**Ruchi Sachdeva (2011).** ***Molecular modeling studies on Aqua (Glycerol) porin(s).*** Ph.D. Thesis. CSIR-IMTECH, Chandigarh/ Jawaharlal Nehru University, New Delhi: India.
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## SUMMARY OF THE THESIS

The structure of C-terminal residues (255-323) was modeled in order to understand the role of C-terminal region on phosphorylation induced regulation of rAQP4 via gating. Molecular dynamics studies were carried out to study structural changes in the phosphorylated rAQP4 in comparison with unphosphorylated. It was observed during simulations that positively charged residues like Lys-109, Arg-260, Lys-263, His-300 and Lys-311, etc. located on the C-terminal region as well as on the cytoplasmic loops connecting transmembrane helices come close to phosphoserine-180. These interactions between phosphoserine-180 and residues (Arg-260 and Lys-263) of C-terminal helix took place at the same side of the cytoplasmic mouth and helped to stabilize the conformation of loop D, thus keeping it away from entrance of channel. MD simulation of unphosphorylated rAQP4 did reveal association of C-terminus and cytoplasmic loops involving residues other than Ser-180 but only in case of monomer C. Although C-terminal region moved towards cytoplasmic mouth of rAQP4 channel but the gap formed between these was larger than that observed in case of phosphorylated rAQP4. Hydrogen and ionic bonds were observed in the residues of three monomers in inter-monomer interactions involving their loop D and C-terminal regions. Though, interaction of phosphoserine-180 with residues of C-terminal region resulted in movement of this region close to the cytoplasmic mouth but it did not cover the mouth of the channel completely. MD simulations of phosphorylated rAQP4 did not reveal any blocking residue that may get inserted into the channel leading to its blockage upon phosphorylation. There was a continuous flow of water molecules from aqueous phase till cytoplasmic

uth in both unphosphorylated and phosphorylated rAQP4. The rate of rmeability of water was similar in phosphorylated and unphosphorylated QP4. Hence, simulations carried out in present study did not reveal gating ffects on rAQP4 water channel due to phosphorylation of Ser-180.

Close examination of molecular dynamics trajectories of all the monomers of rAQP4 revealed intermittent disruption of flow of water across the aquaporin. In our attempt to investigate the reasons of this disruption of flow of water, a detailed analysis of molecular dynamics trajectories of rAQP4, bAQP1 and hAQP5 showed that side chain of histidine residue of loop B protruded into the lumen of aquaporin, thereby constricting the pore to a narrow diameter that did not allow water molecules to pass through. The dihedral angles of side chain of histidine did undergo changes but these did not interrupt the flow of water across the channel. The backbone conformational changes (especially in $\psi$) in histidine and its neighboring residue up to more than 60° were observed during MD simulations of rAQP4. Similar backbone conformational changes in the corresponding residues of loop B of bAQP1 and hAQP5 were observed during MD simulations. These conformational changes in histidine and its neighboring residues caused the movement of loop B towards lumen of channel resulting in blockage of the flow of water across aquaporins. Thus, orientation of histidine side chain per se does not seem to block the water flux. The movement of loop B was found to be affecting the water flow in all monomers of rAQP4, a couple of monomers of bAQP1 and hAQP5. It was further observed that conserved ion pair between Glu and His and the interaction of Glu with Ser of loop B apparently controlled the permanent displacement of loop B into the lumen of aquaporin.

Lead molecules against human AQP4 were identified using docking based virtual screening of zinc database. Initially, molecules which do not have drug like properties were filtered out in first phase of screening itself. The filtered molecules were docked onto hAQP4 binding site that was defined by amino acid residues lying in extracellular vestibule upto channel's mouth. Docking was performed in two phases utilizing different search efficiencies. Top ranking ligands based on high GoldScore and ASP were obtained. Resulting top scoring ligands were further assessed based on strength of binding using X-Score. Clustering analysis of top 400 ligands showed variations in set of interacting partners in hAQP4. These ligands were characterized based on a few descriptors such as number of hydrogen bonds formed, percentage of accessible volume of extracellular mouth of hAQP4 and buried surface area of the ligand. Such characterization helped in further differentiating the lead molecules in terms of number of interactions and extent of blocking the channel's mouth. Based on the descriptors, top 400 ligands were divided into two classes. Class I contained eleven ligands that were found to interact with hAQP4 residues present in the extracellular vestibule and channel's mouth. One of the aromatic rings of these ligands was inserted into the mouth, thereby blocking its access. Both class I & class II ligands exhibited a few hydrophobic contacts that have been reported in case of known inhibitors. In addition, some new interactions of ligands with residues of loop C of hAQP4 were observed in our study. Both class I and II ligands were analyzed for having the probability of interacting with non target proteins. Based on this, three ligands were predicted to interact with other

proteins. Thus, these were removed form the list of lead molecules. The resulting lead candidates found against hAQP4 can be further characterized using molecular dynamics simulation followed by binding free energy calculations.