30 July 2020

Supporting Information

Alternative N-terminal regions of *Drosophila* myosin heavy chain II regulate communication of the purine binding loop with the essential light chain

Marieke J. Bloemink[1,3], Karen H. Hsu[2], Michael A. Geeves[1*] and Sanford I. Bernstein[2*]

[1]Department of Biosciences at the University of Kent, Canterbury, Kent CT2 7NJ, United Kingdom, [2]Department of Biology, Molecular Biology Institute, and SDSU Heart Institute at San Diego State University, San Diego, California 92182-4614, [3]current address: Biomolecular Research Group, School of Natural and Applied Sciences, Canterbury Christ Church University, Canterbury CT1 1QU, United Kingdom.

Running title: *Myosin alternative N-terminal domains influence kinetics*

*Corresponding authors: M. Geeves: M.A.Geeves@kent.ac.uk; S. Bernstein: sbernstein@sdsu.edu

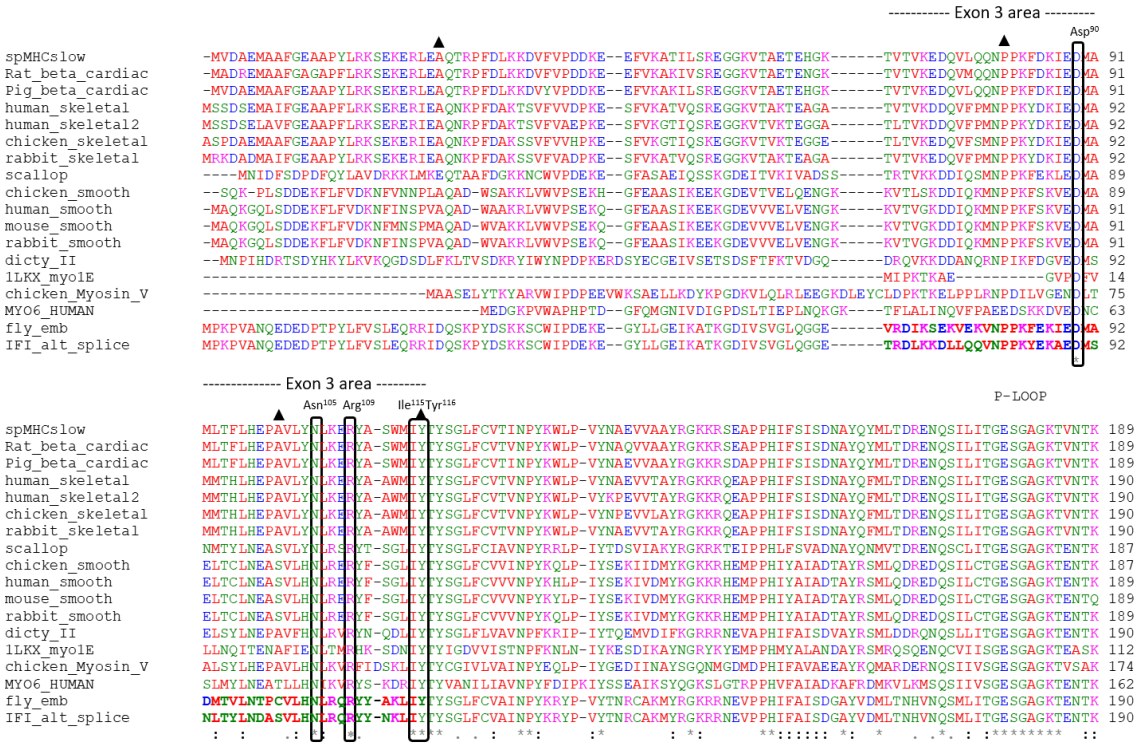

Figure S1: Sequence alignment of the N-termini of various myosins. Conserved residues in the exon 3-encoded region are labelled explicitly by rectangles. "*" indicates that the residues are identical in all sequences in the alignment. ":" represents conserved substitutions, whereas "." indicates semi-conserved substitutions. The corresponding human β-myosin cardiomyopathy sites in the exon 3-encoded domains (Pro81, Ala100 and Tyr115) and the short N-terminal helix (Ala26) are indicated with a ▲.

Interaction between the exon 3 region and the purine binding loop depends on conformational state of the myosin head (Figures S2/S3).

