Supporting Information

Nanothermometry reveals calcium-induced remodeling of myosin

Eric R. Kuhn[1†], Akshata R. Naik[1†], Brianne E. Lewis[2], Keith M. Kokotovich[1], Meishan Li[3], Timothy L. Stemmler[2], Lars Larsson[3], Bhanu P. Jena[1*]

[1]*Department of Physiology, School of Medicine;* [2]*Department of Pharmaceutical Science, College of Pharmacy; Wayne State University, Detroit, MI 48201, USA*
[3]*Department of Physiology and Pharmacology, Karolinska Institutet, SE-171 77 Stockholm, Sweden*

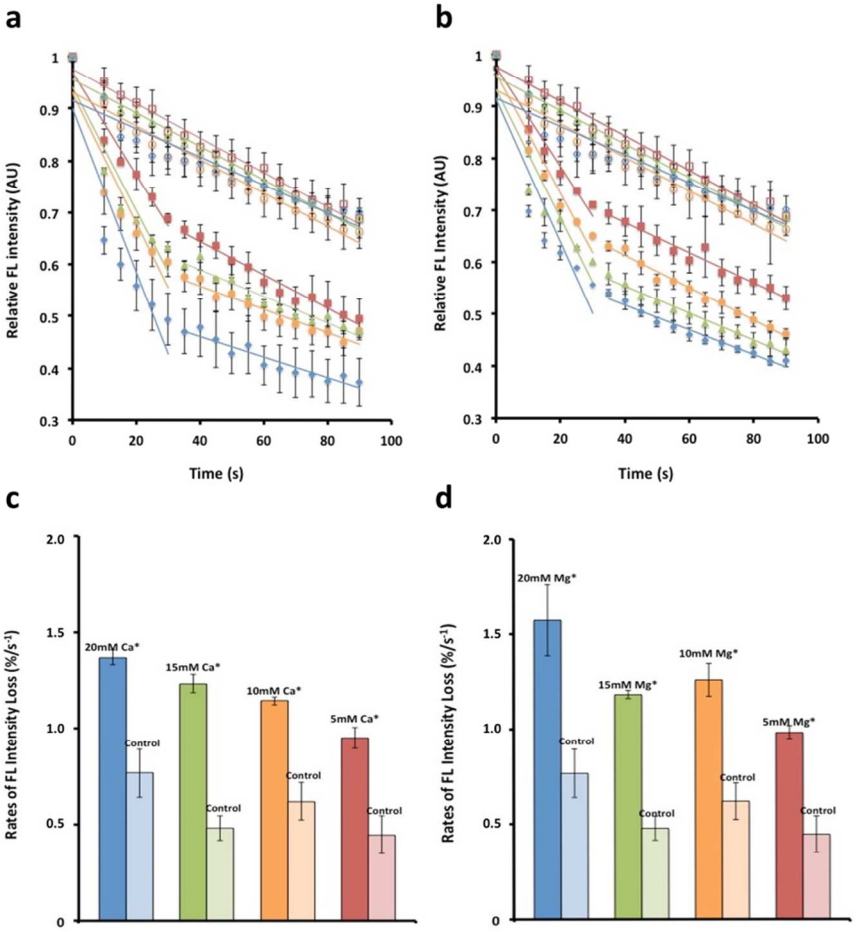

Figure 1S. Cadmium telluride quantum dots adhered to myosin molecules function as nanoscale thermometers, enabling the detection of heat loss from the motor protein on binding to calcium or magnesium. Similar to BC myosin (Figure 1), binding of calcium to rabbit skeletal (RS) myosin lowers the energy state of the molecule. (a) Cadmium telluride (CdTe) quantum dots used as molecular thermometers in this study are able to detect mK changes in temperature of the myosin molecule. CdTe quantum dots associated with the myosin molecule are able to detect changes in temperature of the molecule as a reflection of fluorescence. The greater the heat loss, the lower the fluorescence[11]. Calcium concentrations used were: 5 mM (solid red squares), 10 mM (solid green triangles), 15 mM (solid orange

