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Calmodulin binding proteins provide domains of local Ca^{2+} signaling in cardiac myocytes

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Abstract

Calmodulin (CaM) acts as a common Ca^{2+} sensor for many signaling pathways, transducing local Ca^{2+} signals into specific cellular outcomes. Many of CaM's signaling functions can be explained by its unique biochemical properties, including high and low affinity Ca^{2+} -binding sites with slow and fast kinetics, respectively. CaM is expected to have a limited spatial range of action, emphasizing its role in local Ca^{2+} signaling. Interactions with target proteins further fine-tune CaM signal transduction. Here, we focus on only three specific cellular targets for CaM signaling in cardiac myocytes: the L-type Ca^{2+} channel, the ryanodine receptor, and the IP_3 receptor. We elaborate a working hypothesis that each channel is regulated by two distinct functional populations of CaM: dedicated CaM and promiscuous CaM. Dedicated CaM is typically tethered to each channel and directly regulates channel activity. In addition, a local pool of promiscuous CaM appears poised to sense local Ca^{2+} signals and trigger downstream pathways such as Ca^{2+} /CaM dependent-protein kinase II and calcineurin. Understanding how promiscuous CaM coordinates multiple distinct signaling pathways remains a challenge, but is aided by the use of mathematical modeling and a new generation of fluorescent biosensors.

Introduction

Calmodulin (CaM) is a central mediator of Ca^{2+} -dependent signaling. CaM acts as the Ca^{2+} sensor for many ion channels and signaling pathways. Here, we will review aspects of CaM's biochemistry and targeting that enable it to transduce local Ca^{2+} signals into specific cellular outputs in cardiac myocytes.

CaM Biochemistry

The biochemistry of CaM has important implications for how it regulates diverse local Ca^{2+} signaling pathways. CaM binds Ca^{2+} with an average apparent $K_d \sim 5 \mu\text{M}$, significantly higher than the range of $0.1\text{--}1 \mu\text{M}$ [Ca^{2+}] generally seen in bulk cytosol of cardiac myocytes. Thus it is initially surprising that CaM is regulated by physiologic [Ca^{2+}], but as we will see, local [Ca^{2+}] near channels that pass Ca^{2+} can be much higher. CaM has a

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dumbbell-like structure, with 2 EF-hand Ca^{2+} binding sites at each end, bridged by a central alpha helix [1, 2]. The 2 Ca^{2+} binding sites at the C-lobe have higher affinity ($K_d \sim 1 \mu\text{M}$), while the 2 Ca^{2+} binding sites at the N-lobe have lower affinity ($K_d \sim 12 \mu\text{M}$) [3, 4]. The N-lobe and C-lobe of CaM also differ in their kinetics of Ca^{2+} dissociation. The N-lobe dissociates Ca^{2+} quickly ($\sim 1000 \text{ s}^{-1}$) while Ca^{2+} dissociates from the C-lobe more slowly ($\sim 10 \text{ s}^{-1}$) [3, 4].

The specific manner in which CaM binds its target proteins can alter CaM's properties. Ca^{2+} dissociates more slowly from proteins that bind preferentially to Ca-CaM; as a result the apparent affinity of Ca^{2+} for CaM is increased. For example, the high affinity of calcineurin for Ca-CaM results in a ~ 30 -fold higher apparent Ca/CaM affinity when bound to calcineurin [5]. This effect brings the EC_{50} of $[\text{Ca}^{2+}]$ for calcineurin activity to $\sim 1 \mu\text{M}$ in the presence of sufficient CaM [5], which is notably in the cytosolic $[\text{Ca}^{2+}]$ range. In contrast, some proteins preferentially bind apoCaM such as neuromodulin and PEP19, which then release prebound apoCaM as Ca^{2+} increases [6]. Thus the binding characteristics of CaM target proteins can tune the range of Ca^{2+} signals sensed.

Using these biochemical properties of CaM, we can begin to infer several properties of its likely functions in cardiac myocytes. The relatively low affinity of Ca^{2+} binding ($K_d \sim 5 \mu\text{M}$) suggests that cytosolic Ca^{2+} transients may not be sufficient to strongly stimulate CaM. If instead CaM is regulated by stronger, local Ca^{2+} signals, it would require pre-localization near a Ca^{2+} source preferentially in the apoCaM form. Based on the examples of neuromodulin and PEP19, we may expect that with increased $[\text{Ca}^{2+}]$, CaM would dissociate and then bind to other targets that preferentially bind to Ca-CaM.