The rigor-like EMB crystal structure (5W1A, Figures S2A/S3A) has contacts between the exon 3 area (Tyr^{110} and Lys^{113}) and the purine binding loop (Asn^{127} and Tyr^{132}). In the pre-power stroke conformation (EMB-1QVI, figure S2B/S3B), the EMB homology structure maintains contacts between the exon 3 area (Tyr^{110} and Tyr^{116}) and the purine binding loop (Pro^{128} and Tyr^{135}). Homology models of the ADP-bound near rigor state (1S5G template, figure S2D/S3D) show that all direct contacts between the exon 3 area and the purine binding loop are lost, whereas in the post-power stroke conformation (EMB-1KK8, figure S2C/3C) contacts are maintained between exon 3 ($Lys^{113,}$ Tyr^{116}) and the purine binding loop (Tyr^{132}, Tyr^{135}). For IFI very similar contacts are found between exon 3 and the purine binding loop (see Figure S3 for summary of interactions). Taken together, the crystal structures and homology models suggest that exon 3 is involved in regulating the conformation of the purine binding loop, as the myosin head goes through the various conformational states during the cross-bridge cycle. However, the interactions between the purine binding loop and the exon 3 area are very similar for IFI and EMB.

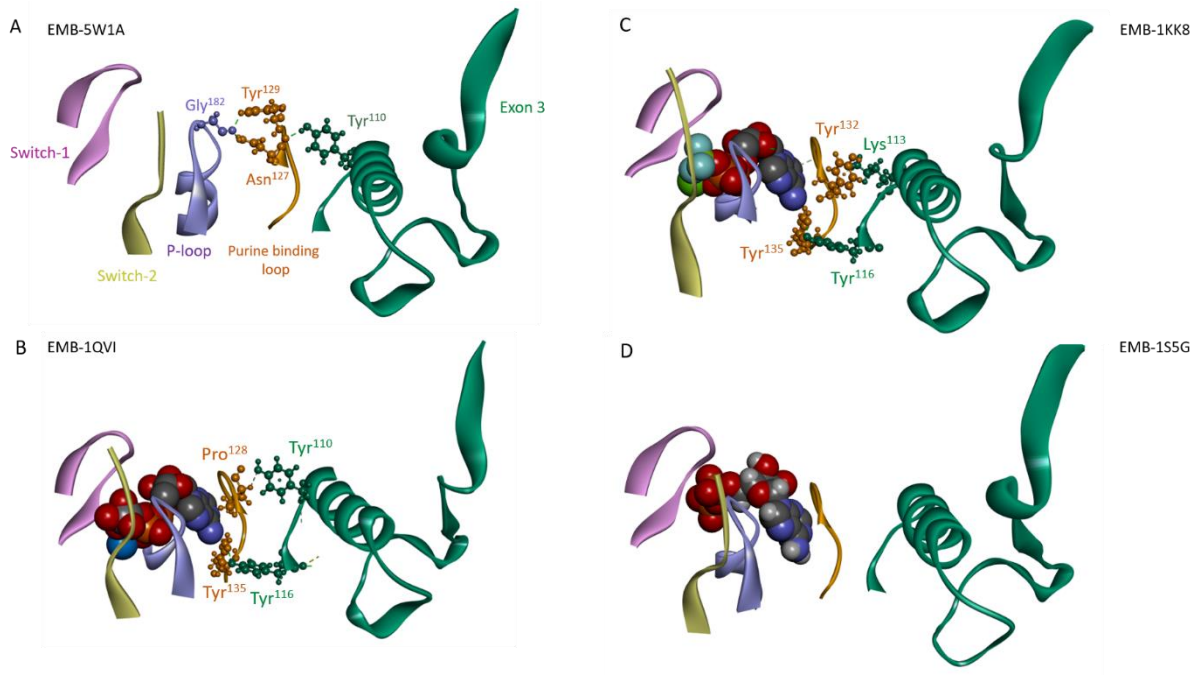

Figure S2: Overview of exon 3 region – purine-binding loop contacts throughout the cross-bridge cycle for EMB. (A) In the near-rigor state exon 3 residue Tyr^{110} interacts with the purine binding loop (PDB: 5W1A). (B) In the pre-power stroke state exon 3 residues Tyr110 and Tyr116 both interact with the purine-binding loop (1QVI used as template). (C) In the post-power stroke state exon 3 residues Lys113 and Tyr116 contact the purine-binding loop (1KK8 used as template). (D) In the ADP-bound near-rigor state no contacts are seen between exon 3 and the purine-binding loop (1S5G used as template).

