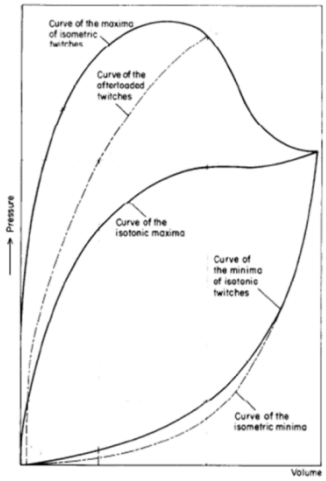
Introduction

Brief literature review on ES isometric vs. work-loop/ isotonic ES curves

A.



B.

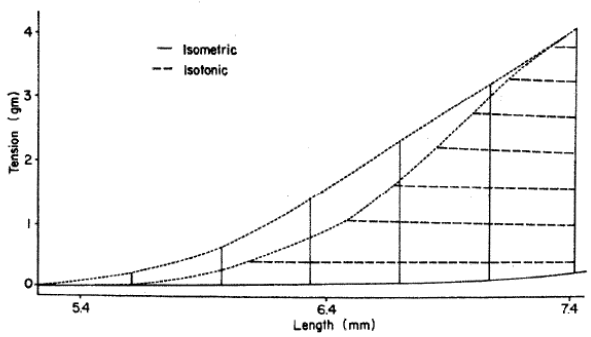


Figure 1

A: Stylised plot of passive (end-diastolic: lower-most two curved lines) and active (end-systolic: upper-most three curved lines) pressure-volume relationships of excised frog heart. Note the separate end-systolic relationship for isometric (solid line) and afterloaded (broken line) twitches. Modified from Figure 3 of Frank (1899), with permission of the Copyright Clearance Center via RightsLink: License Number 3840330537202. B: Example of the relative difference in end-systolic curves between isometrically and isotonically contracted rabbit papillary muscle. Reproduced from Figure 7 of Brady (1967), with permission of Oxford University Press.

Methods

How the model works. Details of the simulations:

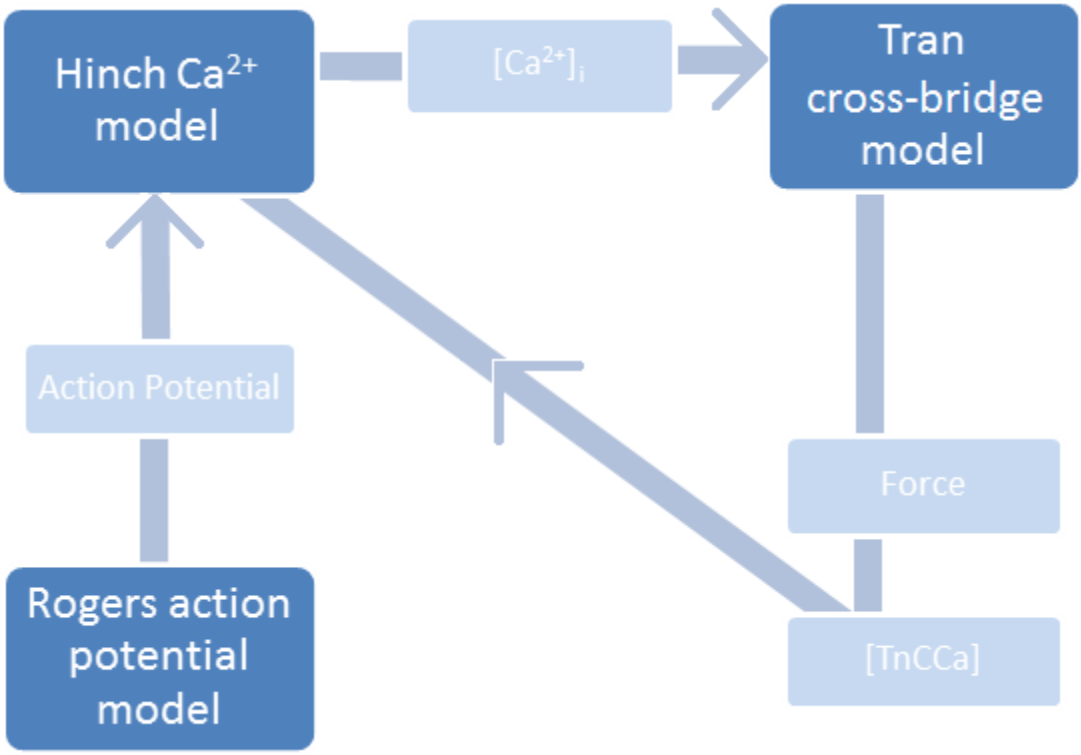


Figure 2

High-level coupling between the Tran et al. (2010) *cross-bridge, the Hinch et al.* (2004) *Ca^{2+}, and the Rogers and McCulloch* (1994) *action potential models.* [TnCCa] represents the intracellular concentration of Ca^{2+} bound to troponin-C.

