REVIEW on cTnC

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Structure and Function of Cardiac Troponin C (TNNC1): Implications for Heart Failure, Cardiomyopathies, and Troponin Modulating Drugs

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Abstract

In striated muscle, the protein troponin complex turns contraction on and off in a calcium-dependent manner. The calcium-sensing component of the complex is troponin C, which is expressed from the TNNC1 gene in both cardiac muscle and slow-twitch skeletal muscle (identical transcript in both tissues) and the TNNC2 gene in fast-twitch skeletal muscle. Cardiac troponin C (cTnC) is made up of two globular EF-hand domains connected by a flexible linker. The structural C-domain (cCTnC) contains two high affinity calcium-binding sites that are always occupied by Ca^{2+} or Mg^{2+} under physiologic conditions, stabilizing an open conformation that remains anchored to the rest of the troponin complex. In contrast, the regulatory N-domain (cNTnC) contains a single low affinity site that is largely unoccupied at resting calcium concentrations. During muscle activation, calcium binding to cNTnC favors an open conformation that binds to the switch region of troponin I, removing adjacent inhibitory regions of troponin I from actin and allowing muscle contraction to proceed. Regulation of the calcium binding affinity of cNTnC is physiologically important, because it directly impacts the calcium sensitivity of muscle contraction. Calcium sensitivity can be modified by drugs that stabilize the open form of cNTnC, post-translational modifications like phosphorylation of troponin I, or downstream thin filament protein interactions that impact the availability of the troponin I switch region. Recently, mutations in cTnC have been associated with hypertrophic or dilated cardiomyopathy. A detailed understanding of how calcium sensitivity is regulated through the troponin complex is necessary for explaining how mutations perturb its function to promote cardiomyopathy and how post-translational modifications in the thin filament affect heart function and heart failure. Troponin modulating drugs are being developed for the treatment of cardiomyopathies and heart failure.

Key words: Hypertrophic cardiomyopathy, dilated cardiomyopathy, heart failure, calcium sensitizer, levosimendan, sarcomere modulators.

Biological context of troponin C

Muscle contraction is produced by the sliding of actin thin filaments against myosin thick filaments. In cardiac and skeletal muscle, thin and thick filaments are organized into highly polarized contractile units known as sarcomeres, which are linked in tandem and bundled together to form myofilaments. This arrangement gives these tissues their characteristic "striated" appearance.

The calcium-dependent contraction of striated muscle is controlled by the thin filament through the action of tropomyosin and the troponin complex, first discovered in 1964[2,3]. The thin filament has a periodicity of about 38.5 nm[1], comprising fourteen actin monomers arranged like a twisted "double string of beads". Tropomyosin is a homodimeric parallel coiled-coil that polymerizes in a head-to-tail manner along the entire length of the thin filament (except at the Z-disc)[4], with each tropomyosin homodimer associated with one troponin complex and seven actin monomers. In the absence of troponin, tropomyosin lies along a positively charged groove [5,6], though it is able to shift or rotate considerably along the filament axis. At resting Ca^{2+} concentrations, the troponin complex anchors tropomyosin into a "blocked" position that sterically hinders the approach of myosin towards actin. An increase in free calcium concentration results in a conformational change within the troponin complex that releases tropomyosin into a "closed" position that allows weak actin-myosin interaction[7]. Strong binding of myosin to actin further shifts tropomyosin to the "open" position.

The troponin complex is made up of three components[8-10]: the calcium binding subunit, troponin C (TnC); the inhibitory subunit, troponin I (TnI); and an elongated protein, troponin T (TnT), that binds both TnC and TnI and anchors the entire complex to tropomyosin. For each of the subunits, homologous genes encode isoforms specific to the different types of striated muscle: cardiac, slow-twitch (type I) skeletal, and fast-twitch (type II) skeletal. The TNNC1 gene (3p21.1) is expressed in both slow skeletal and cardiac tissue (we will refer to it as cardiac troponin C, cTnC), whereas the TNNC2 gene (20q12-q13.11) encodes fast skeletal troponin C (hereafter referred to as sTnC)[11-15]. During development, both cTnC and sTnC are expressed in embryonic skeletal muscle, but expression of the cTnC gene is subsequently turned off during the transition to fast-twitch muscle[16,17,18].

The Ca^{2+}-dependent regulation of striated muscle contraction by troponin has been a fascinating area for biochemical and biophysical study since its discovery more than 50 years ago. TnC expresses well in *E. coli* and is the only soluble globular protein of the sarcomeric thin filament. Moreover, its role within the thin filament has been extensively studied, using well-established methods to reconstitute actin, tropomyosin, and troponin into functional filaments[10,19]. This review will focus on cardiac troponin C, highlighting its structure and function within the troponin complex. In recent times, the cardiac troponin complex has become important for understanding the pathogenesis, diagnosis, and treatment of cardiac diseases, especially as a target for the design of cardiac drugs. Underscoring its critical function, cTnC is highly conserved (96.8%–99.4% = 1–6 sequence differences) across 61 known TnC sequences that have been cloned from 41 vertebrate and invertebrate species to date[20].

Structure of cardiac troponin C

Troponin C is an 18-kDa member of the EF-hand Ca^{2+}-binding protein family, first described in the X-ray crystal structure of parvalbumin in 1973[21]. The family derives its name from a characteristic helix-loop-helix motif, in which six residues contribute oxygen ligands to define an octahedral Ca^{2+}-binding site: 1(X), 3(Y), 5(Z), 7(-Y), 9(-X), and 12(-Z). Most of the ligands are polar amino acid sidechains, whereas the residue in the –Y position contributes a backbone carbonyl oxygen, and the sidechain in the –X position often indirectly coordinates Ca^{2+} via a bridging water molecule. The –Z position is almost always glutamate, which provides the only bidentate ligand, a carboxyl group, which changes the coordination geometry from octahedral to pentagonal bipyramidal. Both sTnC and cTnC comprise four EF-hand helix-loop-helix motifs as potential Ca^{2+}-binding sites (I-IV), except that site I in cTnC is inactive due to an insertion (V28) and two key Ca^{2+}-binding amino acid substitutions (D29L and D31A) (see Figure 1).

The first three-dimensional structures of fast skeletal TnC were solved by X-ray crystallography in 1985, full-length turkey sTnC[22] and full-length chicken sTnC[23]. sTnC is organized into two domains, each containing two Ca^{2+}-binding EF-hands. In both structures, the two Ca^{2+}-binding sites of the N-terminal domain (sNTnC) were unoccupied, while two Ca^{2+} ions were bound to the C-terminal domain (sCTnC) (Figure 2A).

Comparison of the two homologous N- and C-domains showed that sNTnC was in a closed state, while sCTnC was in an open state, leading to the suggestion that Ca^{2+} binding to sNTnC would lead to a structural transition[24], causing helices B+C to rotate away from helices N+A+D and exposing a large hydrophobic patch. A short anti-parallel β-sheet formed between EF-hands I and II (centered at position 8, see Figure 1) acts as a hinge for these sub-domain movements. This closed-to-open transition was confirmed in 1995 with the NMR solution structure of sTnC in the fully Ca^{2+}-saturated state [25,26].

Subsequent X-ray structures of sNTnC•$2Ca^{2+}$ and sTnC•$4Ca^{2+}$ have been reported[27,28]. The X-ray structures provide precise details of the metal coordination sites not obtainable from solution NMR methods. The main difference between the X-ray and solution structures of sTnC is found in the central linker connecting the N- and C-domains. The linker forms a rigid α-helix in the crystal forms, but it is unstructured and flexible in solution, highlighting a key caveat in the interpretation of X-ray crystal structures, that crystal packing contacts can induce structure in otherwise flexible regions. There is extensive solution NMR data on isolated TnC to suggest that the two domains can adopt a wide range of orientations: ^{15}N relaxation measurements showing that the linker region is very dynamic and that the two domains tumble independently[29], and paramagnetic relaxation enhancement (PRE) studies measuring the range of distances between domains[30].

The NMR solution structure of cTnC•$3Ca^{2+}$ was determined in 1997[31]. Like sTnC, cTnC also adopts a dumbbell shape with a flexible linker (Figure 2B). As expected the structural C-domain of cTnC binds two Ca^{2+} ions and adopts an open conformation. A comparison of the regulatory domain of cTnC (cNTnC) in both the apo and Ca^{2+}-bound states[31,32] revealed that cNTnC remains strikingly closed in both the absence and presence of calcium, unlike sNTnC. This is likely a consequence of a defunct site I. In sNTnC, a site I-disrupting mutation E41A similarly abolished the link between Ca^{2+}-binding and transition to the open state[33].

