PROCHECK G-factor was −1.99 and ERRAT environment profile was good.

Figure 5: Ramachandran’s map of Potassium channel protein built using MODELLER software. The plot calculations on 3D model of Potassium channel computed with the PROCHECK program.
Table 3: Ramachandran plot calculations on 3D model of potassium channel protein computed with the PROCHECK program

<table>
<thead>
<tr>
<th>Stereo-chemical parameter</th>
<th>Calculated values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residue in most favored regions [A,B,L]</td>
<td>87.7%</td>
</tr>
<tr>
<td>Residue in the additionally allowed zones [a,b,1,p]</td>
<td>9.8%</td>
</tr>
<tr>
<td>Residue in the generously regions [-a,-b,-1,-p]</td>
<td>1.8%</td>
</tr>
<tr>
<td>Residue in disallowed regions</td>
<td>0.6%</td>
</tr>
<tr>
<td>Non-glycine and non-proline residues</td>
<td>100.0</td>
</tr>
</tbody>
</table>

SUPERIMPOSITION OF 1ORQ WITH POTASSIUM CHANNEL PROTEIN

The structural superimposition of Cα trace of template and Potassium channel protein is shown in Fig 6. The weighted RMSD of Cα trace between 1ORQ and Potassium channel protein with respect to Cα was 1.82 Å which further indicated that the homology model was reliable. This model was used for the identification of active site and for docking of the inhibitors with the protein potassium channel.

![Figure 6: Interactions between the dimer subunits. Hydrogen bonds are indicated with green dots.](image)

SECONDARY STRUCTURE PREDICTION

Amino acid sequences of template, and final Potassium channel were aligned using CLUSTALW. Given their PDB files, secondary structures were also analyzed and compared by the SWISS PROTEIN DATABANK VIEWER software suite; (http://www.expasy.org/spdbv) [31] (The secondary structures of template and final Potassium channel are highly conserved which showed that final structure is highly reliable as shown in figure 3 and was further used for active site identification.

ACTIVE SITE IDENTIFICATION OF POTASSIUM CHANNEL PROTEIN

After the final model was built, possible binding sites of Potassium channel were searched based on the structural comparison of template. Since, Potassium channel from Human and the 1ORQ are well conserved at both sequence and structure their biological function should be identical. In fact, from the structure-structure comparison of template, and final refined models of Potassium channel using SPDBV program it was found that secondary structures are highly conserved and the residues in the model built, Tyr 157, Gly 156, Thr 154, Ile 155 (YGTI motif) are conserved with the active site of template. Thus, we suggest that uptake of K⁺ will be similar for both Potassium channel of human and 1ORQ of bacteria. The final stable structure of Potassium channel protein obtained was shown in Figure 3.

PROTEIN-PROTEIN DOCKING

The model built was docked with itself using HEX software. It works on Fourier-based approach for the fast calculation of correlations using spherical harmonics. It was found that Leu, Ala, Arg, Ile are the important residues
for the hydrogen bonding interactions between the subunits forming K+ channel as shown in Figure 6 and Table 4. The final subunits complex was shown in Figure 6.

Table 4: Hydrogen bonding interactions between dimer subunits

<table>
<thead>
<tr>
<th>S.No</th>
<th>Hydrogen Bonding Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sub unit 1</td>
</tr>
<tr>
<td>1</td>
<td>Leu 38-----------------------------Ala 58</td>
</tr>
<tr>
<td>2</td>
<td>Ala 58-----------------------------Ala 55</td>
</tr>
<tr>
<td>3</td>
<td>Ala 55-----------------------------Ile 51</td>
</tr>
<tr>
<td>4</td>
<td>Arg 30-----------------------------Ile 51</td>
</tr>
<tr>
<td>5</td>
<td>Ile 68-----------------------------Arg 42</td>
</tr>
</tbody>
</table>