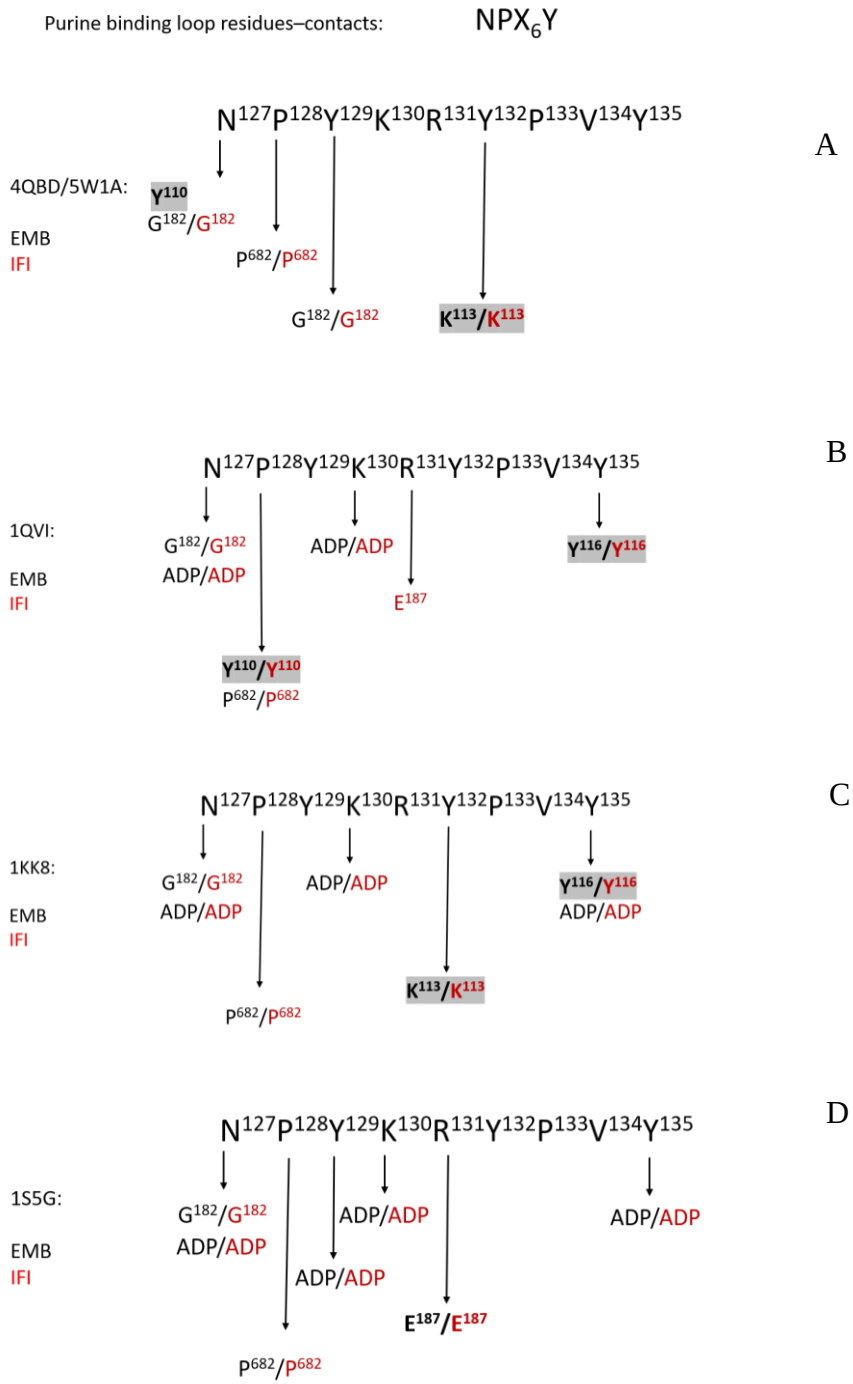

Figure S3 Overview of purine binding loop contacts for EMB and IFI. The purine binding loop is shown at the top of each panel (residues 127-135). Interacting EMB residues are shown below in black, IFI residues are in red. Shaded residues are in the exon 3 area. (A) **rigor-like state** using 5W1A EMB crystal structure as template for IFI. (B) **pre-power stroke state** using 1QVI as template for both EMB and IFI (C) **post-power stroke** state using 1KK8 as template (D) **ADP-bound near-rigor state** using 1S5G as template. In addition to contacts with exon 3 residues (Tyr110, Lys113 and Tyr116), the purine binding loop interacts with the P-loop (Gly182, Glu187) and the bound nucleotide (ADP).