Structure and function of cardiac troponin C within the troponin complex

Binding of Ca^{2+} to the regulatory NTnC domain is the key event that links cytoplasmic calcium influx to muscle contraction. Ca^{2+} binding allows NTnC to interact

with the switch region of troponin I, removing the adjacent inhibitory region of TnI from its binding site on actin. It is primarily the inhibitory region of TnI[34], and to a lesser degree, its C-terminal tail[35,36], that anchors the troponin-tropomyosin complex to the blocked position that prevents actin-myosin interaction and shuts off muscle contraction in the absence of Ca^{2+}.

The closed-to-open transition observed in sNTnC suggests a mechanism for Ca^{2+}-mediated regulation, whereby Ca^{2+} binding is coupled to the opening of a "sticky" hydrophobic surface. This is the central paradigm for calmodulin, the ubiquitous Ca^{2+}-sensing protein that binds and regulates a plethora of targets via its versatile hydrophobic surfaces[37]. Indeed, sNTnC binds to the switch region of fast skeletal TnI via the corresponding hydrophobic surface exposed by Ca^{2+} binding[38]. Despite its significant homology to sNTnC, it was initially uncertain whether cNTnC would bind its target in a similar mode, since its Ca^{2+}-bound form is predominantly closed. Nevertheless, the solution NMR structure of cNTnC•Ca^{2+}•$cTnI_{147-163}$ revealed an open state for cNTnC[39]. The bound $cTnI_{147-163}$ switch peptide adopts an α-helical conformation spanning residues 149-159, which wedges into the AB helical interface and inserts Ile148 and Met153 into the hydrophobic pocket to stabilize the open conformation of cNTnC (Figure 3).

Most of the structural work on cTnC has focused on its place in the cardiac troponin complex. However, cTnC is also the isoform for troponin C in slow-twitch skeletal muscle, forming a complex with slow skeletal TnI (ssTnI) and slow skeletal TnT (ssTnT). In fact, ssTnI is also present in the fetal heart, where it is postulated to allow the troponin complex to function in a more acidic environment[40]. The Ca^{2+} affinity of cTnC is reduced under acidic conditions through direct competition between H^+ and Ca^{2+} ions. However, replacement of cTnI in cardiac muscle with ssTnI restores calcium sensitivity at low pH[41]. This acid-resistance was found to depend on a single amino acid substitution: the replacement of Ala162 in cTnI with the corresponding His130 in ssTnI[42]. The positively charged imidazole ring of His130 forms a salt bridge with Glu19 on the surface of cNTnC, stabilizing the cNTnC-ssTnI complex and restoring Ca^{2+}-binding affinity under acidic conditions[43]. In the complex, His130 has a pKa of ~6.7[44], becoming more positively charged as the intracellular pH drops below 7. Recent NMR studies of cNTnC•Ca^{2+} bound to $fsTnI_{115-131}$, as well as cNTnC•Ca^{2+} bound to A162H-$cTnI_{147-163}$, have confirmed the

importance of this electrostatic interaction[45]. The interaction is also present in the X-ray structure of the fast skeletal core troponin complex[46], suggesting that it contributes to pH insensitivity in fast skeletal muscle as well. Some have advocated cardiac gene therapy to introduce the TnI A162H mutation in patients with severe cardiac ischemia[47], since the Ca^{2+} sensitivity of the ischemic heart is reduced by cytosolic acidosis [40].

While the regulatory NTnC domain is generally accepted to play the crucial role of turning muscle contraction on and off in response to calcium, the structural CTnC domain keeps troponin C firmly anchored to the rest of the troponin complex throughout the cardiac cycle. The X-ray crystal structure of the cardiac troponin complex (Figure 4), $cTnC{\bullet}3Ca^{2+}{\bullet}cTnI_{33\text{-}209}{\bullet}cTnT_{183\text{-}288}$ [48], is indispensible for understanding the spatial relationships between troponin C, I, and T. (Note that the numbering of cTnI and cTnT is inconsistent throughout the literature. For cTnI, discrepancies arise because the N-terminal methionine residue is removed and replaced by an acetyl group, and it is variably included in the numbering. In this review, we will not include the initiator methionine so the first residue is Ala1. For cTnT, alternatively spliced forms result in varying lengths. We will use the numbering for the 288-residue version of human cTnT used in the crystal structure[48].) The overall core domain structure is dominated by α-helical elements, most notably the IT arm (consisting of cCTnC, $cTnI_{41\text{-}135}$ and $cTnT_{225\text{-}276}$). $cTnI_{41\text{-}135}$ forms two long α helices, $cTnI_{41\text{-}79}$ and $cTnI_{88\text{-}135}$. $cTnI_{41\text{-}60}$ binds to the hydrophobic cleft of cCTnC, and then residues 62-80 continue the long helix well beyond cCTnC. Residues 80-87 form a loop that interacts with cTnT, and then $cTnI_{88\text{-}135}$ proceeds back towards the opposite face of cCTnC while forming a coiled coil with $cTnT_{225\text{-}276}$. $cTnT_{225\text{-}276}$ is positioned between cCTnC and $cTnI_{88\text{-}135}$, with $cTnT_{259\text{-}270}$ binding extensively to the Ca^{2+}-bound EF-hands III and IV of cTnC.

Limited proteolysis of troponin T (rabbit fast skeletal) yields two fragments, TnT1 and TnT2[49,50], with TnT2 featured in the troponin X-ray crystal structure. Binding studies of TnT2 have shown that both[51,52] the region N-terminal[53] and C-terminal[54] to the IT arm ($cTnT_{225\text{-}276}$) contribute to the interaction with tropomyosin, suggesting that the IT arm is rigidly fixed to tropomyosin. The C-terminus of cTnT, $cTnT_{275\text{-}288}$, is also immediately adjacent to the $cTnI_{128\text{-}147}$ inhibitory region, and both are essential for maintaining the blocked state of actin-tropomyosin[55]. On the other hand, one group has reported that the

region corresponding to $cTnT_{182-215}$ interacts with actin and contributes to the activation of actomyosin ATPase[56]. Thus, the IT arm and regions immediately adjacent to it are key for binding the troponin complex to tropomyosin as well as for stabilizing the inactive blocked state or the active open state of troponin-tropomyosin on actin.

Work performed prior to the publication of the X-ray structure of the cardiac troponin complex in 2003 suggested a possible interaction between the $cTnI_{128-147}$ inhibitory region and the structural cCTnC domain. An NMR structure of $cCTnC•2Ca^{2+}•cTnI_{128-147}$[57] shows residues L134-K139 of cTnI forming a helix upon interaction with the E and H helices of $cCTnC•2Ca^{2+}$. However, since this arrangement of $cTnI_{128-147}$ on cCTnC would be displaced by $cTnI_{41-60}$, and $cTnI_{88-135}$ is positioned on the opposite side of cCTnC as part of the IT arm (Figure 4), the observed cCTnC-$cTnI_{128-147}$ interaction is unlikely to occur within the context of intact troponin complex. This example illustrates how binding studies on simplified systems need to be interpreted with caution.

Although N- and C-domains of troponin C are connected by a flexible linker, there are additional intermolecular interactions within the whole troponin complex that can fix the relative domain orientations of cTnC. The domain positions have recently been described using Förster resonance energy transfer (FRET) in reconstituted cardiac troponin complex [58] as well as with fluorescence polarization in intact thin filament[59].

In cardiac TnC, the orientation of cNTnC relative to cCTnC is fixed by the direct interaction of the N-terminal 31-amino acid cardiac-specific region of cTnI with cNTnC [60-62]. This region is critical to the regulation of calcium sensitivity in the cardiac sarcomere. Phosphorylation of cTnI in response to β-adrenergic sympathetic stimulation was first identified in 1976[63]. β-adrenergic stimulation leads to production of cyclic AMP and activation of protein kinase A, which phosphorylates cTnI Ser22 and Ser23 (in addition to many other targets)[65,66]. Other kinases have been found to phosphorylate this site as well including PKC, PKD1, and PKG[67], showing Ser22/Ser23 to be an important regulatory node for integrating multiple signaling pathways. Phosphorylation at this site has been consistently shown to decrease the calcium sensitivity of the cardiac sarcomere[64].

