Online Supplemental data for Song et al., "Differential Integration of Ca-Calmodulin Signal in Intact Ventricular Myocytes at Low and High Affinity Ca-Calmodulin Targets".

Each of the below reactions were included in both dyadic cleft and cytosolic compartments, with compartment-specific total concentrations as specified in Table 3.

Ca binding to CaM and CaM buffering

The models of CaM and CaM buffering used here, which are integrated into the dyadic cleft and cytosolic compartments of the Shannon-Bers EC coupling model Ref (1), were developed and validated in Ref (2). Reaction fluxes were in units of [μM sec^{-1}], for reactions of Ca binding to CaM (as shown in Figure 6A) and described below. Parameter values are listed in Table 1.

$$Reaction_{02} = k_{02}[Ca]^2[CaM] - k_{20}[Ca_2CaM] \quad \text{(Eq. 1)}$$

$$Reaction_{24} = k_{24}[Ca]^2[Ca_2CaM] - k_{42}[Ca_4CaM] \quad \text{(Eq. 2)}$$

$$Reaction_{02B} = k_{02B}[Ca]^2[CaMB] - k_{20B}[Ca_2CaMB] \quad \text{(Eq. 3)}$$

$$Reaction_{24B} = k_{24B}[Ca]^2[Ca_2CaMB] - k_{42B}[Ca_4CaMB] \quad \text{(Eq. 4)}$$

$$Reaction_{0B} = k_{0Bon}[CaM][B] - k_{0Boff}[CaMB] \quad \text{(Eq. 5)}$$

$$Reaction_{2B} = k_{2Bon}[Ca_2CaM][B] - k_{2Eoff}[Ca_2CaMB] \quad \text{(Eq. 6)}$$

$$Reaction_{4B} = k_{4Bon}[Ca_4CaM][B] - k_{4Boff}[Ca_4CaMB] \quad \text{(Eq. 7)}$$

Differential equations (units in [μM $msec^{-1}$]) for concentrations of free CaM, Ca_2CaM, and Ca_4CaM, computed separately for each compartment (dyadic cleft and cytosol).

$$\begin{aligned}[CaM] = {} & [CaM_{TOT}] - [Ca_2CaM] - [Ca_4CaM] - [CaMB] - [Ca_2CaMB] \\ & - [Ca_4CaMB] - [CaMBsCaM] - [Ca_2CaMBsCaM] - [Ca_4CaMBsCaM]\end{aligned} \quad \text{(Eq. 8)}$$

$$[B] = [B_{TOT}] - [CaMB] - [Ca_2CaMB] - [Ca_4CaMB] \quad \text{(Eq. 9)}$$

$$\frac{d[Ca_2CaM]}{dt} = 10^{-3}\left\{Reaction_{02} - Reaction_{24} - Reaction_{2B} - Reaction_{2BsCaM}\right\} \quad \text{(Eq. 10)}$$

$$\frac{d[Ca_4CaM]}{dt} = 10^{-3}\left\{Reaction_{24} - Reaction_{4BsCaM} - Reaction_{4B}\right\} \quad \text{(Eq. 11)}$$

$$\frac{d[CaMB]}{dt} = 10^{-3}(Reaction_{0B} - Reaction_{02B}) \quad \text{(Eq. 12)}$$

$$\frac{d[Ca_2CaMB]}{dt} = 10^{-3}(Reaction_{02B} + Reaction_{2B} - Reaction_{24B}) \quad \text{(Eq. 13)}$$

$$\frac{d[Ca_4CaMB]}{dt} = 10^{-3}(Reaction_{24B} + Reaction_{4B}) \quad \text{(Eq. 14)}$$

BsCaM-2 and BsCaM-45 activation

Model of CaM binding to BsCaM-2 or BsCaM-45. Parameters listed in Table 2. Differential equation in units of [μM $msec^{-1}$].

$$[BsCaM] = [BsCaM_{TOT}] - [CaMBsCaM] - [Ca_2CaMBsCaM] - [Ca_4CaMBsCaM] \quad \text{(Eq. 15)}$$

$$Reaction_{02BsCaM} = k_{02BsCaM}[Ca]^2[CaMBsCaM] - k_{20BsCaM}[Ca_2CaMBsCaM] \quad \text{(Eq. 16)}$$

$$Reaction_{24BsCaM} = k_{24BsCaM}[Ca]^2[Ca_2CaMBsCaM] - k_{42BsCaM}[Ca_4CaMBsCaM] \quad \text{(Eq. 17)}$$

$$Reaction_{0BsCaM} = k_{BsCaM\,0on}[CaM][BsCaM] - k_{BsCaM\,0off}[CaMBsCaM] \quad \text{(Eq. 18)}$$