circles), and 20 mM (solid blue diamonds). Color coded corresponding controls were empty symbols. This calcium dependent drop in fluorescence is a measure of heat release. Real-time fluorimetry demonstrates the extent and rate of this heat release. (b) There is similarly, a magnesium dependent drop in fluorescence, however, to a lesser extent than calcium. (c, d) Rate of fluorescence loss in the first 30 sec following addition of calcium or magnesium. (c) Note that the rate of heat release in the presence of increasing calcium concentration is 30% greater than is observed with magnesium (d). (n=3, *p< .05)

Table 1S. Secondary structure fit parameters of bovine cardiac (BC) myosin in the presence and absence of various increasing concentrations of calcium. Note the decrease in alpha helical content with in the presence of increased calcium. There is an approximate 30-33% dose dependent decrease in helical content in the presence of various calcium concentrations compared the absence of calcium. An average of 30 scans were collected in total for each sample from triplicate preparations for each concentration of calcium used.

Table 1S

Secondary structural fit parameters of bovine calf myosin in $CaCl_2$

BC myosin + ionic condition	α	β	*O*	*U*	fit[a]
0mM $CaCl_2$	79.4	6.8	4.7	9.2	0.03
5mM $CaCl_2$	54.9	9.5	16.2	19.3	0.05
10mM $CaCl_2$	52.0	10.7	17.0	20.3	0.06
15mM $CaCl_2$	55.4	8.4	18.4	17.8	0.05
20mM $CaCl_2$	52.7	10.4	17.5	19.2	0.05

Abbreviations used: α, alpha-helix; β, beta sheet; *O*, other; *U*, unordered.

[a]Fit: goodness of fit parameter expressed as normalized spectral fit standard deviation (nm).

Table 2S. Secondary structure fit parameters of rabbit skeletal (RS) myosin in the presence and absence of various increasing concentrations of calcium. Note the decrease in alpha helical content with in the presence of increased calcium. There is an approximate 11-28% decrease in helical content in the presence of various calcium concentrations compared the absence of calcium. An average of 30 scans were collected in total for each sample from triplicate preparations for each concentration of calcium used.

Table 2S
Secondary structural fit parameters of rabbit skeletal myosin in $CaCl_2$

RS myosin + ionic condition	α	β	O	U	fit[a]
0mM $CaCl_2$	55.6	10.7	14.2	19.5	0.05
5mM $CaCl_2$	50.0	10.9	12.4	15.0	0.05
10mM $CaCl_2$	48.5	12.4	18.3	21.5	0.05
15mM $CaCl_2$	40.4	15.0	21.1	23.5	0.05

Abbreviations used: α, alpha-helix; β, beta sheet; O, other; U, unordered.
[a]Fit: goodness of fit parameter expressed as normalized spectral fit standard deviation (nm).