Recently, we have used solution NMR spectroscopy to study $cTnI_{1-73}$ in complex with $cTnC•3Ca^{2+}$[68]. In this complex, $cTnI_{39-60}$ binds to cCTnC, stabilizing an α helix in $cTnI_{41-67}$ and a type VIII turn in $cTnI_{38-41}$ that brings $cTnI_{19-37}$ into close proximity with the

negatively charged surface of cNTnC (opposite the hydrophobic surface that binds the switch peptide, $cTnI_{148-158}$). The interaction with $cTnI_{19-37}$ is electrostatic in nature, which allows $cTnI_{1-37}$ to maintain a disordered state even while in complex with cNTnC. In contrast to a previous model[69] for the cNTnC-$cTnI_{1-37}$ complex, the interaction does not induce secondary structure in $cTnI_{1-37}$, it does not favor the open state of cNTnC, and it does not directly affect the Ca^{2+}-binding affinity of cNTnC. However, it does fix the positioning of cNTnC relative to the rest of the troponin complex. We propose that this domain orientation is optimal for cNTnC binding to the $cTnI_{148-158}$ switch region, and this is how $cTnI_{19-37}$ indirectly increases the Ca^{2+} affinity of cNTnC within the context of the cardiac thin filament. Binding of $cTnI_{148-158}$ stabilizes the Ca^{2+}-bound open conformation of cNTnC, thus increasing its Ca^{2+} binding affinity. The cNTnC domain is released by protein kinase A (PKA) phosphorylation of cTnI Ser22 and Ser23, which disrupts the electrostatic interactions between $cTnI_{19-37}$ and cNTnC.

The domain positioning of cNTnC relative to cCTnC is stabilized by the electrostatic interaction between cNTnC and $cTnI_{19-37}$, but it is fully defined by additional weak interactions: a contact between Ala7 in the N helix of cNTnC and Ala42/Ser43 of the $cTnI_{41-80}$ helix bound to cCTnC, as well as an interaction between the negatively charged C-terminal residue Glu161 of cCTnC and positively charged residues in the cTnC interdomain linker. These interactions are present in the X-ray crystal structure of the cardiac troponin complex[48], and they bring together the N helix of cNTnC, the interdomain linker, and the C-terminus of cCTnC in the presence of unphosphorylated $cTnI_{19-43}$ to define the interdomain orientation of cTnC.

Energetics of binding and conformational transitions

The calcium binding affinity of cNTnC must be exquisitely tuned to the cytosolic Ca^{2+} fluctuations within the cardiomyocyte. The cardiomyocyte free cytosolic Ca^{2+} concentration during diastole is about 100 nM, reaching a peak of about 1 μM during systole[70]. The half-activating concentration of free Ca^{2+} in cardiac myofilaments is typically 1-4 μM[71,72].

The calcium binding affinity of the regulatory cNTnC domain is determined by the relative stability of the Ca^{2+}-bound state versus the apo state. In Ca^{2+}-binding EF-hand

proteins that undergo a closed-to-open conformational transition, Ca^{2+} binding is driven by electrostatic interactions as well as entropically favorable release of water molecules[73]. This is offset by the exposure of a large hydrophobic patch upon transition to the open state. In cNTnC, the fact that EF-hand I is defunct means that there is no positive cooperativity of Ca^{2+} binding (at least for the isolated cNTnC domain), and the transition to the open state is less favorable, so that the closed state predominates in the absence of cTnI switch peptide. The structure of cNTnC in the closed conformation was recently highlighted by an X-ray structure of cNTnC in complex with Cd^{2+}, providing for the first time a high resolution model of EF-hand II metal ion coordination in the closed state. Most of the Ca^{2+}-coordinating interactions observed in the open state[48] (see Figure 1) were intact in the closed state, except that D67 (the 3Y position of EF-hand II) and S69 (position 5Z) do not participate in the coordination of Cd^{2+} [74]. This partial coordination may account for the increased rate of Ca^{2+} release in the closed state when compared to the cTnI switch peptide-stabilized open state (see below).

cNTnC is predominantly in the closed state when it is bound to Ca^{2+}. However, it is now apparent that Ca^{2+}-bound cNTnC exists in a dynamic equilibrium between closed and open forms (Figure 3). The opening involves movement of the BC helices as a unit away from the NAD helices unit. MD simulations have detected a concerted tilting motion of helices B and C away from helix A, corresponding to the opening motion of cNTnC•Ca^{2+} [75]. NMR amide proton relaxation dispersion experiments reveal that many residues in the hinge regions of cNTnC experience a conformational exchange process with a time scale of ~30 μs [76,77]. It was estimated that the open state is populated at about 20%[78]. This value is consistent with recent solution NMR paramagnetic relaxation enhancement (PRE) data from four spin-labeled monocysteine constructs of isolated cTnC, suggesting that the open state is populated ~27% in the calcium-saturated cTnC[79][79], compared with ~0% in the apo state. Together these data support a model in which Ca^{2+} binding creates a dynamic equilibrium between the 'closed' and 'open' structural states to prime cNTnC for subsequent binding of the $cTnI_{148-158}$ switch region.

Hydrophobic binding of $cTnI_{148-158}$ to the large hydrophobic pocket of cNTnC stabilizes its open state, increasing its Ca^{2+} binding affinity. Using fluorescent IAANS labeling at Cys35 and Cys84, a Ca^{2+} binding K_D of about 2 μM was determined for cNTnC

in the context of isolated cTnC[80]. When cTnC was combined with cTnI and cTnT to make full troponin complex, K_D of cNTnC became 0.3 μM, indicating about one order of magnitude tighter Ca^{2+} binding. This is due to a slowing of Ca^{2+} release, from >1000 s^{-1} from isolated cTnC at 15°C[81] to 122 s^{-1} in the presence of cTnI and 33-42 s^{-1} in the presence of cTnI and cTnT[82].

Solution NMR spectroscopy was also used to assess the binding of Ca^{2+} and $cTnI_{147-163}$ to cNTnC at 30°C[83]. K_D of ~20 μM and ~150 μM were determined for Ca^{2+} and $cTnI_{147-163}$ peptide binding, respectively. The Ca^{2+} release rate was estimated to be 5000 s^{-1} in the absence of cTnI. The off rate of $cTnI_{147-163}$ was about 5000 s^{-1}, suggesting a rapid equilibrium between bound and free states.

A subsequent fluorescence study suggested that IAANS labeling at Cys35 and Cys84 altered the Ca^{2+}-binding properties of cNTnC, so a monocysteine C35S/T53C/C84S mutant was put forward[84]. This mutant was used to study the Ca^{2+} binding characteristics of cTnC in the context of cardiac troponin complex (cTn) versus slow skeletal troponin complex (ssTn), as well as in the presence of actin, tropomyosin, and myosin S1 subfragment. In the isolated cardiac complex, a Ca^{2+} K_D of 0.65 μM was measured, compared with 0.25 μM in the slow skeletal complex. The higher Ca^{2+} affinity of the ssTn complex can be expected from the enhanced affinity of the ssTnI switch region for cTnC. Upon addition of thin filament (actin and tropomyosin), the Ca^{2+} K_D of cTn increased to 5 μM, explainable by the binding of $cTnI_{128-147}$ to actin, making the $cTnI_{148-158}$ switch region less available for binding to cNTnC. Addition of myosin S1 fragment (to troponin complex + actin + tropomyosin) changed the Ca^{2+} K_D of cTn to 0.78 μM, likely because the formation of actomyosin rigor complexes shifts the thin filament to an open state that releases $cTnI_{128-147}$ from actin. These studies demonstrate how the Ca^{2+} binding affinity of cNTnC is exquisitely dependent on the availability of $cTnI_{148-158}$ switch region for binding. $cTnI_{148-158}$ is tethered to actin via adjacent segments[34] when the thin filament is in the blocked state, but released and available for binding cNTnC in the thin filament closed or open states. (Note that cNTnC itself has closed and open states that are distinct from the thin filament states, for which “closed” refers to the positioning of tropomyosin when cNTnC is bound to calcium, and the “open state” refers to a further shift of tropomyosin when myosin forms strong cross-bridges to actin.)

There are no known post-translational modifications of cTnC that modulate its calcium affinity. However, there are numerous phosphorylation sites on cTnI[85] that impact the calcium affinity of cTnC, the most important of which is Ser22/23 in humans. We propose that many of these phosphorylation sites, including Ser22/23, impact cNTnC calcium binding by modulating the availability of $cTnI_{148-158}$ switch peptide for binding (the interaction between the unphosphorylated cardiac-specific N-terminal extension and cNTnC orients it optimally for binding $cTnI_{148-158}$, thus increasing the effective concentration of $cTnI_{148-158}$). Moreover, modifications to TnT, tropomyosin, actin, or myosin can also impact the blocked-closed-open equilibrium of the thin filament to impact $cTnI_{148-158}$ availability and calcium sensitivity.

