Supporting Information

Fast Pressure Jumps Can Perturb Calcium and Magnesium Binding to Troponin C F29W

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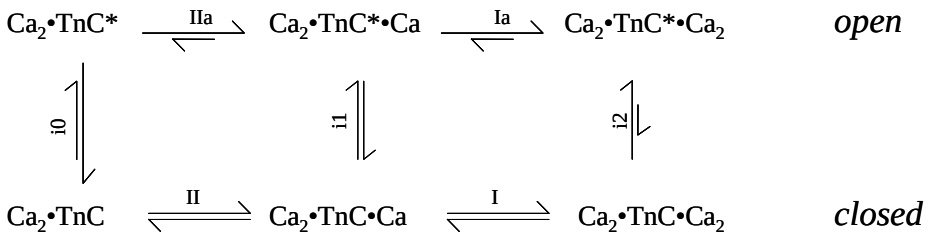
The following two sections show the results of testing the ability of the two models shown to reproduce or explain features of the data resulting from pressure jump, equilibrium fluorescence and stopped-flow experiments. The scheme numbers are the same as in the main manuscript. Where figures or equations are referred to, an 'S' means that they are in this document, otherwise the main manuscript is being directly referenced.

Section S1 – Cooperative N-Terminal Calcium Binding:
Based on the Monod-Wyman-Changeux (MWC) scheme (scheme 3):



Scheme 3

The MWC model explains cooperativity as being the result of the dependency of both the calcium binding and the isomerization steps on the calcium occupancy of the N-terminal sites. In scheme 3, the degree of cooperativity is governed by the ratios between the equilibrium constants on opposite sides of the squares. For this work a value of 10 was chosen for this factor, thus $K_{IIa}/K_{II} = K_{Ia}/K_I = 10$, which implies, through the theory of detailed balance, that $K_{i1} = 10.K_{i0}$ and $K_{i2} = 10.K_{i1}$ (see Table S1 for values of rate constants). Kinetic modeling to scheme 3 was carried using Berkeley-Madonna software (www.berkeleymadonna.com). The system of differential equations was set-up using the chemical reactions module and included the Ca/EGTA system to simulate the pCa buffered environment. We ran a simulation of a 350 bar pressure increase at pCa 5.52. The result approximated the measured transient (Fig. S1).

We started from the simplest assumptions, *i.e.* that there is a single isomerization that is accompanied by a change in fluorescence and a volume change. This model was insufficient to account for the data as shown in Fig. S1 so that adding a single volume change onto the calcium binding step was necessary. The volume changes that were used in this pressure jump simulation were +6 $mL.mol^{-1}$ for the isomerization into the *open* configuration and -9 $mL.mol^{-1}$ for a single calcium binding to either N-terminal site. The sum of these $\Delta V°$ values by any route going from the apo *closed* state (Ca_2•TnC) to the fully calcium bound, *open* state (Ca_2•TnC*•Ca_2) is thus -12 $mL.mol^{-1}$. The fluorescence change between the *open* and *closed* configurations required was a factor of 4, because the values of the constants used imply that the *open* and *closed* conformers cannot be completely occupied by either saturation with or removal of calcium. Thus, in Table S1 'ΔF' refers to the microscopic change in fluorescence whereas in section S2 'Q' is the observed change in fluorescence. The simulated transient did not account for phase 0, so this was added to Fig. S1 for the sake of clarity.

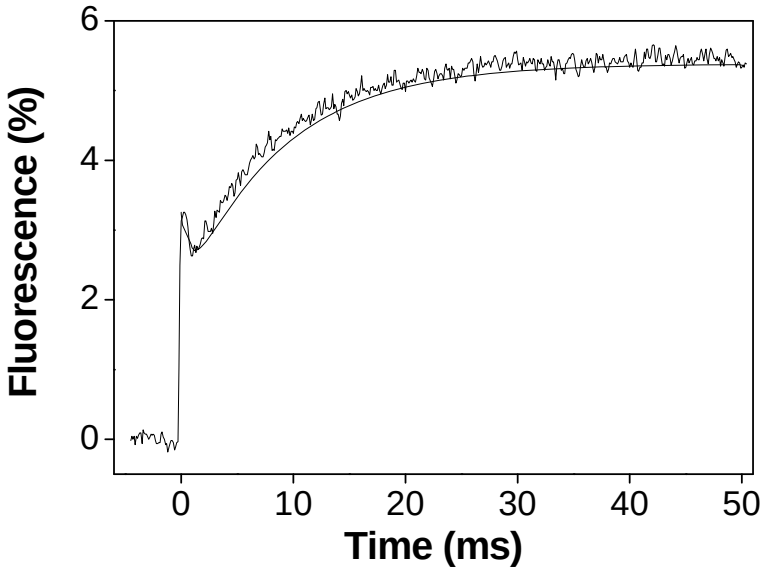


Fig. S1 Fluorescence transient resulting from a 35.0 MPa pressure increase applied to 10 µM TnC F29W at pCa 5.52 (noisy curve) and a simulation based on scheme 3 (smooth curve). The values of the model components are given in Table 1.