Interactions of the SH1-SH2 helix with the exon 3 region are conserved for IFI and EMB.

The EMB crystal structure in the rigor-like state (5W1A) shows that the exon 3 region has no direct contacts with any of the other variable domains in the myosin head (Figure 1A). However, the SH1-SH2 helix is wedged between the exon 3 and exon 9 (relay loop) regions and makes contacts with both variable domains. Since *Drosophila* EMB and IFI share the same SH1-SH2 sequence, the two variable regions could potentially interact differently with this element, thereby altering the myosin properties. An overview of interactions between SH1-SH2 and the exon 3/9 regions is summarized in Figure S4 (see below) In addition to conserved exon 3 residues L^{97} and N^{98}, two variable residues between EMB and IFI (A^{92}/S^{92} and M^{94}/L^{94}) interact with the SH1-SH2 region in the rigor-like state (left panel) and the pre-power stroke state (middle panel). However, for both residues it is the backbone oxygen that is involved in the contacts with the SH1-SH2 element, and thus not expected to significantly change the exon 3 – SH1-SH2 interaction. Overall for the three conformational states of the myosin molecule investigated here (near-rigor, pre-power stroke and post-power stroke state), the homology models show very similar interactions between the two Drosophila myosin isoforms, indicating that the interaction of exon 3 with SH1-SH2 is highly conserved for EMB and IFI.

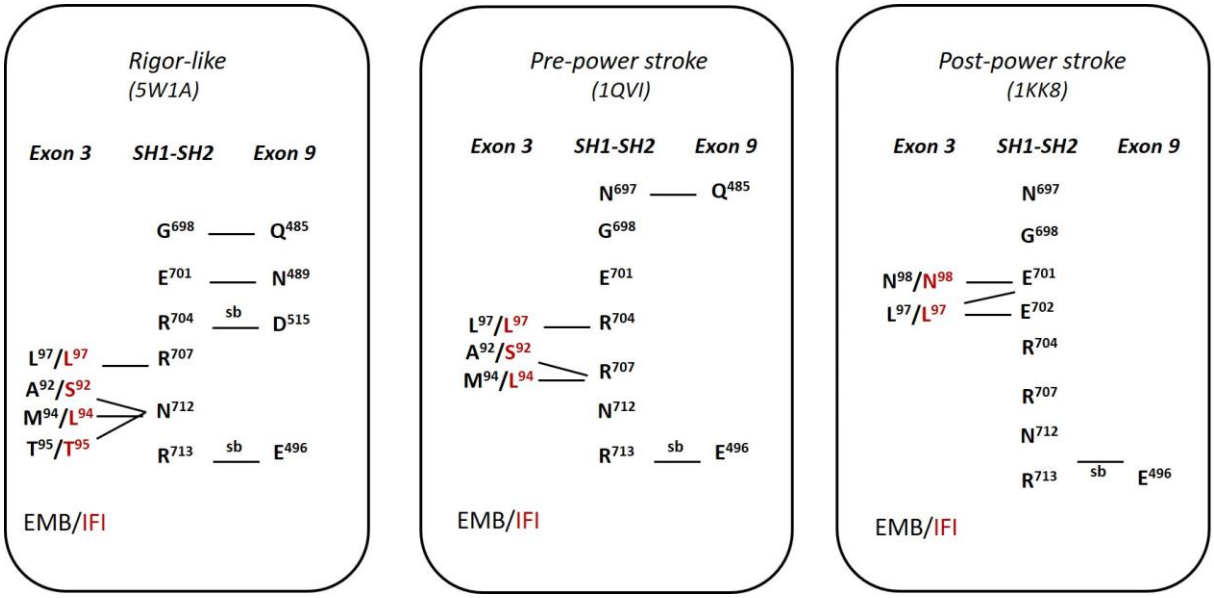

Figure S4: Interactions of the SH1-SH2 helix with the exon 3 region are conserved for IFI and EMB. Residues of SH1-SH2 and the exon 9-encoded relay loop are shown in black for both EMB and IFI. Exon 3 residues are shown in black (EMB) and red (IFI). Left panel: Rigor-like state using the EMB crystal structure (5W1A) as template for IFI. Middle panel: pre-power stroke state for IFI and EMB homology models based on scallop crystal structure (1QVI). Right panel: Post-power stroke state of IFI and EMB homology models based on scallop crystal structure (1KK8).

