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MscL Gating In Liposomes

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functional mammalian isoforms have been discovered so far with different functional and pharmacological properties. In our study, eight subtypes of the voltage gated sodium channel were tested in parallel on the automated patch clamp system QPatch HT. The new clone screening feature developed for QPatch 16 and QPatch HT allows running up to eight different cell lines (clones or subtypes) at the same time, thus ensuring that the exact same conditions (temperature, Ringer's, pH etc.) are applied for each of the cell lines tested. $Na_v1.1$, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7 and 1.8 were tested together in a range of experiments on QPatch HT. All but $Na_v1.2$ and $Na_v1.8$ were co-expressed with the $\beta 1$ subunit. Three types of experiments were designed to explore 1) TTX sensitivity, 2) IV-relationship for activation and inactivation, and 3) recovery from inactivation, for the entire panel of Na_v channel subtypes in a single experiment. It was shown that QPatch experiments using the cell clone screening feature together with the QPatch Assay Software data analysis package, enables the experimenter to obtain IC_{50} values for TTX, IV-relationships and time constants for recovery from inactivation which are very similar to manual patch clamp data, for all Na_v subtypes, thus successfully distinguishing one subtype from another.

Mechanosensitive Channels

1300-Pos Board B144

Analysis of Gating Process Associated with Water Permeation of the E-coli Mechanosensitive Channel MscL Using Molecular Dynamics Simulations

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The bacterial mechanosensitive channel of large conductance MscL is constituted of homopentamer of a subunit with two transmembrane inner and outer α -helices, and its 3D structure of the closed state has been resolved. The major issue of MscL is to understand the gating mechanism driven by tension in the membrane. Although several models for the opening process have been proposed with Molecular Dynamics (MD) simulations, as they do not include MscL-lipid interactions, it remains unclear which amino acids sense membrane tension and how the sensed force induces channel opening. We performed MD simulations for the mechano-gating of MscL embedded in the lipid bilayer. Upon tension generation in the bilayer, Phe78 in the outer helix was dragged by lipids, leading to a tilting of the helices. Among amino acids in the outer helix facing the bilayer, Phe78 at the water-lipid interface showed the strongest interaction with lipids, thus may work as a major tension sensor. Neighboring inner helices cross each other in the inner leaflet, forming the most constricted part of the pore. As tension increases, the crossings move toward the cytoplasm associated with an expansion of the constricted part. During the movement, a hydrophobic water block environment around the constricted part was broken followed by water penetration and permeation. We modeled G22N mutant, known to have an ability to permeate ions without increasing membrane tension, and performed 5 ns equilibrium simulations. We analyzed movements of water molecules around the block and found that the asparagine substitution resulted in spontaneous water flow due to a hydrophilic side chain of asparagine, leading to partial channel opening. Thus a change in the environment around the most constricted part from hydrophobic to hydrophilic promotes MscL opening.

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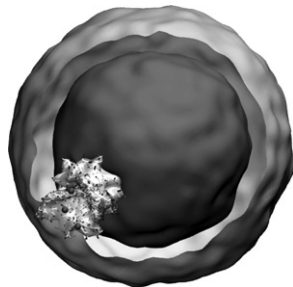
MscL Gating In Liposomes

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Mechano-sensitive channels function as safety valves of a cell by controlling the permeability of the plasma membrane. They are non-selective and respond rapidly to sudden changes in the tension of the membrane. During a hypo-osmotic shock mechano-sensitive channels sense the growing turgor pressure and start to gate, thereby releasing the tension and preventing the rupture of the cell membrane.

Mechano-sensitive channel of large conductance (MscL) forms a large, non-selective channel when activated. The crystal structure of MscL in its deactive state extracted from *Mycobacterium tuberculosis* has given insights into the possible activation mechanism of MscL and has enabled the tentative mapping



of the closed-open transition pathway by molecular dynamics simulations. Nonetheless, the role and importance of e.g. membrane curvature, cytoplasmic helix-bundle and directional ion flux are still unclear.

Liposomes, i.e. tiny lipid vesicles, offer unprecedented possibilities to study the effects of membrane curvature and directional ion flux on MscL gating. We have studied in near-atomistic detail liposome embedded Tb-MscL using the recently developed MARTINI coarse-grained model for biomolecules. Various pressure gradients were inflicted across the liposomal membrane to map the tension-activation response of MscL and to obtain fully activated channels capable of rapid release of liposomal stress.

1302-Pos Board B146

Multi-scale Modelling Of Tb-MscL Gating In Its Native Environment

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Here we explore the possibility of combining Molecular Dynamics (MD) simulation together with Monte-Carlo simulation in order to predict the gating pathway for a tension-gated pore protein, the Mechanosensitive Channel of Large Conductance from *Mycobacterium Tuberculosis* (Tb-MscL). To mimic its native environment, we embed the channel protein in a native-like lipid membrane, itself first equilibrated by MD, and the whole system is then equilibrated using MD, followed by rigid cluster decomposition by FIRST software and Monte-Carlo simulation of channel opening using FRODA software. Our goal was to explore in a more atomistic level protein-lipid interactions that were explored by continuum models, and uncover the role played by various components of the protein-membrane system during the channel gating. Our results suggest that protein-lipid interactions are necessary in order to produce an asymmetric motion of channel subunits, which was observed in previous experimental studies.

1303-Pos Board B147

Spandex Proteins: Mechanosensitive Closed-closed Transitions Suitable for Osmoprotector and for Tension Damper Functions in Large Membrane Proteins

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Large membrane proteins are potentially more expansible than bilayers. We therefore term membrane proteins with stochastic tension-sensitive transitions between closed states "spandex proteins" and ask what design features might allow spandex proteins to act as tension relievers. Spandex is modeled with two states (contracted, expanded). Its barrier state location strongly impacts the timescale of the expansion transition. Expansion depends on spandex concentration, with the apparent midpoint tension shifting to larger tensions as the membrane density of spandex increases.

In a cell, there are two ways spandex might be advantageous. In the case of an abrupt tension increase, spandex expansion could reduce bilayer tension enough to prevent unnecessary opening of osmotic valve channels. To achieve this safely, a protein that expands at a very precise tension before the channels can open is required. This requires a large spandex protein, whose barrier is located close to the expanded state, ensuring that if tension is high, the spandex will react rapidly. Secondly, spandex proteins could be used to maintain a steady bilayer tension. However, a single species of spandex could not be both a good partner for osmotic valves and a good tension damper. For reliable tension damping, the spandex tension midpoint must equal the target tension. To ensure the spandex reacted rapidly to tension fluctuations, its barrier would need to be located halfway between the contracted and expanded states. Also, the larger the change in area of the protein, the more precisely the target tension will be maintained in the bilayer. The concentration needed depends on the strain amplitude that is to be dealt with. We discuss possible interactions among the tension sensitive closed-closed and closed-open transitions of different bacterial membrane proteins. *NSERC funded.*

1304-Pos Board B148

A Site-directed FRET Confocal Microscopy Approach for Studying Conformational Changes in the Mechanosensitive Ion-channels, MscL and MscS

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Bacterial mechanosensitive channels act as safety valves that protect cells from hypo-osmotic shock by opening under membrane tension to relieve pressure within the cell. Although the crystal structures of two such ion channels - the mechanosensitive channels of large (MscL) and small (MscS) conductance - are known, the mechanism by which bilayer deformations are transduced into channel opening is still being worked out. Here we describe a method to study conformational changes associated with the channel opening (of both