Supporting Information for

Molecular effects of Familial Hypertrophic Cardiomyopathy-related mutations in the TNT1 domain of cTnT

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Components of complete cTn model

Compilation of known and predicted structures of cTn complex. This reflects the inclusion of PDB ID 2JPW and 1YTZ into our existing model.[33]
*= 137-147 in cTnI of PDB entry 1J1E is homologous to 104-114 in sTnI of PDB entry 1YTZ.

	Structural Source	(by Residue Number)		
cTn subunit (Accession number)	PDB 1J1E (chain ID)	PDB 2JPW (Model No.)	PDB 1YTZ (chain ID)	PSIPRED
cTnT	---	---	---	1-201
(X74819; 288 rr.)	202-276	---	---	---
	---	---	---	277-288
	(chain E)	---	---	---
cTnI	---	1-31	---	---
(M64247; 210 rr.)	---	---	---	32-39
	40-136	---	---	---
	---	---	137-147*	---
	148-191	---	---	---
	---	---	---	192-210
	(chain F)	(Model 1)	(chain I)	---
cTnC	1-48	---	---	---
(NM_003280; 161 rr.)	---	---	---	49-50
	51-161	---	---	---
	(chain D)	---	---	---

Substitutions to contributing structures

Changes made to PDB ID 2JPW and 1YTZ in order for primary sequences to comply with human cTn.

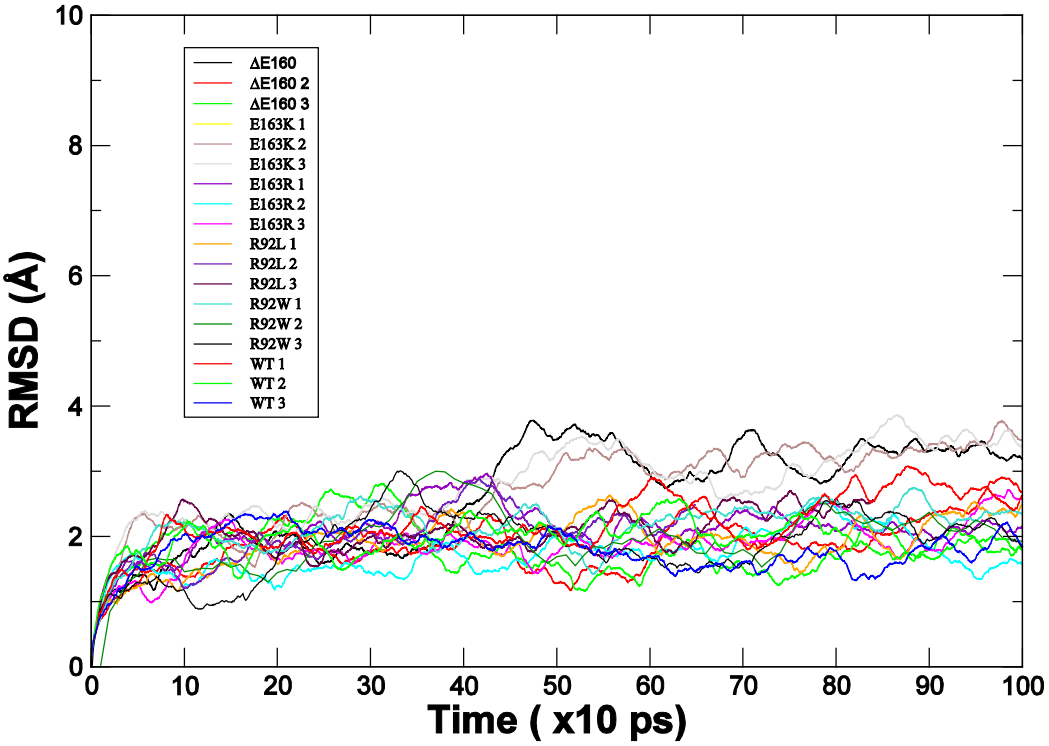
Destination in hcTnI	Structural Source	Changes Made
1-31	res. 1-32 PDB 2JPW	res. 4: E to G res. 10: G to R res. 13: Q to R res. 19: V to I res. 25: A deleted
137-147	res. 104-114 PDB 1YTZ	res. 110: P to T

