NONLINEAR ELASTICITY OF A CROSSBRIDGE IN SARCOMERE LATTICE

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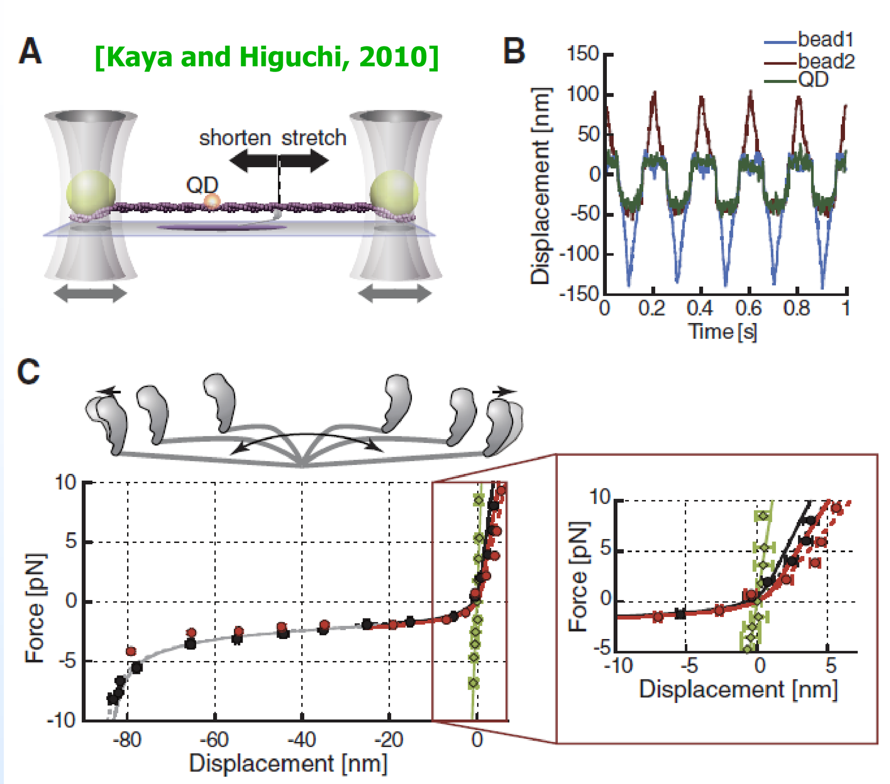
Abstract

Crossbridge elasticity is an essential determinant of strain dependent transition rates in the actomyosin cycle. Recent estimates of myosin stiffness range from 1.5 to 3.2pN/nm and are much larger than most previous estimates used in sliding filament models. These higher stiffnesses limit thermally-induced motions on unattached myosin heads, affect transition rates associated with power stroke and narrow the parabolic parts of energy landscapes. This in turn raises the energy barriers between actomyosin states reducing the probability of strain dependent transitions between them. Estimates of crossbridge stiffness derived from a study of its parts (S2, the lever arm, the "neck region") could be helpful in informing this issue. We used the known atomic structures of crossbridge components in molecular dynamic simulations (CHARMM) to estimate the elasticity of the individual components. We then used nonlinear finite element analysis to estimate the crossbridge stiffness under a range of tensile and compressive forces in the context of the 3-D sarcomere lattice. Using estimated axial and lateral stiffnesses for S2 (of 60 pN/nm, and 0.01 pN/nm respectively), and a bending stiffness S1 of 3 pN/nm, we computed force displacement relationships for crossbridges under tension and in compression. As expected, crossbridge stiffness under tension was slightly below 3 pN/nm at any force. In contrast, stiffness under compression falls about 3-fold at 1 pN, and more than an order of magnitude at forces exceeding 3-4 pN. Consequently, the energy landscape is asymmetric and skewed toward negative crossbridge strains. Our data agree well with recent measurements of nonlinear cross-bridge compliance (Kaya et al., Science 329:686-688) and quantitatively define departures from these measurements in terms of azimuthal departures of the S1-S2 plane from the axial axis of myosin filament and increased inter-filament lattice spacings. Supported by: R01s AR048776 and DC 011528

