Complex	% α-helix	% β-sheet
EH	62.9	3.3
$E_{NT2}H_{NT}$	75.6	1.7
E_{CT1}	15.0	35.0
$E_{CT1}H_{CT}$	42.6	9.8

Figure S1: K2D2 estimation of secondary structure content from CD data. Molar differential absorbance values observed between 200 and 240 nm were used to estimate the secondary structure content of the various complexes using the K2D2 server (http://www.ogic.ca/projects/k2d2/). The higher estimated α-helical content of $E_{NT2}H_{NT}$ compared to EH supports its delineation as the N-terminal coiled-coil domain (see RESULTS for details). The secondary structure content for $E_{CT1}H_{CT}$ is similar to that found by NMR using the CSI protocol (Fig. 5), suggesting that the E_{CT1} fold is stabilized upon binding H_{CT}. The apparent over-estimation of β-sheet and under-estimation of α-helix in isolated E_{CT1} is likely due to its partial destabilization in the absence of H.

Figure S2A-C

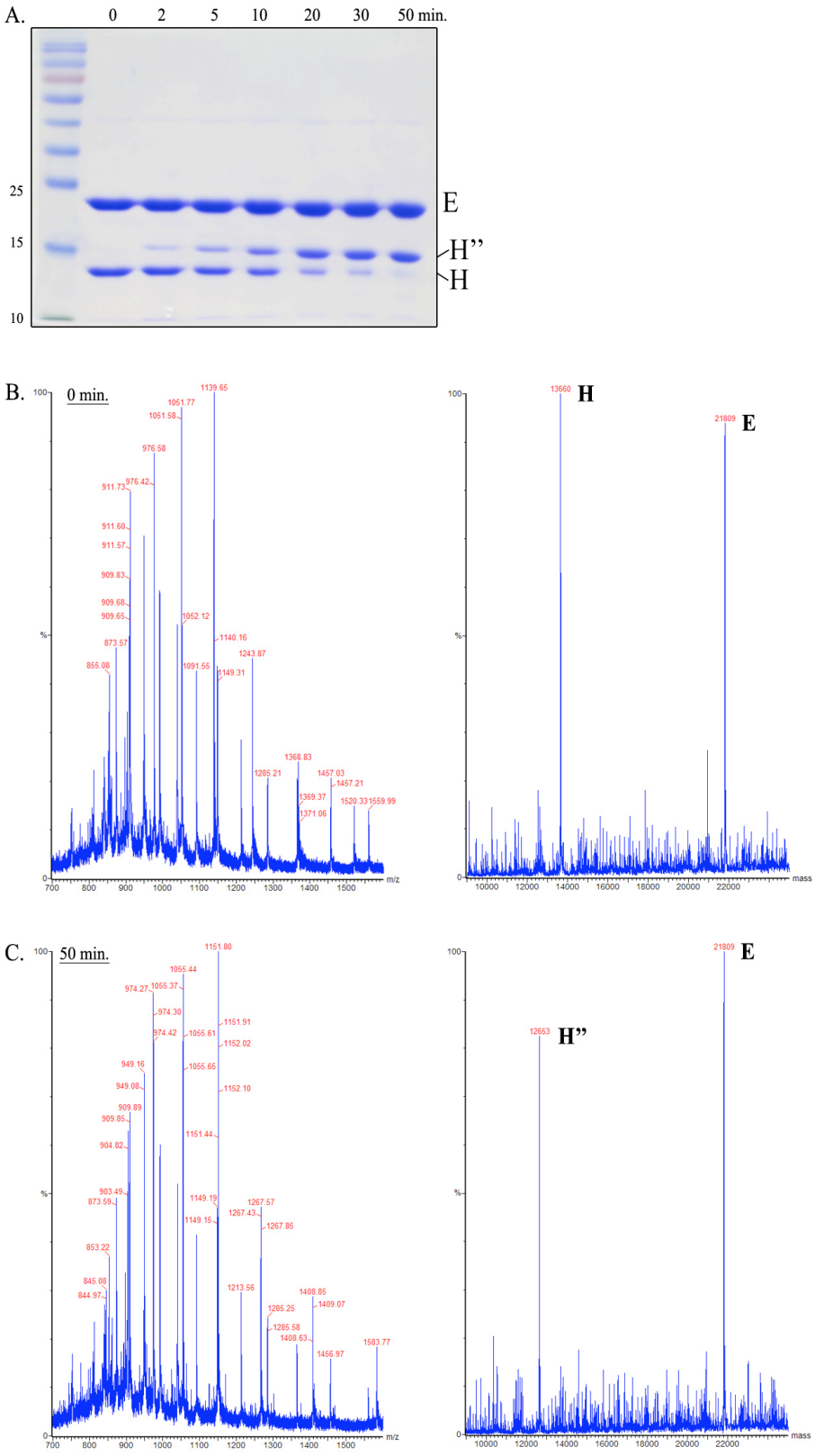

Figure S2D-F

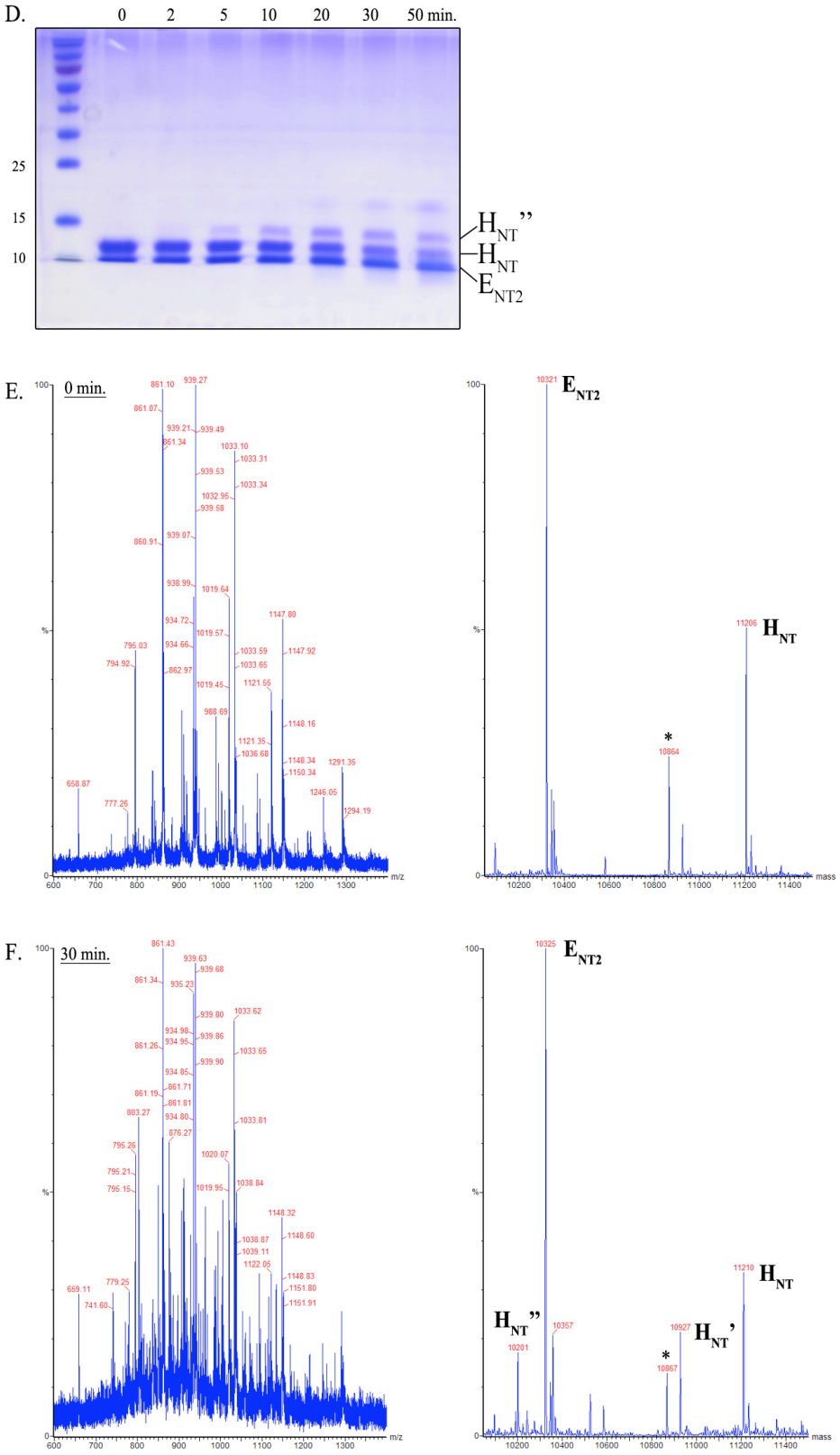
D.
0
2
5
10
20
30
50 min.
25
15
10
H NT ”
H NT
E NT2
E.
0 min.
E NT2
H NT
F.
30 min.
E NT2
H NT ”
H NT ’
H NT
m/z
mass