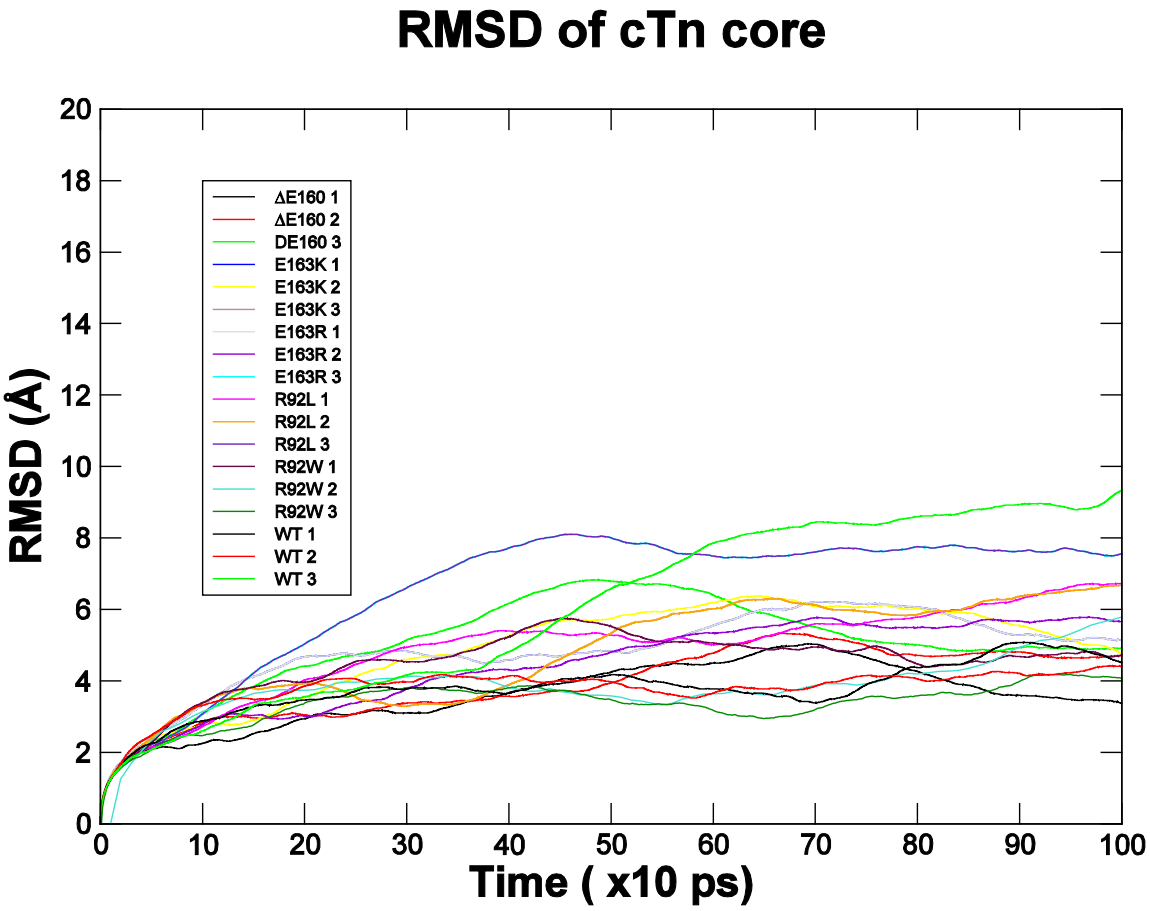
Rmsd data

Average rmsd (rounded to nearest Å) for the 1 ns production runs

cTnT variant	TNT1	cTn core
WT	2, 2, 2	4, 4, 6
R92L	2, 2, 2	5, 4, 5
R92W	2, 2, 2	5, 6, 4
ΔE160	2, 2, 2	5, 4, 4
E163K	3, 3, 3	7, 6, 5
E163R	2, 2, 2	5, 4, 7

We analyzed the stability of our molecules by measuring the rmsd of TNT1 (residues 70-170 cTnT) and the core of cTn (residues 205-288 cTnT, all of cTnC, and 1-159 of cTnI) with respect to their minimized structures. We eliminated hypervariable regions of cTnT[25] and cTnI[29,30] whose fluctuations appear critical to signal transduction but less important for structural stability. The rmsd as a function of time for our simulations quickly steadied for TNT1 and the cTn core, ranging from two to three Å for TNT1 and four to seven Å for the cTn core, as noted in the table above. The graphs for each are included below. We ensured that rgyr measurements for TNT1 were measured only once the region was structurally stable.


RMSD of TNT1
RMSD (Å)
Time (x10 ps)
ΔE160
ΔE160 2
ΔE160 3
E163K 1
E163K 2
E163K 3
E163R 1
E163R 2
E163R 3
R92L 1
R92L 2
R92L 3
R92W 1
R92W 2
R92W 3
WT 1
WT 2
WT 3
0
2
4
6
8
10
0
10
20
30
40
50
60
70
80
90
100


RMSD of cTn core
RMSD (Å)
Time (x10 ps)
ΔE160 1
ΔE160 2
DE160 3
E163K 1
E163K 2
E163K 3
E163R 1
E163R 2
E163R 3
R92L 1
R92L 2
R92L 3
R92W 1
R92W 2
R92W 3
WT 1
WT 2
WT 3
0
2
4
6
8
10
12
14
16
18
20
0
10
20
30
40
50
60
70
80
90
100

Calcium coordination data

Data for Figure 5. Coordinating oxygens interacting with calcium were based on analysis of WT simulations. No new interactions between site II atoms and calcium occurred during the course of any WT or mutant simulations.

