Segmental isotopic labeling of HIV-1 capsid protein assemblies for solid state NMR

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Supplementary Material, consisting of Tables S1-S2 and Figures S1-S4

Table S1: Conditions for 3D NCACX and 3D NCOCX spectra of segmentally-labeled HIV-1 CA assemblies. The MAS frequency was 12.00 kHz in all measurements.

sample	spectrum	magnetic field	^{13}C-^{13}C mixing period	t_1 and t_2 increments	number of t_1 and t_2 values	scans per FID
CTD-labeled CA	NCACX	14.1 T	30 ms	160.6 μs, 134.0 μs	40, 30	64
	NCOCX	14.1 T	60 ms	160.6 μs, 134.0 μs	40, 30	48
NTD-labeled CA	NCACX	14.1 T	20 ms	174.2 μs, 174.2 μs	36, 36	96
	NCOCX	14.1 T	70 ms	174.2 μs, 174.2 μs	36, 36	64
NTD-labeled CA	NCACX	17.5 T	70 ms	82.6 μs, 94.4 μs	96, 53	24
	NCOCX	17.5 T	100 ms	82.6 μs, 247.8 μs	96, 20	72

Table S2: Chemical shift assignments for HIV-1 CA assemblies from 3D solid state NMR spectra of segmentally labeled samples. Assignments are shown only for residues that did not have unambiguously assigned signals in earlier work by Bayro *et al*. (J. Mol. Biol. 426, 1109-1127, 2014). For C198, unambiguous assignments were reported by Bayro *et al*., but were different due to a different oxidation state. Chemical shifts are in parts per million, relative to 2,2-dimethyl-2-silapentane-5-sulfonate for ^{13}C and liquid ammonia for ^{15}N.

residue	N	Cα	CO	Cβ	Cγ
G106	103.1	45.8	174.2		
I141	117.4	56.6	176.5	38.3	29.6
V142	117.2	67.1	179.1	32.2	22.44
S146	117.4	55.7	176.75	63.71	
R173	120.6	59.2	179.1	32.2	26
A174	121.5	53.7	179.3	18.6	
E175	118.6	60.2	176.8	30.05	
C198	123.8	59.1	175.6	28.77	
E212	121.5	60.09	177.76	30.77	
E213	117.9	60.18	177	30.54	