Step #	k_+	k_-	K	$\Delta V°$ (mL.mol^{-1})	ΔF
i0	375 s^{-1}	3750 s^{-1}	0.1	+6	4
i1	375 s^{-1}	375 s^{-1}	1	+6	4
i2	375 s^{-1}	37.5 s^{-1}	10	+6	4
II	75 s^{-1}.µM^{-1}	750 s^{-1}	10 µM	-9	0
IIa	75 s^{-1}.µM^{-1}	75 s^{-1}	1 µM	-9	0
I	75 s^{-1}.µM^{-1}	750 s^{-1}	10 µM	-9	0
Ia	75 s^{-1}.µM^{-1}	75 s^{-1}	1 µM	-9	0

Table S1 Parameter values used for simulations using the MWC scheme. Forward rate constants and volume changes are defined as starting in the apo-*closed* state and moving towards the calcium bound-*open* state.

Using the same model, we simulated the pCa jump experiment as shown in Fig. 6 (Fig. S2).

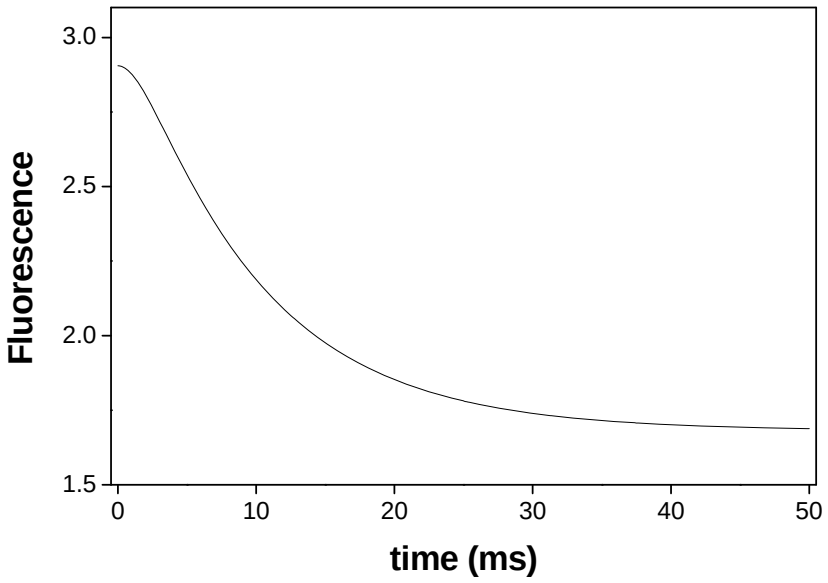


Fig. S2 Results of a simulation of a pCa jump from 4 to 6.2 on 10 µM TnC F29W. The simulated data was well-fitted by a double exponential (A_1 = +27 %, k_1 = 617 s^{-1}, A_2 = -150 %, k_2 = 108 s^{-1}). The values of the model components are given in Table 1.

The resulting simulated transient (filled circles) was well fitted by a double exponential (Smooth curve - A_1 = +27 %, k_1 = 617 s^{-1}, A_2 = -150 %, k_2 = 108 s^{-1}). Although the amplitudes and observed rate constants resulting from this fit were not the same as was observed in the real stopped-flow pCa jump experiment, they do share the property that the ratio $-A_1/A_2 \sim k_2/k_1$, which is indicative of the accumulation of an optically silent intermediate. This property is dependent on the condition $k_{-i2} < k_{-IIa}$ as this ensures that the first step is optically silent. The value of k_{-i0} was adjusted to 3750 (as opposed to making k_{+i0} =3 7.5) in order to allow the apo N-terminal sites to re-equilibrate quickly during the pCa jump simulation. Thus this MWC scheme is capable of explaining a result normally associated with a linear scheme.

Section S2 – Calcium/Magnesium Competitive Effects:
Based on the following scheme for competitive Ca/Mg binding:

$$\begin{array}{ccccc} TnC & \overset{1}{\longleftrightarrow} & Ca_2{\cdot}TnC & \overset{2}{\longleftrightarrow} & Ca_2{\cdot}TnC^*{\cdot}Ca_2 \\ \updownarrow Mg & & & & \\ Mg_2{\cdot}TnC & & & & \end{array}$$

