Supporting Material

Evidence for Pre- and Post-Power Stroke of Cross-Bridges of Contracting Skeletal Myofibrils

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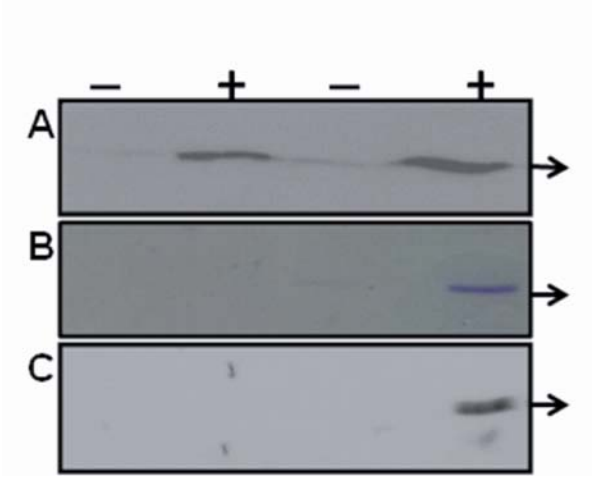


Fig. 1S. Characterization of recombinant LC1 protein by SDS-PAGE and Western Blot analysis. (A) Western blot of the 25-kDa LC1 protein with an anti-LC1 antibody after induction (+) and with no induction (-) by IPTG in E.coli M15 cells (two separate batches). (B) SDS-PAGE of the 25-kDa LC1 protein purified on a Ni-affinity column and stained with Coomassie blue (16% SDS-PAGE). (C) Western blot of the same 25-kDa protein band as in panel B labeled with a rabbit polyclonal LC1 antibody (AbCam, CA). Arrows indicate MW = 25 kDa.

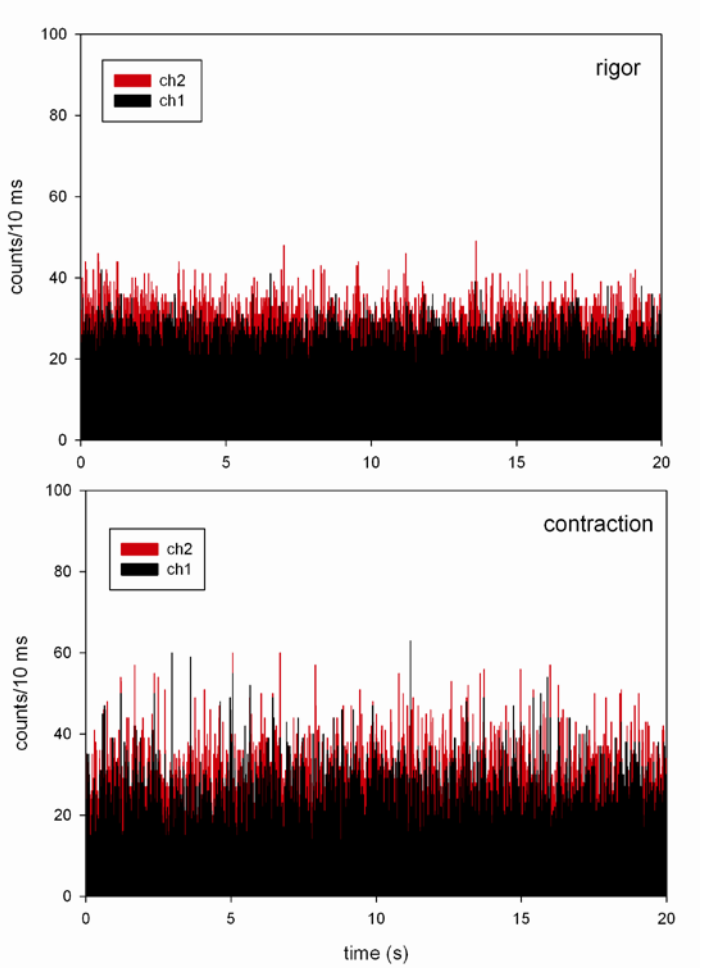


Fig. 2S. *Typical time course of polarized intensity of rigor (**A**) and contracting (**B**) psoas muscle myofibril. This is a bar plot, where the vertical scale is the number of counts during 10 msec. Ch1 (black) and Ch 2 (red) are the fluorescence intensities polarized perpendicular ($I_{\perp}$) and parallel ($I_{\parallel}$) to the myofibrillar axis, respectively. The excitation polarization is ‖ to myofibrillar axis.*