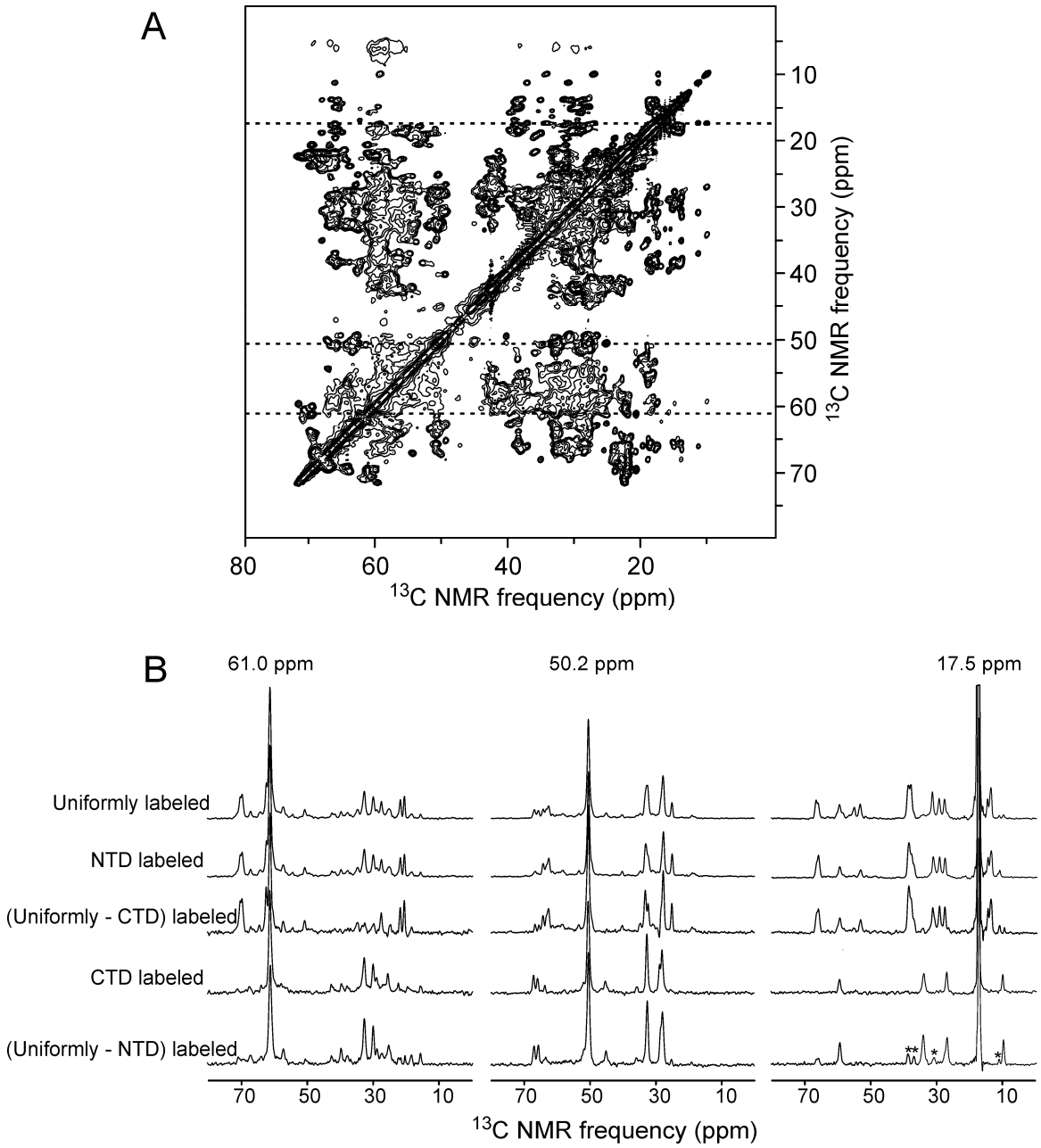

Figure S1: (A) Aliphatic region of the 2D solid state ^{13}C-^{13}C NMR spectrum of fully ^{15}N,^{13}C-labeled wild-type CA assemblies. Contour levels increase by successive factors of 1.7. (B) 1D slices at three ^{13}C chemical shift values from 2D ^{13}C-^{13}C spectra of of fully labeled and segmentally labeled CA assemblies, as well as 2D difference spectra. Asterisks indicate peaks in the difference between spectra of fully labeled CA assemblies and NTD-labeled CA assemblies that are not observed in the spectrum of CTD-labeled CA assemblies. These "extra" peaks are attributable to the reducing conditions used to prepare segmentally labeled samples, resulting in the absence of a C198-C218 disulfide bond in the segmentally labeled samples that is present in the fully labeled sample.

