work we present a structure for the open and closed states of the voltage-gated proton channel based upon a homology model from the Shaker channel, which has been using fluorinated new experimental data from the labs of Peter Lars. We will also present the results of hydration studies and internal and external salt bridges in the open and closed states and what they reveal about the stabilization of the two structures.

1423-Pos Board B315
Graded Tuning of Phosphatase Activity of VSP Coupled with the Intermediate State of the Voltage Sensor
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Voltage-sensing phosphatase (VSP) consists of the voltage sensor domain and the cytoplasmic phosphatase region. We have shown that the movement of the voltage-sensing domain (VSD) is coupled to the phosphatase activity over a wide range of membrane potential (Sakata et al., 2011). However, it still remains elusive how the phosphatase activity is regulated by the movement VSD at the level of single molecule. One possible idea is that the number of phosphatase activities is attained when VSD is in a fully activated state, and the number of molecules in the active state as the membrane potential becomes more positive. The other probability is that the enzyme-defective mutant or the protein with mutation in the linker between VSD and the phosphatase domain also showed two-step movements of the VSD. Measurements of the phosphatase activity of Dr-VSP(156R/165R) revealed that both transitions of VSD activation increase the phosphatase activity. These suggest that the full activation of the voltage sensor is not necessary to exhibit the phosphatase activity, and the phosphatase activity at the single protein level could be graded dependent upon the magnitude of the movement of the VSD.

1424-Pos Board B316
Correlating Residue Coevolution and Function in a Conserved Voltage-Sensing Domain
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The voltage-sensing domain (VSD) is a 4-helix transmembrane (TM) regulatory domain that undergoes a conformational change in response to a change in TM potential. While originally identified in voltage-gated cation channels, the VSD has since been observed in voltage-gated proton channels and voltage-sensing lipid phosphatases. Therefore, sensitivity to a change in TM potential appears to rely on an evolutionarily unique and structurally conserved domain. Moreover, VSDs have been fine-tuned by evolution to sense a wide range of polarization states. Here, we develop a robust hidden Markov model (HMM) of the VSD sequence for the purpose of detecting and aligning remote homologues. Then, we use statistical coupling analysis and molecular dynamics simulations to correlate highly-conserved residues and residue pairs with the atomic-level details of VSD structure, function, and tunability.

1425-Pos Board B317
Oligomeric States of Full Length Influenza a Virus M2 Proteins on Biological Membranes
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Influenza a viruses have a viral matrix protein M2 that serves as a proton channel and a pH sensor. M2 has two states: an open state from the matrix protein M1. Although X-ray crystallography and NMR studies using truncated proteins concluded that M2 stably forms a tetrameric channel that opens at acidic pH, the oligomeric state of the full length protein on biomembranes is not clear yet. In the present study, we examined the oligomeric state of full length M2 protein on the plasma membrane of living cells by using fluorescence resonance energy transfer (FRET) among the M2 proteins labeled with fluorophores by the coiled-coil method. Contrary to previous models, M2 formed dimers at neutral pH and the dimers were converted to tetramers at pH 4.9. The tetramerization and channel activity were completely inhibited in the presence of the antiviral Amantadine hydrochloride (Am) at low pH. In contrast, the S31N mutant resistant to Am formed dimers independent of pH and the presence of Am, and the channel activity was not blocked by Am. These results indicate that the resistance of the S31N mutant could be attributed to its ability to conduct protons as dimers without forming tetramers.

References

1426-Pos Board B318
Amantadine Analogs that Inhibit Mdck Cell Infection by Influenza a with M2(S31N)
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A mutation of Ser31 to Asn has become dominant globally in human infections by influenza A since 2005, rendering the virus resistant to the FDA approved prophylactics, amantadine and rimantadine. Attempts to identify alternative M2 blockers have reportedly been futile so far. Here we report that infection of cultured Madin-Darby canine kidney cells by Influenza A/California/04/2009 (H1N1 swine flu), which bears the S31N mutation, is not blocked effectively by amantadine (115 μM EC50) nor rimantadine (56 μM EC50), but is effectively blocked by 11 amantadine variants. EC50s range from 1-37 μM. 8 of these 11 compounds were previously reported to block influenza A with wild type M2. As another control, an H3N2 strain of influenza A with wild type M2 (Strain Victoria from ATCC) was found to be effectively blocked by amantadine (3 μM EC50) and rimantadine, as expected. We suggest that the amantadine variants block viral reproduction by blocking the S31N strain of the M2 proton channel.

1427-Pos Board B319
Direct Observation of the Rotary Motion of Fo-F1-ATP Synthase Driven by Proton Motive Force
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Fo-F1-ATP synthase (F1(FoF1)) is a rotary motor protein which carries out ATP synthesis by coupling to the rotary motion driven by the proton motive force (pmf) across the membrane. F1(Fo) consists of two molecular motors; ATP-driven motor (F1) and proton-driven motor (Fo). In 1997, we for the first time observed the ATP-driven rotation of F1(Fo), and have thoroughly elucidated its operating principle as a rotary motor protein in this decade. In contrast, the proton-driven rotation of F1(Fo) has never been observed yet. This is mainly due to the defects of pmf generation system and its interference with the detection system for rotary motions. In this year, to resolve these technical issues, we fixed these defects one by one, and eventually developed the experimental system which enabled to monitor the amplitude of pmf and the rotation of F1(Fo), simultaneously. In this system, we used the pH-sensitive fluorophore; pHrodo, for the measurement of proton gradient across the membrane, which is the main component of pmf. In addition, to directly observe the rotary motion of F1(Fo), we attached the gold nanoparticle to the rotor part and its rotation was visualized by the total internal reflection dark-field illumination system (TIRDFD). In the presence of pmf, F1(Fo) showed the clockwise rotation when viewed from Fo to F1, and accelerated its rotational rate depending on the amplitude of pmf, which was essentially consistent with the previous biochemical study. In addition to the rotational rate, we obtained various information related to the "dynamics", such as the stepwise rotary motion coupled with the proton transport and ATP synthesis, which provide a clue for further understanding of energy conversion mechanism of F1(Fo) in the physiological condition.

1428-Pos Board B320
Single Proton Pump Activity Measurements on Single Vesicles for a Quinol Heme-Copper Oxidase
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Heme-copper oxidases are key elements in the respiratory chain pumping protons cellular membrane (1, 2) and generate the electrical and chemical potential that drives ATP synthesis. Understanding the precise proton pumping mechanism has been a central theme in bioenergetics research over the last decades (1, 2). However crucial mechanistic details underlying heme-copper oxidase function and regulation remain masked in conventional techniques due to ensemble averaging.