Figure S2: Limited trypsin proteolysis of full length EH & $E_{NT2}H_{NT}$. A 1 mg/ml solution of EH (A-C) or $E_{NT2}H_{NT}$ (D-F) was incubated with trypsin (at 5 μg/ml) for 0, 2, 5, 10, 20, 30, and 50 minute intervals at room temperature. (A) SDS-PAGE of the EH digest time course. A fragment of H (H'') was first visualized at 2 minutes with a majority of H converting to this fragment by 50 minutes while subunit E remained intact during this time period. ESI-TOF MS analysis of the (B) undigested and (C) 50 minute digest reactions. The multiple charge envelope mass spectra are shown on the left while the deconvoluted spectra are on the right. As can be seen in the deconvoluted spectrum for the digested sample (right spectrum in part (C)), essentially all H subunit is converted to H'' within 50 minutes. The observed difference in mass for H and H'' is 1005 Da, close to the predicted mass loss (1008 Da) due to removal of the N-terminal 5 residues (including the linker GPKVP). Observed masses for E and H in (B) are 21809 (expected 21854) and 13660 (expected 13688), respectively. Observed mass for H'' in (C) was 12653 (expected 12679). (D) The digest of $E_{NT2}H_{NT}$ exhibits a similar trend, with H_{NT} (top band) being fragmented by 5 minutes and increasingly so over the time course, while E_{NT2} remains largely intact. Comparison of ESI-TOF MS spectra of the (E) undigested and (F) 30 minute digest reactions indicates the presence of two H_{NT2} truncations, one that coincides with the cleavage site discussed above (H_{NT2}''; 1009 Da. mass loss observed, 1008 Da predicted) and a second that is consistent with truncation after the Lys in the linker (H_{NT2}'; 284 Da. mass loss observed; 284 Da. predicted). A contaminant peak (*) is present in both reactions (its observed mass of 10864 Da does not match any of the expected trypsin cleavage products of either H_{NT} or E_{NT2}). Observed masses for E_{NT2} and H_{NT} in (E) are 10321 (expected 10327) and 11206 (expected 11211), respectively. Observed masses for $H_{NT'}$ and $H_{NT''}$ in (F) are 10927 (expected 10929) and 10201 (expected 10203), respectively. Mass spectra were obtained on a Q-TOF Micro (Waters) in positive ion mode from a 1:10 dilution of above samples in a 50:50:0.1 water:acetonitrile:formic acid solution. The spectrometer was calibrated with horse heart myoglobin (Sigma; for spectra in (B-C)) or 0.1 % phosphoric acid (for spectra in (E-F)).