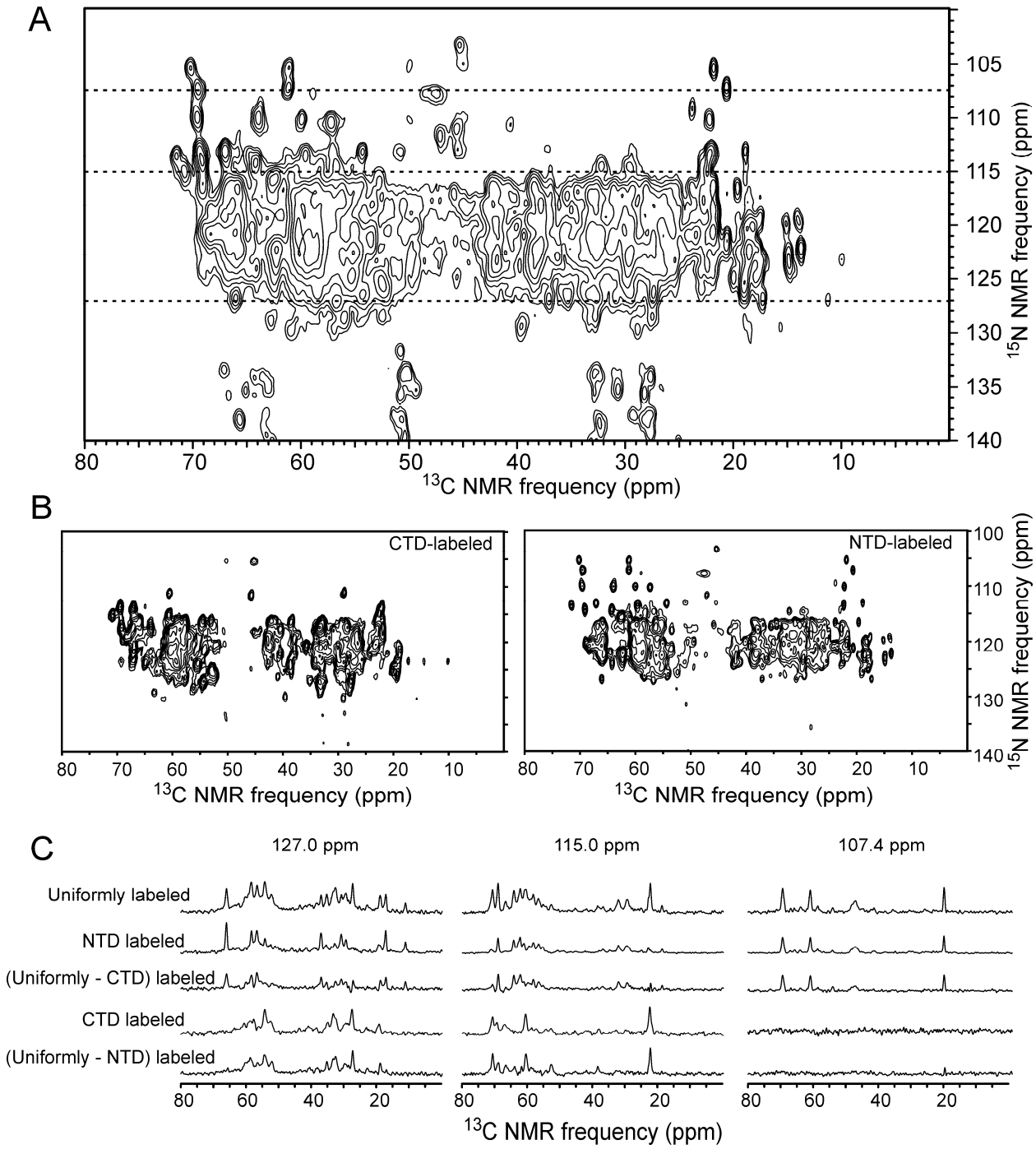

Figure S2: (A) Aliphatic region of the 2D NCACX spectra of fully labeled CA assemblies. Contour levels increase by successive factors of 1.5. (B) Aliphatic regions of 2D NCACX spectra of segmentally labeled CA assemblies. (C) 1D slices at three ^{15}N chemical shift values from 2D NCACX spectra of fully labeled and segmentally labeled CA assemblies, as well as 2D difference spectra.