Results

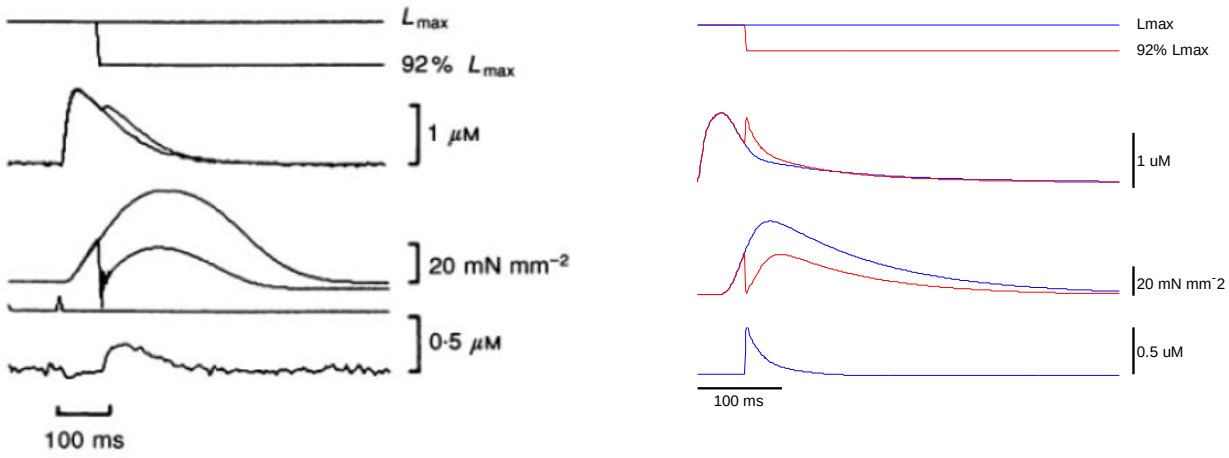


Figure 3
Experimental quick-release shortening of ferret papillary muscle (left; (Kurihara & Komukai, 1995)) and the simulated quick-release shortening of a single sarcomere (right).

The combined Tran-Rice-Hinch (TRH) model is used to examine the role Ca2+ plays in the end-systolic force-length relation of a cardiomyocyte contraction. But before any data is collected, we test the model's ability to replicate experimentally collected data. In doing so, we verify the combined model is mechanistically accurate.

This paper focuses on the interplay between cardiomyocyte force, sarcomere length, and intracellular Ca2+. Accordingly, model validation is focused on assessing the mechanisms responsible for force-dependent Ca2+ binding to troponin.

The experiment conducted Kurihara et al provides data for model validation. In this paper, tension-dependent changes of the intracellular Ca2+ transient are analysed by imposing quick-releases on isometric contractions. The sudden length change from a quick release results in an immediate drop in developed force and subsequent surge of intracellular [Ca2+]. Kurihara et al attribute this behaviour to _____ .

Replicating the Kurihara experiment with the combined model generates quantitatively similar results. A quick-release change in length causes a decrease in force and increase in Ca2+. The extent of the Ca2+ surge and redevelopment of force depends on the timing of the quick release, as expected. Figure 3 shows the comparison between experimental and model data.

