due to a decrease in the rate of phosphate release. The rate of actin gliding, ATP-induced actomyosin dissociation, and ADP release. The IQ motifs. The R156W mutation only moderately affects the rates of ATP hydrolysis. We have used an optical tweezer assay that allows us to apply forces in various directions relative to the motor’s movement along an actin filament.

631-Pos Board B431
Direct Observation of the Myosin Power Stroke and its Reversal
Claudia Veigel, Christopher Batters, Christopher P. Toseland, James R. Sellers.
Cell locomotion and division, organelle trafficking or signal amplification in hearing are complex forms of cellular motility that require strong coordination of the myosin motors involved. The most basic mechanism of coordination is the direct mechanical interaction of individual myosin motor heads, leading to modification and regulation of their mechano-chemical cycles. We have used an optical tweezer-based assay to study the mechanical response of a single myosin-V motor head to a range of loads. We found that the response to be non-linear, including reversibility of the force-generating conformational change (power stroke) of single myosin-V motor heads at intermediate forces. By applying load to the head shortly after binding to actin, we found that at 2-4 pN the power stroke could be reversed and the head fluctuated between an actin-bound pre- and a post-power stroke conformation. Load-dependent mechanical instability might be critical to coordinate the heads of processive, dimeric myosin-V. Non-linear response to load leading to coordination or oscillations amongst motors might be relevant for many cellular functions, including those that involve other members of the myosin superfamily.
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Allosteric Tuning of Myosin 5a Motor Activity
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Myosin 5a is a processive vesicle transporter capable of taking multiple steps without detachment from actin. Its translocation activity, which powers cargo transport to micrometer distances, requires a range of biochemical adaptations. In this study we engineered the activity of myosin 5a by introducing mutations into two key regions of the motor domain. G227 is located at the entrance of the nucleotide binding pocket. This position is occupied by Gly only in highly processive vertebrate myosin 5a and 5b isoforms, whereas other myosin 5 isoforms and myosins from other classes possess larger amino acids at this position. Our results show that the G227A mutation in myosin 5a causes a change in the rate-limiting step, which is ADP release in the wild type enzyme. In the mutant, a structural change taking place after ATP hydrolysis and before ADP release becomes rate limiting. The ADP release rate constant is much higher than that of the steady-state ATPase activity. Surprisingly, however, the mutant displays even higher steady-state actin attachment ratio than wild-type myosin 5a. The other region mutated in this study is the interface between the N-terminal and converter subdomains. In myosin 2, a repulsive interaction in this interface (K84-R704 in Dictyostelium myosin 2) exerts a kinetic tuning effect during the hydrolytic cycle, as determined in earlier studies. In wild-type myosin 5a this repulsive interaction is absent as the positive charge is missing at the position homologous to K84. This hypothesis is supported by optical trap studies of a myosin 1b construct, which demonstrated that myosin I is exquisitely sensitive to forces opposing its motion (Laakso et al., Science. 321 (5855):133-136). We tested this model by conducting single-molecule studies of the motor activity of Acanthamoeba myosin Ic (AM1c). Specifically, we used an optical trap assay that allows us to apply forces in various directions relative to the motor’s movement along an actin filament.