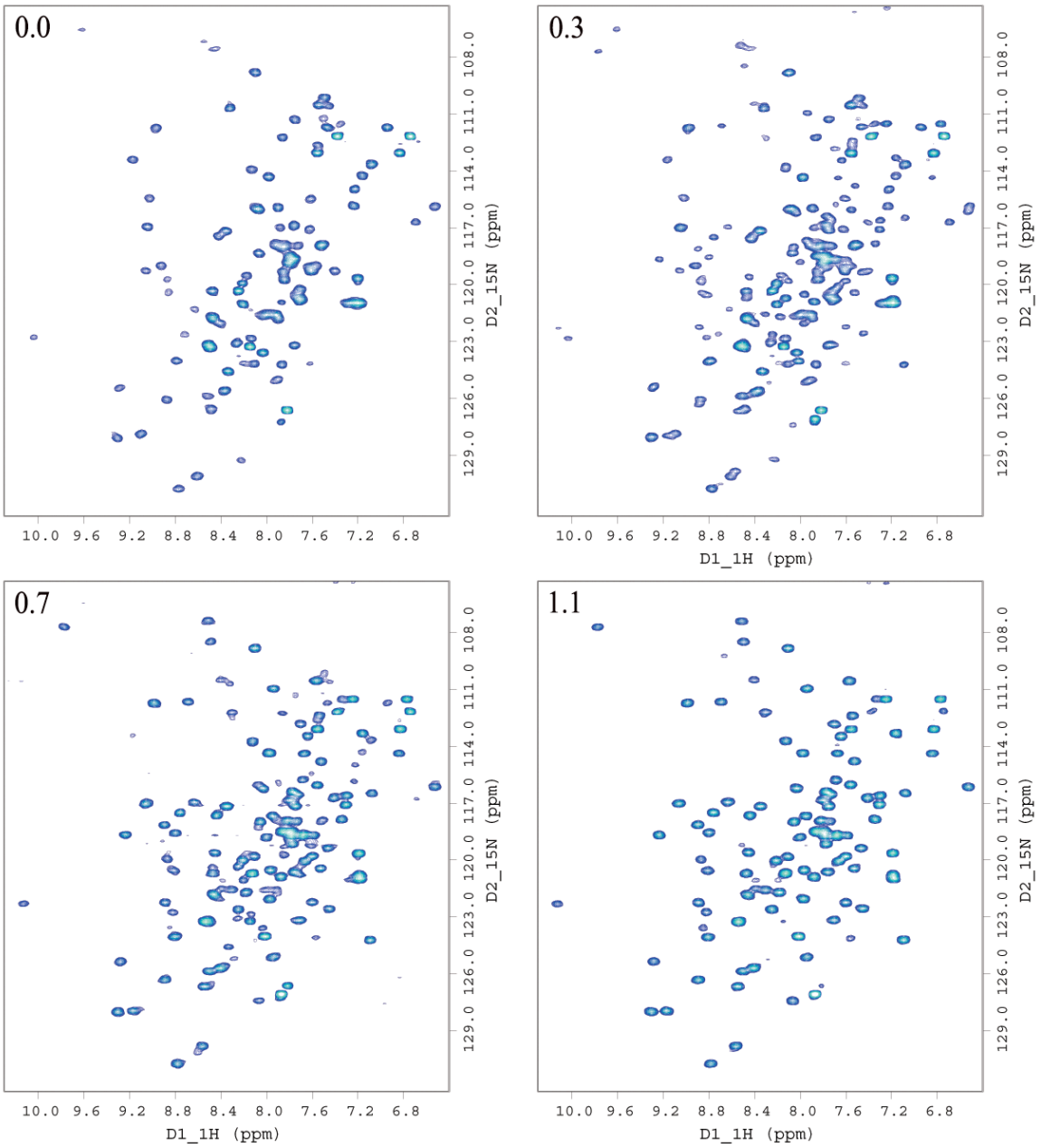

Figure S3: Titration of ^{15}N-labeled E_{CT1} with unlabeled H subunit as observed by ^{15}N-HSQC NMR spectroscopy. 2D ^{1}H/^{15}N-HSQC NMR spectra taken at 298 K in 25 mM sodium phosphate (pH 7.0), 0.5 mM EDTA, and 0.02% NaN_3. The molar ratio of H to E_{CT1} for each titration point is noted in the upper left portion of the spectra. The concentration of ^{15}N labeled E_{CT1} was ~0.5 mM. The dramatic improvement in spectral quality upon addition of H was found to be also achieved using only the C-terminal residues of H, 91-111 (H_{CT}; see Fig. 4B).