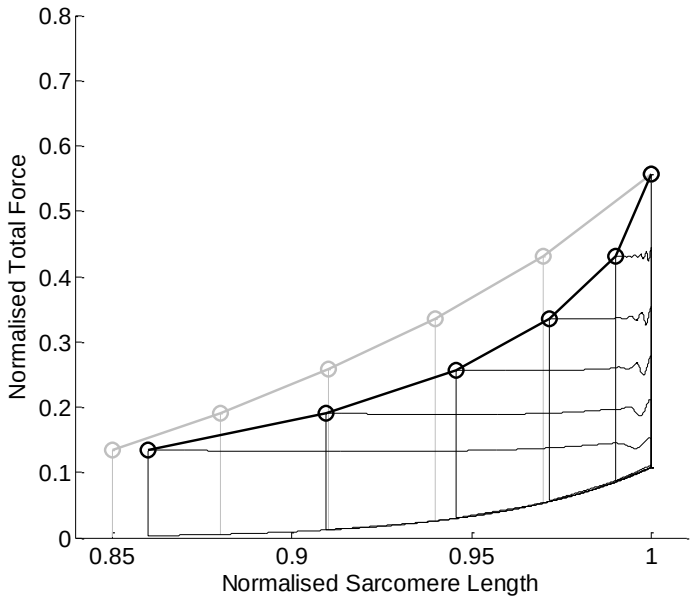


Figure 4

Two distinct, contraction-mode dependent end-systolic curves are an emergent property of the combined model. The Isometric end-systolic curve (grey) lies to the left of the work-loop end-systolic curve (black).

From the validated model emerges two, contraction-mode dependent ES curves (Figure 4). To understand why the location of the end-systolic force-length relation is contraction-mode dependent, it is helpful to consider the factors that determine end systolic length for each of the contraction types:

1) **Isometric:** The sarcomere-lengths for the isometric contractions (grey, Figure 4) are fixed during the contraction. The specific value is predetermined by the user.

2) **Work-loop:** The end-systolic sarcomere lengths for work-loop contractions (black, Figure 4) depend on the sarcomere's ability to maintain force. During the flat-top, isotonic phase, a sarcomere length will decrease as long as the total generated force is greater than the afterload.

Sustained force, then, is critical in determining the location of work-loop end-systolic, force-length curves. Equation 1 shows how force is calculated in the TRH model.

$$force = kxb \cdot SOVFThick \cdot (xXBpostr \cdot XBpostr + xXBprer \cdot XBprer) \qquad [1]$$

Force is proportional to the fractional occupancy of the strongly-bound states (XBprer and XBpostr) multiplied by the average distortion of these states (xXBprer and xXBpostr). $SOVF_{thick}$ is a scaling factor for the contribution of sarcomere geometry to the number of recruitable crossbridges (Rice et al., 2008). "kxb" is also a scaling factor that is set to 1 in for all simulations discussed in this paper.

Two main mechanisms determine whether total force is above or below the afterload value:

1) The overlap fraction (SOVFThick in equation [1])

The number of recruitable crossbridges varies with sarcomere length (Rice *et al.*, 2008).

However, an additional mechanism exists that enables a cardiomyocyte to generate different levels of force for the same sarcomere length. We see this in figure 4 where the same end-systolic sarcomere length is capable of generating vastly different end-systolic force levels (depending on contraction-mode).

Sarcomere length, therefore, is not the limiting factor in force production for a work-loop contraction. Thus, our attention will be focused on the second mechanism at play in generating and maintaining force.

2) The proportion of XBs that occupy a force-producing state.

A sarcomere generates force via strongly bound crossbridges. In equation 1, strongly-bound crossbridges are represented by two states, XBpostr and XBprer. The flux of crossbridges going in and out of these strongly bound states depends on intracellular [Ca^{2+}].

$[Ca2+]_i$ significantly influences the proportion of XBs in a permissive or non-permissive state. Only the XBs that move from a nonpermissive (N_{XB}) state to a permissive (P_{XB}) state can be recruited for cycling and force development (see figure below ADD figure label). (citation… Ca2+ affects activation)

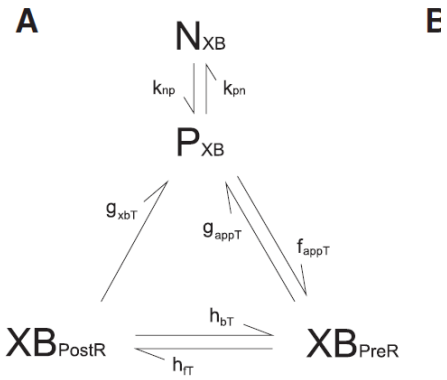


Figure label

We suspect that differences in the intracellular Ca2+ transients can sufficiently explain the presence of two distinct, contraction-mode dependent end-systolic curves due to [Ca^{2+}]'s role in determining crossbridge state.