Introduction

Cross-bridge Stiffness

Coupling between biochemical cycle and sarcomere mechanics is defined by the strain dependence of the actomyosin cycle, which is dependent upon the cross-bridge compliance. This compliance in turn resides in the S2, crossbridge "neck" region (stretching) and lever arm (bending). The most recent experiments report values of myosin stiffness from 1.5 to 3.2pN/nm which are much larger than previous estimates used in sliding filament models. Recently Kaya and Higuchi, 2010, measured force-displacement relationship of a crossbridges interconnecting actin and myosin filaments:



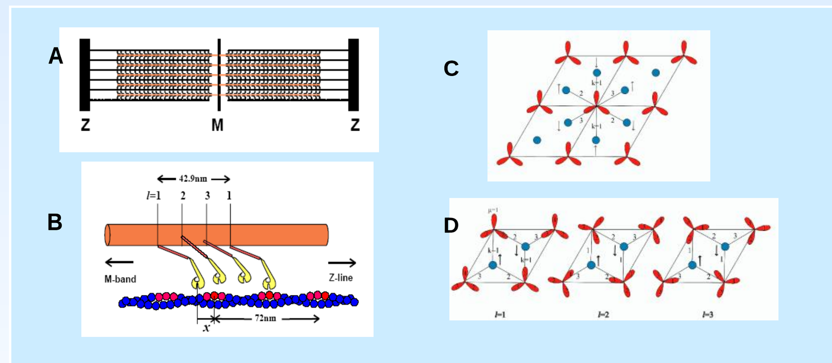
Measurement of crossbridge stiffness. (A) Optical trap system for a single-myosin stiffness measurement. (B) Time course of displacement of beads and quantum dot on an actin filament (C) Force-displacement curve of single myosin in two rigor states.

The cross-bridge stiffness extracted from these measurements is highly nonlinear in compression. This nonlinear behavior could challenge current understanding of mechanochemisry of actin-myosin state transitions in sarcomere lattice.

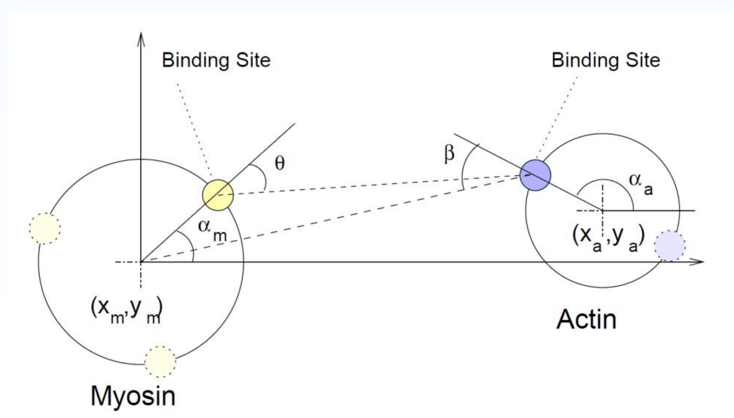
Methods

Nonlinear Crossbridge Stiffness in Sarcomere Lattice

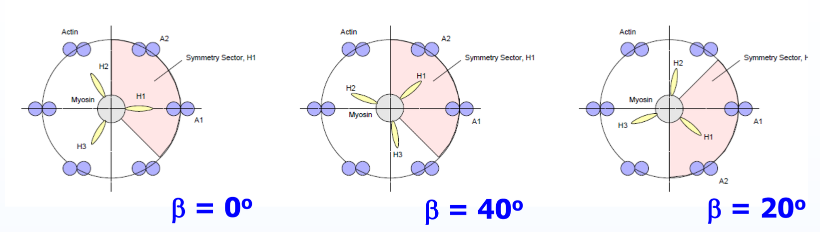
In sarcomere lattice crossbridges are constrained by geometry of sarcomere lattice and position of myosin lever arm. We hypothesize that nonlinear crossbridge stiffness is strongly compromised by lattice constrains. Consequently the mechnochemistry of each crossbridge will be modulated by geometry of bound crossbridge and its state.



