Ammonium permeability ($P'_{NH3}$) was measured by following the internal compartment alkalinisation, after submitting ghosts to inward ammonium gradients. Alkalinisation rate constants (k) were deduced from stopped-flow analysis of pH variations at 15°C, allowing the determination of $P'_{NH3}$ by using the simplified equation $P'_{NH3}=k.r/3$ (r: radius of spheric ghosts). The $P'_{NH3}$ values were significantly different between Ctl and OHSt samples ($1.7 \pm 0.11$ vs $0.71 \pm 0.004$ $\mu m.s^{-1}$). Additionally, water permeability was measured in the same OHSt resealed ghosts but containing 8mM 6-carboxyfluorescein (6-CF), after submitting them to an osmotic gradient (150mosm/kg/$H_2O$ mannitol). The osmotic water permeability (Pf) values deduced from the fluorescence quenching at 15°C were similar between Ctl and OHSt samples ($0.035 \pm 0.005$ vs $0.035 \pm 0.04$ $cm.s^{-1}$).
In conclusion, the F65S mutation of RhAG induces a reduction of ammonia flux resulting in an alteration of pHi regulation. The decrease of ammonia conductance through the mutated RhAG can result from loss of hydrophobicity and/or from structural modifications inside the pore of the channel.

**2957-Pos Board B62**
**Molecular Modeling of the ATP-Synthase Motor F0 Subunit of Escherichia Coli and Proton Translocation**
**Megan Scoppa**, Margaret Cheung.
An all-atom model based on the $ac_{10}$ subunit of *Escherichia coli* Adenosine-Triphosphate (ATP) synthase complex was created using steered molecular dynamics along with parallel computer programming designed for the ability to view larger biomolecular systems. The salt bridge which is required for proton translocation and thus, proton motive force (PMF), was formed during initial minimization and equilibration simulations. Using physiological parameters and experimental data allows for a unique chance to view this nanoscale motor's rotational mechanism and will also supply more information about the elusive a-c subunit interface.

**2958-Pos Board B63**
**All-Atom Molecular Dynamics Simulation of Multidrug Efflux Transporter AcrB**
**Tsutomu Yamane**, Mitsunori Ikeguchi.
In E. coli, it is known that the tripartite multidrug efflux system (AcrB/ AcrA/ TolC) exists, and AcrB resides in the inner membrane region and take part in substrate recognition and energy transduction for drug export through proton transfer. Recently, x-ray structures provided that AcrB forms trimeric protein where each subunit is different conformation, ''binding state'', ''extrusion state'' and ''access state''. Especially, only extrusion state subunit has different side chain conformation of residues (Asp407, Asp408 and Lys940), which are essential for proton translocation (protonation site). These results suggest that drugs are exported by a three-step structural change involved in proton motive force which is inferred to be caused by change of protonation states of the protonation site residues. However, the structural change process which involved in proton motive force is still unclear. In the present study, we performed 100 ns all-atom molecular dynamics (MD) simulations of three types of AcrB-membrane-water systems which are different from protonation state of Asp407 and/or Asp408 in extrusion state protomer. During all of the 100 ns MD simulations, the global structures of each subunit were conserved. However, in extrusion state protomer, the conformation of the protonation site residues were changed to those of other state protomers only when Asp 407 and 408 residues were deprotonated. In this presentation, we will discuss the structural change and proton transfer mechanism.

**2959-Pos Board B64**
**Pathways to Exit a Receptor: Agonists and Delta-Oppioid Studied via Computer Simulations**
Francesca Collu, Matteo Ceccarelli, **Paolo Ruggerone**.
The importance of delta-opioid receptors as target of a large number of drugs is well recognized, but the molecular details of interaction and action of the compounds are largely unknown. In an effort to shade some light on this important issue we performed an extensive computational study on the interaction of two compounds, clozapine and desmetilclozapine, with a delta-opioid receptor. According to experiments, the lacking of a single methyl group in desmetilclozapine with respect of clozapine makes the former more active than the latter, providing a system well suited for a comparative study. We investigated the escape route of the two drugs from the receptor using molecular dynamics simulations and metadynamics. Our results point out that prolonged interactions of the compounds with specific residues of the receptor do not correlate directly with their activity, having clozapine the longest interactions if compared with desmetilclozapine but being also less active. The action of the compound is related to the spatial distribution of the affinity sites it visits during its permanency. Additionally, the role of long-resident water molecules is discussed. Such information might be useful to provide hints and insights that can be exploited in more structure-and-dynamics-oriented drug design.