$$Reaction_{2BsCaM} = k_{BsCaM\,2on}[Ca_2CaM][BsCaM] - k_{BsCaM\,2off}[Ca_2CaMBsCaM] \quad \text{(Eq. 19)}$$

$$Reaction_{4BsCaM} = k_{BsCaM\,4on}[Ca_4CaM][BsCaM] - k_{BsCaM\,4off}[Ca_4CaMBsCaM] \quad \text{(Eq. 20)}$$

$$\frac{d[CaMBsCaM]}{dt} = 10^{-3}\left(Reaction_{0BsCaM} - Reaction_{02BsCaM}\right) \quad \text{(Eq. 21)}$$

$$\frac{d[Ca_2CaMBsCaM]}{dt} = 10^{-3}\left(Reaction_{2BsCaM} + Reaction_{02BsCaM} - Reaction_{24BsCaM}\right) \quad \text{(Eq. 22)}$$

$$\frac{d[Ca_4CaMBsCaM]}{dt} = 10^{-3}\left(Reaction_{4BsCaM} + Reaction_{24BsCaM}\right) \quad \text{(Eq. 23)}$$

Table 1. Ca/CaM binding and CaM buffering parameters.

Parameter	Value	Units	Description	References
$\mathbf{k_{20}}$	10	sec^{-1}	2 Ca dissociation f. CaM	(3)
$\mathbf{k_{02}}$	k_{20}/9.67	$\mu M^{-2}\ sec^{-1}$	2 Ca association w. CaM	(4, 5)
$\mathbf{k_{42}}$	500	sec^{-1}	2 Ca dissociation f. CaM	(3)
$\mathbf{k_{24}}$	k_{42}/575	$\mu M^{-2}\ sec^{-1}$	2 Ca association w. CaM	(4, 5)
$\mathbf{k_{0Boff}}$	0.0014	sec^{-1}	CaM dissociation f. Buff	(6)
$\mathbf{k_{0Bon}}$	k_{0Boff}/0.202	$\mu M^{-1}\ sec^{-1}$	CaM association w. Buff	(6)
$\mathbf{k_{2Boff}}$	k_{0Boff}/100	sec^{-1}	Ca_2CaM dissociation f. Buff	(6)
$\mathbf{k_{2Bon}}$	k_{0Bon}	$\mu M^{-1}\ sec^{-1}$	Ca_2CaM association w. Buff	-
$\mathbf{k_{4Boff}}$	k_{0Boff}/100	sec^{-1}	Ca_4CaM dissociation f. Buff	(6)
$\mathbf{k_{4Bon}}$	k_{0Bon}	$\mu M^{-1}\ sec^{-1}$	Ca_4CaM association w. Buff	-
$\mathbf{k_{42B}}$	k_{42}	sec^{-1}	2 Ca dissociation f. CaMBuff	detailed balance
$\mathbf{k_{24B}}$	k_{24}	$\mu M^{-2}\ sec^{-1}$	2 Ca association w. CaMBuff	-
$\mathbf{k_{20B}}$	k_{20}/100	sec^{-1}	2 Ca dissociation f. CaMBuff	detailed balance
$\mathbf{k_{02B}}$	k_{02}	$\mu M^{-2}\ sec^{-1}$	2 Ca association w. CaMBuff	-

Table 2. BsCaM-2 and BsCaM-45 reaction parameters.

Parameter	Value	Units	Description	References
$\mathbf{k_{BsCaM2\text{-}4off}}$	0.05	sec^{-1}	Ca_4CaM dissoc. f. BsCaM2	(7)
$\mathbf{k_{BsCaM45\text{-}4off}}$	0.05*45/1.5	sec^{-1}	Ca_4CaM dissoc. f. BsCaM45	(7, 8)
$\mathbf{k_{BsCaM4on}}$	$k_{BsCaM4off}$/1.5e-3	μM^{-1} sec^{-1}	Ca_4CaM assoc. w. BsCaM	(7)
$\mathbf{k_{BsCaM2off}}$	1.62e4*$k_{BsCaM4off}$	sec^{-1}	Ca_2CaM dissoc. f. BsCaM	MLCK: (9, 10)
$\mathbf{k_{BsCaM2on}}$	$k_{BsCaM4on}$	μM^{-1} sec^{-1}	Ca_2CaM assoc. w. BsCaM	-
$\mathbf{k_{BsCaM0off}}$	1.62e4*$k_{BsCaM2off}$	sec^{-1}	CaM dissoc. f. BsCaM	MLCK: (9, 10)
$\mathbf{k_{BsCaM0on}}$	$k_{BsCaM2on}$	μM^{-1} sec^{-1}	CaM assoc. w. BsCaM	-
$\mathbf{k_{20BsCaM}}$	1.6	sec^{-1}	2 Ca dissoc. f. BsCaM	MLCK: (11-13)
$\mathbf{k_{02BsCaM}}$	$k_{20BscaM}$*1.62e4/575	μM^{-2} sec^{-1}	2 Ca assoc. w. BsCaM	detailed balance
$\mathbf{k_{42BsCaM}}$	1.6	sec^{-1}	2 Ca dissoc. f. BsCaM	MLCK: (12, 13)
$\mathbf{k_{24BsCaM}}$	$k_{42BscaM}$*1.62e4/9.75	μM^{-2} sec^{-1}	2 Ca assoc. w. BsCaM	detailed balance

Table 3. Dyadic cleft and cytosolic total concentrations.