In contrast to cNTnC, cCTnC binds Ca^{2+} with very high affinity. In isolated cTnC, the Ca^{2+} binding K_D is about 40 nM[86], decreasing to about 3 nM in intact troponin complex[80]. The very high affinity of CTnC is a consequence of the instability of its apo state. A closed-to-open transition does not occur in skeletal or cardiac CTnC, which appears to form a molten globule in the absence of Ca^{2+}[87,88]. Ca^{2+} binding induces the formation of well defined tertiary structure, demonstrating that the sequence of CTnC is optimized for the open conformation, but not the closed. $cTnI_{39-60}$ binds to Ca^{2+}-saturated cTnC with very high affinity[83,89]. An affinity of 3 nM was measured for $cTnI_{38-57}$ via surface plasmon resonance and ELISA[90]. The affinity for intact cTnI in the presence of cTnT is likely even stronger, given the additional contacts formed within the IT arm.

Mg^{2+} is chemically similar to Ca^{2+} and able to compete for the same binding sites in many EF-hand-containing proteins[91]. A classic equilibrium dialysis study of Ca^{2+}- and Mg^{2+}-binding in fast skeletal TnC showed that Mg^{2+} competes for the high affinity Ca^{2+} binding sites in the structural CTnC domain, whereas the lower affinity sites in the regulatory NTnC are Ca^{2+}-specific[92]. (The same study also identified two non-competitive Mg^{2+} sites.) More recent fluorescence studies, however, have demonstrated that Mg^{2+} is also able to compete with Ca^{2+} in the NTnC domain, reducing the apparent calcium affinity by about a factor of 2 at a Mg^{2+} concentration of 1-3 mM[93]. Thus, the presence of Mg^{2+} in the muscle cytoplasm would have a calcium desensitizing effect, since Mg^{2+} itself does not trigger muscle contraction. Compared to Ca^{2+}, Mg^{2+} binding tends to favor the closed conformation[91,93], so cNTnC cannot bind cTnI switch peptide (in the open

conformation) in the absence of Ca^{2+}. In contrast, cCTnC is able to adopt an open conformation and bind $cTnI_{33-80}$ when loaded with Mg^{2+} instead of Ca^{2+}, as demonstrated by an NMR structure [94,95].

Hypertrophic cardiomyopathy-associated mutations

Hypertrophic cardiomyopathy (HCM) is a common genetic disease, having an estimated prevalence of ~1:500[96,97]. Although there are some rare infiltrative causes, HCM is generally a disease of the sarcomere, with a sarcomeric mutation identifiable in about half of affected patients. Mutations in the myosin heavy chain β and cardiac myosin binding protein-C are most common, followed by mutations in cardiac troponin I and troponin T[98,99]. One challenge in identifying definitive mutations is that many mutations have incomplete penetrance. As the name suggests, the most striking feature of HCM is abnormal hypertrophy of the ventricles, most classically prominent in the interventricular septum. The hypertrophy can be so severe that it causes a left ventricular outflow tract obstruction. The hypertrophied ventricles are also prone to ventricular arrhythmias that can cause sudden death. One challenge in the treatment of HCM is identifying patients at greatest risk of sudden death who would benefit from placement of an implantable cardioverter defibrillator. Other common complications include atrial fibrillation, diastolic dysfunction, myocardial ischemia, and mitral regurgitation[100]. To date, 7 mutations associated with HCM (A8V, L29Q, A31S, C84Y, Q122AfsX30, E134D, D145E) and 7 mutations associated with dilated cardiomyopathy (DCM) (Y5H, Q50R, E59D/D75Y, M103I, D145E, I148V, G159D) in cTnC have been reported. A recent review[101] has summarized the biochemical characterization of these mutations, but we will briefly survey them here.

The first publication of a TNNC1 mutation associated with cardiomyopathy was in 2001[102]. An L29Q mutation was identified in a 60-year-old male HCM patient with 15 mm thickness of the septal and posterior left ventricular wall (normal is <11 mm and 13 mm is the minimum in adults required for HCM diagnosis). Ca^{2+} sensitivity of the L29Q-cTnC mutant has been reported to be decreased[103], increased[104,105], or unchanged[106]. These discrepancies have been suggested to be at least partly due to the different model systems used in these studies, although the same range of divergent results have also been

reported for isolated protein components. Given the wide range of experimental findings in the absence of further supportive genetic evidence, it is possible that L29Q represents an incidental polymorphism.

An important study describing HCM mutations in troponin C was published in 2008, in which 1025 HCM patients were screened for mutations in TNNC1, and all positive hits were further screened at fifteen other known HCM susceptibility loci[107]. Four patients were identified with missense mutations in TNNC1 and no other locus, and these had left ventricular maximum wall widths ranging from 19 to 26 mm. The same authors later identified another mutation, A31S, in a five-year-old boy with a history of ventricular fibrillation and a mean ventricular wall thickness of 20 mm [108]. None of these patients had affected family members with the same mutation. The five mutations, A8V, A31S, C84Y, E134D, and D145E were expressed recombinantly in troponin C and exchanged into porcine muscle fibers. All of the mutants except E134D were associated with increased calcium sensitivity[109-112], so E134D may be a clinically insignificant variant. The observed increases in calcium sensitivity are consistent with what is observed in HCM-associated mutations involving other thin filament proteins [99]. It seems plausible that increased calcium sensitivity of the thin filament leads to increased muscle activation and exuberant muscle hypertrophy.

HCM-associated mutations in cNTnC highlight the potential mechanisms by which the calcium affinity of cNTnC can be increased. The A8V mutation was found to increase calcium sensitivity of reconstituted thin filaments, particularly when cTnI was unphosphorylated [113]. Ala8 is part of the N helix, which plays the key role in defining the domain orientation of cNTnC relative to the rest of the troponin complex, so it is possible that the A8V mutation stabilizes the positioning of the cNTnC domain. Ala31 is located in the loop region of EF hand I, so the A31S mutation may favor the conformation of this loop when cNTnC is in the open form. Cys84 contacts the switch region of cTnI in the open conformation and the BC loop in the closed conformation, so the C84Y mutation may either stabilize the open complex bound to cTnI or destabilize the closed conformation of cNTnC. In summary, we propose that the A8V, A31S, and C84Y increase the Ca^{2+}-binding affinity of cNTnC by favoring the active form of cNTnC, either by stabilizing the activating orientation of the cNTnC domain, shifting the

conformational equilibrium from the closed to open state, or directly enhancing binding to the cTnI switch peptide.

HCM-associated mutations identified in the cCTnC domain have far more destabilizing effects than those identified in the cNTnC domain. Asp145 is Ca^{2+}-coordinating residue 5(Z) of EF-hand IV, and the D145E mutation was found to drastically reduce the Ca^{2+} binding affinity of cCTnC[112,114],. Another HCM-associated mutation with a profound effect on the C-domain is Q122AfsX30, in which a frameshift mutation obliterates the last half of cCTnC and replaces it with 30 mistranslated residues[115]. This mutation was identified in a patient with histologically confirmed HCM after sudden death at the age of 19. The mutation was also identified in other family members with HCM. How these drastic mutations in the structural cCTnC domain contribute to HCM is currently unknown.

Dilated cardiomyopathy-associated mutations

Familial dilated cardiomyopathy (DCM) causes thin dilated ventricles associated with a decreased left ventricular ejection fraction. Compared with HCM, there is a wider variety of mutations, both sarcomeric and non-sarcomeric, that give rise to familial DCM, though DCM is more rare, with an estimated prevalence of ~1:5000 [99,117].

G159D was the first DCM mutation reported[118]. It co-segregated with five affected family members, including the proband, who developed heart failure at the age of 21 and received a heart transplant two years later. A subsequent study identified a sixth family member who developed severe systolic heart failure requiring a heart transplant at the age of 3[119]. Thus, the genetic evidence supporting G159D as a causative mutation in DCM is the most extensive of any TNNC1 mutation. G159D-cTnC was shown to have similar Ca^{2+}-binding affinity to wildtype-cTnC in isolation, but a reduced Ca^{2+} affinity was observed in intact muscle fiber experiments [120-123]. Further studies demonstrated that G159D blunted the effect of cTnI phosphorylation at Ser22 and Ser23 [124-126]. Gly159 is located near the C-terminus of cTnC, where we postulate that the G159D mutation disrupts the delicate positioning of cNTnC relative to cCTnC.