Once activated near a high $[\text{Ca}^{2+}]$ source, what is the expected "range of action" for Ca-CaM? Persechini and Stemmer [7] used simple diffusion calculations to estimate the average diffusion distance of CaM. Assuming 1-dimensional diffusion, mean diffusion length L can be estimated as $L = \sqrt{2Dt}$, where D is the diffusion coefficient and t is the time for diffusion. Based on a CaM diffusion coefficient of $5 \mu\text{m}^2/\text{sec}$ and the slow Ca^{2+} dissociation from the C-lobe ($t = 1/10 \text{ sec}$), it was calculated that CaM could diffuse $\sim 1 \mu\text{m}$ before losing all its Ca^{2+} [7]. This would imply that CaM would have sufficient time to diffuse throughout a half-sarcomere on the timescale of a heartbeat. However, most Ca-CaM targets require Ca^{2+} bound to both N- and C-lobe of CaM. Based on the faster Ca^{2+} dissociation from the N-lobe (1000 s^{-1}), the range of action of the Ca_4CaM reduces to just $0.10 \mu\text{m}$, similar to the radius of the dyadic cleft [8]. This calculation suggests that Ca-CaM is a local signal.

How does consideration of target proteins affect CaM's range of action? In the case of an immobile CaM target, CaM's range of action will be dictated by the rate of target association. Using an estimated $\sim 20 \mu\text{M}$ free CaM targets [9] and an association rate of Ca_4CaM for CaMKII of $66 \mu\text{M}^{-1}\text{s}^{-1}$ [10], we can estimate that Ca-CaM will diffuse about $L = \sqrt{2 * 5 / (66 * 20)} = 0.087 \mu\text{m}$ before binding a target. This indicates that CaM can diffuse only to local target proteins before losing its N-lobe Ca^{2+} .

In the case of a mobile CaM target, CaM may be able to extend its range of action. Due to the high affinity of calcineurin for Ca-CaM (K_d 28 pM, [11]), the expected dissociation rate of Ca^{2+} from the Ca/CaM/calcineurin complex falls from 1000 s^{-1} to 0.2 s^{-1} [9]. Together with an estimated diffusion coefficient of $2 \mu\text{m}^2/\text{sec}$ this yields a significantly larger $\sim 4.5 \mu\text{m}$ diffusion distance for Ca/CaM/calcineurin. This property may help explain how CaM is able to translocate to the nucleus while retaining activity, as has been reported with endothelin-1 stimulation [12]. While the above calculations are only rough estimates, they provide rules of thumb for what may be quantitatively reasonable within the cellular

environment. Using this knowledge we can begin to interpret studies focused on CaM and its regulation of targets in cardiac myocytes.

CaM availability and signals in cardiac myocytes

Total CaM concentration is $\sim 6 \mu\text{M}$ in isolated cardiac myocytes [13, 14]. While this may appear to be plenty to activate its targets, more than 90% of CaM in the cell is prebound, even at resting $[\text{Ca}]_i$. Indeed, studies in other cell types have indicated that free CaM is only $\sim 50 \text{ nM}$, making CaM a limiting factor in Ca^{2+} signaling [7]. Wu and Bers used null-point titration approaches with fluorescently labeled CaM to assess free CaM levels in myocytes [15]. Consistent with studies in other cell types, they found that free CaM is $50\text{--}75 \text{ nM}$. CaM was highly bound at the sarcomeric z-line/T-tubule location, with enhanced CaM binding and markedly decreased dissociation rates when $[\text{Ca}^{2+}]$ was elevated [15]. This indicates an excess of CaM binding targets, which on average preferentially bind Ca-CaM. Indeed, using the kinetics of CaM binding from that study, we estimated the total amount of CaM binding proteins in myocytes to be at least $26 \mu\text{M}$ [9]. With only $\sim 1\%$ free CaM in the cell, most CaM targets are likely to require locally prebound CaM for effective signaling.

Fluorescent reporters of CaM binding have revealed new insights into the dynamics of CaM signals in the cell. FRET reporters of Ca-CaM binding [16] have been adapted to directly measure Ca-CaM signals in adult cardiac myocytes [14]. In electrically stimulated myocytes, Ca-CaM signals oscillated on a beat-to-beat basis in correlation with cell contractions. However, detection of measurable signals was facilitated by coexpression of exogenous CaM, further emphasizing the limited CaM availability in myocytes [14].

