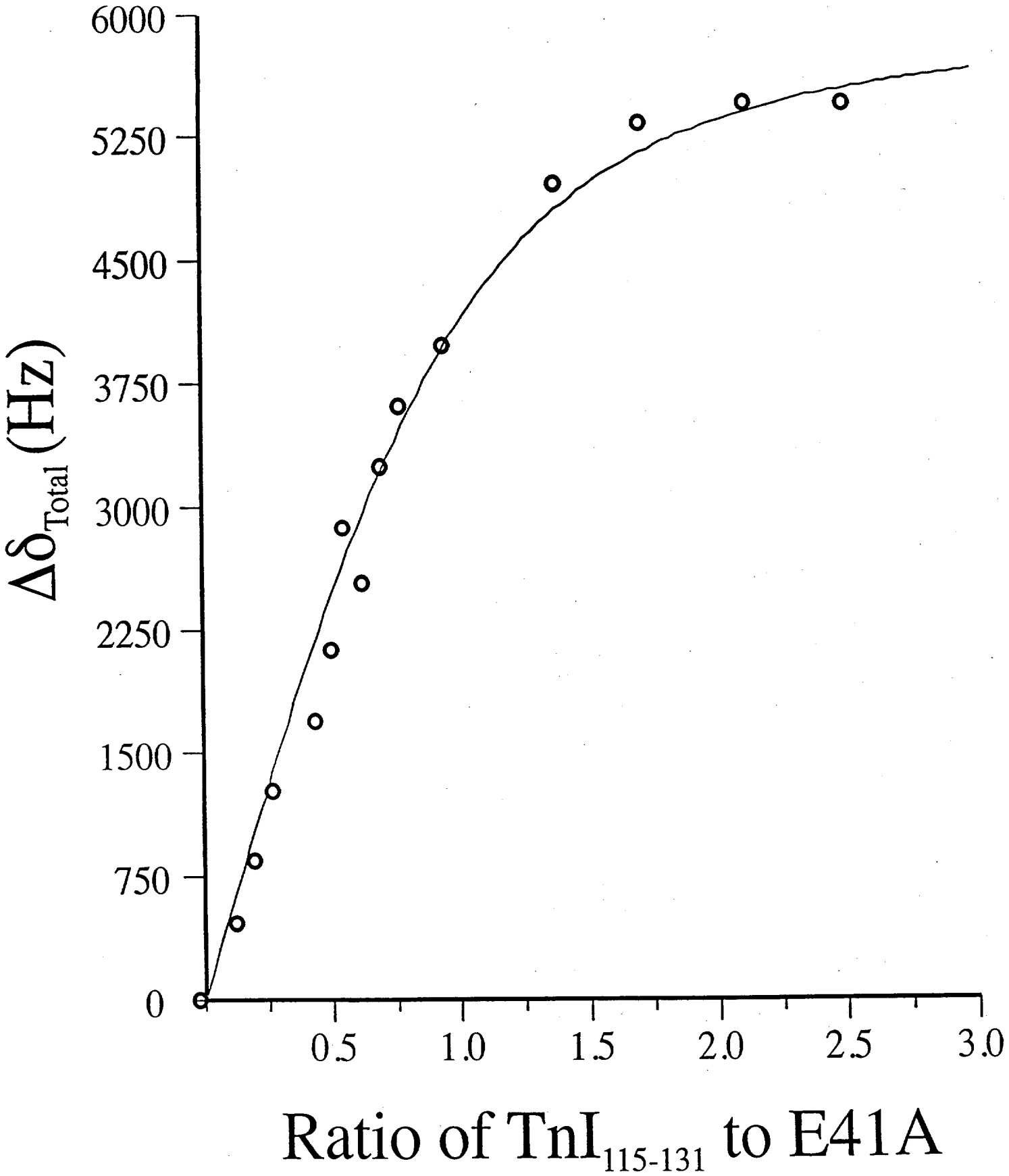
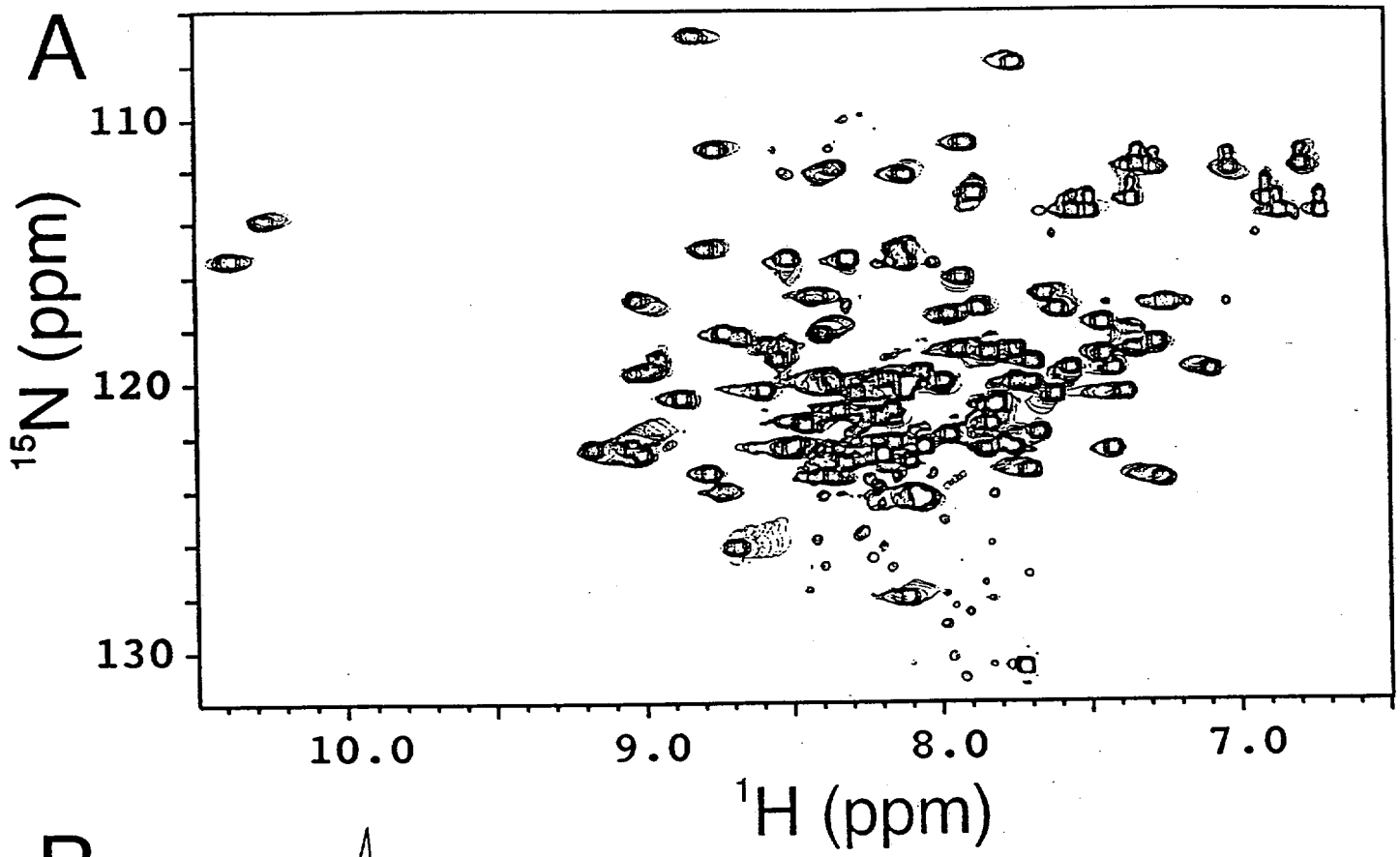
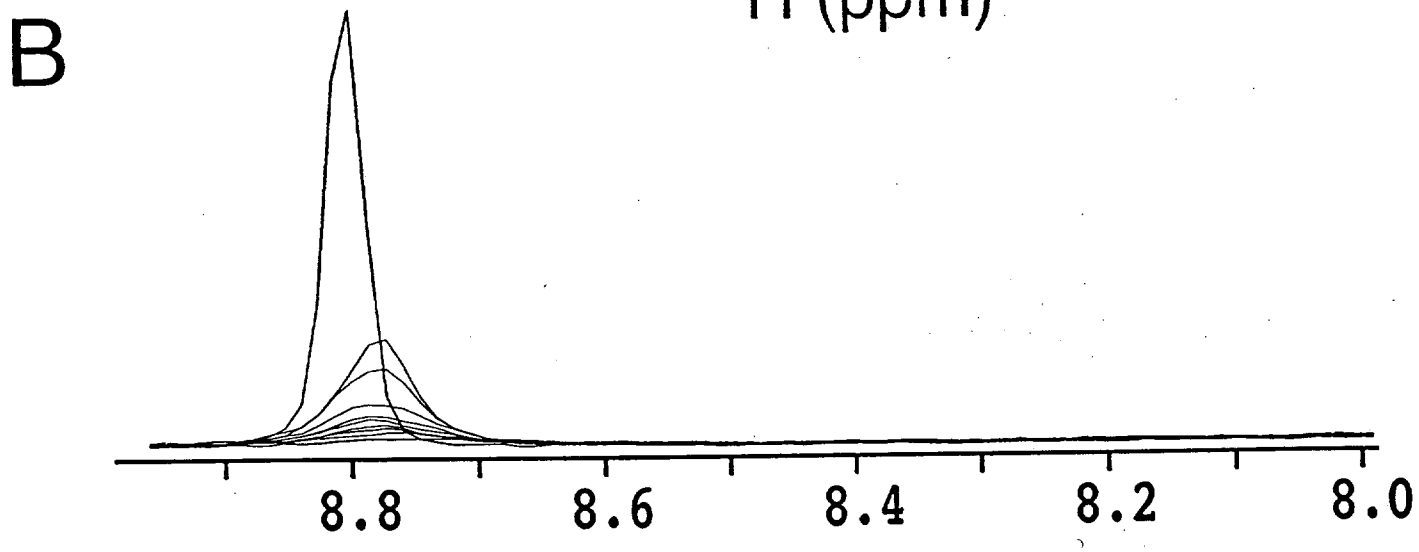
Figure 1S. Binding curve determined from the $\Delta\delta_{total}$ of 2D {^{1}H,^{15}N}-HSQC NMR spectral changes of E41A-sNTnC•2Ca^{2+} upon addition of $sTnI_{115-131}$. The curve is an average of 82 residues, which exclude Ser^{15}, Asp^{30}, Asp^{36}, Val^{65}, and Glu^{76} due to spectral overlap, Pro^{53} due to the lack of an amide hydrogen resonance, Ala^{1} and Ser^{2} presumably due to rapid exchange with solvent, and residues involved in site I. The determined dissociation constant was 300 ± 100 μM.