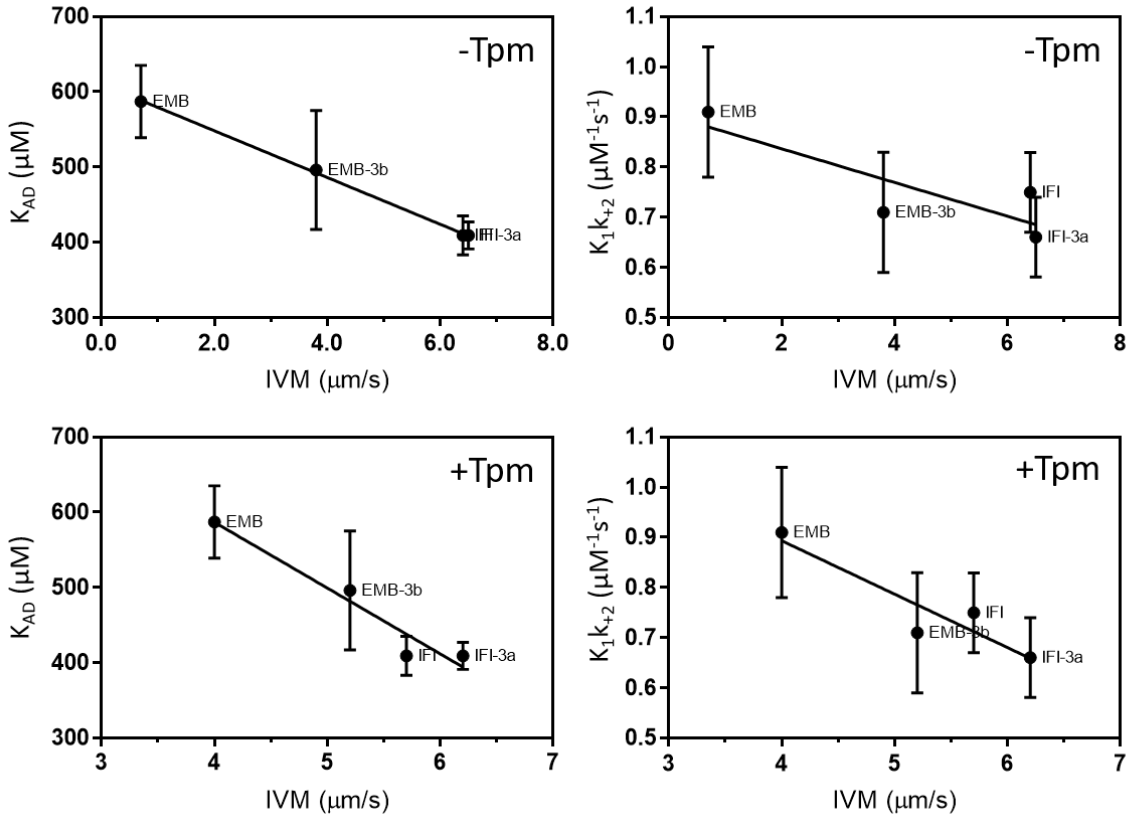

Figure S5: Comparison of kinetic data with *in vitro* motility (IVM) as a function of K_{AD} or K_1k_{+2} for *Drosophila* myosin isoforms IFI, IFI-3a, EMB and EMB-3b. Left two panels: K_{AD} correlation with motility; linear fits give slopes of -32 ± 1 (R^2=0.99) and -88 ± 15 (R^2=0.94) in the absence (-Tpm) and presence (+Tpm) of tropomyosin, respectively. Right two panels: K_1k_{+2} correlation with motility; slopes of -0.03 ± 0.01 (R^2=0.72) and -0.11±0.03 (R^2=0.86) without and with tropomyosin respectively were determined. IVM data is from Swank *et al* 2003.

References for supplementary materials:

Swank, D. M., Knowles, A. F., Kronert, W. A., Suggs, J. A., Morrill, G. E., Nikkhoy, M., Manipon, G. G., and Bernstein, S. I. (2003) Variable N-terminal regions of muscle myosin heavy chain modulate ATPase rate