Order-Disorder-Order Transitions Mediate the Activation of Cholera Toxin - *Supplementary Data*

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CTA1:     MNDDKLYRADSRPPDEIKQSGGLMPRGQSEYFDRGTQMNINLYDHARGTQTGFVRHDDGY
CTA1-T2:  MNDDKLYRADSRPPDEIKQSGGLMPRGQSEYFDRGTQMNINLYDHARGTQTGFVRHDDGY
CTA1-T4:  MNDDKLYRADSRPPDEIKQSGGLMPRGQSEYFDRGTQMNINLYDHARGTQTGFVRHDDGY

CTA1:     VSTSISLRSAHLVGQTILSGHSTYYIYVIATAPNMFNVNDVLGAYSPHPDEQEVSALGGI
CTA1-T2:  VSTSISLRSAHLVGQTILSGHSTYYIYVIATAPNMFNVNDVLGAYSPHPDEQEVSALGGI
CTA1-T4:  VSTSISLRSAHLVGQTILSGHSTYYIYVIATAPNMFNVNDVLGAYSPHPDEQEVSALGGI

CTA1:     PYSQIYGWYRVHFGVLDEQLHRNRGYRDRYYSNLDIAPAADGYGLAGFPPEHRAWREEPW
CTA1-T2:  PYSQIYGWYRVHFGVLDEQLHRNRGYRDRYYSNLDIAPAADGYGLAGF
CTA1-T4:  PYSQIYGWYRVHFGVLDEQLHRNRG