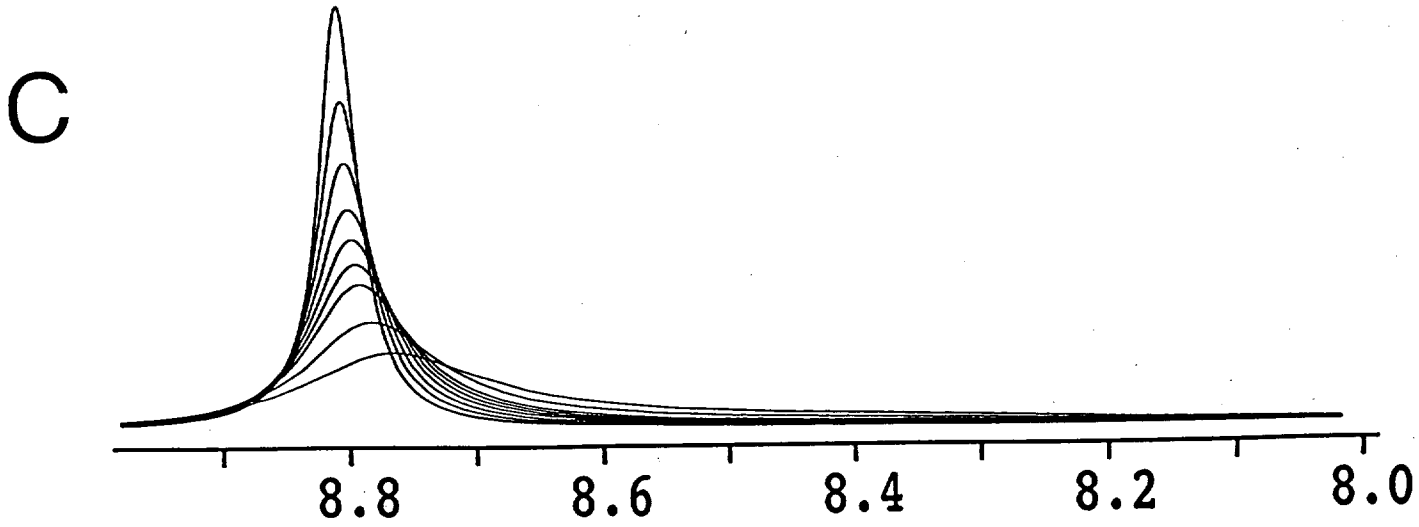
Figure 2S. (A) Contour plot of 2D {^{1}H,^{15}N}-HSQC NMR spectra of apo E41A-sNTnC showing the effect of $sTnI_{115-131}$ addition. The apo E41A-sNTnC spectrum before the addition of peptide is shown with multiple contours while each addition of $sTnI_{115-131}$ are indicated by a single contour. (B) 1D-trace of Gly^{43} throughout the titration of $sTnI_{115-131}$, showing the progressive line broadening effect.