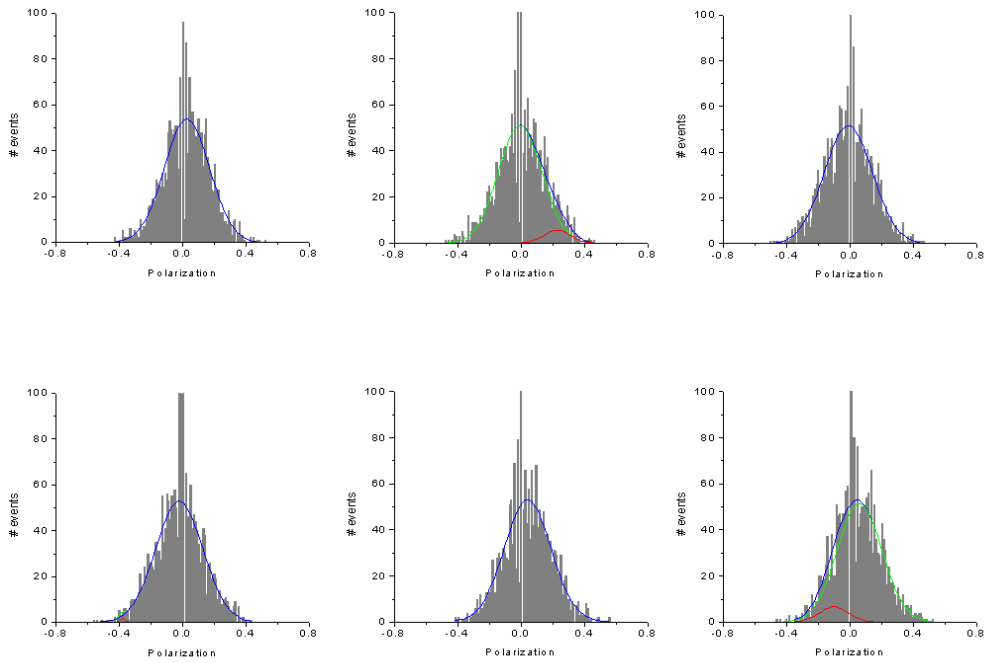


Fig. 3S*. Effect of EDC showing that cross-linking does not induce second peak. Uncross-linked myofibrils in rigor. The second peak constitutes (clockwise, starting from upper left): 0, 6.4, 0, 0.3, 0 and 8.3% of the total signal. Red: minor peak, green: major peak, blue: the sum of minor and major peaks.*