The double mutant E59D/D75Y was identified in one patient [127]. The same study also found that rat cardiomyocytes transfected with D75Y mutation, but not E59D, had

greatly reduced contractility. Mutant D75Y-cTnC appears to have reduced Ca^{2+}-binding affinity when tested in isolation, but the reduction in affinity appears more pronounced when it is incorporated into intact troponin complex or reconstituted thin filaments[123]. Asp75 is located position 11 of EF-hand II, though it is not directly involved in coordinating Ca^{2+}. It is possible that the D75Y mutation favors the closed conformation of EF-hand II.

The Q50R mutation was identified in a 16-month-old with dilated cardiomyopathy, whose mother also had peripartum cardiomyopathy. The maternal grandmother and two of her sisters had a history of dilated cardiomyopathy as well, and the mutation co-segregated with disease in at least four family members across three generations[128]. Thus, the genetic evidence supporting Q50R as a DCM-causative mutation is strong, though no biochemical studies have been published to date. Q50 is located in the BC loop between EF-hands I and II, in a position where the Q50R mutation may produce an electrostatic repulsion with R147 of cTnI, disrupting binding of the switch region, $cTnI_{148-158}$.

The remaining DCM-associated mutations in TNNC1 were identified in a study[129] that examined a cohort of 312 patients with DCM. A total of 14 genes were sequenced, identifying mutations in up to 27% of the patients. There were four DCM-associated TNNC1 mutations identified: Y5H, M103I, D145E, and I148V. Y5H was detected in one patient, who developed heart failure at two weeks of age and later received a heart transplant at age of 15. Neither parent had DCM or the Y5H mutation. The father and the proband both had a mutation identified in MYH7, the beta myosin heavy chain, so it is possible that Y5H was only an incidentally observed mutation. All of the TNNC1 mutations identified in the study yielded small changes in calcium sensitivity, but Y5H had the largest Ca^{2+} desensitizing effect and also reduced the effect of PKA phosphorylation[109]. Tyr5 is located in helix N of cNTnC, where it packs against Arg83 of helix D. It is possible that the Y5H mutation creates an electrostatic repulsion that destabilizes helix N and disrupts positioning of the cNTnC domain. The M103I mutation was detected in a patient and a sister both with DCM [129]. However, this family had two additional sisters without this mutation, but who had cardiac conduction abnormalities and/or syncope. Met103 is located in helix E of EF-hand III and binds to $cTnI_{57-64}$. It is

interesting to note that there is an isoleucine at the homologous position in skeletal TnC, so the M103I mutation would not be expected to cause a major perturbation. I148V was identified in a single patient with DCM[129]. Ile148 is located in the EF-hand IV loop region and is part of the small β-sheet at the base of the hydrophobic pocket. Other EF-hand proteins contain a valine at this site, so I148V is a relatively conservative substitution. Thus, M103I and I148V mutations would be expected to have only a minor impact on cCTnC structure and function, so it is uncertain whether these are truly clinically significant. In contrast, the D145E mutation, also independently identified in an unrelated HCM study (see section on HCM above)[107], drastically reduces the calcium affinity of cCTnC. It is possible that this mutation primarily causes HCM, but HCM can progress towards a DCM phenotype in later stages.

The genetic evidence behind some DCM-associated TNNC1 mutations is excellent. However, unlike thin filament HCM mutations, for which there is a consensus about increased myofilament calcium sensitivity, there is less agreement regarding the functional impact of DCM mutations. Although some studies document a decrease in calcium sensitivity, this has not been consistently demonstrated. One interesting observation in a number of DCM-associated mutations in the thin filament is an insensitivity to the effect of PKA-mediated phosphorylation of Ser22/Ser23 in cTnI[130]. Phosphorylation of cTnI is an important mechanism by which the calcium sensitivity of the cardiac sarcomere is regulated. Dephosphorylation of cTnI has been observed in heart failure of various etiologies, including DCM and ischemic cardiomyopathy[131]. A non-phosphorylatable R21C mutation in cTnI (it abolishes the PKA RRXS phosphorylation motif) was associated with hypertrophic cardiomyopathy and diastolic dysfunction[132,133]. It is possible that the insensitivity to cTnI phosphorylation seen in some DCM mutants is indicative of a structural derangement whereby positioning of the regulatory cNTnC domain by unphosphorylated cTnI is either unachievable or ineffective. In this case, the predominant defect would be a decrease in calcium sensitivity for which dephosphorylation of cTnI would be unable to compensate.

Drugs that bind the regulatory domain, cNTnC

A number of small molecules have been found to bind to cardiac troponin C (see

Figure 5). Of these, levosimendan is the most widely studied to date, licensed in some South American and European countries for use as a positive inotropic agent in acute decompensated systolic heart failure. Levosimendan has been shown to improve cardiac output and decrease symptoms of heart failure[134]. However, its use has been limited by associated cardiac arrhythmias and hypotension, and recent Phase III trials demonstrated no improvement in overall mortality[135,136], though some meta-analyses suggest some mortality benefit[137]. Levosimendan has been suggested to bind to the regulatory domain of troponin C, cNTnC, but its exact mechanism of action has remained controversial[138,139]. One study failed to detect evidence of levosimendan binding to cTnC[140], though small amounts of covalent adduct were detected after prolonged incubation, likely formed through levosimendan's reactive malononitrile moiety (see Figure 5). It was later suggested that Cys84 is necessary for the covalent binding of levosimendan with cTnC [139,141], and this residue was changed to serine to produce a cysteineless mutant in some studies. Nevertheless, even with intact Cys84, no group has yet produced uniform stable cTnC•levosimendan complex for structural analysis. Moreover, the active metabolite of levosimendan, OR-1896[142], does not possess the reactive malononitrile group and is unlikely to form a covalent conjugate. On the other hand, levosimendan does inhibit type 3 phosphodiesterase (PDE3) with nanomolar affinity[142], and there are many other PDE3 inhibitors that act as positive inotropes and calcium sensitizers[143], all of which also cause hypotension and arrhythmias as undesirable side effects[144].

A levosimendan analogue (DFBP-O) was shown to bind to cNTnC-cTnI$_{147\text{-}163}$ and increase the Ca^{2+} sensitivity of skinned cardiac trabeculae[145]. The structure of cNTnC•Ca^{2+}•cTnI$_{147\text{-}163}$•DFBP-O shows that DFBP-O binds to the interface between cNTnC and cTnI$_{147\text{-}163}$ to enhance the binding of cTnI$_{147\text{-}163}$ to cNTnC (see Figure 6). Using the structure of cNTnC•Ca^{2+}•cTnI$_{147\text{-}163}$•DFBP-O, a novel Ca^{2+} sensitizer 4-(4-(2,5-dimethylphenyl)-1-piperazinyl)-3-pyridinamine (NCI147866) was discovered using computational drug screening and verified by solution NMR [146]. NCI147866 binds to both cNTnC•Ca^{2+} and the cNTnC•Ca^{2+}•cTnI$_{147\text{-}163}$ complex. Its presence increases the affinity of switch peptide to cNTnC by approximately a factor of two. Its affinity for cNTnC•Ca^{2+}•cTnI$_{147\text{-}163}$ is comparable to that of DFBP-O (K_D 380 μM for both).

A CaM antagonist, W7 (N-(6-aminohexyl)-5-chloro-1-naphthalene sulfonamide)

was found to inhibit the maximum ATPase activity and tension development in both skeletal and cardiac muscle fibers[147,148]. NMR titration studies have shown that W7 reduced the affinity of cTnI switch peptide for cNTnC from 154 μM to over 2000 μM[149]. An NMR structure of the ternary complex cNTnC•Ca^{2+}•$cTnI_{144-163}$•W7 (see Figure 6) revealed that W7 binding causes a considerable displacement of the cTnI switch peptide from its preferred binding site[149]. Thus, W7 acts as a troponin inhibitor and Ca^{2+} desensitizer by increasing the off rate of the cTnI switch peptide. Its mechanism of action stands in contrast to DFBP-O and NCI147866, which both act as troponin activators by enhancing the binding of cTnI switch peptide to cNTnC.

Bepridil is a Ca^{2+} channel blocker and a calmodulin (CaM) antagonist previously marketed as an anti-anginal agent. Bepridil increases the Ca^{2+} sensitivity of myofilaments, increasing actomyosin ATPase activity and force generation[150]. The X-ray structure of cTnC•3Ca^{2+}•3bepridil provides detailed structural data on bepridil binding[151]. In this structure, three bepridil molecules stack together, but one molecule is bound to the hydrophobic pocket of cNTnC and another to the hydrophobic pocket of cCTnC, bringing the two domains together. The NMR structure of the cNTnC•Ca^{2+}•$cTnI_{147-163}$•bepridil ternary complex reveals a binding site for $cTnI_{147-163}$ primarily located on the A/B interhelical interface and a binding site for bepridil in the hydrophobic core of cNTnC•Ca^{2+} [152]. Bepridil seems to slightly weaken the binding of $cTnI_{147-163}$ by displacing cTnI Ile148, but not to the same extent as W7.