There are clear, contraction-mode dependent differences in intracellular [Ca^{2+}] (grey vs. black Figure 5) as well as variation of transient shape within each individual contraction type.

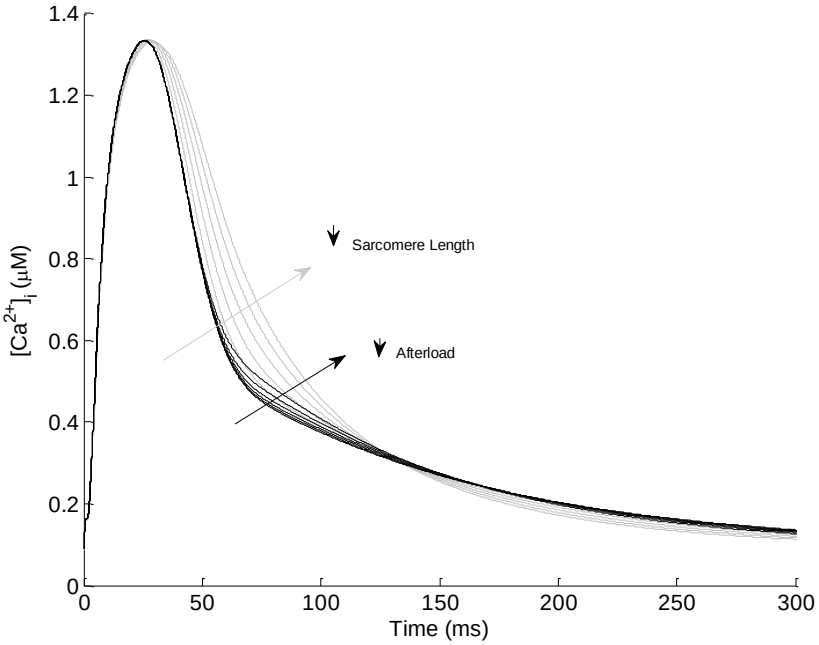


Figure 5

The shape of the intracellular [Ca^{2+}] transient depends on the contraction mode (gray vs. white). Additionally, sarcomere length (for isometric contractions-grey) and afterload (for work-loop contractions-black) affect the width of the intracellular [Ca^{2+}] transient

Notable information from Figure 5:

1) **Variation in Isometric Ca2**+: Initial SL affects Ca2+ width (before t=48ms / shortening)
2) **Variation in Work-loop Ca2+:** For lower afterloads, the isotonic phase of a work-loop contraction is characterized by a correspondingly lower rate of Ca2+ binding to troponin-C. This appears as a 'bump' in the intracellular Ca2+ transient during sarcomere shortening.
3) **General difference between Isometric and WL Ca2+:** Intracellular Ca2+ levels, for the most part, decrease quicker in work-loop contractions (steep black lines, Figure 5) than in isometric contractions (less-steep grey lines, Figure 5). Intracellular Ca2+ levels affect XB activation. A lack of activated XBs resulting from a quick drop-off in intracellular Ca2+ could affect the sarcomere's ability to maintain force.

We believe the relative lack of free [Ca2+] in work-loop contractions (Figure 5) is the main determining factor in the location of the ES point. The physical number of XBs in the thick/ thin filament overlap region would allow for the generation of force sufficient for continued isotonic shortening, but we believe the lack of free [Ca2+] causes the sarcomere to stop shortening 'prematurely'.

To test our hypothesis, isometric Ca2+ transients are inserted into WL contractions with the belief that the 'wider' isometric Ca2+ transients will allow for further isotonic shortening within work-

loops. We expect a leftward shift of the ES curve and ultimately aim to unite the isometric and work-loop ES curves.

