PNAS
www.pnas.org

Supplementary Information for

Revealing the mechanism of how Cardiac Myosin-Binding Protein C N-terminal Fragments Sensitize Thin Filaments for myosin binding

Alessio V. Inchingolo[1], Samantha Beck Previs[2], Michael J. Previs[2], David M. Warshaw[2*] and Neil M. Kad[1,*]

[1]School of Biological Sciences, University of Kent, Canterbury, CT2 7NH, UK. [2] Department of Molecular Physiology and Biophysics, Cardiovascular Research Institute, University of Vermont, Burlington, Vermont 05405 USA

[*] To whom correspondence should be addressed.

This PDF file includes:

www.pnas.org/cgi/doi/10.1073/pnas.1816480116

Derivation of proportional population of the A_{Cl}.M state.

$$A_B + Ca^{2+} \overset{K_{Ca}}{\longleftrightarrow} A_{Cl} + M \overset{K_2}{\leftrightarrow} A_{Cl}.M$$

The proportion of the A_{Cl}.M state of the thin filament is derived from the chemical equation above, as below:

$$pA_{Cl}.M = \frac{A_{Cl}.M}{A_B + A_{Cl} + A_{Cl}.M}$$

$$= \frac{M \cdot [Ca^{2+}]}{K_1 \cdot K_2 + [Ca^{2+}] \cdot K_2 + M \cdot [Ca^{2+}]}$$

Determining the intensity of a single fluorophore

To quantify the fluorescence intensity of a single fluorophore, we fitted a series of fluorescent spots seen at low calcium conditions where binding was sparse and therefore, more likely to derive from a single molecule. In addition, by keeping the frame rate and illumination power constant throughout all the acquisitions using Cy3-labelled cMyBP-C fragments, we ensured that the fluorescence emission from Cy3 remained approximately the same (1). These values for Cy3-C0C3 binding to thin filaments at low calcium (0.1 μM, pCa 7, Fig. 3a left panel) are shown as a histogram in Figure S2. This histogram was fitted to six Gaussian distributions using least squares in Microsoft Excel. We selected the maximum number of distributions for fitting by increasing the number of Gaussians until an additional Gaussian led to it being overlaid on top of another peak with no substantial improvement of fit. The intensity and the mean of the Gaussian distributions were fit independently while their standard deviation was constrained to one value for all the distributions, to reduce the number of degrees of freedom used in the fitting.

The mean values for each of the Gaussian components follow a linear relationship with the number of molecules within each fluorescence spot, with a slope of 372.4, corresponding to the intensity increase for the addition of another fluorophore (inset in Fig. S1). This value was used as a scaling term for the number of Cy3 molecules in all other conditions.