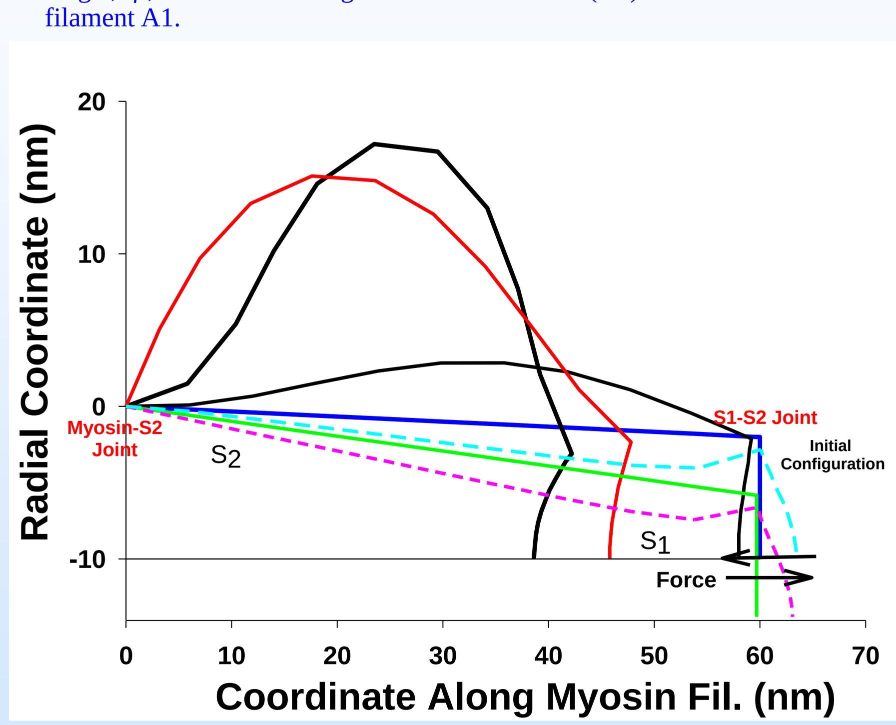
Three dimensional arrangement of actin and myosin filaments: matching of myosin crowns and pitch of actin binding sites



Azimuthal angles of myosin crown turn, α_m, relative to angular position of actin site , α_a .



Azimuthal position of myosin crown relative to lattice of actin filament. Angle, β, denotes the angle between head 1 (H1) and axis of actin filament A1.

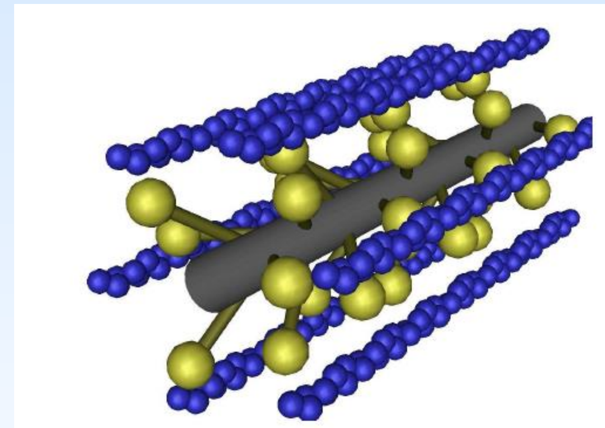


Deformed crossbridge configurations under compression (*solid lines*) and tension (*dashed lines*). Initial configuration is shown as *solid blue line*. Compressed configurations show large bending and buckling of S2 and bending of S1. Under tension, both S1 and S2 are bended. Degree of bending depends strongly of the stiffness of S1-S2 joint. The stiffness of myosin S2 joint strongly influences both compressive and tensile modes of deformation.

Results

Overview

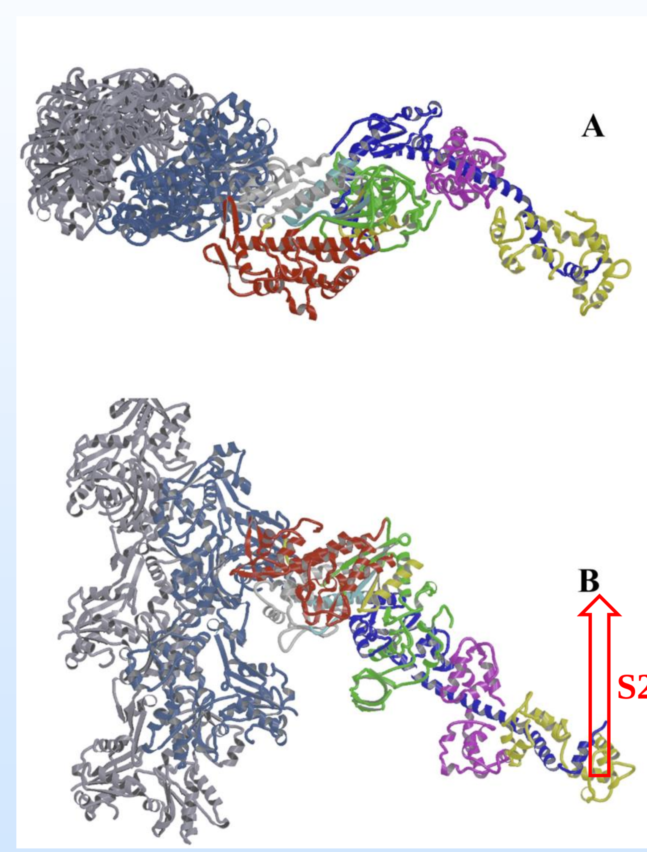
We quantitatively estimated crossbridge stiffness for a crossbridge in Rigor and pre-stroke configuration. We varied range of stiffnesses of S1-S2 and myosin-S2 joints and quantitatively assessed the effect of various relative positions in 3-D of myosin bound to actin.