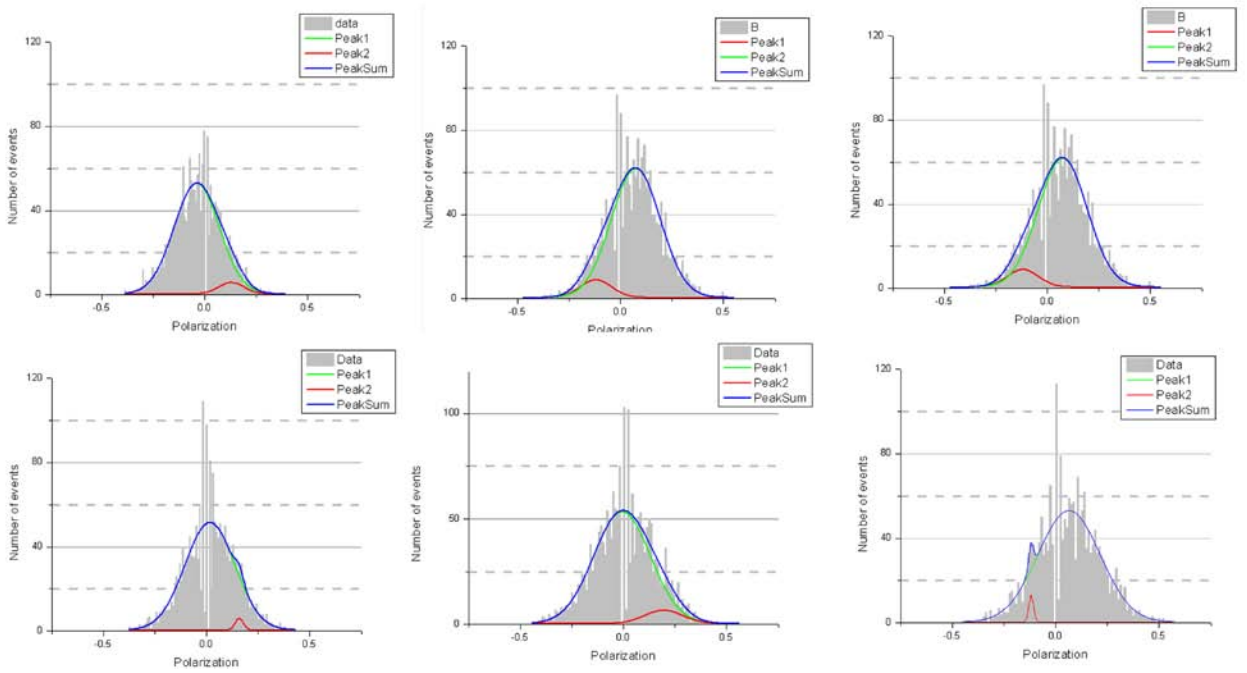
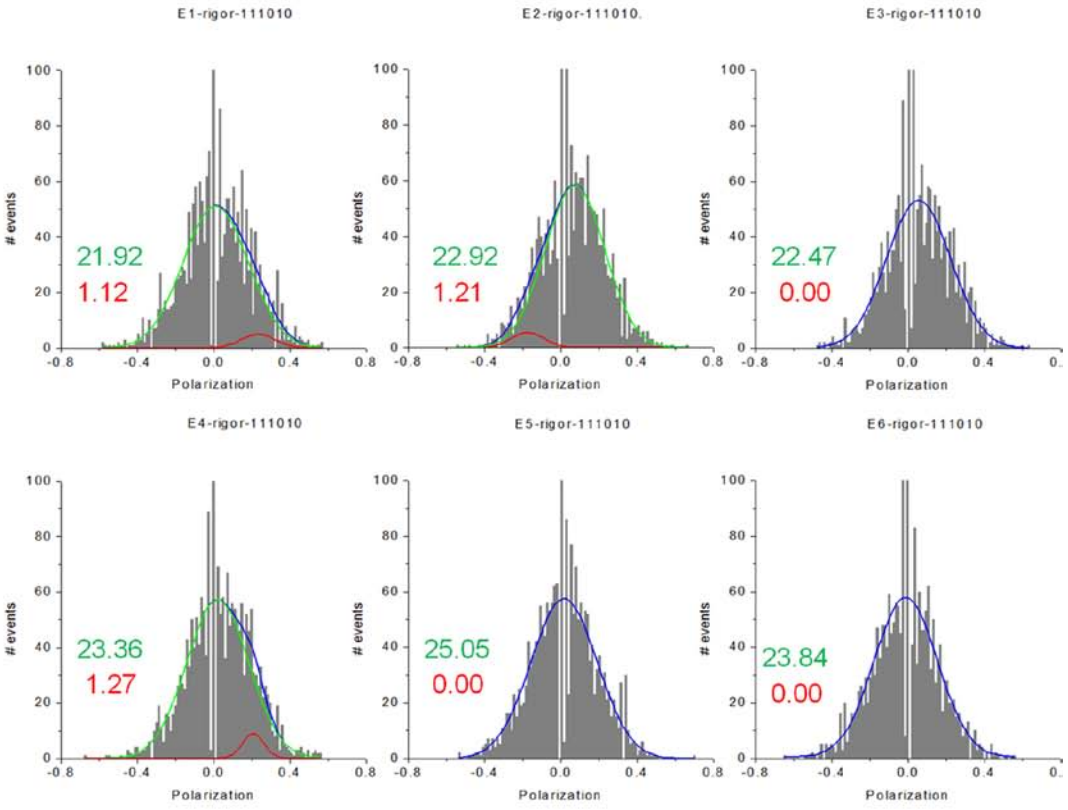