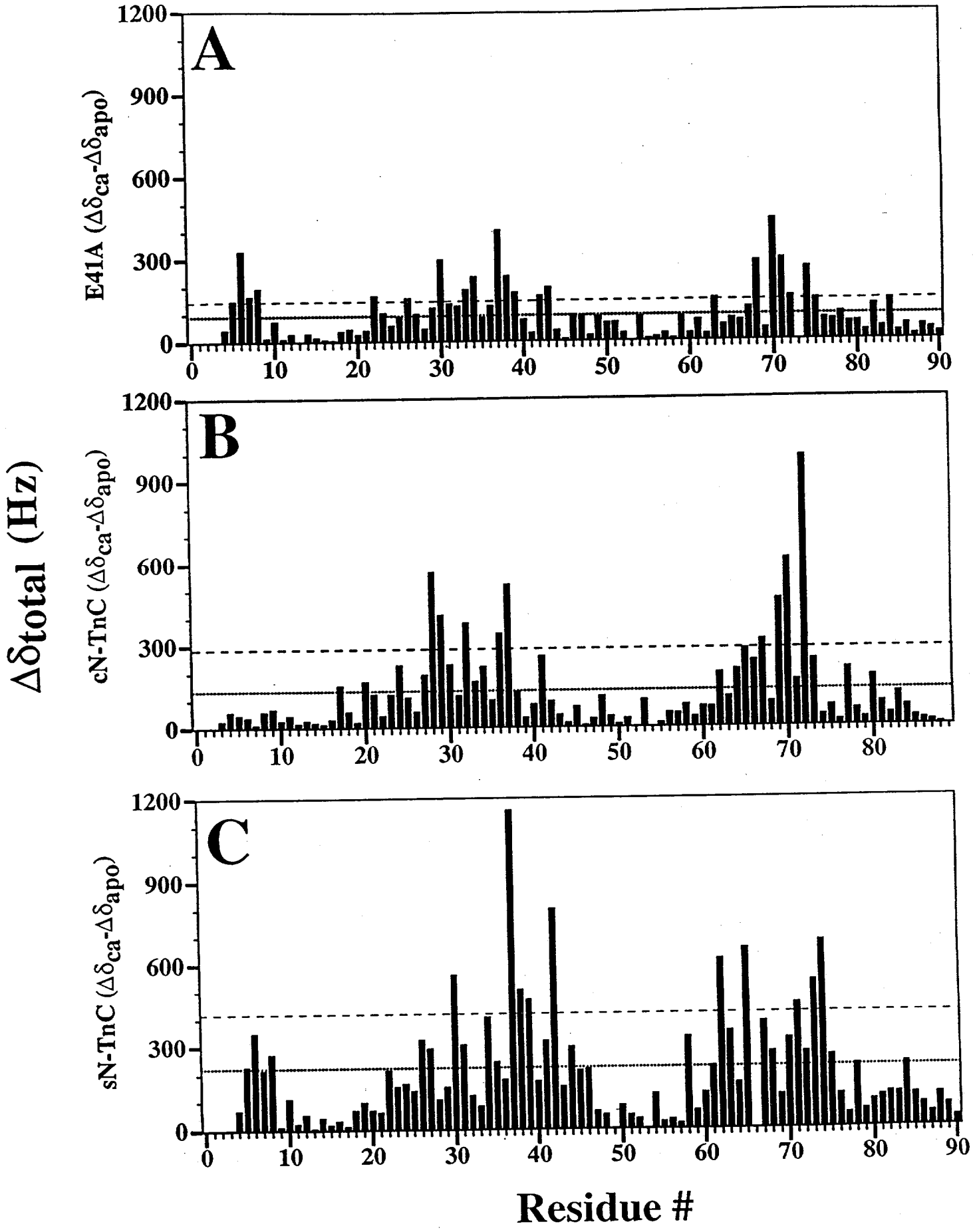
Figure 3S. The total change in chemical shift ($\Delta\delta_{total}$ in Hz) for each backbone amide pair in E41A-sNTnC (A,D), cNTnC (B,E) and sNTnC (C,F) upon addition of calcium (A-C), or TnI peptide (D-F). The thin dashed lines indicate one standard deviation above the average.

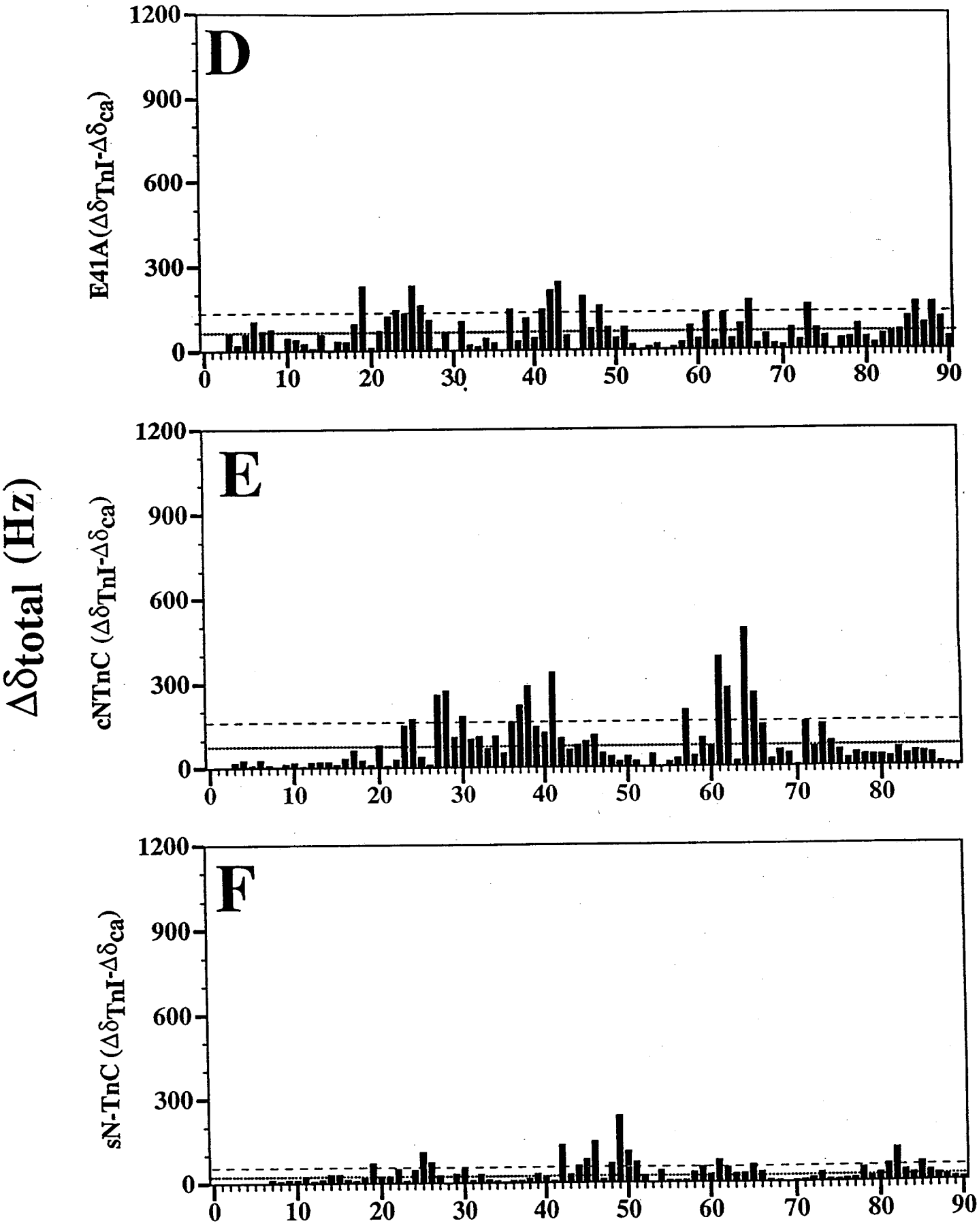

6000
5250
4500
3750
3000
2250
1500
750
0
$\Delta\delta_{Total}$ (Hz)
0.5
1.0
1.5
2.0
2.5
3.0
Ratio of $TnI_{115-131}$ to E41A


A
110
120
130
^{15}N (ppm)
10.0
9.0
8.0
7.0
^{1}H (ppm)


B
8.8
8.6
8.4
8.2
8.0


C
8.8
8.6
8.4
8.2
8.0


A
B
C
E41A ($\Delta\delta_{ca}$-$\Delta\delta_{apo}$)
cN-TnC ($\Delta\delta_{ca}$-$\Delta\delta_{apo}$)
sN-TnC ($\Delta\delta_{ca}$-$\Delta\delta_{apo}$)
$\Delta\delta$total (Hz)
Residue #
0
300
600
900
1200
0
10
20
30
40
50
60
70
80
90


D
E41A(ΔδTnI-Δδca)
E
cNTnC (ΔδTnI-Δδca)
F
sN-TnC (ΔδTnI-Δδca)
Δδtotal (Hz)
0
300
600
900
1200
0
10
20
30
40
50
60
70
80
90