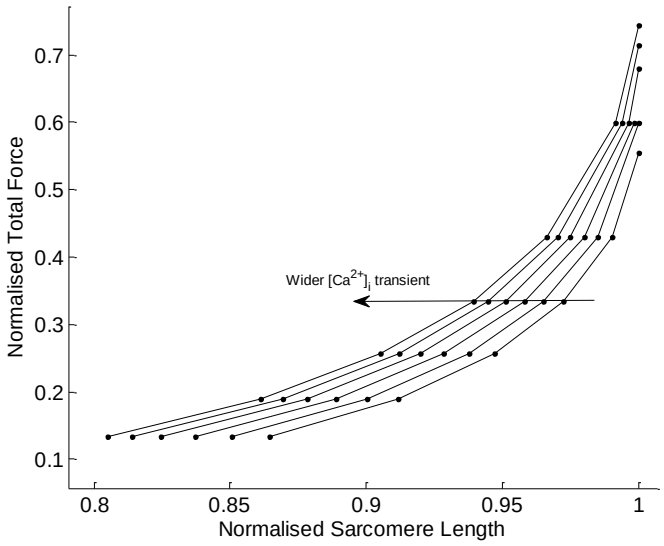


Figure 6

Inserting isometric [Ca^{2+}] transients into work-loop simulations cause a leftward shift in the work-loop end-systolic force-length curve. Wider [Ca^{2+}] transients result in a larger leftward shift.

Figure 6 shows that inserting wider, isometric [Ca2+]$_i$ into work-loop contractions cause a leftward shift in work-loop end-systolic force-length curves. The duration/ width of the fixed intracellular [Ca^{2+}] transient determines how far the ES curve moves. A wider transient corresponds to more isotonic shortening, and a larger leftward shift (Figure 6).

Tailoring the [Ca^{2+}] transient shape to the afterload of individual work-loops affords us the ability to specify the end-systolic length. With this ability, the isometric and work-loop end systolic curves can be united (Figure 7).

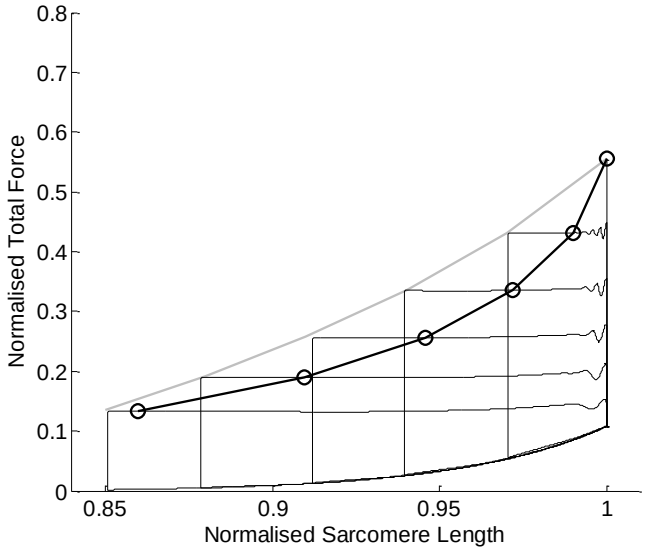
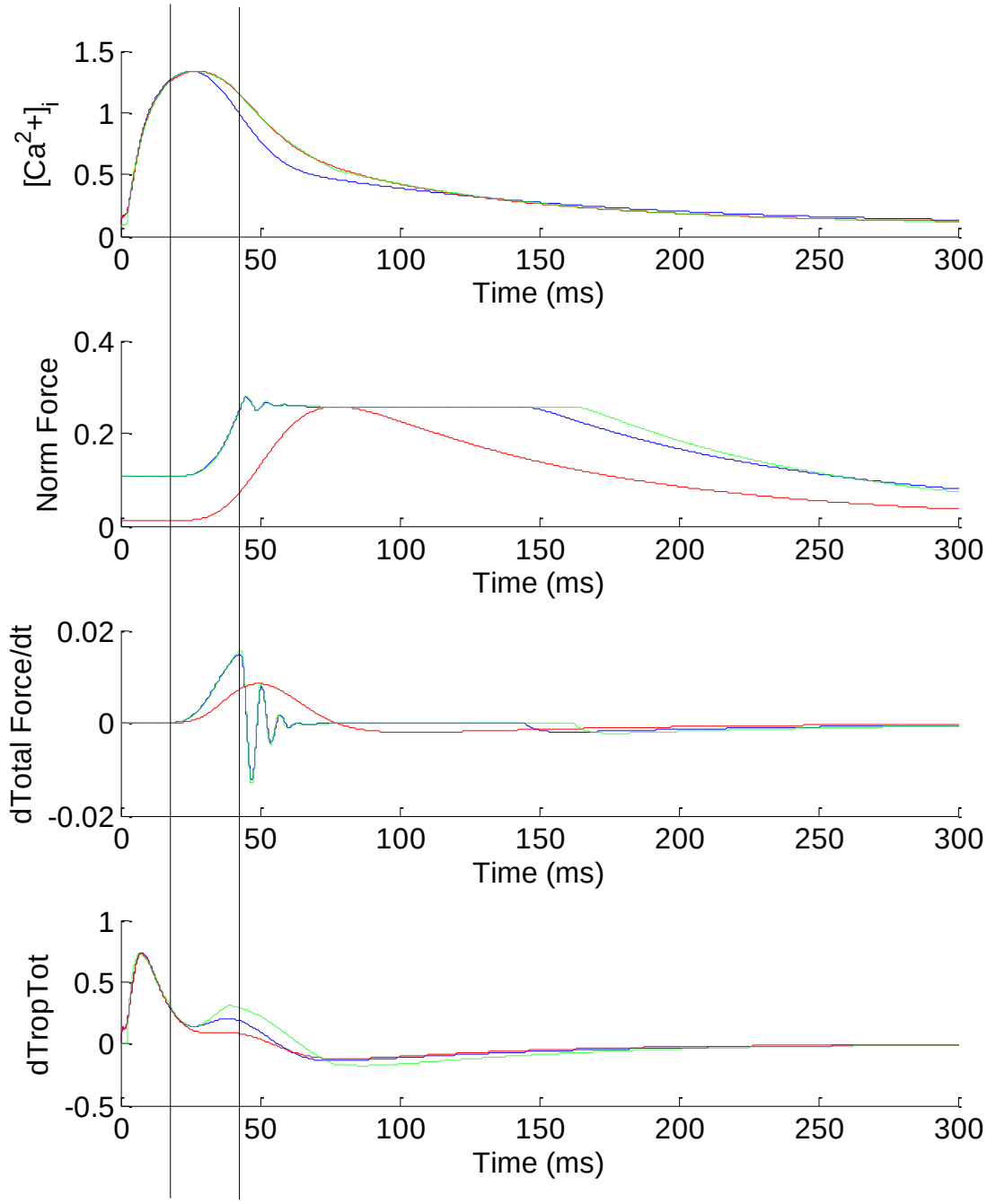