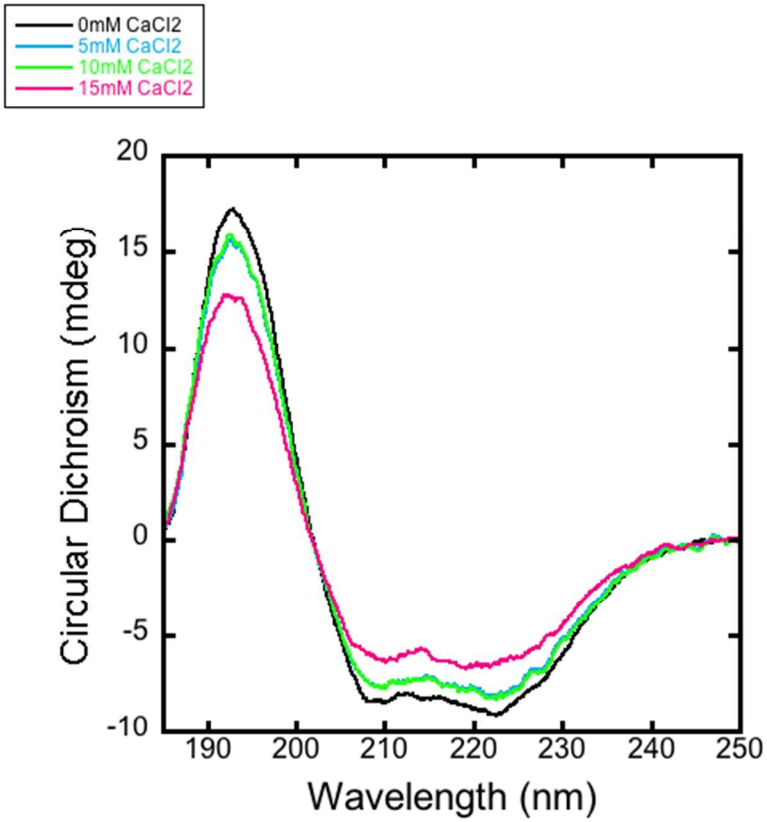

Figure 2S. Circular dichroism (CD) spectroscopy of rabbit skeletal (RS) myosin confirms unwinding of the alpha helix. An average of 30 scans were collected in total for each sample from triplicate preparations. Note the loss of alpha helical content in the presence of calcium.

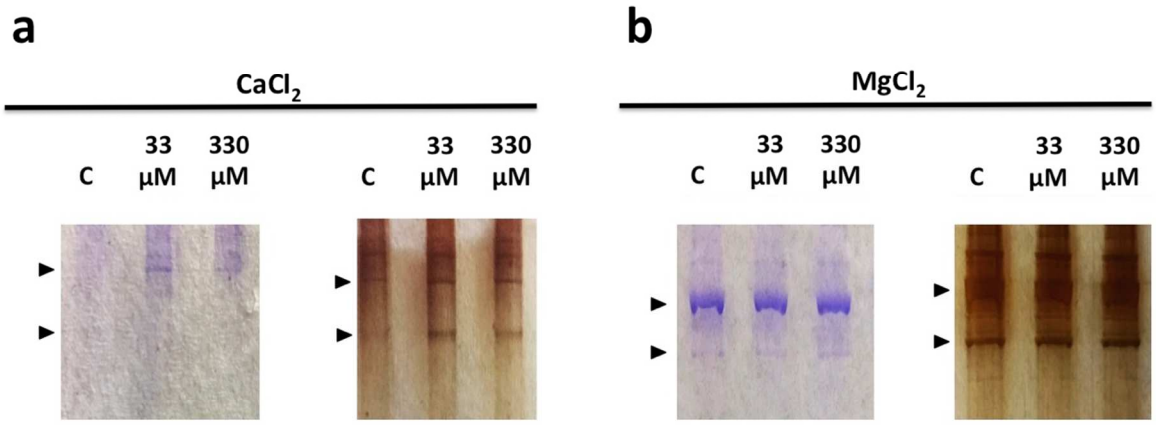

Figure 3S. Calcium mediated structural changes in myosin enable close interaction between the molecules as indicated by their ability to cross link in the presence of paraformaldehyde (PFA). (a) Coomassie stained gel of resolved PFA cross-linked myosin molecules in the absence and presence of 33 µM and 330 µM calcium. Note the myosin molecules shown in the lower arrowhead and the complexed form in the upper arrowhead pointing to the higher molecular weight complex observed only in the presence of calcium. This is also seen in the right image of the same gel stained with silver. (b) Magnesium, in contrast to calcium, does not show any significant differences in the interactions between myosin molecules.