In summary, cNTnC is an eminently “druggable” target with a large hydrophobic cavity already known to bind a number of compounds, albeit at low affinity. Moreover, cNTnC-binding compounds are able to act as troponin activators or inhibitors, depending on their effect on cTnI switch peptide binding. (It is preferable to refer to these compounds as troponin modulators rather than calcium sensitizers/desensitizers to distinguish their mechanism of action from other compounds that act through signaling pathways, like levosimendan.) Cardiomyopathy-associated mutations in troponin demonstrate that minute changes in troponin can have a dramatic effect on cardiac function and phenotype, so it is very possible that drugs targeting troponin will play a role in treating cardiomyopathies as well as heart failure in general. For fast skeletal troponin, a number of troponin activators developed by Cytokinetics are already at

clinical and pre-clinical stages. We are currently working to develop the first high affinity cardiac troponin modulators.

Compounds that bind to the structural domain, cCTnC

As noted above, bepridil also binds to the structural domain of cardiac troponin C, cCTnC, being a promiscuous molecule that also interacts with calmodulin and calcium channels. However, bepridil does not bind to cCTnC in the presence of cTnI. The hydrophobic patch of cCTnC is capable of binding a number of other compounds well known for their promiscuity: EMD 57033, resveratrol, and green tea catechin EGCg (epigallocatechin gallate) (see Figure 5). Of these, resveratrol and EGCg have been highlighted recently as an example of "PAINS" (pan-assay interference compounds)[153]. Thus, although these compounds have demonstrated activity as calcium sensitizers or desensitizers, it is uncertain whether binding to cCTnC or some other target accounts for their observed activity.

EMD 57033 is the (+)-enantiomer of EMD 53998. While the (-)-enantiomer, EMD 57439, is a PDE3 inhibitor, EMD 57033, enhances the Ca^{2+}-sensitivity of both myofibrillar ATPase and force development in skinned muscle fibers[154-156]. EMD 57033 exerts a direct effect on actomyosin, increasing its ATPase activity. A recent study of EMD 57033 binding to myosin suggested that its binding site involved the SH3-like subdomain of the N-terminal domain [157]. Direct binding of EMD 57033 to the C-domain of cTnC has been detected though tyrosine fluorescence [158] and solution NMR spectroscopy [140,159]. In the high resolution structure of cCTnC•2Ca^{2+}•EMD 57033[89], the drug molecule is oriented such that the chiral group of EMD 57033 fits deep in the hydrophobic pocket and makes several key contacts with the protein (see Figure 7). EMD 57033 is completely displaced by the binding of $cTnI_{34-71}$, but it is compatible with $cTnI_{128-147}$ binding[159]. This suggests that EMD 57033 does not bind to cTnC in the context of intact troponin complex, but its interaction may become a factor if cCTnC becomes dissociated from the rest of the IT arm. In such a circumstance, EMD 57033 may even act as a calcium sensitizer by stabilizing the open conformation of cCTnC and priming it to bind $cTnI_{128-147}$. However, there is currently no evidence that this ever occurs *in vivo*.

The polyphenolic compounds EGCg[160] and resveratrol[161] were also found to bind to

cCTnC, but not cNTnC. While EGCg is found in green tea, resveratrol is found in red wine. The NMR solution structures of cCTnC•2Ca^{2+}•EGCg[160] and cCTnC•2Ca^{2+}•resveratrol[161] show that these compounds bind to a similar pocket as EMD 57033 (see Figure 7). EGCg binding is more shallow than EMD 57033 binding, and unlike EMD 57033, EGCg can still bind cCTnC in the presence $cTnI_{34-71}$, although the affinity is very weak, with a K_D of about 1.8 mM. An examination of the cCTnC complexes presented in Figure 7 suggests that EGCg and $cTnI_{128-147}$ bind more superficially to cCTnC than $cTnI_{34-71}$ and the other drugs.

Summary

Over the past 40 years, significant progress has been made toward understanding the structure and function of cardiac troponin C. cTnC plays a critical role in the heart, coupling its electrical pacing system to its mechanical apparatus through the binding of Ca^{2+} ions. Calcium plays a dual role in cTnC, stabilizing the structural C-domain (via two high affinity binding sites), as well as activating the regulatory N-domain (via a single low affinity binding site), the key calcium sensor of cardiac contraction. cNTnC is a dynamic molecule, allowing for effective regulation of cardiac calcium sensitivity. First of all, cNTnC undergoes a rapid closed-to-open equilibrium, with the open state being the biologically active form that binds the troponin I switch peptide. Troponin modulating drugs can act by binding the open state, with troponin activators enhancing the affinity for the switch peptide and troponin inhibitors blocking this interaction. The cNTnC domain as a whole is dynamic, being connected to cCTnC and the rest of the troponin complex by a flexible linker. Stabilizing or disrupting the orientation of cNTnC via weak electrostatic interactions is an important regulatory mechanism readily impacted by phosphorylation of troponin I. Finally, the availability of the troponin I switch peptide as the thin filament alternates between blocked, closed, and open states, is an important downstream modulator of cNTnC function that can be impacted by modifications in myosin, actin, tropomyosin, and the rest of the troponin complex. All of these dynamic processes (the closed-to-open equilibrium, domain positioning, and switch peptide availability) are readily impacted by cardiomyopathy-causing mutations. Understanding

the dynamic structural biology of cardiac troponin C within the thin filament will impact not only the treatment of the heritable cardiomyopathies, but also the treatment of heart failure of any etiology.

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FIGURES

Figure 1: Amino acid sequence comparison of human cTnC and sTnC. Helices are denoted by white bars below the sequences. In cTnC, residues 87-92 comprise the inter-domain helix. Calcium-coordinating EF-hand positions 1, 3, 5, 7, 9, and 12 are highlighted in cyan. Note, however, that EF-hand I in cTnC is defunct. Position 8 of each EF-hand is marked with an asterisk, denoting the hydrogen bonding central β-sheet-forming residue in each EF-hand.

Figure 2: Structures of sTnC and cTnC: A. sTnC•2Ca^{2+} (X-ray, pdb 5TNC); B. sTnC•4Ca^{2+} (X-ray, pdb 2TN4); C. cTnC•3Ca^{2+} (NMR, pdb 1AJ4). Ca^{2+} ions are shown as spheres.

Figure 3: Comparison of the Ca^{2+}- and cTnI switch peptide-induced structural changes in cNTnC and sNTnC. The pdb code for the structures are cNTnC•apo (1SPY), cNTnC•Ca^{2+} (1AP4), cNTnC•Ca^{2+}•cSp (1MXL), sNTnC•apo (1TNP), sNTnC•2Ca^{2+} (1AVS), sNTnC•2Ca^{2+}•sSp (1YTZ). Ca2+ ions are shown as spheres. Cardiac switch peptide (cSp) and fast skeletal switch peptide (sSp) are shown in red in the far right complexes.

Figure 4: X-ray structure of cardiac troponin core complex, cTnC•3Ca^{2+}•$cTnI_{34-161}$•$cTnT_{221-276}$ (pdb 1J1E). cTnC is colored in green and the 3 bound Ca^{2+} are represented by magenta spheres, cTnI is colored in blue, and cTnT is colored in red. Regions N-terminal and C-terminal to $cTnT_{221-276}$ are known to anchor the entire complex to tropomyosin and have been drawn in as squiggles to show that their structures are unknown. $cTnI_{136-145}$ and $cTnI_{162-209}$ are known to interact with actin to maintain the blocked state of the thin filament. These regions are shown as blue squiggles to signify that they are unstructured in the calcium-activated state, but acquire an as-of-yet undetermined structure bound to actin in the blocked state.

Figure 5: Chemical structures of compounds known to bind to cardiac troponin C.

Figure 6: Structures of cNTnC in complex with drugs and/or cTnI peptides: cNTnC•Ca^{2+}•cSp (pdb 1MXL) is colored blue, cNTnC•Ca^{2+}•cSp•bepridil (pdb 1LXF) is colored gold, cNTnC•Ca^{2+}•cSp•DFBP-O (pdb 2L1R), cNTnC is colored teal, and cNTnC•Ca^{2+}•cSp•W7 (pdb 2KRD) is colored brown. The cardiac switch peptide (cSp) is colored red, Ca^{2+} ions are shown as red spheres, and drugs are shown as sticks.