Scheme 1

where Ca_2•TnC and Mg_2•TnC have the appropriate metals bound to their structural binding sites (III and IV) whereas Ca_2•TnC*•Ca_2 has both sets of sites filled with calcium. The following equation was used to calculate the occupancy of the high fluorescence state (Ca_2•TnC*•Ca_2):

$$\frac{[Ca_2{\cdot}TnC^*{\cdot}Ca_2]}{[TnC]_{TOTAL}} = \frac{[Ca]^2}{\left([Ca]^2 + K_1^2\right)} \cdot \frac{[Ca]^{n_2}}{\left([Ca]^{n_2} + K_2^{n_2}\right)} \qquad \text{S1}$$

where n_2 is the Hill coefficient for calcium binding to the N-terminal sites. Note that this equation includes the following assumptions:

- The fluorescence change is exclusive to cooperative calcium binding to the N-terminal sites.
- The value of K_1 is defined by the pCa position of the trough minimum in the amplitude plot of Fig. 8 or Fig. S4 (open triangles), thus pK_1 = 6.5.
- Cooperative binding at the C-terminal sites (steps 'Mg' and '1') is 'perfect' third order, *i.e.* there is no significant occupancy of any intermediate or mixed binding states (Ca•TnC, Mg•TnC or CaMg•TnC). This is justified by the fitted Hill coefficient values close to 2 in the magnesium titration experiment (Fig. 10).

The normalized fluorescence data (F) obtained in the absence of magnesium was fitted using the following logistic sigmoid form of Equation S1:

$$F(\mathrm{Ca}) = 1 + \frac{1}{1+10^{2(\mathrm{pCa}-\mathrm{p}K_1)}} \cdot \frac{(Q-1)}{1+10^{n_2(\mathrm{pCa}-\mathrm{p}K_2)}} \qquad \text{S2}$$

where Q is the observed increase in fluorescence upon saturating the N-terminal sites with calcium. The fit yielded values of 2.96 for Q, 5.76 for pK_2 and 1.22 for n_2 (Fig. S3). Only n_2 was significantly changed from the simple Hill fit as shown in Fig. 9. The data obtained in the presence of 2 mM magnesium was fitted to Equation S2 using fixed values from the previous fit (defining calcium binding) but allowing pK_1 to vary. The value we obtained was 5.62. Equation 2 was used to estimate the magnesium affinity from these results and a value of K_{Mg} = 300 µM was obtained. Note that the apparent increase in cooperativity observed upon addition of magnesium was replicated using this simple adjustment.

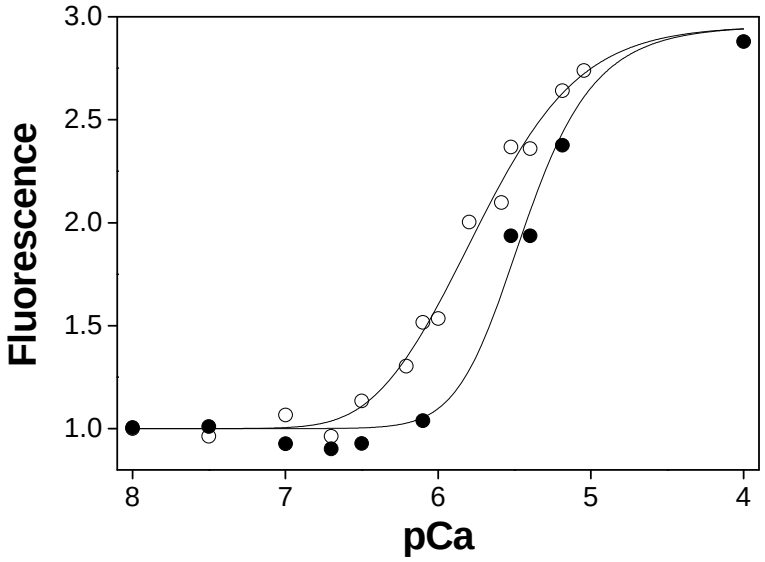


Fig. S3 Equilibrium fluorescence of 10 µM TnC F29W in the presence (filled circles) and absence (empty circles) of 2 mM magnesium. Fits to Equation S1: In the absence of magnesium Q = 2.96, pK_1 = 6.5 (fixed), pK_2 = 5.76, n_2 = 1.2. In the presence of magnesium all values fixed to previous except pK_1 = 5.62.

The expected amplitudes from pressure jump experiments (increasing pressure) were calculated in the presence and absence of magnesium using scheme 1 (Fig. S4), by calculating the changes in the affinity constants using equation 4 and using the change in fluorescence calculated according to equation S2 to provide estimates of the amplitude as a function of pCa. The value of $\Delta V_1°$ was set using the value calculated from the MWC modelling (-12 mL.mol^{-1} - see section S1). Utilizing the other values directly from the model above yielded volume changes of +25 mL.mol^{-1} for calcium binding to the C-terminal sites and +35 mL.mol^{-1} for magnesium binding to the C-terminal sites.