Local CaM regulation of ion channels

Calmodulin has been shown to regulate a variety of ion channels in cardiac myocytes. Here, we will focus on CaM's regulation of L-type Ca^{2+} channel, ryanodine receptor and IP_3 receptor as these have been well studied and exhibit multiple modes of CaM regulation. A key emerging theme for these channels is regulation by both "dedicated" and "promiscuous" CaM (Figure 1). "Dedicated" CaM binds stoichiometrically to the channel (even at resting $[\text{Ca}]_i$) and participates in direct channel regulation. On the other hand, "promiscuous" CaM refers to additional CaM that is "in the neighborhood" (probably in association with some nearby protein) that participates indirectly in channel regulation, often via kinases or phosphatases.

The L-type Ca^{2+} channel (LTCC or $\text{Ca}_v1.2$) is the main entry point for Ca^{2+} flux into the cardiac myocyte, triggering excitation-contraction coupling. A key negative feedback for the LTCC is Ca^{2+} -dependent inactivation. In contemporaneous studies, both Peterson et al. and Zuhlke et al. used mutant CaM unable to bind Ca^{2+} to show that CaM is the primary Ca^{2+} sensor for LTCC Ca^{2+} -dependent inactivation [17, 18]. Both studies also demonstrated that CaM is constitutively tethered to an apoCaM binding site on the carboxyl tail of $\text{Ca}_v1.2$ (the α_{1C} subunit). Peterson et al. further used mutant CaMs unable to bind Ca^{2+} to either the N or C-lobe of CaM to show that Ca^{2+} binding to the C-lobe is the main sensor for Ca^{2+} -dependent inactivation [17]. This provided clear evidence of a "dedicated" CaM that regulates Ca^{2+} -dependent inactivation of the LTCC. Subsequently, Alseikhan et al. extended these studies in adult ventricular myocytes, again finding that the C-lobe of CaM regulates inactivation [19]. Eliminating LTCC Ca^{2+} -dependent inactivation with CaM mutants profoundly prolonged the action potential duration, demonstrating a powerful physiological role for this negative feedback [19].

Ca^{2+} channels have been a prototype system for how CaM can decode complex Ca^{2+} signals. DeMaria et al. examined CaM regulation of the neuronal P/Q-type Ca^{2+} channel

(Ca_v2.1), again using CaM mutants deficient in either N-lobe or C-lobe binding [20]. Unlike Ca_v1.2, Ca_v2.1 exhibits both Ca²⁺/CaM-dependent facilitation and inactivation without requiring kinases. Intriguingly, they found that dedicated CaM mediates both processes: N-lobe CaM selectively regulates Ca_v2.1 inactivation while C-lobe CaM selectively regulates Ca_v2.1 facilitation [20]. Combining this information with the differential sensitivity of inactivation and facilitation to the Ca²⁺ buffer EGTA, they proposed an intriguing conceptual model where the C-lobe of CaM selectively detects local Ca²⁺ while the N-lobe of CaM selectively detects global Ca²⁺. This interesting dual local-global signaling by CaM on Ca_v2.1 producing opposite I_{Ca} effects has been further studied experimentally and theoretically by the Yue laboratory [21, 22]. These studies establish an intriguing paradigm for local vs. global Ca²⁺ sensing by CaM that may extend to other CaM-binding proteins. It is important to note however, that in contrast to Ca_v2.1, the cardiac Ca_v1.2 exhibits strong C-lobe CaM-dependent inactivation but weaker N-lobe-dependent effects. Ca_v1.3 (expressed in sinoatrial node and atrial myocytes [23]) exhibits strong C-lobe and N-lobe CaM-dependent inactivation but both are regulated by local Ca²⁺ [21].

Apart from the central role of a dedicated CaM in LTCC inactivation, “promiscuous” CaM plays additional regulatory roles for LTCCs. Mori et al. fused wildtype or mutant CaMs to the LTCC to evaluate the stoichiometry of CaM at the LTCC [24]. Mutant tethered CaM blocked Ca²⁺-dependent inactivation, indicating that a single dedicated CaM is involved in this process. Varying the length of the CaM tether, they also inferred that there may be ~25 CaM molecules within a 40 nm hemisphere of the LTCC [24]. This indicates a significant pool of promiscuous CaM (at far higher concentration than the 50–100 nM freely diffusible CaM). This local CaM may sense local Ca²⁺ signals and trigger downstream signaling.

