of them is high pore dynamics and unknown shape of the pore. The other problem comes from comparable diameters of the probe molecules and electropore, both within nanometer scale. The molecular diffusion in such systems is affected by hindrance posed by the entropic barrier and molecular interactions. Therefore, a classical Nernst-Planck equation with a bulk value of the diffusion constant is very imprecise. More advanced modeling is necessary, which takes into account more physical representation of the nanopore shape, charge distribution, interactions between molecules, ions and pore walls. The comparable scale of the pores and probes should be reflected in an effective diffusion constant value. The impact of such improvements on the results, applied to a nanopore, is demonstrated by means of the Poisson-Nernst-Planck model with adjustable diffusion coefficient. The results show a significant discrepancy of the results from simplified and more advanced models.

2793-Pos
Determinant of Cation Blocking Behavior in Aquaporin-1
Hui Li.
University of Utah, Salt lake city, UT, USA.
The most extensively studied member of the aquaporin family, wildtype (WT) aquaporin-1 (AQP1), has been shown to effectively block cations from permeation across the cell membrane, thus maintaining the osmotic pressure of the cell. Recent experiments have suggested the essential role that the Selectivity Filter (SF) region of the channel plays in blocking cation flux. In the present study, the potential of mean forces for permeation of sodium cation (Na^+), potassium cation (K^+), and classical hydronium cation (H_3O^+) (without the possibility of Grotthuss proton shuttling) are characterized for a series of AQP1 mutants. The free energy barriers for conducting hydrated excess protons (H^+), which diffuse via the Grotthuss mechanism, are characterized as well, by utilizing the multi-state empirical valence bond (MS-EVB) method. The maximum cation conductance is calculated using the Poisson-Nerst-Planck theory. The present study reveals the key role of the SF domain in cation gating and provides insight into the subtle mechanism of proton permeation mutants of the AQP1 channel.

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2794-Pos
Loop 1's Role in a Novel Step on the ADP Release Pathway of Smooth Muscle
Justin Decarreau, Lynn Chrin, Chris Berger.
University of Vermont, Burlington, VT, USA.
Smooth muscle myosin has two N-terminal isoforms that result from alternative splicing of loop 1. Loop 1 contains a seven amino acid insert (QGPSFSY) in one isoform (SM-B) that is absent in the other (SM-A). It has been shown that the presence of the insert causes a two-fold increase in the rate of in-vitro actin sliding velocity and actin-activated ATPase activity (Rovner et al., 1997, Muscle Res Cell Motil 18:103). Based on these results and its proximity to the active site, it was hypothesized that loop 1 plays a role in modulating the release of ADP (Spudich, 1994, nature 372:515). However, little is known about the conformation of loop 1 in different nucleotide states, as it is absent in crystal structures. To further investigate the role of loop 1 in modulating ADP release we have inserted a single tryptophan residue into the interior of loop 1 in the SM-B isoform to monitor its dynamics. In combination with stopped-flow kinetics to monitor the release rate of mant-ADP from the motor domain, we have observed three steps in the ADP release mechanism, one of which is a unique transition that occurs before ADP release and following opening of the active site. Significantly, this previously undetected kinetic step appears to arise from a specific change in the state of loop 1. This is the first time a role of loop 1 in the ADP release mechanism has been directly identified and may account for the functional differences observed between two isoforms of smooth muscle myosin.

