the redistribution of a cell surface, electric field receptor (EFR) in the presence of an applied EF. We are investigating the electromigration model that depends on electrically generated forces in the plane of the plasma membrane and parallel to the electric field vector, specifically, electrophoretic and electro-osmotic forces. Electrophoretic force drives net negatively charged macromolecules to the anode while electro-osmotic flow of water, in the boundary layer, drives macromolecules with large extracellular domains to the cathode. We have derived a model based on these opposing forces, to predict the relative concentration of surface proteins over space and time, allowing us to test the redistribution of known plasma membrane surface macromolecules in applied EFs and under controlled conditions. The model closely describes accumulation of a net negatively charged, GPI-anchored, fluorescent protein to the cathode under different field strengths, showing that redistribution reaches steady-state within minutes, significantly faster than cathodally migrating cells turn toward the cathode. The model is useful for determining the most likely candidates for the EFR on many different cell types.

**1029-Pos**

**Analysis of Ion Channel Dynamics by Single Molecule Tracking in Live Cells**

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Protein dynamics play an important role in signal transduction in association with their activation mechanisms, functions, and so on. Single molecule tracking technique have been widely used for investigating diffusion behavior of protein in live cells. Especially membrane proteins have been studied since they are important to understand cell responding to the surroundings. However, not many ion channel proteins such as AMPAR, NMDAR, etc., have been monitored due to their complex system. Here, we observed ionotropic glutamate receptor in neuroblastoma SH-SY5Y cell using single-molecule imaging. The diffusion coefficient of the receptor was significantly low because the receptor has four subunits (tetramers) and each subunit possesses a four transmembrane domain. Furthermore, we also analyzed interaction between subunits using single molecule tracking, further investigation of the protein dynamics of the membrane proteins such as ionotropic receptors, their structure and protein activation mechanism will be possible.

**1030-Pos**

**Gating Mechanism of a Potassium Channel, Experimental and Theoretical Studies**

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Inwardly-rectifying potassium (Kir) channels are transmembrane proteins that regulate membrane electrical excitability and $K^+$ transport in many cell types generating and controlling the membrane resting potential. The function of this channel although simple is primordial for physiological processes such as the creation and the propagation of the neuronal action's potential, the regulation of cellular volume, the muscular contraction and the cardiac pulse.

Their physiological importance is highlighted by the fact that genetically inherited defects in Kir channels are responsible for a wide-range of channelopathies including Andersen's syndrome a pathology that can cause periodic paralysis or serious heart problems. To date unfortunately, this disease does not have any effective treatment.

The goal of these studies is to understand how the channel gates and what are the conformational changes required. Our work focused on the KirBac3.1 channel, homologous to Kir2.1 human. We started with known crystallographic data from the KirBac3.1WT and two mutants S129R and W46R. We explored conformational changes using Molecular Dynamics using Excited Normal Modes (MDeNM), a new method which gives access to vibrational motions (Molecular Dynamics) and global motions (Normal Modes) at the same time. MDeNM successfully opened the closed state of the protein giving the opportunity to identify the key motions involved in the gating such as the role of the cytoplasmic domain, the slide-helix as well as the transmembrane helices during the opening of the channel. In parallel, HDX-MS Spectrometry studies had been performed and gave important information on the flexibility of the protein. Experimental and theoretical studies were compared for validation.

In addition, human Kir2.1 was purified and imaged with a Titan Krios microcope. The structure will help to understand the gating mechanism of this human channel.

**1031-Pos**

**Dynamic Coupling of the Aromatic Rotamer Conformation with the Bacteriorhodopsin Photocycle as Revealed by the Chemical Shift Assisted QM/MM Calculations**

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Aromatic residues are well known to be highly conserved in G-protein-coupled receptors (GPCRs) and play very important roles in activating the global toggle switch through a local rotamer switch mechanism. However, little is to know about the mechanism of the highly conserved aromatic residues during the photocycle of the microbial rhodopsin family proteins. In this work, tyrosine 185 (Y185), one of the highly conserved aromatic residues within the retinal (Ret) binding pocket of bacteriorhodopsin (bR) was used as an example to calculate its rotamer conformation in the inactive and active states during the bR photocycle by the automated fragmentation quantum mechanics/molecular mechanics (AF-QM/MM) method, assisted by the chemical shifts of the Ret chromophore obtained by 2D solid-state NMR correlation experiments. Our results showed that Y185 not only underwent a rotamer configurational changes during the bR photocycle, but also affected the protein dynamics and the proton-pumping efficiency through a rotamer switch mechanism. In the AF-QM/MM calculations, the whole protein was divided into three regions, the core region, buffer region and remaining region. The protonated Schiff base, including the Ret chromophore and K 216, is defined as the core region. The buffer region is defined by the residues within the retinal binding pocket and some other residues within 2.5 Å of the core region where the contacting atoms are hydrogen, and the residues within 4.0 Å where at least one of the contacting atoms is non-hydrogen. The remaining part of the protein is defined as the rest region. Both the core region and its buffer region were treated by QM calculations, and the remaining region was calculated at the MM level as background charges.

**1032-Pos**

**Exploring the Hydrophobic Barrier of Human K2P Channel TWIK1 with Steered MD Simulations**

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We used steered molecular dynamics simulations to explore the hydrophobic barrier within the two pore domain potassium channel TWIK-1. This hydrophobic barrier is formed by Leucine 146 and Leucine 261 that lie below the selectivity filter on the cytoplasmic side of the protein. Experimental studies have shown that mutations of Leucine 146 and 261 allow more water to permeate to the selectivity filter and increase ion conduction (*Nature Commun.,* 5, 4377, 2014). We use a collective variable (CV) that mimics tension by applying forces on lipids in the vicinity of the channel based on their proximity to explore the role of membrane interactions in modulating the hydrophobic barrier. Our group has studied the mechanosensitive channel of large conductance using a similar approach. By applying a harmonic potential to this CV, we observed different solvent accessibility to the selectivity filter. We found that TWIK-1 interacts strongly with lipids on the cytoplasmic side of the membrane through hydrogen bonding, which is higher compared to the periplasmic side. Positive amino acid residues Lysine (176, 275, 278) and Arginine (155, 162, 163, 171) alone contribute to one third of the hydrogen bond made by cytoplasmic side with protein. Furthermore, we explored the effects of mutating the hydrophobic L146 and L261 residues to Asparagine on solvent accessibility to the channel's selectivity filter and compare to the steered CV model.

## Posters: Transcription

**1033-Pos**

**Nuclear Myosin VI Stabilizes RNA Polymerase II in Transcription Factories**

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While it is widely accepted there is temporal regulation of gene expression, more recently there has been increase studies of spatial regulation. Indeed, transcription is known to occur within discrete foci throughout the nucleus with RNA Polymerase II arranged in hubs or factories. The factories bring together several genes and arrange the chromatin to enable simultaneous gene expression. The hubs are estimated to contain 4-30 RNAPII molecules in a region