**2960-Pos Board B65**
**Computational Studies of Translocon-Assisted Processes of Membrane Protein Insertion and Translocation**
**Anna Rychkova**, Arieh Warshel.
Membrane proteins make up about 30% of all the proteins in the body and represent more than 50% of drug targets. The protein-conducting channel, called translocon, is responsible for protein-membrane integration and the understanding of the mechanism of translocon-associated membrane protein folding has a significant biological and pharmaceutical interest.
The translocon is a very large multidomain protein complex and the structural information about the protein-translocon complex is very tentative. Thus brute force all atom MD computer simulations are not expected to be very useful at this stage. In order to advance this challenging direction we introduce a unique coarse grain (CG) method with extended electrostatic treatment. This model allowed us to explore the energetics of the insertion of transmembrane helixes into lipid bilayer trough the translocon and to study the controversial question of the charged residue location in the membrane. More recently we stated to use the CG model in exploring the effect of key mutations that allow the secretion of proteins with defective or absent signal sequences. The preliminary insight provided by this study will also be described.

**2961-Pos Board B66**
**An Atomistic Gating Mechanism of the AMPA-Subtype Glutamate Receptor**
**Hao Dong**, Huan-Xiang Zhou.
Glutamate receptors mediate fast excitatory synaptic transmission in the central nervous system. Agonist binding to an ionotropic glutamate receptor (iGluR) triggers the opening of an ion channel, allowing the flow of cations across the membrane. Although the structures of the isolated extracellular ligand binding domain (LBD) in different states have been determined, it remains unclear how agonist binding is transmitted to open the channel. Here, based on targeted molecular dynamics simulations, we propose an atomistic mechanism for how the closure of the clamshell-like LDB upon agonist binding leads to channel activation. Simulations of an AMPA-subtype iGluR, including both the LBD and the transmembrane domain (TMD) in explicit water and membrane, started with the LBD in the antagonist-bound form. The LBD was then targeted to move toward the agonist-bound form, mimicking agonist binding. The resulting LBD closure was propagated to the TMD, and the pore formed by the M3 helices widened, signifying channel opening. The link peptides between the S2 half lobes of the LBD and the M3 helices of TMD were critical for the communication between the two domains. The M3 helices were rigid, and pore-widening resulted from an increase in tilt angle. At the same time, the whole M3 helix bundle translated upward. The detailed mechanism of coupling and communication between the extracellular and membrane-spanning domains elucidated here may serve to guide new experiments on the glutamate receptor and motivate the use of our methodology for the study of other neurotransmitter receptors in the central nervous system.

**2962-Pos Board B67**
**Modeling KCNQ1 Channel and KCNE1 Interactions**
Xuanyu Meng, Yu Xu, Hongxing Zhang, Gea-Ny Tseng, **Meng Cui**.
KCNQ1 is the pore-forming component of the slow delayed rectifier (Iks) channel in human heart. Although KCNQ1 can function as a voltage-gated (Kv) channel on its own, it requires KCNE1 to modulate its functions. To understand the mechanism of how KCNE1 modulate KCNQ1, molecular simulations are employed to model the interactions between KCNQ1 and KCNE1. A homology model of KCNQ1 was constructed based on the crystal structure of Kv1.2-Kv2.1 paddle chimera channel (PDB entry: 2R9R) template. For KCNE1, we used a NMR structure as an initial conformation, and manually adjusted the orientations of its N- and C-terminal domains to avoid their folding into membrane. Then 100ns molecular dynamics (MD) simulations were conducted to sample possible conformations of KCNE1 in POPC lipid bilayers. The cluster analysis was conducted on KCNE1 tansmembrane domain (KCNE1-TMD) from 40ns to 100ns simulation period. Five representative structures of KCNE1-TMD were selected, and used for docking simulations to KCNQ1. We performed 2 dimensional Brownian Dynamics (2D-BD) protein-docking simulations on each representative structure of KCNE1-TMD and KCNQ1 homology model. After selecting compact complexes by using a distance-based filter to remove loose contact complexes, a series of experimental restraints were applied to select one final KCNQ1/KCNE1-TMD complex structure. The N- and C- terminal domains of KCNE1 were reconstructed to generate the whole complex of KCNQ1/KCNE1, and followed by 100ns MD simulations in explicit membrane environment. For comparison, we also conducted 100ns MD simulations on KCNQ1 alone. Based on the two simulation studies, we analyzed the dynamics of the KCNQ1 channel with and without KCNE1 present through a systematic examination of hydrogen bond network patterns and ion permeation pathway of the channel. The results could provide us helpful insights of the molecular mechanism of how KCNE1 modulating KCNQ1 channel.