	65 OD2	69 OG	71 O	76 OE1	76 OE2
Average					
WT	3.5	4.7	3.1	3.0	3.0
ΔE160	2.5	3.1	2.7	2.8	2.7
E163K	5.3	6.6	3.6	4.9	4.3
E163R	3.9	5.6	3.3	3.1	3.0
Sim1					
WT	2.53	3.06	2.70	2.86	2.64
ΔE160	2.60	3.61	2.70	2.82	2.72
E163K	4.94	7.34	4.02	4.14	2.60
E163R	4.20	5.19	2.71	3.66	3.01
Sim 2					
WT	2.55	4.50	2.67	2.81	2.69
ΔE160	2.51	2.81	2.70	2.78	2.65
E163K	2.59	2.78	2.68	3.48	2.98
E163R	2.55	4.90	3.31	2.81	2.90
Sim 3					
WT	5.47	6.67	4.07	3.40	3.51
ΔE160	2.50	2.80	2.71	2.76	2.69
E163K	8.44	9.64	5.84	7.17	7.31
E163R	4.06	6.70	4.10	2.83	2.91

We performed three separate 1 ns simulations with varying initial conditions for WT and each mutant and took the average distances of the oxygens that interact with calcium in site II (residues 54-90) of cTnC. By subtracting the mutant distances from the WT, we see the average change in distances between the interacting oxygens and calcium. These values are graphed in Figure 5. A positive difference means that the distance between the mutant oxygen and calcium has decreased, and vice versa. We can see that the distances for ΔE160 tend to decrease, while the distances for E163K and E163R tend to increase. This implies that once calcium is bound to site II cTnC of ΔE160, it is less likely to leave than if calcium were bound to WT. The opposite is true for E163K and E163R; it is more likely that calcium bound to site II of E163K and

E163R will dissociate than if it were bound to WT. Therefore, more calcium would be required to activate the thin filaments of E163K and E163R than WT while less would be required for the same degree of activation of ΔE160 mutants than WT. This directly corresponds to preliminary date of calcium-sensitivity where E163K and E163R were found to decrease calcium-sensitivity and ΔE160 was found to increase calcium sensitivity with respect to WT.[51]

Rgyr and R-IVM data

Data for Figures 3 and 7

	EC_{50}	*n*, Hill coefficient	Flexibility: (range)
WT(54)	7.08 ± 0.020	1.80 ± 0.10	0.056 (0.041 - 0.085)
R92L	7.02 ± 0.010	1.20 ± 0.40	0.071 (0.051 - 0.084)
R92W	6.90 ± 0.010	1.50 ± 0.50	0.067 (0.051 - 0.195)
WT(18)	7.58 ± 0.001	2.62 ± 0.04	0.057 (0.041 - 0.085)
ΔE160	7.99 ± 0.020	2.37 ± 0.11	0.042 (0.027 - 0.057)
E163K	7.28 ± 0.002	1.58 ± 0.01	0.085 (0.063 - 0.290)
E163R	6.93 ± 0.010	1.64 ± 0.04	0.067 (0.047 - 0.075)

WT(54) represents R-IVM data collected at high ionic strength.[26] WT(18) represents R-IVM data collected at low ionic strength.[51] Flexibility is reflected by the variance (σ^2) of the rgyr. Data for the flexibility of all three trajectories are shown. A Pearson correlation was performed to determine the general relationship between flexibility and calcium properties measured by R-IVM. It should be noted that a Pearson correlation assumes a linear relationship. Due to the limited amount of data points, the possibility of a non-linear relationship cannot be excluded.

Site II E-F hand sequence alignment

Alignment and assignment of canonical E-F hand positions to site II cTnC.

E-F hand position				1		3		5		7		9			12		
Residue number	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78
Residue name	D	E	V	D	E	D	G	S	G	T	V	D	F	D	E	F	L

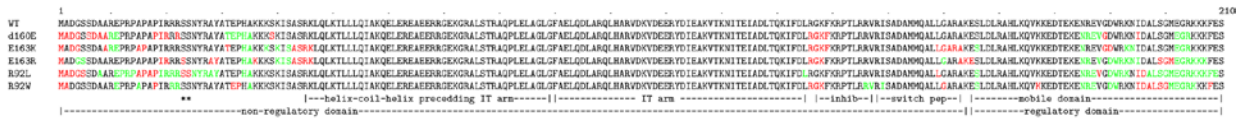
A canonical E-F hand has seven oxygen-calcium interactions resulting from six residues at positions 1, 3, 5, 7, 9, and 12. Underlined numbers indicate residues that require water bridges to mediate oxygen-calcium interactions. Due to the implicit solvation of our model these interactions cannot be accurately simulated.

Secondary structural changes

Secondary structure analysis of mutations resulting in common pathway of TNT1 mutationally-induced changes throughout cTn. Sequences for WT and mutants ΔE160, E163K, E163R, R92L, and R92W are aligned for each subunit of cTn.

cTnC structural changes

cTnI structural changes



cTnT structural changes

This analysis represents the data for Figure 9. Each line represents the average change in secondary structure per residue over the three simulations of that particular variant. The mutation site for each variant is underlined. Residue letters are colored as a function of their average change in secondary structure with respect to WT. Black = no change; red = decrease in secondary structure; green = increase in secondary structure. 100 ps snapshots of cTn were taken during each simulation. These snapshots were aligned by their respective residues and compared with WT. A change in secondary structure was defined as a 33% or greater difference in the structural sample for each residue.