Supplementary Figures

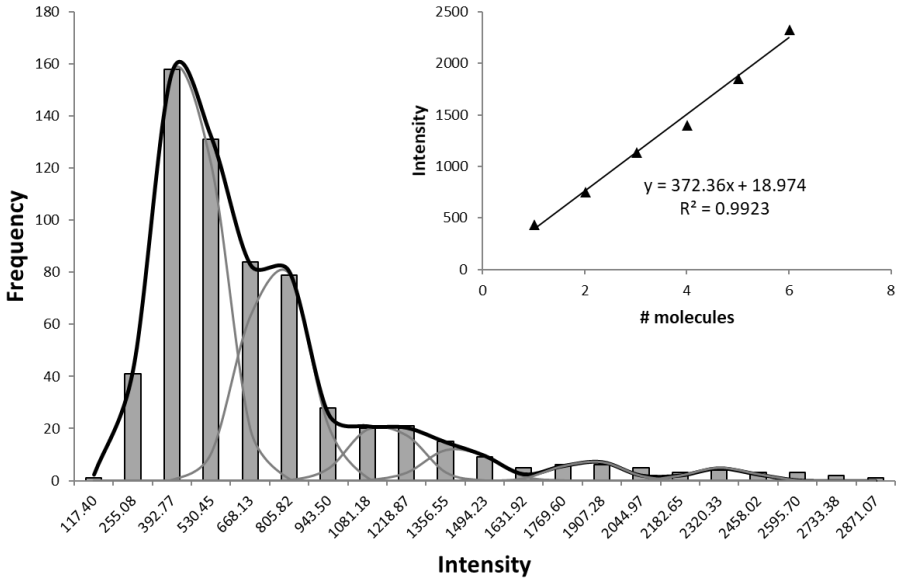

Fig. S1. Determining the fluorescence intensity of a single Cy3-C0C3: a) A histogram of fluorescence spot intensities (bars) were fitted to a sum of Gaussian distributions (individual component Gaussians are shown in grey and the sum is the dark line) of fixed standard deviation. The inset shows the peak value for each Gaussian distribution plotted against a linear increasing scale, equivalent to the number of molecules present in that peak. The slope of this linear plot provides the fluorescence intensity of a single Cy3 molecule as 372.4 arbitrary units.

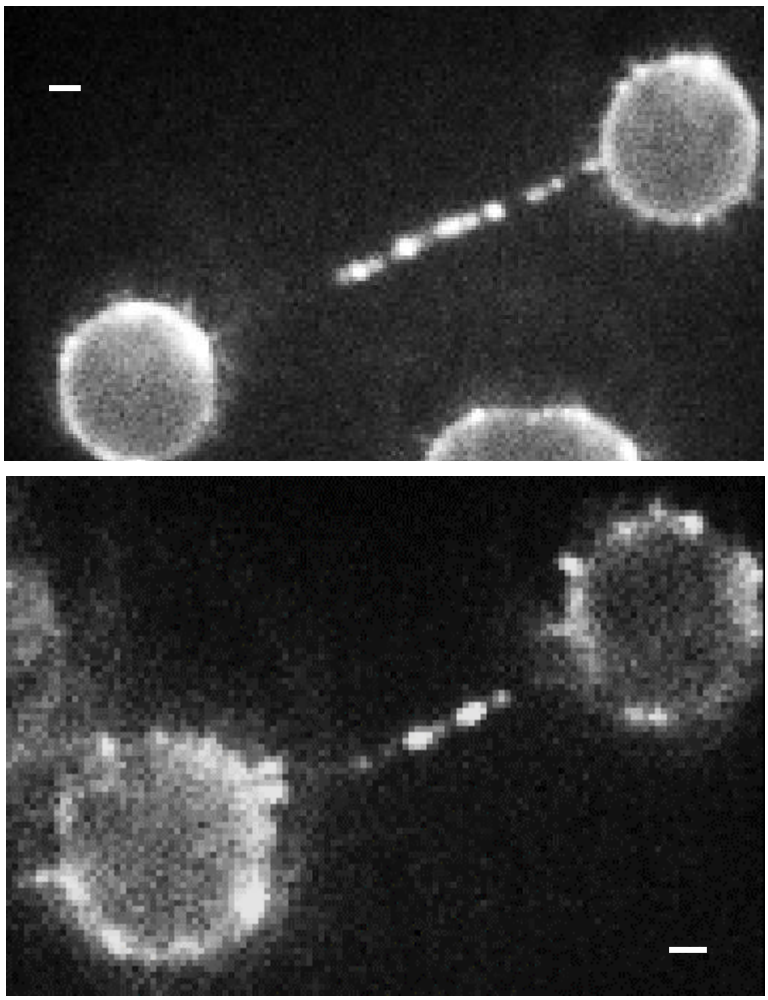

Fig. S2. Punctate clustering of Cy3-C0C3 on thin filaments at high calcium: Two representative images showing the punctate binding of C0C3 to the thin filament. Large regions of undecorated actin are apparent suggesting that the binding of C0C3 is not random; instead binding of one C0C3 promotes the binding of others locally. The scale bars indicate 1 μm.