CTA1:     IHHAPPGCGNAPR
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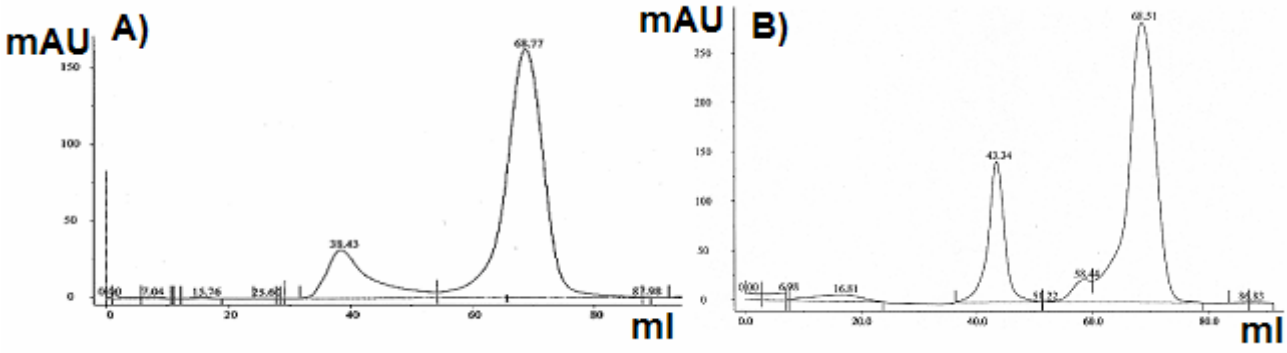
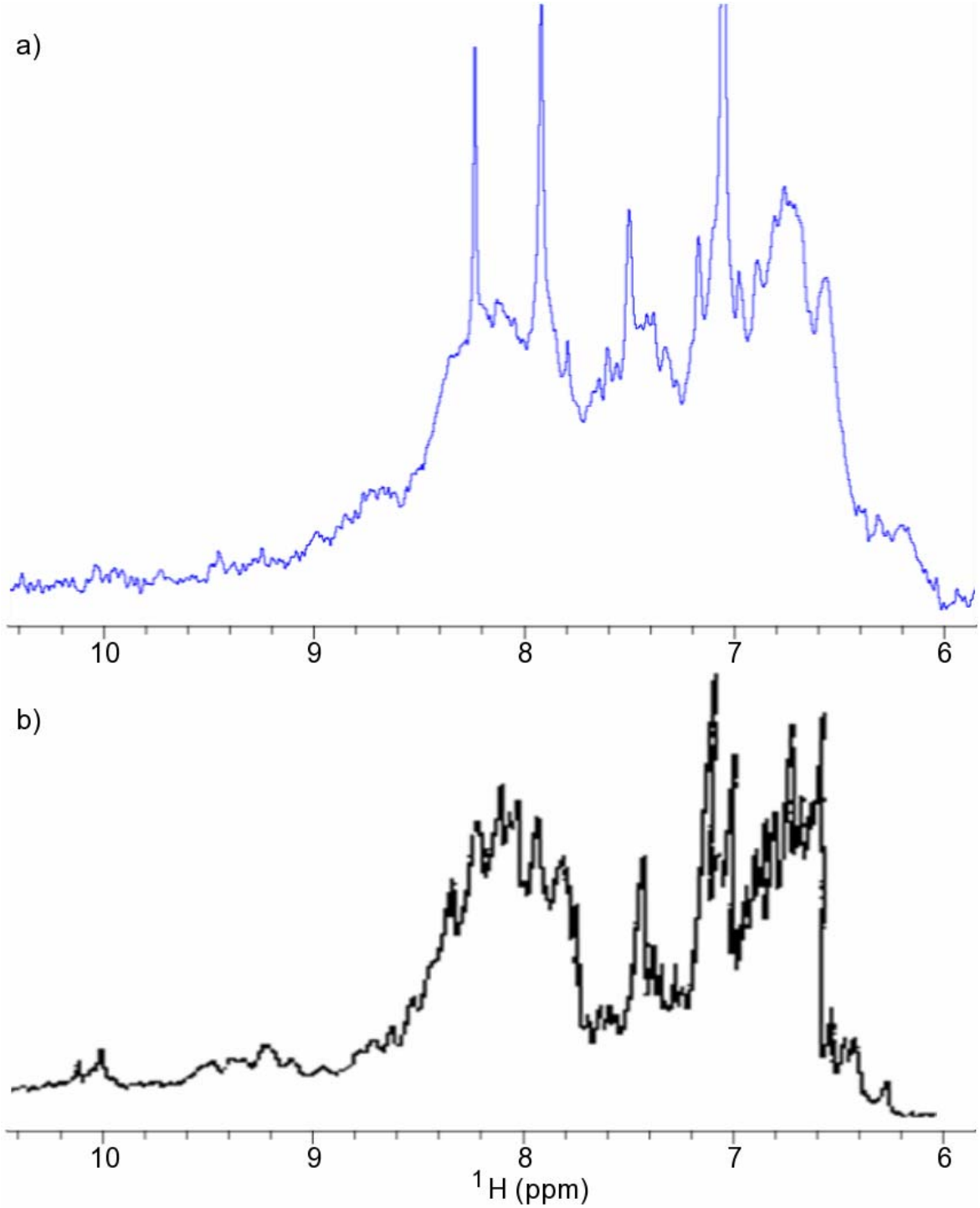
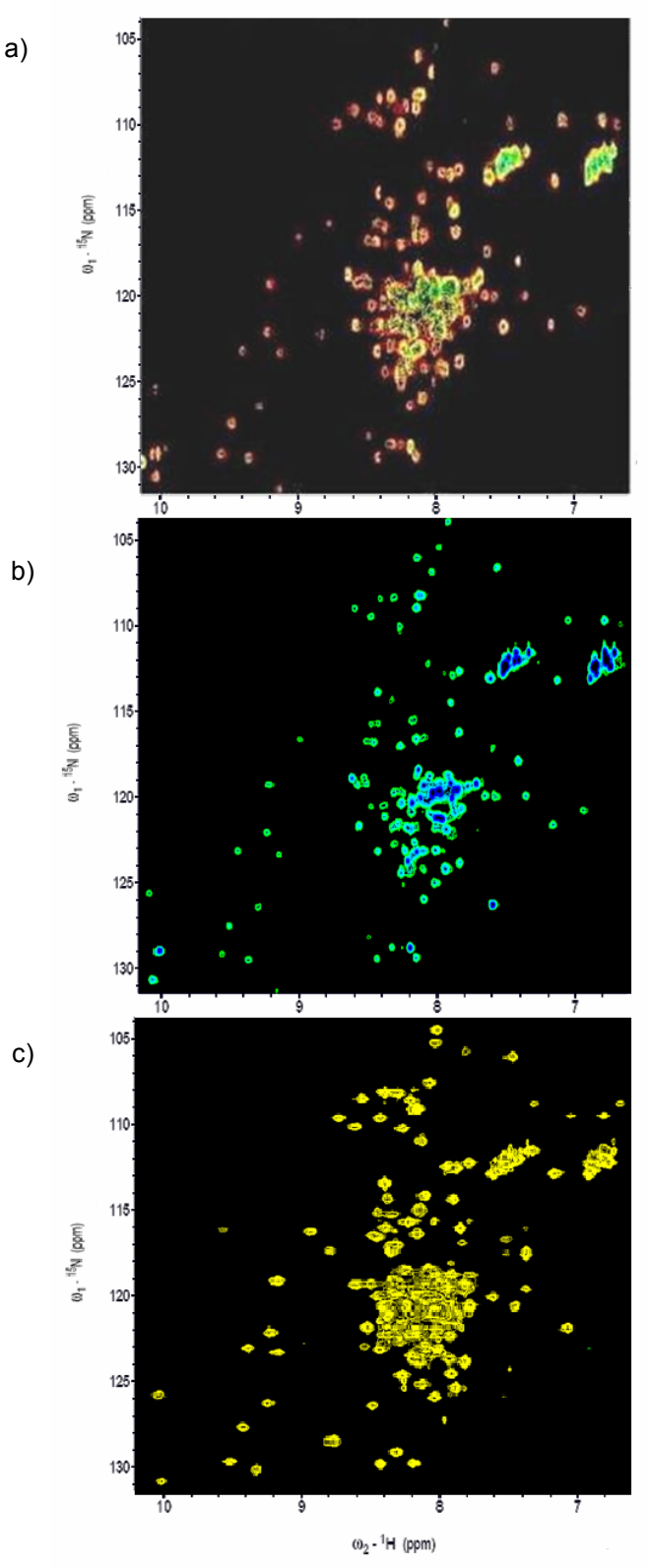
Supplementary Figure 1: a) Free CTA1 Constructs used for C-terminal truncation studies. CTA1 full (N1-R192), CTA1-T2 (N1-F167) and CTA1-T4 (N1-G144). The six C-terminal residues that are deleted in CTA1 (N1-G186) are boxed.