Fig. 4S. *Double Gaussian fit to the rigor data of text Fig. 6, showing that the second Gaussian adds little to the rigor data. EDC cross-linked myofibrils. The fit was made with Origin v. 8.5, which allows "forcing" the second Gaussian on the data. The numbers are given in order: area, center width height in clockwise order, starting from upper left. Upper Left: minor peak (red): 0.82462, 0.1283, 0.1228, 5.35773; major peak (green) 14.37968, -0.03971, 0.21856, 52.49544. Upper Middle: minor peak (red) 1.20808, 0.0335, 0.11703, 8.2366; major peak (green): 21.71532, 0.06157, 0.31485, 55.03056. Upper Right: minor peak (red) 1.57167, -0.1198, 0.14996, 8.36239; major peak (green) 18.24836, 0.07354, 0.23784, 61.21869. Lower Left: minor peak (red) 0.26789, 0.15766, 0.0393, 5.43849; major peak (green) 15.46251, 0.01562, 0.24175, 51.03383. Lower Middle: minor peak (red) 1.4487, 0.19353, 0.18414, 6.27738; major peak (green) 18.23233, -0.00736, 0.27435, 53.02543. Lower Right: minor peak (red) 0.36099, -0.11915, 0.02241, 12.85047; major peak (green) 19.83176, 0.0639, 0.3003, 52.69175.*


E1-rigor-111010
E2-rigor-111010.
E3-rigor-111010
E4-rigor-111010
E5-rigor-111010
E6-rigor-111010
events
Polarization
21.92
1.12
22.92
1.21
22.47
0.00
23.36
1.27
25.05
0.00
23.84
0.00