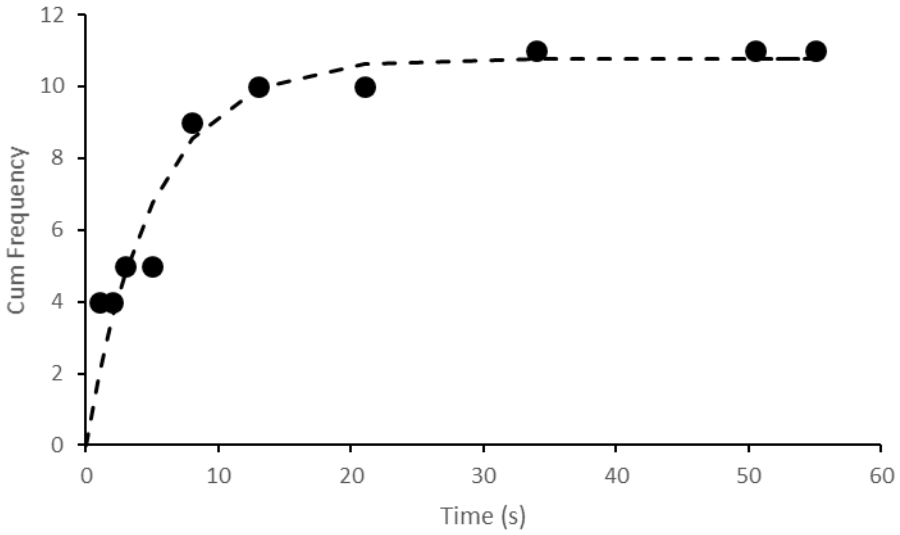

Fig. S3. Cumulative frequency histogram of Cy3-C0C3 on thin filaments at high calcium: The attached lifetimes of single (determined by intensity), static Cy3-C0C3 molecules bound to thin filaments at pCa 4 (high calcium) plotted as a cumulative frequency histogram. These data were fitted to an exponential function to yield the lifetime of attachment as 5.07 ± 3.05 s (>95% CI).

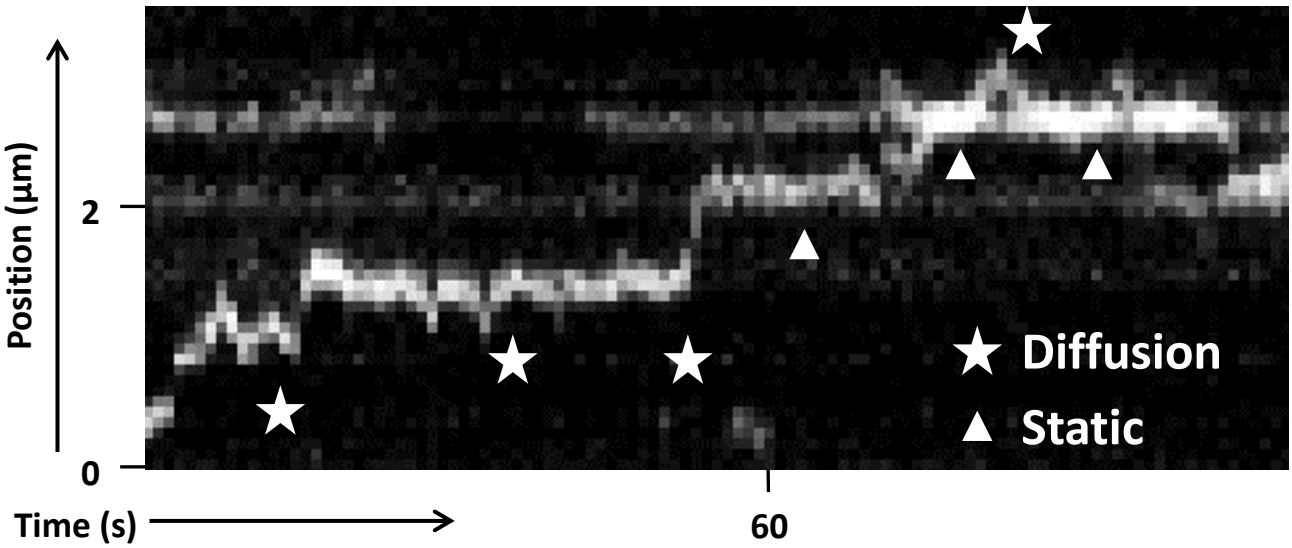

Fig. S4. Cy3-C0C3 switches between static binding and diffusion on thin filaments at low calcium: The motion of Cy3-C0C3 is complex including periods when statically bound (triangles) and diffusing (stars). The static periods may represent the formation of an incomplete binding interface.