Supplementary Figure 2: Preparative gel filtration FPLC chromatography traces of refolded CTA1 (N1-G186) by solubilization in a) 6 M Guanidine-HCl and b) 8 M Urea denaturation solutions. From standard molecular weight markers, the expected retention volume of the 21.1 kDa monomer peak was about 69 mL for both samples. Sample volume was 2 mL. Note the presence of a shoulder peak in the urea sample at 58.4 mL. Guanidine refolding was preferred due to the absence of a residual dimeric species, which was present in the urea refolded samples (shoulder peak at 58.4 mL).

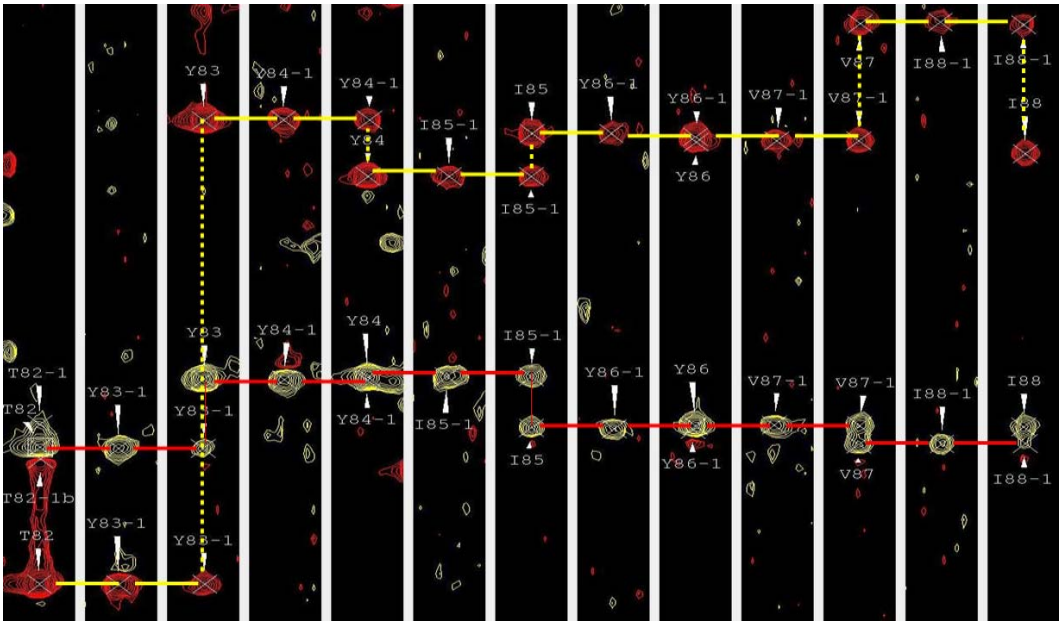


Supplementary Figure 3: Both soluble expressed NhisCTA1 and inclusion body refolded CTA1 show similar intensity variations of both the random coil resonances and dispersed resonances as evident from the expansion of the amide region within their 1D-^{1}H NMR spectrum. a) 6 μM soluble expressed CTA1 (1-192) (E110:112D) obtained from an extended incubation of the CTA1:ARF6-GTP complex (O'Neal *et al*. *Science* 2005) in 10 mM EDTA; the CTA1 domain was then purified by dilution and Ni-NTA affinity chromatography and exchanged into 10mM dTris, pH 7.2, 200 mM NaCl and 6% v/v $^{2}H_2O$; number of scans = 8192. (Note some solvent peaks remain) b) ~100 μM inclusion body (refolded) CTA1 (CTA1 1-186) in the same NMR buffer; number of scans = 32.

