Supplementary material

for

Structure and dynamics of the acidosis-resistant A162H mutant of the switch region of troponin I bound to the regulatory domain of troponin C

by

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Supplementary figure 1. Titration of cNTnC into ^{15}N -cTnI_{A162H}. Overlap of ^1H , ^{15}N correlation spectra for the addition (maroon to green) of unlabelled cNTnC to ^{15}N -cTnI_{A162H} (residues 144-173) used to aid the assignment of cChimera_{A162H}. The insert shows chemical shift mapping of cTnI residues that did not experienced significant chemical shift changes (146, 170, 171, 173) or underwent fast exchange (145, 147, 148, 149, 153, 155, 160, 165, 167, 168, 169) or intermediate exchange (164) in green, and those that underwent slow exchange (150, 151, 152, 154, 156, 157, 158, 159, 161, 162, 163, 166) in maroon.

Supplementary figure 2. Determination of pK_a for H162 in cChimera_{A162H}. **A.** Overlap of ^1H , ^{15}N correlation spectra of cChimera_{A162H} for the pH range from 5.81-8.40 (red to green), residues in the cTnl_{A162H} region close to H162 that experienced large chemical shift perturbations are labeled in bold, the insert shows the location of those residues in the present structure. **B.** Curve fitting, pK_a, and pK_a^{app} values for the residues labeled bold in **A**, the error indicates the standard deviation of the fit.

Supplementary Table 1. Parameters of the NMR spectra acquired for the assignment of cNTnC•cTnI_{A162H}.

Experiment name (Varian)	Solvent	Nucleus in x/y/z dimension	¹ H frequency (MHz)	nt ^a	x-points ^b	y-points ^b	z-points ^b	x-sw ^c	y-sw ^c	z-sw ^c	Mixing time (ms)
gNhsqc	H ₂ O	¹ H/ ¹⁵ N	500	4	512	128		8398	2432		
ghn_cacb	H ₂ O	¹ H/ ¹³ C/ ¹⁵ N	500	16	512	59	32	8385	10056	2026	
gcbca_co_nh	H ₂ O	¹ H/ ¹³ C/ ¹⁵ N	500	16	512	59	32	8385	10056	2026	
gChsqc	H ₂ O	¹ H/ ¹³ C	500	4	512	192		8398	12066		
ghc_co_nh	H ₂ O	¹ H/ ¹ H/ ¹⁵ N	500	16	512	72	32	8385	8385	2026	
gc_co_nh	H ₂ O	¹ H/ ¹³ C/ ¹⁵ N	500	16	512	64	32	8385	10056	2026	
ghnha	H ₂ O	¹ H/ ¹ H/ ¹⁵ N	600	8	512	128	32	8398	7198	1823	
gnoesyChsqc	D ₂ O	¹ H/ ¹ H/ ¹³ C	600	16	512	64	64	8398	5999	4525	100
gnoesyNhsqc	H ₂ O	¹ H/ ¹ H/ ¹⁵ N	600	8	512	128	48	8398	7198	1823	100
tndqcosy	D ₂ O	¹ H/ ¹ H	600	64	2048	512		8398	5999		
tnnoesy	D ₂ O	¹ H/ ¹ H	600	32	2048	512		8398	8398		100
zdpsitocsy	D ₂ O	¹ H/ ¹ H	600	32	2048	512		8398	8398		60
CNfiltocsy	H ₂ O	¹ H/ ¹ H	800	128	4096	256		11990	11998		60
CNfilnoesy	H ₂ O	¹ H/ ¹ H	800	128	4096	512		11990	11998		150
CNfilnoesy*	H ₂ O	¹ H/ ¹ H	800	128	4096	512		11990	11998		150
gChmqcnoesy_Cfilt	D ₂ O	¹ H/ ¹ H/ ¹³ C	800	16	2048	72	40	11990	7998	4022	150

^a Number of scans, ^b number of complex points, ^c spectral width (Hz), * acquired at pH 7.4.

Supplementary Table 2. Structural statistics for final ensemble of 20 structures.

NOE restraints	
Total	1282
Intramolecular for cNTnC-Ca ²⁺	
Short range ($ i-j \leq 1$)	858
Medium range ($2 \leq i-j \leq 4$)	167
Long range ($ i-j \geq 5$)	83
Intramolecular for cTnI _{A162H}	140
Intermolecular between cNTnC and cTnI _{A162H}	34
intermolecular between cNTnC and Ca ²⁺	8
Dihedral restraints	
For cNTnC	157
For cTnI _{A162H}	13
PROCHECK validation	
RMSD from average (backbone atoms N, CA, C, O)	2.1 Å ^a , 0.9 Å ^b
ϕ , ψ in allowed regions of Ramachandran plot	98.7 % ^a , 100% ^b
Z-score ^c (all)	0.12 ^a , 2.90 ^b
Z-score ^c (ϕ , ψ)	0.90 ^a , 3.82 ^b

^a For all residues, ^b for ordered residues: 3-11, 14-27, 38-48, 54-64, 74-85, 150-157, ^c with respect to mean and standard deviation for a set of 252 x-ray structures < 500 residues, resolution ≤ 1.80 Å, R-factor ≤ 0.25 , and R-free ≤ 0.28 ; a positive value indicates a “better” score.