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

supernatant and pellet fractions. Co-sedimentation of NMR-unP with isolated molluscan thin filaments revealed that in this case the interaction was Ca^{2+}-dependent. NMR-unP slightly inhibited the Mg^{2+}-ATPase activity of actomyosin reconstructed from molluscan myosin and rabbit F-actin. In contrast, NMR-P as well as intact phosphorylated myorod increased actomyosin Mg^{2+}-ATPase activity of about 1.5-3 fold depending on the experimental conditions. This finding was supported by a 3-fold higher binding affinity of NMR-P for myosin filaments with comparison of that of NMR-unP. Taken together these results implicate that myorod, a thick filament protein of molluscan catch muscle, can modulate actin-myosin interaction in a phosphorylation-dependent manner.

2798-Pos

Smooth Muscle Tropomyosin Forms Semi-Rigid End to End Polymers

Duncan Sousa[1], Anthony Cammarato[2], Jason-Pingcheng Li[1], Xiaochuan Li[1], William Lehman[1].
[1]Boston University School Medicine, Boston, MA, USA, [2]San Diego State University/Burnham Institute, San Diego, CA, USA.

Variation in the structural mechanics of tropomyosin isoforms may govern differences in their affinity and positioning on F-actin. Such differences may influence the access of actin-binding proteins along the sides of actin filaments and also the cooperativity of actin-myosin interactions. Here, smooth and striated muscle tropomyosin were rotary shadowed and compared by electron microscopy. EM shows that cardiac and skeletal tropomyosin primarily consist of 40 nm long single molecules, whereas smooth muscle tropomyosin is a mixture of varying length chains of end-to-end linked molecules found together with single molecules. The tendency of smooth muscle tropomyosin to polymerize reflects greater end-to-end interaction, possibly required on smooth muscle thin filaments, which lack troponin to stabilize this interaction. Measurement of the apparent persistence length (PL) of single smooth muscle tropomyosin molecules and the chain-like polymers yield indistinguishable values, which are comparable to those that we find for cardiac tropomyosin. The semi-rigidity of smooth muscle tropomyosin polymers may ensure a high degree of positional fidelity of tropomyosin on smooth muscle thin filaments, despite the lack of troponin (*cf*. Lehman *et al*., 2009). It is unlikely, however, that stiff, polymerized superhelical chains of tropomyosin can bind directly to F-actin. However, *in vitro* an equilibrium may yield sufficient single smooth muscle tropomyosin molecules or short chains to bind. *In vivo,* actin and smooth muscle (or cytoskeletal) tropomyosin may copolymerize or, alternatively, G-actin may polymerize on a scaffold of tropomyosin chains. Thus differing mechanisms of thin filaments assembly may be related to tropomyosin end-to-end binding strength.

2799-Pos

Airway Smooth Muscle Dynamics are Governed by the Imposed Rate of Strain

Guillaume Lenormand, Madavi Oliver, Srboljub M. Mijailovich, James P. Butler, Jeffrey J. Fredberg.
Harvard School of Public Health, Boston, MA, USA.

It is commonly believed that the time scale governing the rheology of airway smooth muscle (ASM) is set by the internal viscosity and elasticity of the muscle. We show here, to the contrary, that this time scale is set by the externally imposed rate of strain.

For any fixed strain rate amplitude (SRA), the elastic modulus of the ASM showed a sigmoidal dependence upon frequency. Remarkably, as the SRA was increased over a range spanning almost four decades, sigmoidal response curves demonstrated little change of shape but shifted dramatically to higher frequencies. As such, the time scale of underlying molecular processes is set not by any internal viscosity, elasticity, or any spontaneous internal rate process, but instead is set by the imposed rate of strain. When the muscle is loaded at a small strain-rate, the molecular dynamics are slow; when loaded at a large strain-rate, the dynamics are fast.

Using numerical computations, we then assessed the contribution of myosin bridge kinetics to this behavior. In the regime where frequency was the highest, a good agreement between data and computations was obtained; ASM dynamics could, therefore, be attributed to forced acto-myosin crossbridge dynamics. But at the lowest frequencies, the slopes differed dramatically and stiffness values differed by an order of magnitude, exposing a new domain of slow dynamics that cannot be accounted for by acto-myosin interactions.