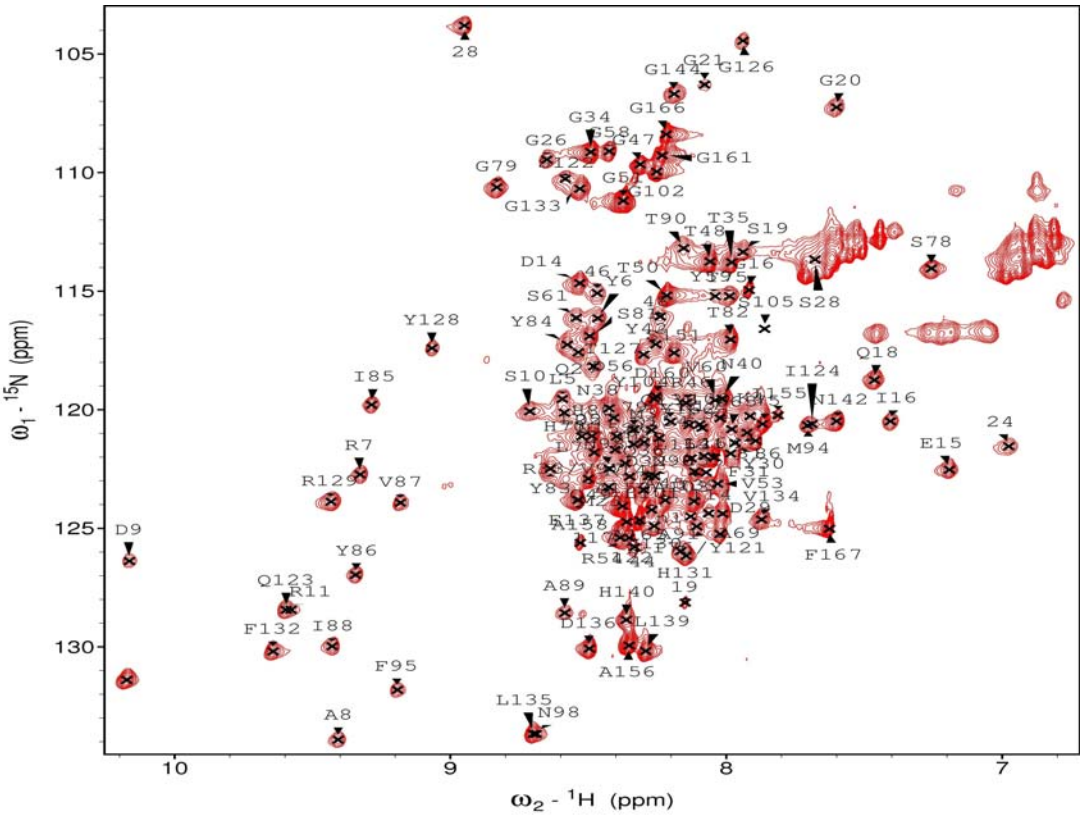


Supplementary Figure 4: ^{1}H/^{15}N HSQC spectral comparison of CTA1 and C-terminal truncation mutants. a) CTA1 (N1-G186), b) CTA1-T2 and c) CTA1-T4. Spectra were acquired at 288 K on the Bruker Avance 800 MHz.

a)



b)