Figure 7. Structures of cCTnC in complexes with drugs or cTnI peptides: cCTnC•2Ca^{2+}•cIp (pdb 1OZS) is colored magenta, cCTnC•2Ca^{2+}•cRp (pdb 1J1E) is colored slate, cCTnC•2Ca^{2+}•EMD57033 (pdb 1IH0) is colored red, cCTnC•2Ca^{2+}•bepridil (pdb 1DTL) is colored orange, cCTnC•2Ca^{2+}•EGCg (pdb 2KDH) is colored blue, and cCTnC•2Ca^{2+}•resveratrol (pdb 2L98) is colored cyan. Ca^{2+} ions are shown as black spheres, inhibitory peptide (cIp, $cTnI_{128-147}$) and regulatory peptide(cRp, $cTnI_{34-71}$) are shown in green, and drugs are shown as sticks.

REVIEW on cTnC
Gene and Gene Wiki

Structure and Function of Cardiac Troponin C

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FIGURES:

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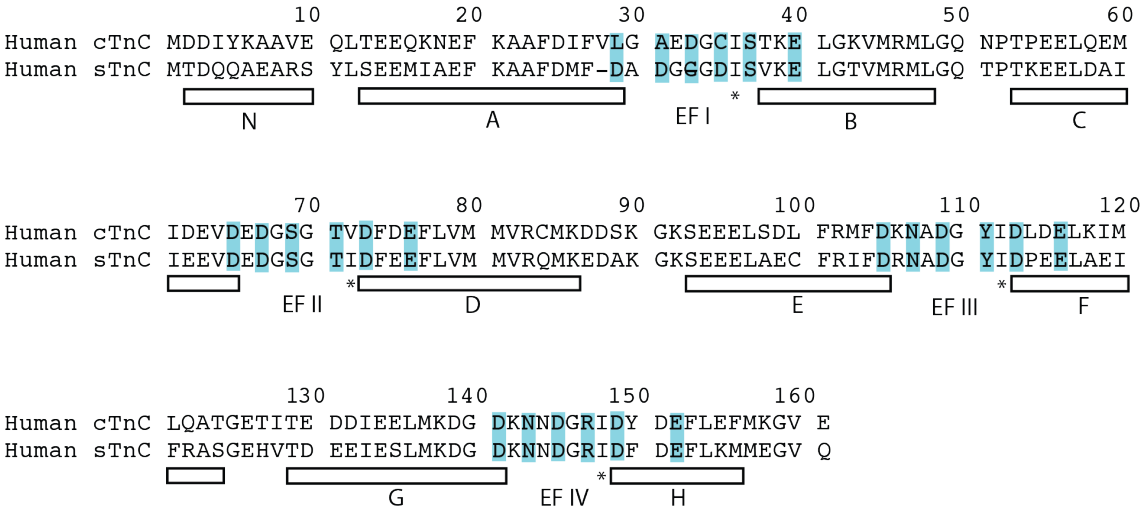

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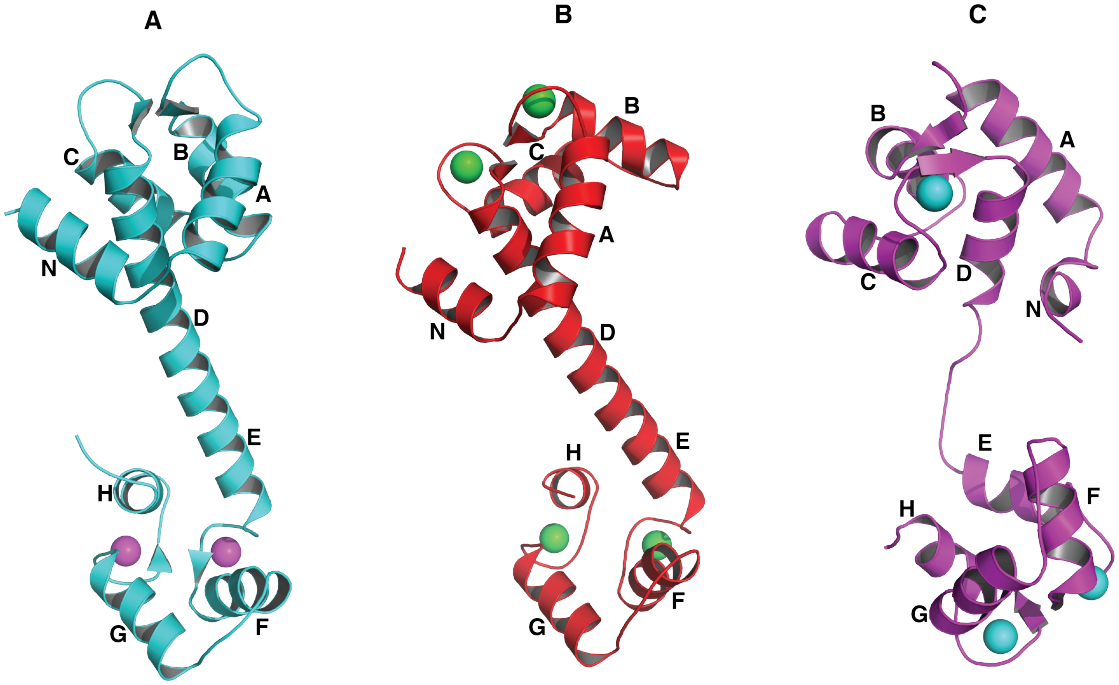

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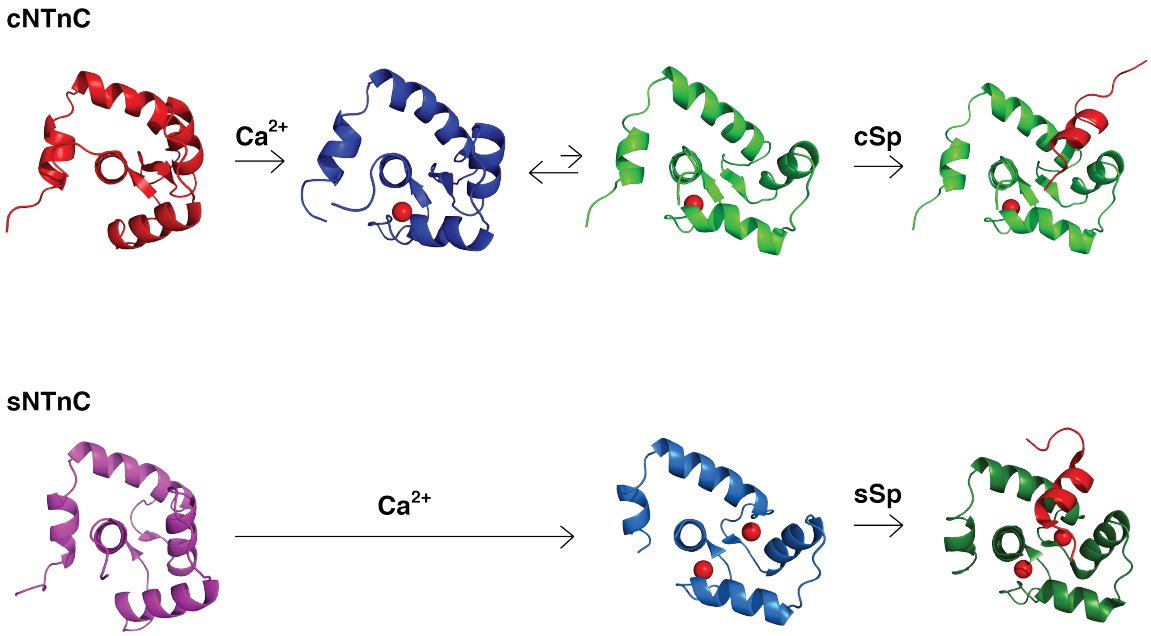