Interestingly, these results unify scale-free dynamics, fluidization, and length adaptation. While this unification is not explained by any traditional physical picture of cell rheology or polymer dynamics, it deepens substantially the analogy between living and inert soft matter, and in doing so, reveals a central role for microstructural fragility.

2800-Pos

Role of Nonlinear Serial Elasticity on Airway Smooth Muscle Contraction

Srboljub M. Mijailovich.
Harvard School of Public Health, Boston, MA, USA.

Both the elevated shortening velocity and maximal shortening capacity of airway smooth muscle (ASM) in asthmatic airways have been associated with airway hyperresponsiveness, even though the isometric force-generating capacity of the muscle is the same as in normal airways. This paradox may be partly explained by the relaxing role of tidal breathing, which is associated with perturbed equilibria of myosin binding. We have developed a theoretical model of airway narrowing to quantitatively assess how and in what degree the observed alterations in ASM contractility and nonlinear ASM serial elasticity (SE) can account for hyperresponsiveness in asthma. The model includes the elasticity and geometry of the lungs, ASM contractility, and the dynamics of breathing. The airway caliber, proportional to ASM length, is dynamically determined by the balance between the airway wall reaction force (AWRF) and ASM contractile force. AWRF depends on the instantaneous difference between pleural pressure and airway pressure at each generation of Weibel's symmetrical bronchial tree, elasticity and geometry of the airway wall, tethering of the airway to the lung parenchyma, and the state of lung inflation. ASM contractile force depends on myosin binding kinetics and the level of ASM activation. From equliriated ASM length the airway resistance is calculated. The model enables simulation of breathing in normal and asthmatic airways exposed to an increasing dose of spasmogen. Increasing the dose causes a contraction of the ASM, narrowing of the airways, and an exponential increase airway resistance. We show that an airway with asthmatic or sensitized muscle (increased level of myosin LC_{20} phosphorylation, by 30-50%) narrows faster and significantly more than a normal airway. These results lead to a plausible mechanism by which the rate of bridge cycling and its regulation may account for airway excessive narrowing in asthma.

2801-Pos

Structural Dynamics of the Dystrophin-Actin and Utrophin-Actin Complexes

Ava Y. Lin, Ewa Prochniewicz, Davin Henderson, James Ervasti, David D. Thomas.
University of Minnesota, Minneapolis, MN, USA.

Dystrophin and utrophin bind actin *in vitro* with similar affinities, but with different molecular contacts. It is proposed that these differences alter the elasticity of dystrophin-actin and utrophin-actin linkages to the sarcolemma, affecting the cell's response to muscle stretches. To test this hypothesis, we have determined the effects of dystrophin and utrophin on the microsecond dynamics of phosphoresescent-labeled actin, using transient phosphorescence anisotropy (TPA). At higher levels of saturation, utrophin was more effective than dystrophin in causing changes to the final anisotropy, correlation time, and initial anisotropy of actin dynamics. The simplest interpretation of these changes is that utrophin restricted the amplitude and increased the rates of actin to a substantially larger extent than dystrophin. Further analysis indicated that the actin-utrophin complex is much more torsionally flexible than the actin-dystrophin complex. We propose that these differences between dystrophin and utrophin in their effects on actin dynamics affect elastic properties of actin-mediated linkages with the sarcolemma. Preliminary data on fragments containing all the proposed actin binding domains (DN-R17/UN-R10) show less of an effect on regulating rotational amplitude and nearly no effect on rotational rate. Future experiments looking at other fragments of dystrophin and utrophin, and constructs with engineered disease-causing point mutations will determine which structural elements of these proteins are critical in determining the flexibility of actin filaments and what level of actin flexibility is physiologically optimal. Finally, to test the hypothesis that different orientation or conformation of the actin binding domain in dystrophin and utrophin contributes to changes in actin dynamics, we are using spectroscopic probes to do direct distance measurements between the 2 Calponin homology actin-binding domain heads to differentiate between the 4 currently proposed models of CH domain conformations.

2802-Pos

Congenital Contracture Syndrome Caused by Mutation in Embryonic Myosin Heavy Chain Characterized by Significant Changes in Adult Muscle Contractility

Alice W. Ward, Anita E. Beck, Michael J. Bamshad, Michael Regnier.
University of Washiington, Seattle, WA, USA.

Congenital contracture syndromes affect 1 out of every 1000 live births, and of those syndromes, distal arthrogryposis (DA), characterized by contractures of