DOCKING OF INHIBITORS WITH THE ACTIVE SITE OF POTASSIUM CHANNEL

Docking of the substrate with Potassium channel was performed using FRED v 2.1 which is based on Rigid Body Shape-Fitting (Open Eye Scientific Software, Santa Fe, NM, USA). This program generates an ensemble of different rigid body orientations (poses) for each compound conformer within the binding pocket and then passes each molecule against a negative image of the binding site. Poses clashing with this ‘bump map’ are eliminated. Poses surviving the bump test are then scored and ranked with a Gaussian shape function. We defined the binding pocket using the ligand-free protein structure and a box enclosing the binding site. This box was defined by extending the size of a cocrystallized ligand by 4 Å (addbox parameter of FRED). This dimension was considered here appropriate to allow, for instance, compounds larger than the cocrystallized ones to fit into the binding site. One unique pose for each of the best-scored compounds was saved for the subsequent steps. The compounds used for docking was converted in 3D with OMEGA (OpenEye Scientific Software, Santa Fe, NM, USA). To this set, the active compounds (generation of multiconformer with Omega) corresponding to the modeled protein were added. To understand the interaction between Potassium channel protein and inhibitors, the Potassium channel-inhibitor complex was generated using the OPENEYE software suite (Open Eye Scientific Software, Santa Fe, NM) (Figure 7). It is evident from the Fig that inhibitors are located in the center of the active site, and is stabilized by hydrogen bonding interactions. The hydrogen bonds present in enzyme-inhibitor complex along with their distances and angles are listed in Table 5. Significant binding key residues in the active site of the model were determined based on the interaction energies of the substrate with residues in the active site of the Potassium channel. This identification, compared with a definition based on the distance from the substrate can clearly show the relative significance for every residue. Table 5 shows the interaction energies including the total, electrostatic and steric energies for all the residues in the active site of enzyme-inhibitor complex. From Table 6 it is evident that Protein substrate complex has large favorable total interaction energies of -26.18, -3.07, -13.17, -47.08, -221.99 with a consensus score of 7 kcal/mol. These results indicate that steric interactions, ligand acceptor - protein donor and all metal interactions, contribution from ligand donors interaction with protein acceptors, aromatic-aromatic interactions, frozen rotatable bond penalty, lipophilic-lipophilic interactions, acceptor-metal interactions, hydrogen bond interactions, penalty for ligand clashes with the protein, ligand hydrogen bond donors and acceptors, ligand non-polar atoms, interactions of ligand metal atoms, interactions of ligand sulphur atoms, lipophilic-polar and polar-polar interactions, clash penalty, piecewise Linear Potential, phenyl interactions with amides, methyl and arylCH groups and shape complimentarity between ligand and protein are important for the protein-substrate complex interaction. Through analysis, it was found that Amiloride is the best inhibitor for Potassium channels.

Table 5: Hydrogen bonds along with their distances and angles between the inhibitors and active site residues of Unknown protein as deciphered using OPENEYE software
Table 6: The total energies of Chemguass score, Chemscore, PLP score and shapeguass score of the best-docked conformations

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Chemgauss Score</th>
<th>Chemscore</th>
<th>PLP Score</th>
<th>Screen Score</th>
<th>Shapeguass Score</th>
<th>Consensus Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiloride</td>
<td>-26.18</td>
<td>-3.07</td>
<td>-13.17</td>
<td>-47.08</td>
<td>-221.99</td>
<td>7</td>
</tr>
<tr>
<td>4-amino pyridine</td>
<td>-22.63</td>
<td>-9.81</td>
<td>-20.47</td>
<td>-30.19</td>
<td>-144.19</td>
<td>12</td>
</tr>
<tr>
<td>3,4-diamino pyridine</td>
<td>-21.68</td>
<td>-9.20</td>
<td>-17.67</td>
<td>-34.99</td>
<td>-162.23</td>
<td>13</td>
</tr>
</tbody>
</table>

In the present study, we constructed a 3D model of Potassium channel protein from human using the MODELLER software and obtained a refined model after energy minimization. The final refined model was further assessed by ERRAT and PROCHECK programs, and the results show that this model is reliable. The stable structure is further used for protein-protein and protein-ligand docking studies. Docking results indicate that conserved amino-acid residues in Potassium channel protein play an important role in maintaining a functional conformation and are directly involved in donor substrate binding. The interaction between the protein and the inhibitors proposed in this study are useful for understanding the potential mechanism of K⁺ uptake. It was also found that Amiloride is a more preferred ligand. This preliminary analysis of the interactions of the potassium channel with the inhibitors suggests that concerted single-file motion of K⁺ ions and water through the selectivity filter occurs. This suggests that a single-filling mechanism is conserved between K⁺ channel structures and may be robust to changes in simulation details. The hydrogen–bonding interactions also play an important role for the stability of the complex. These computational results provide some new insights into the possible role of glycine in the K⁺ and importance of Potassium channel protein in various processes of the Human and may lead to a better understanding of the structures, dynamics of this family of ion channels.
As is well known, hydrogen bonds play an important role for the structure and function of biological molecules, especially for the Potassium channel protein catalysis. The results indicate that Leu 38, Ala 58, Ala 55, Arg 30 and Ile 68 of subunit1 is interacting with Ala 58, Ala 55, Ile 51; Ile 51 and Arg 52 of subunit2. Hence, Lys 160, Thr 151,161, and Ile 155 are important for strong hydrogen bonding interaction with the inhibitors. PkA calculations of the Potassium channel protein suggest that E17, 108, Y114, 141, 31, 103, D89, 133, C27, 28, 29, R21, 30, 39, 32, 42, 37, 84, K43,107 are predicted to be ionized. Further more Normal mode analysis of the protein suggests that Arg 168, Glu 17, Ile 19, Ile 20, Leu 166, Gly 167 are important residues responsible for largest amplitude of motion. From docking studies it is noticeable that, Amiloride, is the most preferred inhibitor and that there is a simple competitive inhibition between the K+ ion and the Amiloride. Further more, Thr161 and Ile 155 residues are involved in inhibitor binding and are conserved among these two enzymes (1ORQ and Potassium channel) and forms hydrogen bonding with the inhibitors.

ACKNOWLEDGEMENTS
The authors are thankful to Dr. Sunil Chawla, Seascape, New Delhi, for providing free academic license of Open Eye scientific software. This work is supported by Department of Biotechnology (DBT), Bioinformatics infrastructure facility (BIF) BT/BI/25/001/2006 Dt. 24.08.2009.

REFERENCES