3D lattice – each myosin filament associates with 6 actin filaments and each actin filament with three myosin filaments arranged in multiple interconnected hexagonal lattices. The force applied in axial direction deforms crossbridges. Using 3D FE model of beam structures we calculated force-displacement relationships. Obtained data were used to calculate crossbridge stiffness as functions of relative axial displacements between myosin S2 junction and position of actin site to which myosin head is bound.

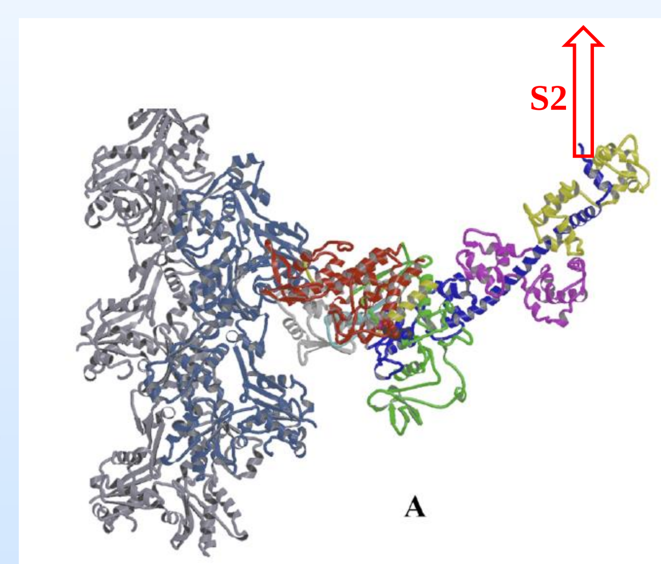
Typical crossbridge deformed configuration obtained from finite element calculations. Deformed are S2 and the lever arm. The motor domain is assumed to be rigid.