Promiscuous CaM plays an important role in CaMKII-dependent LTCC facilitation. LTCC facilitation is blocked by CaM or CaMKII inhibition [25–27]. Further, LTCC facilitation is blocked by the fast BAPTA Ca²⁺ buffer but not the slower EGTA, indicating that this is a very localized Ca²⁺/CaM signal [28]. As a CaMKII holoenzyme has 10–12 CaM-binding subunits, this would appear to require a significant local pool of promiscuous CaM, consistent with the experimental inference above [24]. Further, the Ca-CaM-dependent phosphatase CaN has recently been found to anchor to the LTCC, where it appears to regulate basal LTCC function and reverse PKC-mediated LTCC phosphorylation [29]. Activation of this localized CaN would also be a target of this pool of promiscuous CaM. Thus, multiple Ca²⁺ signaling pathways are localized to the LTCC, where they appear to rely on high local Ca²⁺ signals and an enriched local pool of promiscuous CaM.

We will not discuss it in detail here, but the cardiac Na channel (Na_v1.5) is also regulated by both dedicated and promiscuous CaM. A dedicated CaM is bound to an IQ domain on the carboxy tail and may also interact with the domain III–IV linker that is critical for voltage-dependent inactivation [30]. CaMKII also associates with Na_v1.5, and via promiscuous CaM, this CaMKII can phosphorylate Na_v1.5 and alter Na current gating in ways that may be arrhythmogenic, as in genetically linked long QT and Brugada syndromes [31, 32]. Notably, we do not expect the cardiac Na channel to be exposed to the same pool of promiscuous CaM as for the Ca channel, because we expect most of the Na channels not to be in the cleft region where Ca_v1.2 and RyR are colocalized [33]. On the other hand, the Na channel, like Na/Ca exchange, is likely to see higher local [Ca²⁺]_i in the submembrane space compared to bulk cytosolic [Ca²⁺]_i, albeit not as high as cleft [Ca²⁺]_i during Ca transients [8, 34].

The RyR, once triggered by Ca²⁺ from the LTCC, releases Ca²⁺ from the sarcoplasmic reticulum, which accounts for most of cytosolic Ca²⁺ transient in ventricular myocytes. Here too CaM plays key roles in regulating Ca²⁺ channel function. The cardiac isoform RyR2 has

been shown to bind 1 dedicated CaM molecule per RyR monomer, which stays bound in both apoCaM and Ca-CaM states [35]. CaM binding to RyR2 inhibits channel opening, and this dedicated CaM may participate in RyR2 inactivation at high $[Ca^{2+}]$ [35]. Defective CaM-mediated RyR inactivation may contribute to RyR leakiness in heart failure. In FK506-treated and heart failure myocytes CaM binding to the RyR is decreased and associated with the increased spark rate that can be reversed by additional CaM [36, 37].

In contrast to the inhibitory role of dedicated CaM, promiscuous CaM plays a key role in stimulating RyR flux via CaMKII-dependent RyR phosphorylation [38, 39]. RyR hyperphosphorylation by CaMKII has been implicated in several aspects of heart failure including RyR leak, decreased SR Ca^{2+} content and arrhythmia [37, 40, 41]. RyR hyperphosphorylation by CaMKII has also been implicated as a contributing mechanism to atrial fibrillation [42].

But where is the local source of the promiscuous CaM regulating RyR? It seems likely to be within the dyadic cleft, but these effects are very unlikely to be mediated by the dedicated CaM molecules prebound to the RyR2 [35]. While CaM is highly concentrated at the T-tubule/z-line location in resting cardiac myocytes [15, 36], it is unknown at this point if or which specific proteins might serve as local reservoirs of promiscuous CaM for either $Ca_v1.2$ or RyR2 (e.g. proteins which might release CaM at higher $[Ca^{2+}]$).