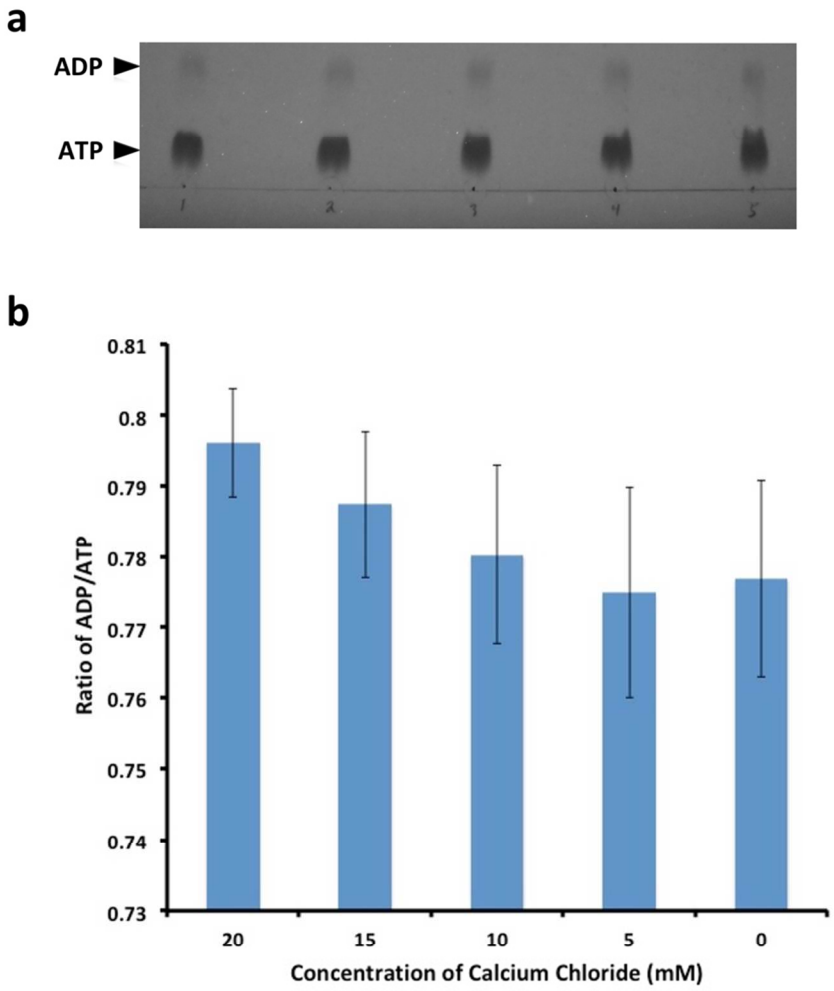

Figure 4S. Thin layer chromatography of ATP hydrolysis demonstrating no significant change in the ratios of ATP to hydrolyzed ADP in the presence of different calcium concentrations. (a) This demonstrates that there is no difference in the total amount of ATP hydrolyzed with different calcium concentrations of 20 mM (1), 15 mM (2), 10 mM (3), 5 mM (4), and 0 mM (5) of calcium chloride. (b) Bar graph showing the ratio of ATP to ADP produced in various concentrations of calcium chloride demonstrating no significant difference among the concentrations. (n=3).

