Harmonic Force Spectroscopy Reveals a Force-Velocity Curve from a Single Human Beta Cardiac Myosin Motor

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macroscopic mechanics of the membrane. The experimental approach is essential in accessing much larger physical properties of membranes altered by the action of proteins. With this approach, we investigate the self-assembly of N-BAR proteins on the membrane and the way protein-membrane interactions lead to the initiation of membrane curvature. We study how the molecular interactions couple to membrane restructuring. Our research also sheds light on the complex role of protein’s subdomains, namely the amphipathic helices, in interacting with the membrane and inducing its curvature. Finally, it gives vital clues how protein self-assembly and crowding affect physical properties of membranes to regulate their shape and dynamics in living cells.

Platform: Muscle: Fiber and Molecular Mechanics and Structure

2288-Plat
Harmonic Force Spectroscopy Reveals a Force-Velocity Curve from a Single Human Beta Cardiac Myosin Motor
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A muscle contracts rapidly under low load, but slowly under high load. This load-dependent motor shortening has been described with a hyperbolic load-velocity curve. Its molecular mechanisms remain to be elucidated, however. During muscle contraction, myosins in thick filaments interact with actin in thin filaments in the sarcomere, cycling between a strongly bound state (force producing state) and a weakly bound state (relaxed state). Huxley and Simmons have previously proposed that the transition from the strong to the weak interaction can be modulated by an external load, i.e., the transition is slow under high load and fast under low load.

We use a new, simple method we call “harmonic force spectroscopy” to extract a load-velocity relationship from a single human beta cardiac myosin II motor (S1). With a dual-beam optical trap, we hold an actin dumbbell over a single myosin molecule that is anchored to the microscope stage, which we oscillate sinusoidally in the direction of the dumbbell. Upon binding of the motor to the actin filament, it experiences an oscillatory load with a mean value that may be directed forward or backward, depending on where the binding took place. We find that the duration of the strongly bound state at saturating [ATP] is exponentially correlated with the mean load applied to the motor. We use a new, simple method we call “harmonic force spectroscopy” to extract a load-velocity relationship from a single human beta cardiac myosin II motor (S1).

In vitro motility assays with surface-adsorbed myosin motor fragments have given important insights into the molecular physiology and pathology of striated muscle contraction and inspired nanotechnological applications e.g. lab-on-a-chip devices. However, to date neither precise localized control of the motor nor introduction of nanoscale three-dimensional geometrical constraints has been possible. This hampers studies of cooperative phenomena and realization of three-dimensional (3D) transport systems. Here, we take critical steps to overcome these limitations, using aluminum oxide coated gallium phosphide nanowires as scaffolds for heavy meromyosin (HMM) adsorption. The wires (diameter: 100-200 nm; height < 5 μm) were either positioned vertically or horizontally on a surface to give hollow nanowires. Upon ATP addition, actin filaments were propelled by HMM on top of the arrays, with filaments spanning inter-wire distances up to 1 μm. The filaments also moved up and down vertical nanowires as detected using sub-wavelength light guiding properties of the nanowires. Motility on top of nanowire arrays holds potential for studies of cooperative phenomena e.g. local enhancement of myosin binding along actin filamentes upon actomyosin interactions. Here we tested whether the low velocity for long filaments seen at uniform low motor densities on flat surfaces may be attributed to loss of cooperative enhancement of myosin head binding locally to actin close to an existing actomyosin crossbridge. Velocity data showing 1/3 the velocity with 1 μm compared to 300 nm inter-wire spacing, in both cases with ~90 HMM molecules per 150 nm wide wire tip (giving high local motor density), argue against this idea. We further demonstrate HMM propelled transport through hollow nanowires of 80 nm inner diameter. Uses of hollow nanowires in fundamental studies of actomyosin and in nanotechnological applications will be considered.

2290-Plat
The Minimal Group Size for Globally Coordinated Stepping of Muscle Myosins Depends on ATP Hydrolysis Free Energy
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In vitro motility assays of muscle myosins, a distinct motile state emerges with increasing number of mechanically coupled myosin binding sites (N). This is explicable by coordinated myosin stepping (CMS): build-up of pre power stroke (PS) myosins is followed by a whole group PS and detachment cascade [1]. In this model, the number of myosins’ mechanical steps increases monotonically by ~6 orders of magnitude dependent on the skew in myosin crossbridge strains (S). Plotting S and the fraction of myosin in the pre and the post PS state (n1,n2) reveals globally coordinated behavior that changes with N. N=5: a quiescent state with high n1 dominates; N=15: a quiescent state and cascading behavior with lowered n1 alternate; N>30: cascading behavior dominates. An accurate continuous model shows two N-dependent stable steady states representing quiescence and cascading. Adding stochastic fluctuations in S lead to N-dependent cascade-like cycles. This suggests global coordination of myosins, which occurs above a minimal myosin group size that depends on ΔG.


2291-Plat
Toward the Realization of a Sarcomere-Like Machine
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We report toward the realization of a synthetic sarcomere-like machine consisting of an array of motor proteins, regularly distributed on an inorganic nano-structured surface, interacting with a single actin filament. The actin filament is a component of a Dual Laser Optical Tweezers system (DLOT, range 0.5-200 pN force and 1-10,000 nm displacement) under either nano-positioner control or force control (Bianco et al. Biophys J. 101:866-874, 2011). The correct polarity of the actin filament (5-15 μm long) is controlled by attaching its barbed end to a trapped bead via gelsolin (Suzuki et al. Biophys J., 70:401-408, 1996). Mechanical measurements have been carried out with a simplified version of the machine, in which the motor proteins (HMM from skeletal muscle of frog or rabbit) are randomly adsorbed on the flat tip of an etched optical fiber (diameter 4 μm), the position of which is controlled by a piezoelectric nano-positioner. In ATP-free solution the rupture force of the single actin-HMM bond (nano-positioner control) is 12.85 ± 0.35 pN. The bond lifetime under a load of 8 pN (force control) has a bi-exponential distribution and the time constant of the major, faster, component is 1 s. If the motor ensemble in rigor (1 mM ATP, 2 mM KCl, [ADP]=0.2 mM) resolving actin sliding velocities by N [2] showed that increasing [Pi] increased the N at which bursts and continuous filament motion emerge. Lowering the rate of P, release reproduced these observations in our detailed mechanochemical model of linearly elastic myosins mechanically coupled via an actin filament [1]. In this model, the number of myosins’ mechanical steps increases monotonically by ~6 orders of magnitude dependent on the skew in myosin crossbridge strains (S). Plotting S and the fraction of myosin in the pre and the post PS state (n1,n2) reveals globally coordinated behavior that changes with N. N=5: a quiescent state with high n1 dominates; N=15: a quiescent state and cascading behavior with lowered n1 alternate; N>30: cascading behavior dominates. An accurate continuous model shows two N-dependent stable steady states representing quiescence and cascading. Adding stochastic fluctuations in S lead to N-dependent cascade-like cycles. This suggests global coordination of myosins, which occurs above a minimal myosin group size that depends on ΔG.