Parameter	Value	Units	References
$\mathbf{CaM_{TOT\text{-}CYT}}$	5.65	μM	(14, 15)
$\mathbf{CaM_{TOT\text{-}DYAD}}$	418	μM	(16, 17)
$\mathbf{B_{TOT\text{-}CYT}}$	24.2	μM	(6)
$\mathbf{B_{TOT\text{-}DYAD}}$	0	μM	-
$\mathbf{BsCaM_{TOT\text{-}CYT}}$	3e-3	μM	-
$\mathbf{BsCaM_{TOT\text{-}DYAD}}$	3.62	μM	(3e-3*Vmyo/Vdyad)

References

1. Shannon, T. R., F. Wang, J. Puglisi, C. Weber, and D. M. Bers. 2004. A mathematical treatment of integrated Ca dynamics within the ventricular myocyte. **Biophys J** 87:3351-3371.
2. Saucerman, J. J., and D. M. Bers. 2008. Calmodulin mediates differential sensitivity of CaMKII and calcineurin to local Ca2+ in cardiac myocytes. **Biophys J, Aug. 8** Epub ahead of print.
3. Klee, C. B. 1988. Interaction of calmodulin with Ca2+ and target proteins. In Calmodulin. P. Cohen, and C. B. Klee, editors. Elsevier, Amsterdam ; New York. 35-56.
4. Linse, S., A. Helmersson, and S. Fors,n. 1991. Calcium binding to calmodulin and its globular domains. **J Biol Chem** 266:8050-8054.
5. Stemmer, P. M., and C. B. Klee. 1994. Dual calcium ion regulation of calcineurin by calmodulin and calcineurin B. **Biochemistry** 33:6859-6866.
6. Wu, X., and D. M. Bers. 2007. Free and bound intracellular calmodulin measurements in cardiac myocytes. **Cell Calcium** 41:353-364.
7. Tran, Q. K., D. J. Black, and A. Persechini. 2005. Dominant affectors in the calmodulin network shape the time courses of target responses in the cell. **Cell Calcium** 37:541-553.
8. Romoser, V. A., P. M. Hinkle, and A. Persechini. 1997. Detection in living cells of Ca2+-dependent changes in the fluorescence emission of an indicator composed of two green fluorescent protein variants linked by a calmodulin-binding sequence. A new class of fluorescent indicators. **J Biol Chem** 272:13270-13274.
9. Persechini, A., and P. M. Stemmer. 2002. Calmodulin is a limiting factor in the cell. **Trends Cardiovasc Med** 12:32-37.
10. Cox, J. A. 1988. Interactive properties of calmodulin. **Biochem J** 249:621-629.
11. Persechini, A., H. D. White, and K. J. Gansz. 1996. Different mechanisms for Ca2+ dissociation from complexes of calmodulin with nitric oxide synthase or myosin light chain kinase. **J Biol Chem** 271:62-67.
12. Kasturi, R., C. Vasulka, and J. D. Johnson. 1993. Ca2+, caldesmon, and myosin light chain kinase exchange with calmodulin. **J Biol Chem** 268:7958-7964.
13. Johnson, J. D., C. Snyder, M. Walsh, and M. Flynn. 1996. Effects of myosin light chain kinase and peptides on Ca2+ exchange with the N- and C-terminal Ca2+ binding sites of calmodulin. **J Biol Chem** 271:761-767.
14. Fabiato, A. 1983. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. **Am J Physiol** 245:C1-14.
15. Maier, L. S., M. T. Ziolo, J. Bossuyt, A. Persechini, R. Mestril, and D. M. Bers. 2006. Dynamic changes in free Ca-calmodulin levels in adult cardiac myocytes. **J Mol Cell Cardiol** 41:451-458.
16. Balshaw, D. M., L. Xu, N. Yamaguchi, D. A. Pasek, and G. Meissner. 2001. Calmodulin binding and inhibition of cardiac muscle calcium release channel (ryanodine receptor). **J Biol Chem** 276:20144-20153.
17. Mori, M. X., M. G. Erickson, and D. T. Yue. 2004. Functional stoichiometry and local enrichment of calmodulin interacting with Ca2+ channels. **Science** 304:432-435.