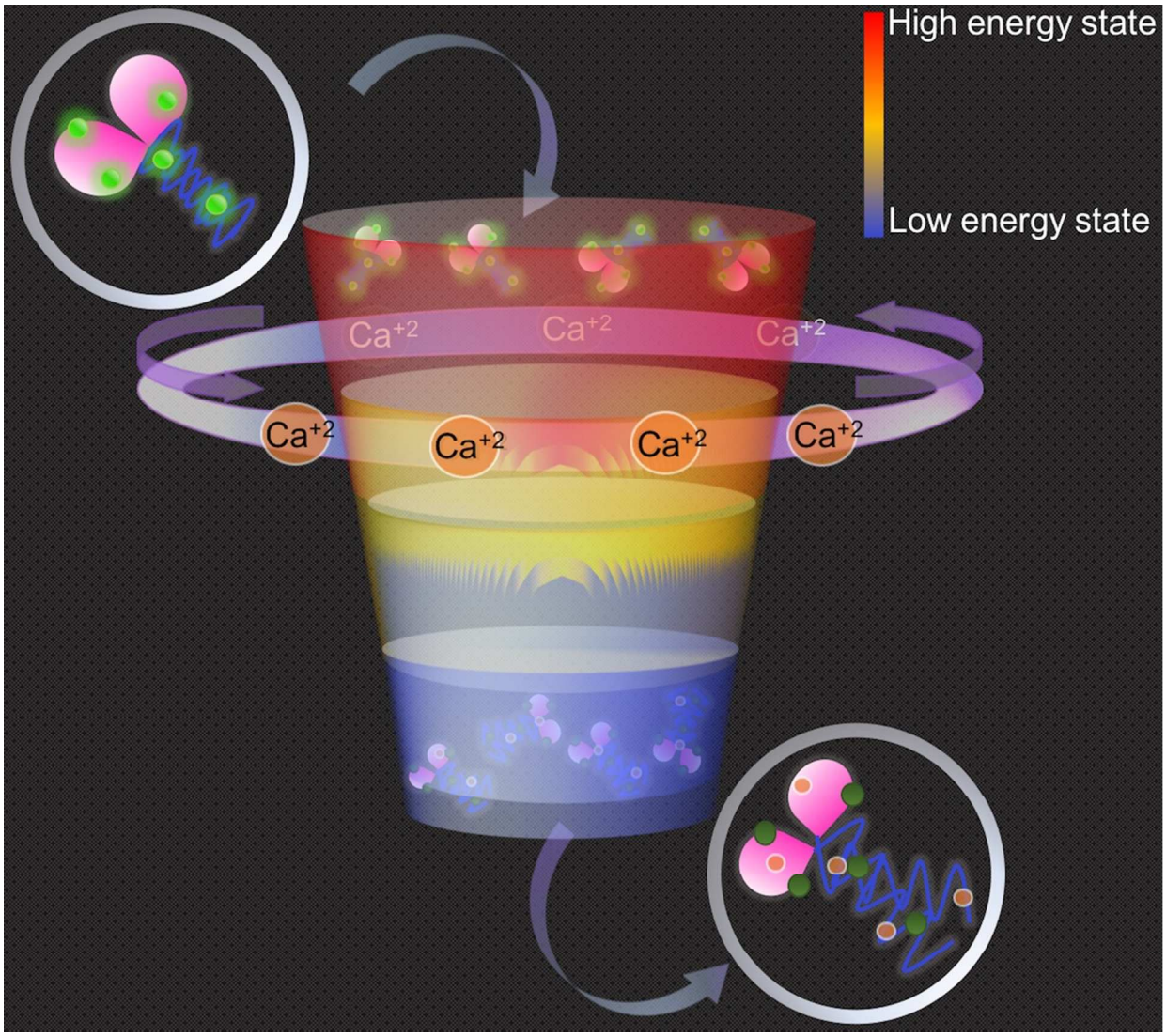

Figure 5S. Schematic drawing showing changes in enthalpy and structure of proteins following binding of ions, that can be detected using quantum dot (QD)-based nanoscale thermometry and circular dichroism spectroscopy (CD) respectively. Note the heat loss (lower right circle with dim QD's compared to the circle in the upper left with bright green QD's) and unwinding of the myosin (from CD studies) following exposure to calcium. Hence in the presence of high calcium, the myosin is present in a more stable low energy state.

Table 3S. Actin on myosin motility assay demonstrating no significant change in velocity at lower concentrations of calcium and a significant change at 5 mM calcium. (n=4; P< 0.02).

Muscle Fiber ID	Concentration of Calcium (mM)				
	0	0.1	0.3	1	5
20171108 F4	0.4499	0.4251	0.4189	0.3731	0.4204
20171109 F2	0.7104	0.7361	0.6064	0.3906	0.3439
20171117 F4	0.6086	0,4778	0.5449	0.3945	0.2877
20171124 F4	0.5072	0.5316	0.4621	0.4814	0.4499
Mean ± SD (μm/s)	0.608±0.083	0.543±0.136	0.508±0.084	0.410±0.049	0.375±0.074
P	0.013				