2795-Pos
A Kinetic Step Involving Loop 1 in Smooth Muscle may Dictate Isoform Specific Differences in ADP Release
Justin Decarreau, Lynn Chrin, Chris L. Berger.
University of Vermont, Burlington, VT, USA.
The actin sliding velocity and ADP release rates in smooth muscle myosin are modulated by the make-up of a flexible surface loop spanning the active site known as loop 1. There are only two motor domain isoforms of smooth muscle myosin and they differ in the presence (SM-B) or absence (SM-A) of a seven amino acid insert (QGPSFSY) in this loop. The presence of this insert leads to a two-fold increase in both actin sliding velocity and ADP release, although the mechanism for this difference is unknown. To investigate the role of this insert in functional differences between the SM-B and SM-A isoforms of smooth muscle myosin we have inserted a single tryptophan residue into loop 1 of both isoforms. The dynamics of loop 1 have been correlated with the kinetics of ADP release using a combination of steady-state fluorescence measurements (i.e., tryptophan emission, FRET, and acrylamide quenching) and stopped-flow kinetics. Using this approach we have already shown that the long loop SM-B isoform displays an extra step in its ADP release pathway that has not been previously observed. Here, we show that the additional transition seen in the long loop SM-B isoform is not observed in the short loop SM-A isoform upon ADP release. Furthermore, the final ADP release step is twice as slow in the short loop SM-A isoform, suggesting that the unique transition observed in the presence of the insert alters loop 1 dynamics in a way as to facilitate ADP release. This alteration of ADP release constitutes a simple and fundamental way to tune the activity of the motor at the molecular level and mechanical function at the physiological level in smooth muscle.

2796-Pos
Mutant Analysis and Computational Analysis of the Essential Light Chain and Regulatory Light Chain Interactions with Respect to Regulation of Smooth Muscle Myosin
Shaowei Ni, Feng Hong, Michael S. Carter, Josh E. Baker,
Kevin C. Facemyer, Christine R. Cremo.
University of Nevada School of Medicine, Reno, NV, USA.
To understand how smooth muscle myosin's (SMM) light chains are involved in phosphorylation dependent regulation of ATP hydrolysis, we expressed single, double and triple amino acid mutants of the heavy chain, regulatory (RLC) or essential light chain (ELC) in the area of the Ca2+-binding loop of the ELC. We mutated this area because of similarities to scallop smooth myosin's Ca2+ regulatory mechanism. Since the ELC is not required to maintain the off state, we reasoned that mutations in this region would specifically alter the activity of the on (phosphorylated) state of SMM. Mutations were made to disrupt hydrogen bonding between the ELC and RLC and the heavy chain (HC) based on scallop crystal structures. All mutant ATPase activities and actin sliding velocities (ASVs) were essentially identical to wild type in the unphosphorylated. For the phosphorylated states, one class of mutant showed normal ATPase activity and ASVs; a second class showed similarly depressed ATPase activity and ASVs; and a third class with differentially depressed ATPase activity and ASVs. We also created recovery mutants that restored (and recoupled) ATPase rates with ASVs. We then used course grain discrete molecular dynamics and force constant profiling to reconcile changes in the interactions of ELC, RLC & HC in that region, with changes at the ATPase site. We created a prepowerstroke smooth muscle myosin model with an ELC Ca2+ loop from scallop that interacted weakly with the RLC and used FlexServe (http://mmb.pcb.ub.es/FlexServ/) to compute changes in flexibility between the native (1QVI) and modified model. We see that changes in the ELC RLC interaction have direct effects on lever arm flexibility and active site protein dynamics.

2797-Pos
Modulation of Actin-Myosin Interaction by N-terminal Unique Domain of Myorod of Molluscan Catch Muscle
Oleg S. Matusovsky[1], Galina G. Matusovskaya[1], Apolinary Sobieszek[2], Nikolay S. Shelud'ko[1].
[1]A.V. Zhirmunsky Institute of Marine Biology FEB RAS, Vladivostok, Russian Federation, [2]Institute for Biomedical Aging Research, Life Science Center, AAS, Innsbruck, Austria.
Myorod, a thick filament protein of molluscan smooth muscle, is an alternative product of the myosin heavy chain gene. It contains the rod domain identical to that of the rod portion of the myosin molecule and a unique N-terminal domain (NMR). We previously reported that myorod is phosphorylated within NMR at Thr141 by vertebrate smooth muscle myosin light chain kinase (Sobieszek et al., *Arch. Biochem. Biophys., 454*: 197-205, 2006). To investigate whether phosphorylation of NMR may affect the actin-myosin interaction, two peptides were synthesized with sequence corresponding to this domain. One of two peptides included a phosphorylated Thr141 (NMR-P) and the other not (NMR-unP). We found that the latter peptide interacted with rabbit and molluscan F-actin causing an aggregation and sedimentation of F-actin at low-speed centrifugation while NMR-P had no effect on the distribution of F-actin in the