Figure 7

By Inserting fixed [Ca^{2+}] transients that are tailored to each work-loop afterload, it is possible to unite isometric and work-loop end-systolic force-length curves.

Discussion


[Ca2+]i
1.5
1
0.5
0
0
50
100
150
200
250
300
Time (ms)
Norm Force
0.4
0.2
0
0
50
100
150
200
250
300
Time (ms)
dTotal Force/dt
0.02
0
-0.02
0
50
100
150
200
250
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Time (ms)
dTropTot
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-0.5
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50
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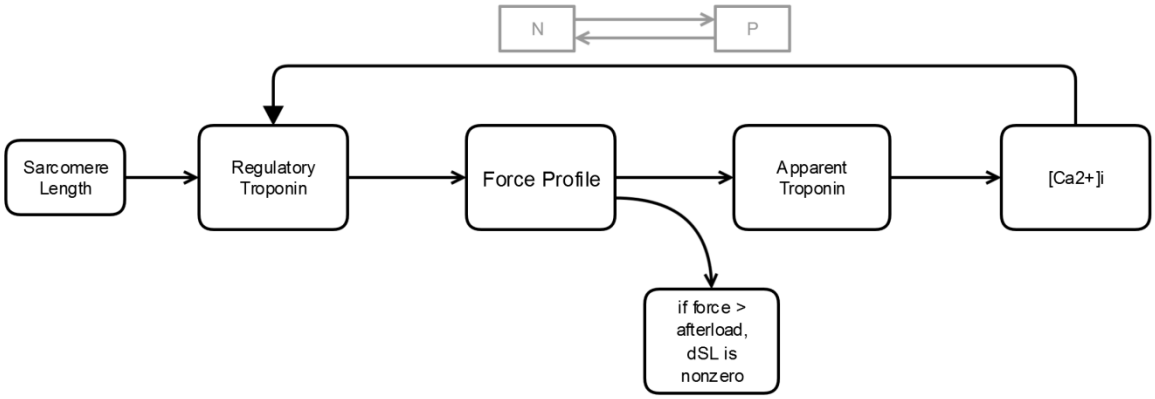
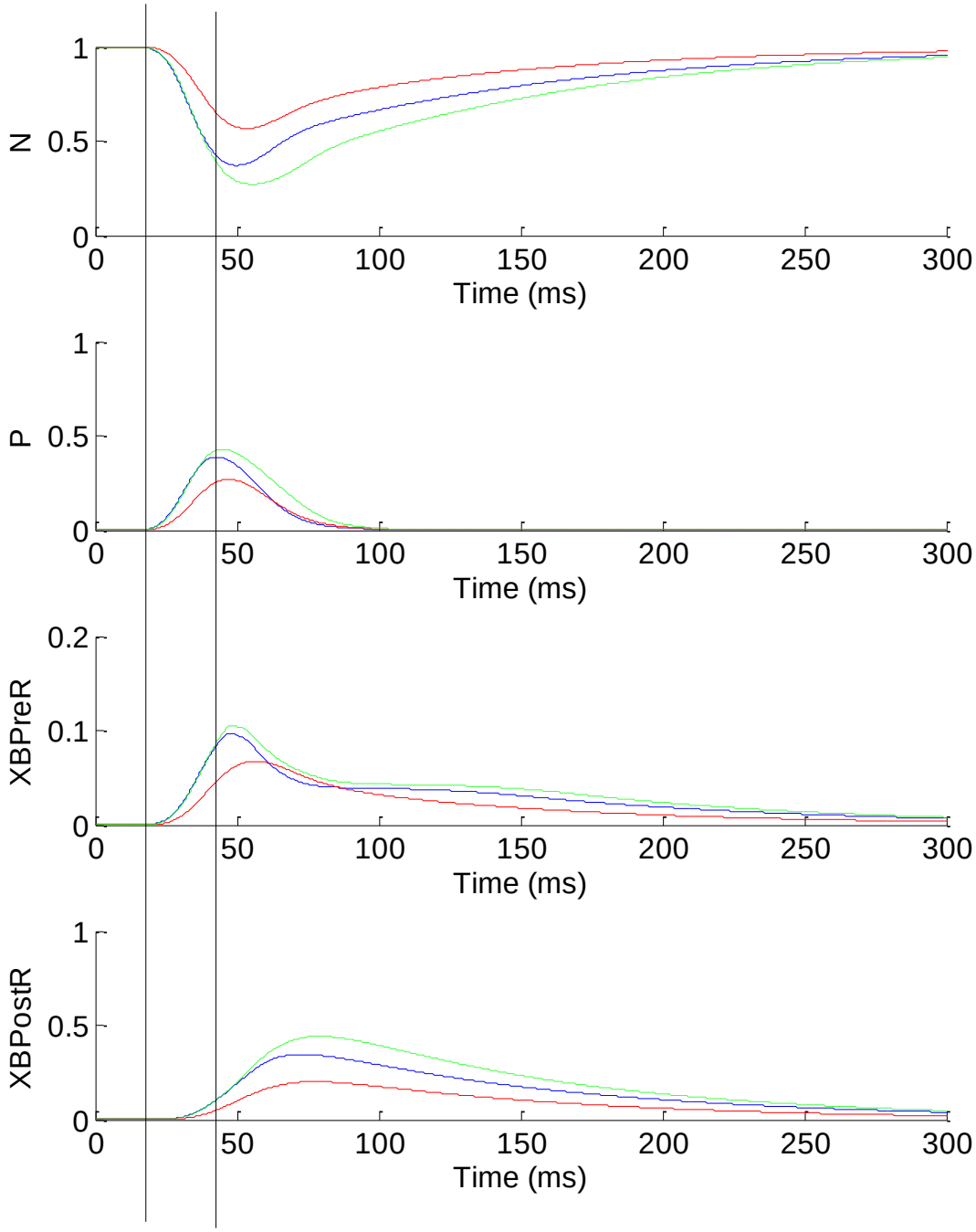
250
300
Time (ms)


N
P
Sarcomere Length
Regulatory Troponin
Force Profile
Apparent Troponin
[Ca2+]i
if force > afterload, dSL is nonzero


N
1
0.5
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Time (ms)
P
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Time (ms)
XBPreR
0.2
0.1
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250
300
Time (ms)
XBPostR
1
0.5
0
0
50
100
150
200
250
300
Time (ms)

Rice JJ, Wang F, Bers DM & de Tombe PP. (2008). Approximate model of cooperative activation and crossbridge cycling in cardiac muscle using ordinary differential equations. *Biophysical journal* **95,** 2368-2390.