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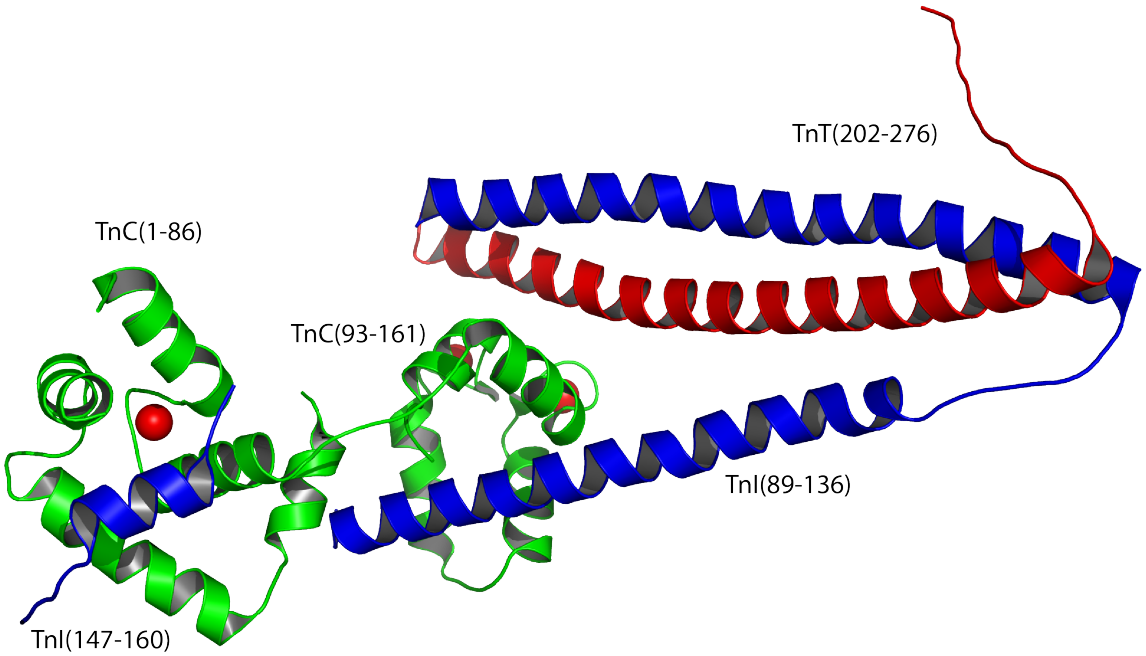
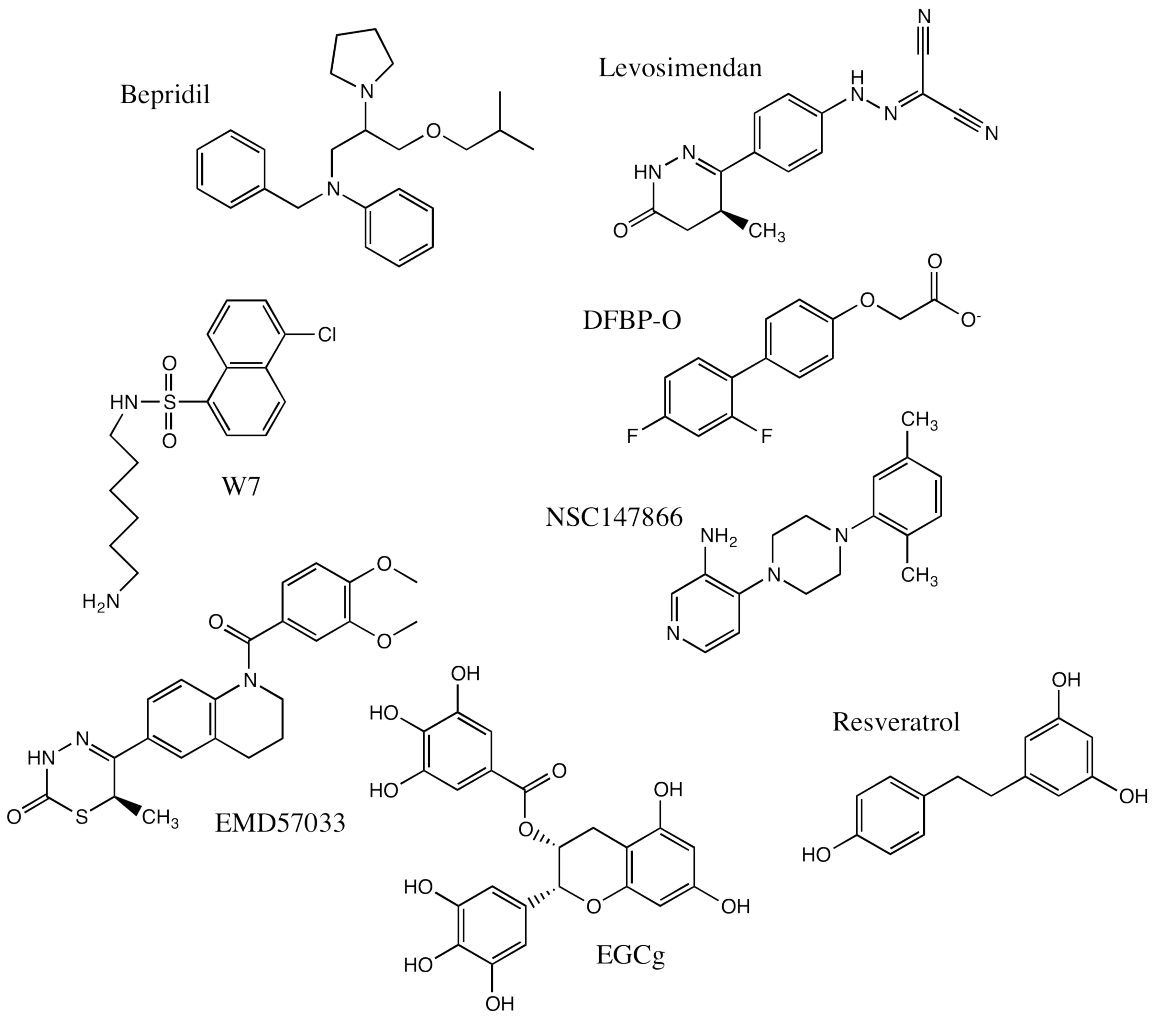


Figure 5: The chemical structures of cardiotonic compounds.



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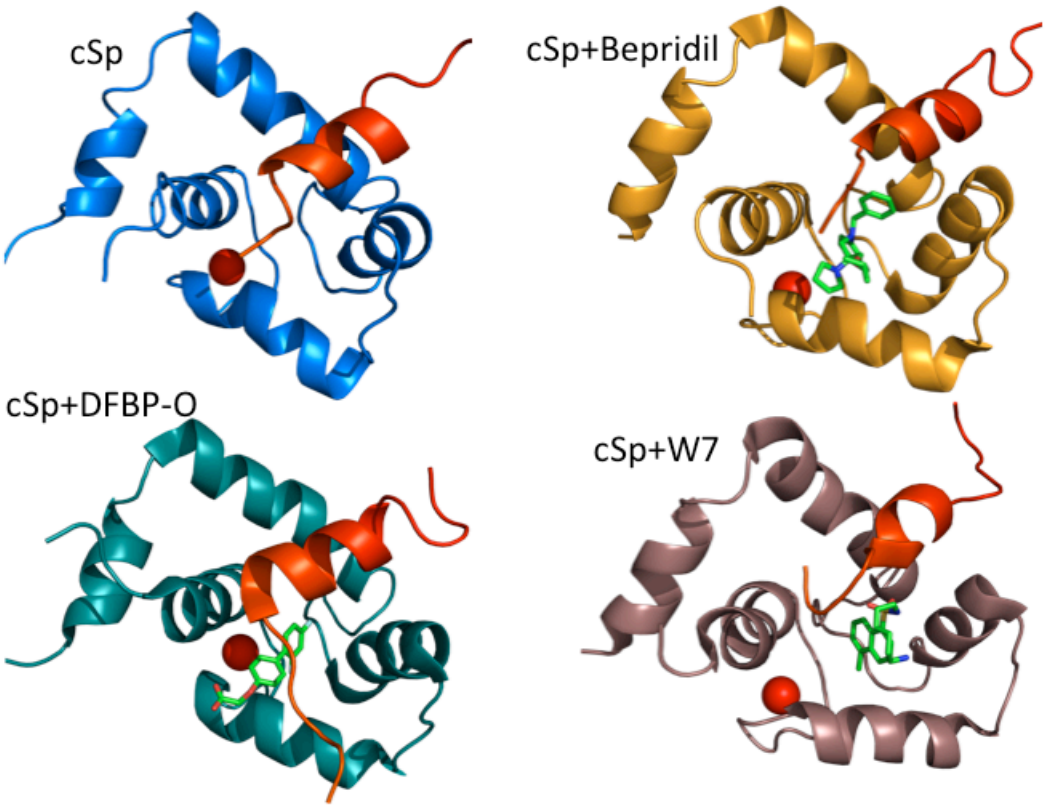

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