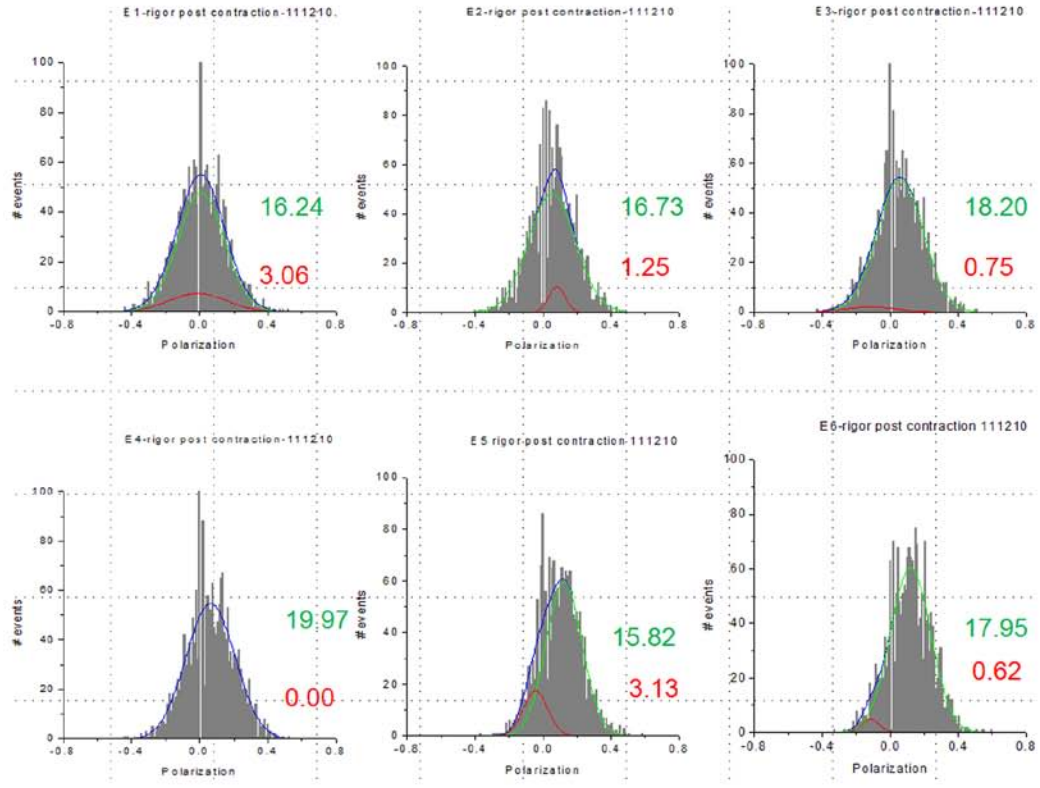

E1-rigor post contraction-111210.
E2-rigor post contraction-111210
E3-rigor post contraction-111210
E4-rigor post contraction-111210
E5 rigor post contraction-111210
E6-rigor post contraction 111210
events
Polarization
16.24
3.06
16.73
1.25
18.20
0.75
19.97
0.00
15.82
3.13
17.95
0.62

Fig. 5S*. Check for reversibility of rigor. The naive rigor was induced by transferring muscle from glycerinating solution (containing ATP and EGTA) to rigor solution and incubating it for 0.5 hrs before making myofibrils (first rigor, top panel). The myofibrils were then contracted and transferred to rigor solution again (second rigor, bottom panel). Double Gaussian fit was then forced by Origin 8.5 program. The second Gaussian (red) constitutes only 2.6% of the major peak (green) in the naivet rigor data and 8.8% in the second rigor data. EDC cross-linked myofibrils. In the contracting data from the same myofibrils (following figure), the second Gaussian (red) constitutes over 50% of the major peak (green). Red and green text indicated area under red and green peaks, respectively.*

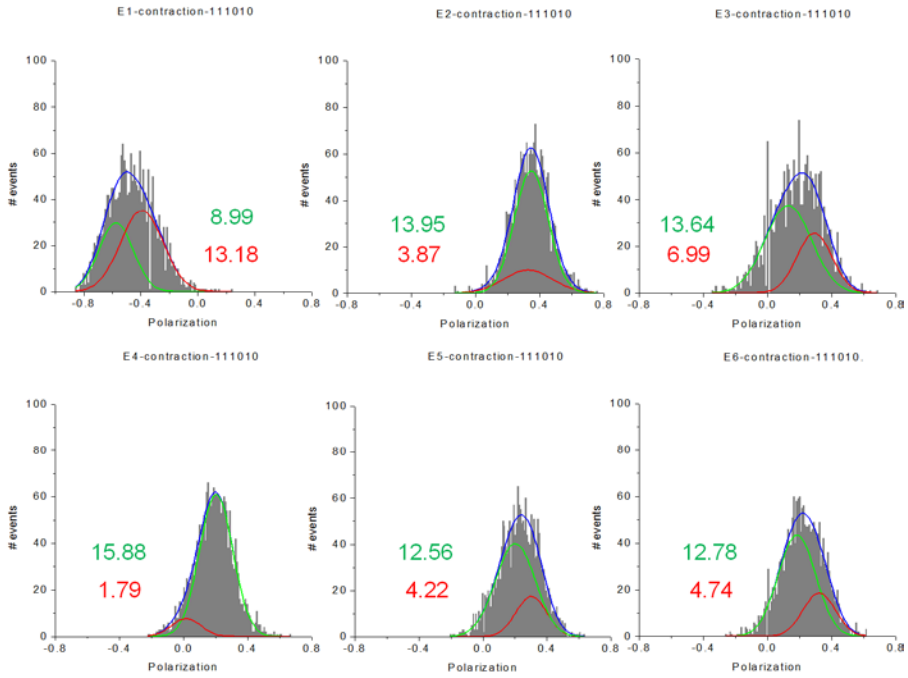


***Fig. 6S**. Distribution of fluctuations of myofibrils contracting after the naive rigor. The same myofibrils as in the previous figure. Parallel polarization of fluorescence (negative of polarization used elsewhere in this paper). The second Gaussian (red) constitutes 51% of the major peak (green). EDC cross-linked myofibrils. Red and green text indicated area under red and green peaks, respectively.*