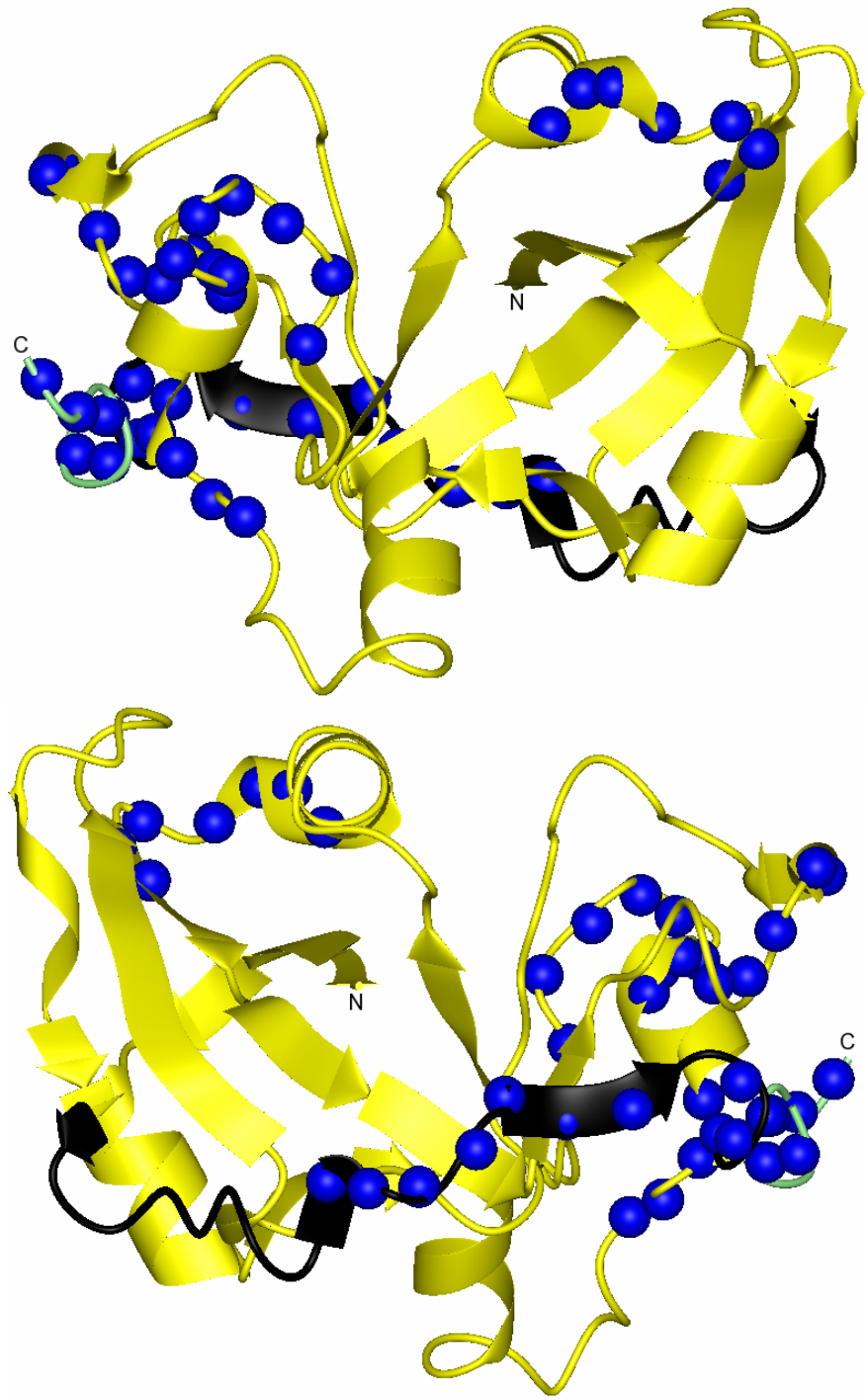


Supplementary Figure 5: Sequence specific assignment of CTA1-T2. a) CTA1-T2 assigned connectivity strip-plot from T82-I88. The HN(CO)CACB and HNCACB were used where Cα (yellow peaks) and Cβ (red peaks) were connected to display a sequential walk. Figure was produced by SPARKY. b) Assigned $^{1}H/^{15}N$ HSQC spectrum of 250 µM CTA1-T2 acquired on Bruker Avance 800 MHz (8 scans at 288 K). BMRB accession number 15162.

Supplementary Table 1: Residues which show intense peak volumes in the $^{1}H/^{15}N$ HSQC spectra (>20X increase over that of the downfield shifted amides).				
D3	A45	L77	G133	A156
K17	R46	H80	D136	A159
G34	G47	S81	E137	D160
T35	T48	T82	Q138	G161
Q36	G51	V100	L139	Y162
N38	F52	L101	R141	L164
N40	V53	G102	S151	A165
L41	R54	A103	L153	G166
		Y104	D154	F167

a)

b)



Supplementary Figure 6: Structural mapping of residues which show intense peak volumes in the NMR Spectra: a) Ribbon diagram of the enzymatic domain from the CTA1:ARF6-GTP crystal structure (pdb# 2A5F) showing residues N1-F167 that correspond to the termini of CTA1-T2, while b) is the same diagram, rotated 180°. The boundaries of the secondary structural elements were based on the CSI data of CTA1-T2 (Figure 5a, main text), with structural region indicated by color: $CTA1_1$ (N1-N142) is shown in yellow, while $CTA1_2$ (residues R143-G161) is shown in black and $CTA1_3$ (Y162-F167, the C-terminus of CTA1-T2) is shown in light green. Amide nitrogen atoms of residues with intense peak volumes (Supplementary Table 1) are shown in blue CPK. Figure was generated in MolMol.