Rigor



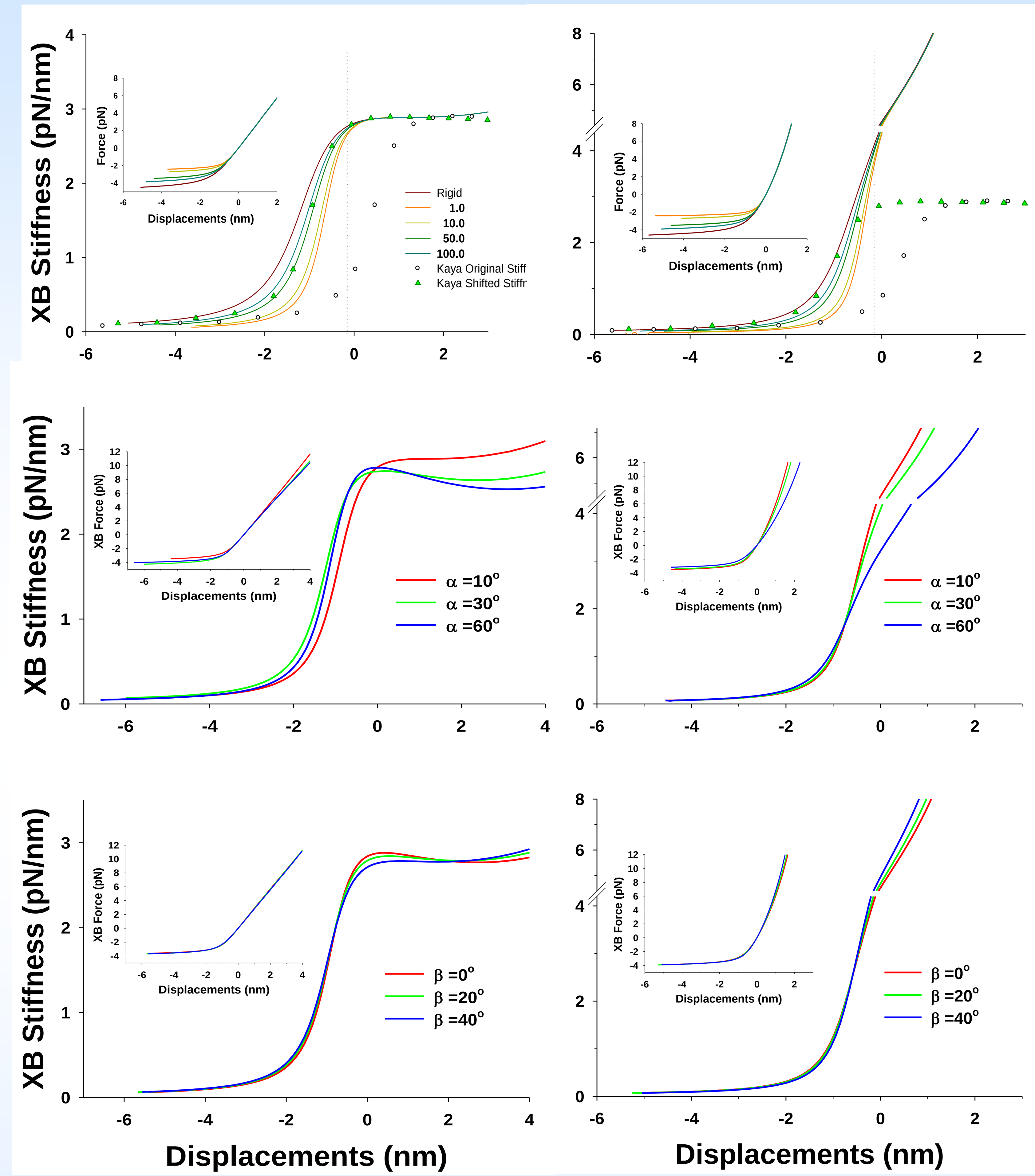
Pre-stroke

[Geeves and Holmes, 2005]



A model of the rigor state (*left panel*) provides 3D geometry of bound myosin to actin filament relative to S2. The model of strongly bound myosin in pre-power stroke state is shown in *right panel*.

Rigor   Pre-stroke



Nonlinear crossbridge dependence of its end to end axial displacement for rigor and strongly bound prestroke configuration. *Upper panel*: Crossbridge dependence of the stiffness of Fmyosin-S2 joint. Kaya's experimental data (after shift) excellently fit data for Fmyosin-S2 joint stiffness of 50 pN·nm and S1-S2 joint stifness of 100 pN·nm. *Middle panel*: Effect of angular position, α, of myosin head bound to actin relative to the plane passing through axial axis of Factin and the position Fmyosin-S2 junction on nonlinear crossbridge stiffness. *Lower panel*: Effect of angular position, β, of alignment of myosin crown with actin filament for $\alpha=60^o$. For $\beta=0$ myosin crown is perfectly aligned with actin filament. The other two crowns take relative angular positions to the associated actin filaments $\beta= 20^o$ and 40^o. Axial rigidity of lever arm is assumed to be $AE_{S1}= 1800$ pN and of S2 $AE_{S2}= 3600$ pN. Bending rigidity of lever arm is assumed to be $AI_{S1}= 1200$ pN·nm^2 and of S2 $AI_{S2}= 600$ pN·nm^2.

Conclusions

The calculated nonlinear crossbridge stiffness shows excellent agreement with Kaya's data but only for rigor configuration

- The nonlinear stiffness in prestroke configuration shows large increase in stiffness for crossbridge stretching due to alignment of lever arm and S2.
- The effect of variation of angle α was significantly larger then the variation of angle β.
- The shift necessary to bring Kaya's data in order to fit the model predictions can be explained by either, the setting zero of force and displacements recorded in Kaya's measurements or unfolding of the myosin head "neck region".