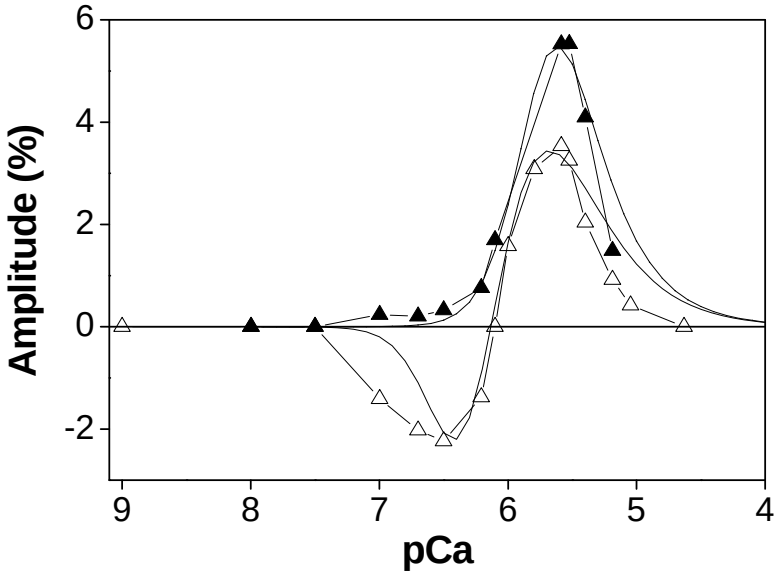


Fig. S4 Fluorescence amplitudes resulting from 35.0 MPa pressure increases over a range of pCa in presence (filled triangles) and absence (empty triangles) of 2 mM magnesium. Smooth curves calculated using scheme 1 with values of $\Delta V°_1$ = +25 mL.mol^{-1}, $\Delta V°_2$ = -12 mL.mol^{-1} and $\Delta V°_{Mg}$ = +35 mL.mol^{-1}.

Conclusions

1. Scheme 3 reproduced an example pressure jump profile with reasonable fidelity.
2. The double-exponential decay observed upon mixing TnC F29W with a high EGTA buffer (pCa jump) is consistent with scheme 3 as well as with sequential calcium dissociation, though the absolute values of the observed rate constant and amplitudes were not correct.
3. Equilibrium binding of calcium and magnesium to TnC F29W is explained by the branched pathway of scheme 1 (Fig. S1).
4. The increase in apparent cooperativity observed upon addition of 2 mM magnesium arises as a result in the shift of the apparent binding constant K_1 to a value comparable to K_2 (Fig. S1).
5. Scheme 1 successfully reproduced the amplitude profiles observed for phase 2 in pressure jump experiments (Fig. S2), providing estimates of the molar volume changes upon cooperative calcium and magnesium binding to the C-terminal sites ($\Delta V_1°$ and $\Delta V_{Mg}°$, respectively), though these values were somewhat dependent upon the value used for $\Delta V_2°$ which was derived from the analysis of scheme 3.

Section S3 – Notes on Consecutive Reactions:

In the section on calcium dissociation from the regulatory binding sites of TnC F29W, the following scheme was used:

$$TnC^{*}{\cdot}Ca_2 \underset{+I[Ca]}{\overset{-I}{\rightleftharpoons}} TnC^{*}{\cdot}Ca \underset{+II[Ca]}{\overset{-II}{\rightleftharpoons}} TnC \qquad \text{Scheme 2}$$

The sum and product of the observed rate constants (λ_1 and λ_2) are given by the following equations:

$$b = \lambda_1 + \lambda_2 = [Ca]\left(k_{+I} + k_{+II}\right) + k_{-I} + k_{-II} \qquad \text{S4}$$

$$c = \lambda_1\lambda_2 = [Ca]^2\left(k_{+I}k_{+II}\right) + [Ca]k_{-I}k_{+II} + k_{-I}k_{-II} \qquad \text{S5}$$

For this system, the rate constants are given by a quadratic equation in λ. Equations S4 and S5 define the terms to be substituted into the quadratic root finder:

$$\{\lambda_1, \lambda_2\} = \frac{b \pm \sqrt{b^2 - 4c}}{2} \qquad \text{S6}$$

The equations for the corresponding amplitudes of two phases, when measured in the third species (TnC) – *i.e.* assuming that step I is optically silent, depend on both the intrinsic and observed rate constants (*1*):

$$A_1 = \frac{k_{+I}[Ca].k_{-I}}{\lambda_1\left(\lambda_1 - \lambda_2\right)}, \quad A_2 = \frac{k_{+I}[Ca].k_{-I}}{\lambda_2\left(\lambda_2 - \lambda_1\right)} \qquad \text{S7}$$

Taking the ratio of the equations in eqn S6 clearly yields eqn 8.

References:

(1) Capellos, C., and Bielski, B. H. J. (1972) *Kinetic systems: mathematical description of chemical kinetics in solution*, Wiley-Interscience, New York.