While both the LTCC and RyR are primarily localized to the dyadic cleft, the IP_3 receptor (IP_3R) forms a distinct local Ca^{2+} domain at the nuclear envelope of ventricular myocytes. In normal ventricular myocytes IP_3R play a more subtle role in excitation-contraction coupling compared with RyRs [43]. Instead, IP_3R is increasingly recognized to contribute more substantially to transcriptional regulation and cardiac hypertrophy [12, 44, 45]. IP_3R s contain binding sites for both apoCaM and Ca-CaM, and it was originally hypothesized that, like the RyR and LTCC, Ca-CaM would act as the Ca^{2+} sensor for channel inactivation [46]. But while CaM is required for Ca^{2+} -dependent inactivation, this functionality is maintained with mutant CaMs unable to bind Ca^{2+} [47]. Removal of the dedicated CaM using an MLCK peptide was shown to eliminate IP_3R activity, indicating that rather than acting as a Ca^{2+} sensor, CaM is as an essential subunit for the IP_3R [48]. However, others later reported that this MLCK peptide may disrupt the IP_3 -dependence of IP_3R activity independently of CaM, and that instead CaM is not essential for IP_3R activation [49]. Thus, the role of dedicated CaM in IP_3R regulation remains controversial.

Promiscuous CaM also seems to regulate the IP_3R via CaMKII. Bare et al. found that in ventricular myocytes the IP_3R was localized mainly to the nuclear envelope where it immunoprecipitated with CaMKII, raising the possibility of a local domain of nuclear Ca^{2+} /CaM/CaMKII signaling [50]. They further found that CaMKII can phosphorylate and inhibit the IP_3R *in vitro*, indicating a potential negative feedback loop [50]. Wu et al. later identified an endothelin- IP_3R -CaMKII pathway that stimulated histone deacetylase nuclear export and activation of the transcription factor MEF2, key regulators of cardiac hypertrophy [12]. In some cases the CaM-dependent calcineurin/NFAT hypertrophic pathway also may work through nuclear IP_3R signaling [44]. While the physiological importance of promiscuous CaM in IP_3R signaling and hypertrophy is becoming clearer, the source and amount of promiscuous CaM available in this compartment is unknown. But again, based on the range of action calculations and the low affinity of Ca^{2+} for CaM we hypothesize that apoCaM needs to bind locally near the IP_3R complex.

Integrating the known mechanisms of CaM regulation for the $Ca_v1.2$, $Na_v1.5$, RyR, and IP_3R together, we see that in each case there is one dedicated CaM per subunit, most often involved in channel inactivation. In addition, there appears to be a local pool of promiscuous

CaM that may be activated by the local Ca^{2+} source, stimulating Ca^{2+} signaling pathways such as CaMKII. All three Ca^{2+} channels are also regulated by CaMKII phosphorylation, forming positive or negative feedback loops.

Local CaM signals modulate kinase and phosphatase activities

While dedicated CaM at ion channels is typically regulated by a local Ca^{2+} signal, it appears that the promiscuous CaM coupled to Ca^{2+} signaling pathways may transduce a wider array of Ca^{2+} signals. CaM binding proteins exhibit a wide range of affinities for CaM, with dissociation constants ranging from 2 pM to 100 nM [11]. As discussed above, the affinity of the target protein for Ca-CaM will tune its Ca^{2+} -dependence, with greater Ca^{2+} sensitivity for proteins that bind Ca-CaM tightly. Song et al. used FRET biosensors with high and low affinities for Ca-CaM in ventricular myocytes to examine the functional consequence of CaM affinity for target proteins. They found when electrically pacing the myocytes, the sensor with lower affinity (similar to CaMKII) exhibited phasic oscillations in Ca-CaM binding, while the higher affinity sensor (similar to NOS or calcineurin) exhibited a qualitatively different integrative component to Ca-CaM binding at high frequencies. Song et al. then used a mathematical model of Ca-CaM activation and protein binding based on biochemical rate constants to predict the differential Ca-CaM binding patterns seen in the myocyte experiments. This indicated that both the slower signal decay and signal integration of the higher affinity sensor could be mechanistically explained by a decreased dissociation rate of Ca from CaM. Similar fluorescent biosensors will be needed to reveal the local dynamic CaM signals at ion channels or activities of endogenous CaM targets.