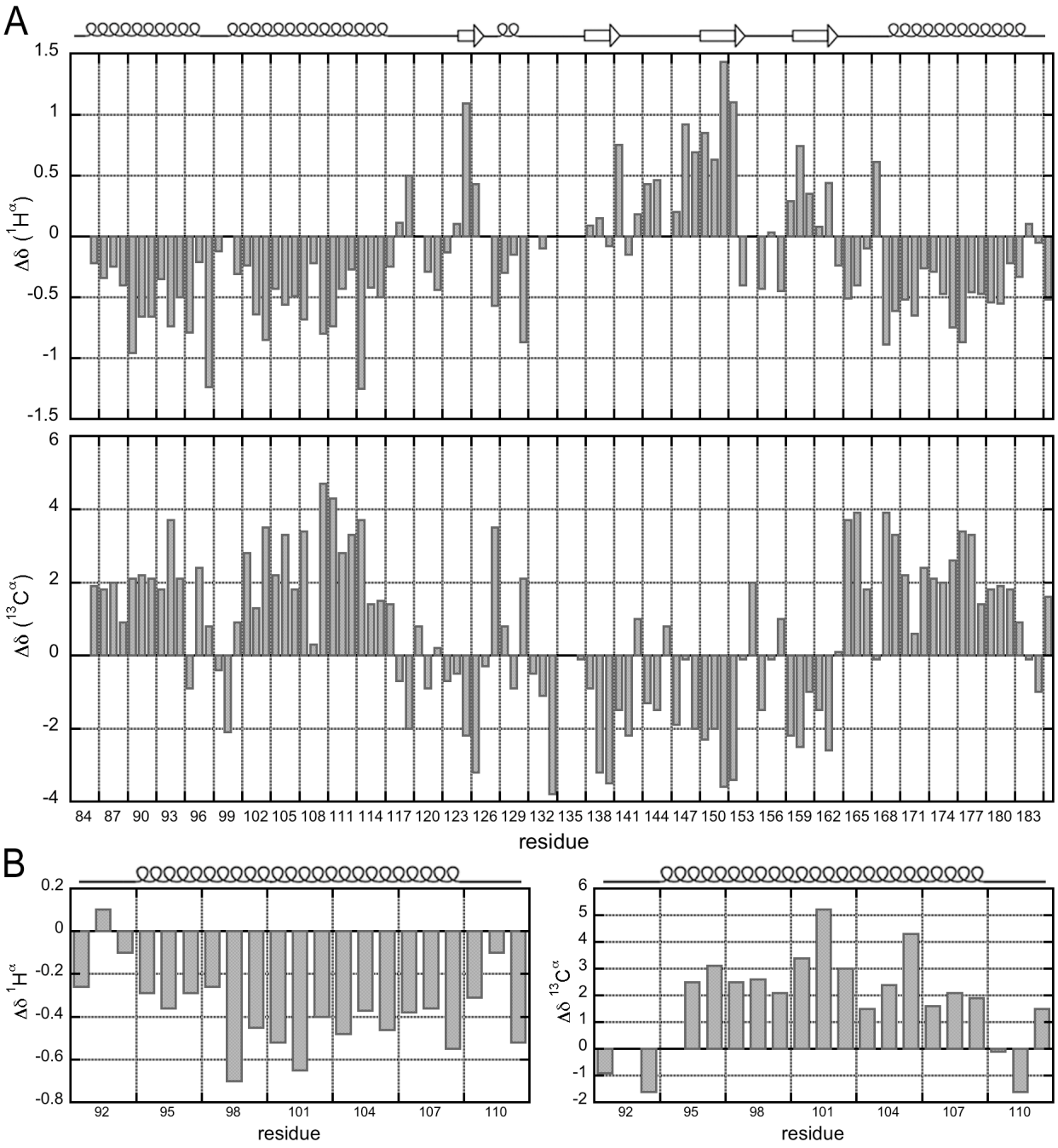

Figure S4: Deviations of $E_{CT1}H_{CT}$ $^1H^\alpha$ and $^{13}C^\alpha$ chemical shifts from random coil values. The differences of $^1H^\alpha$ and $^{13}C^\alpha$ chemical shifts from random coil values (17) are plotted for (A) E_{CT1} and (B) H_{CT} in the $E_{CT1}H_{CT}$ complex.