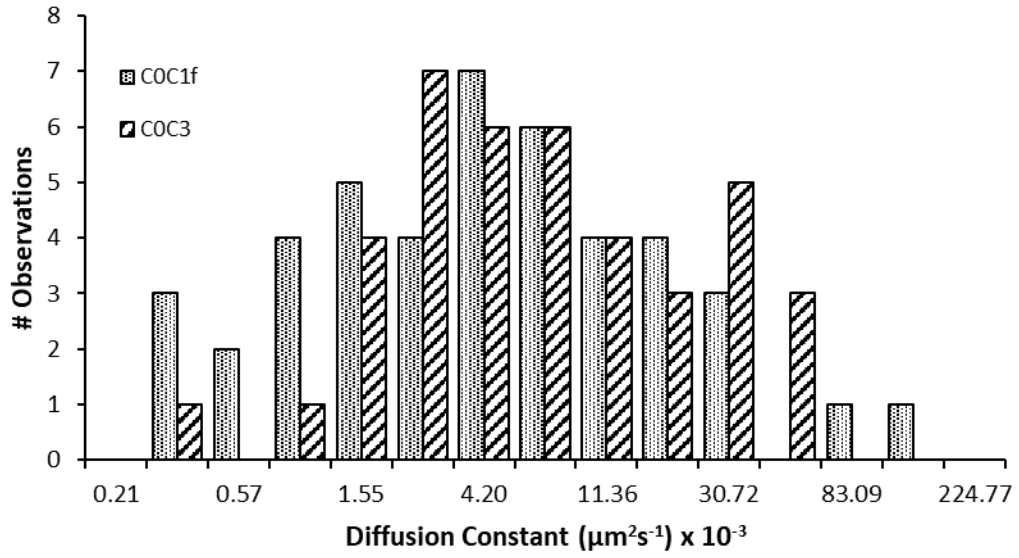

Fig. S5. Log-Normal distribution of diffusion constants N-terminal cMyBP-C fragments: Diffusion constants determined for Cy3-C0C3 (diagonal stripes) or Cy3-C0C1f (dappled shading) at low calcium show normal distributions on a logarithmic scale.

Table S1. Diffusional characteristics for N-terminal cMyBP-C constructs versus calcium concentration.

	Cy3-C0C3				Cy3-C0C1f				Cy3-C0C1*	
	0.1 μM Ca^{2+}		100 μM Ca^{2+}		0.1 μM Ca^{2+}		100 μM Ca^{2+}		0.1 μM Ca^{2+}	
	D ($\mu m^2/s$) $x10^{-3}$	α	D ($\mu m^2/s$) $x10^{-3}$	α	D ($\mu m^2/s$) $x10^{-3}$	α	D ($\mu m^2/s$) $x10^{-3}$	α	D ($\mu m^2/s$) $x10^{-3}$	α
Mean	6.28	0.98	0.68	1.36	4.06	1.00	2.95	0.88	1.13	0.71
SEM	1.24	0.07	0.21	0.16	0.90	0.07	1.15	0.08	0.40	0.09
n	32	33	5	6	39	39	15	15	9	12

**Due to insufficient binding, diffusion data were not acquired for C0C1 at 100 μM Ca^{2+}*
Distributions of representative diffusion constants are shown in Fig. S5

References

1. Walcott S & Kad NM (2015) Direct Measurements of Local Coupling between Myosin Molecules Are Consistent with a Model of Muscle Activation. *PLoS Comput Biol* 11(11):e1004599.