To examine the local CaM signals regulating CaMKII and calcineurin, we developed a mathematical model incorporating many of the above mechanisms for calmodulin activation, buffering and regulation of CaM targets CaMKII and calcineurin into a compartmental model of excitation-contraction coupling [9]. The model predicted large beat-to-beat Ca-CaM oscillations in the dyadic cleft, but diminutive Ca-CaM signal in the cytosol due to both the extensive CaM buffering and lower cytosolic $[\text{Ca}^{2+}]$. Indeed, the Ca-CaM signals reported by Maier et al. and Song et al. required co-expression of CaM to keep overall CaM levels at or slightly above normal myocyte levels [14, 51]. The model predicted that CaMKII activity would strongly oscillate beat to beat in the dyadic cleft due to high Ca^{2+} and Ca-CaM signals, while the limited cytosolic Ca-CaM would prevent CaM-dependent CaMKII activity in that compartment (Figure 2) [9]. This may explain why the majority of CaMKII substrates have CaMKII bound locally, although it is still unclear how cytosolic CaMKII substrates such as phospholamban may be effectively phosphorylated.

The very high affinity of calcineurin for Ca-CaM was predicted to have very different consequences, with frequency-dependent integration of calcineurin activity in the cytosol but saturated calcineurin activity in the dyadic cleft even at low frequencies (Figure 2) [9]. Consistent with these predictions, calcineurin/NFAT signaling in atrial and neonatal ventricular myocytes has been reported to correlate closely with the frequency of global Ca^{2+} oscillations [52, 53]. Whether dyadic calcineurin activity is saturated or dynamically regulated by Ca^{2+} signals has not yet been shown experimentally, but calcineurin is tethered to the LTCC and is sensitive to Ca^{2+} buffers [29]. Thus there may be two distinct functional populations of calcineurin in myocytes: constitutively active calcineurin anchored near Ca^{2+} channels and frequency-dependent calcineurin in the cytosol. However, more direct experimental studies are needed to validate these predictions.

The differences between regulation of CaMKII and calcineurin are illustrative of the diverse modes of Ca-CaM signaling. For example, while both CaMKII/HDAC and calcineurin/NFAT hypertrophic pathways are activated by overexpression of the LTCC β subunit, the

SERCA inhibitor thapsigargin enhanced NFAT translocation while inhibiting HDAC translocation [54]. Although both CaMKII and CaN can localize to Ca²⁺ channels, more quantitative localization experiments are needed to determine whether their distinct signals are explained by localization.

Conclusions

Despite being the Ca²⁺ sensor for many different pathways, CaM is able to transduce variations in the location and magnitude of Ca²⁺ signals into specific cellular outputs. At the biochemical level, the different Ca²⁺ sensitivities and kinetics of CaM's C- and N-lobes, together with the affinity of the target protein for Ca-CaM are key determinants of how this signal will be processed. In most cases, CaM acts either as a dedicated CaM that is tethered to its target or as promiscuous CaM, which is activated and then binds new partners. This working hypothesis provides an organizing principle for understanding spatial signaling involving Ca²⁺ and CaM in cardiac myocytes. The mechanisms and signal processing for dedicated CaM at the LTCC and other voltage-dependent Ca²⁺ channels has been extensively studied, providing a prototype for the complex behaviors possible. In most other cases, there are known functional roles for dedicated CaM in regulating ion channels but the precise mechanisms and signal processing involved are less clear. The mechanisms of promiscuous CaMs are more difficult to ascertain, as in most cases direct measurements have not yet shown whether the CaM is activated locally or diffuses from more distant sources. However, the quantitative biochemistry and computational modeling studies strongly indicate a requirement for locally activated CaM by high magnitude Ca²⁺ signals in most cases. Exceptions may occur for proteins with very high affinity for Ca-CaM, which may be able to overcome the combined obstacles of high CaM buffering and lower trigger Ca²⁺ signals in the cytosol.

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Highlights

- Calmodulin (CaM) acts as a local Ca^{2+} sensor with a limited range of action.
- Ca^{2+} -sensitive ion channels are regulated by dedicated and promiscuous CaMs.
- CaM modulates the sensitivity of CaMKII and calcineurin to local Ca^{2+} signals.

Figure 1. Regulation of cardiac ion channels by dedicated and promiscuous calmodulin (CaM). Abbreviations: CaN, calcineurin; CaMKII, CaM-dependent protein kinase II; LTCC, L-type Ca²⁺ channel; Na_v1.5, voltage-dependent Na⁺ channel; RyR, ryanodine receptor; IP₃R, IP₃ receptor.

Figure 2. Computational model predictions of CaMKII and CaN activities in A) dyadic cleft and B) cytosol of a ventricular myocyte. Adapted from [9].