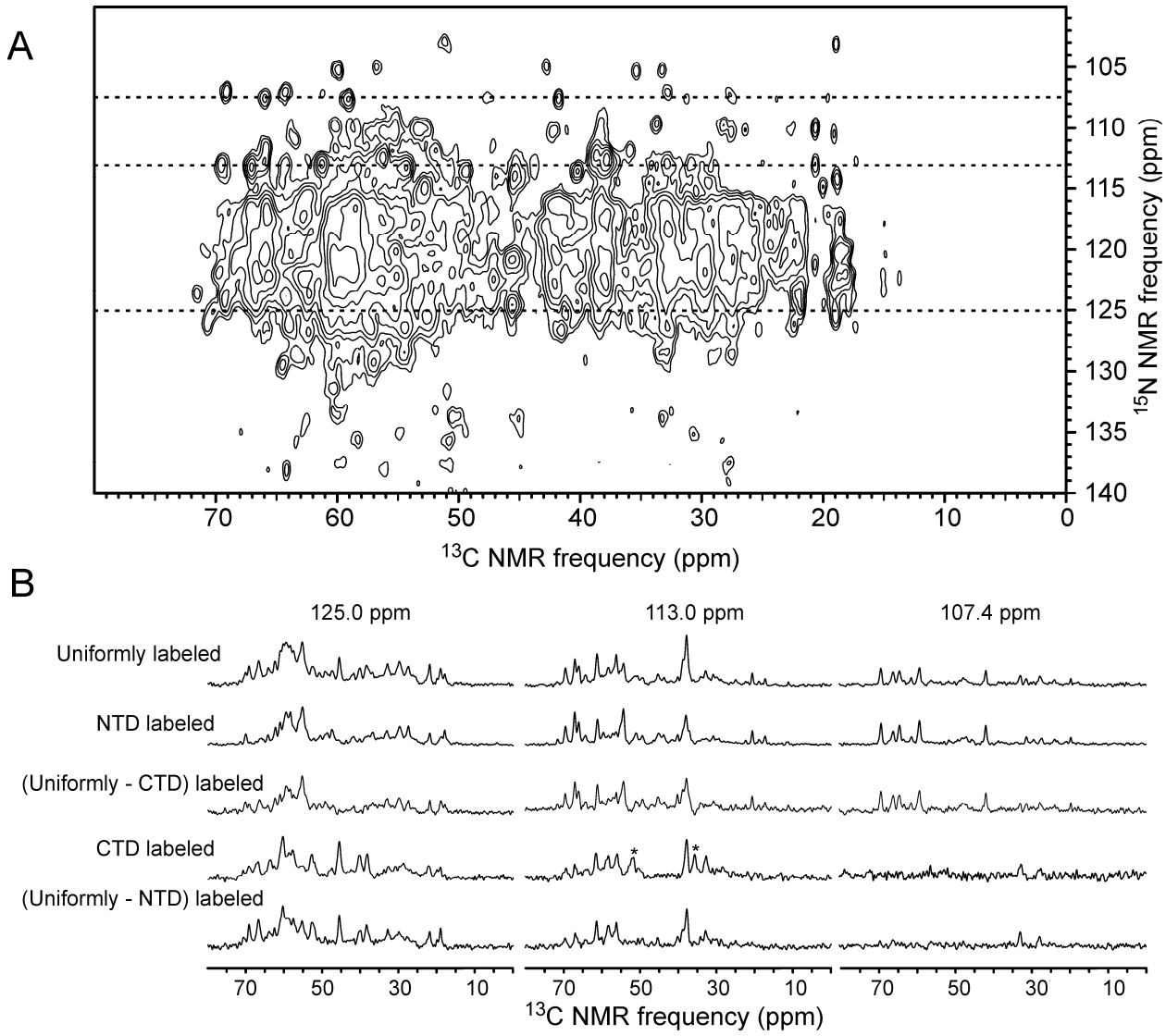

Figure S3: (A) Aliphatic region of the 2D NCOCX spectra of fully labeled CA assemblies. Contour levels increase by successive factors of 1.5. (B) 1D slices at three ^{15}N chemical shift values from 2D NCOCX spectra of fully labeled and segmentally labeled CA assemblies, as well as 2D difference spectra. Asterisks in panel B indicate "extra" peaks, which we tentatively assign to I150, of which the C_α chemical shift may be altered by the S149C substitution.

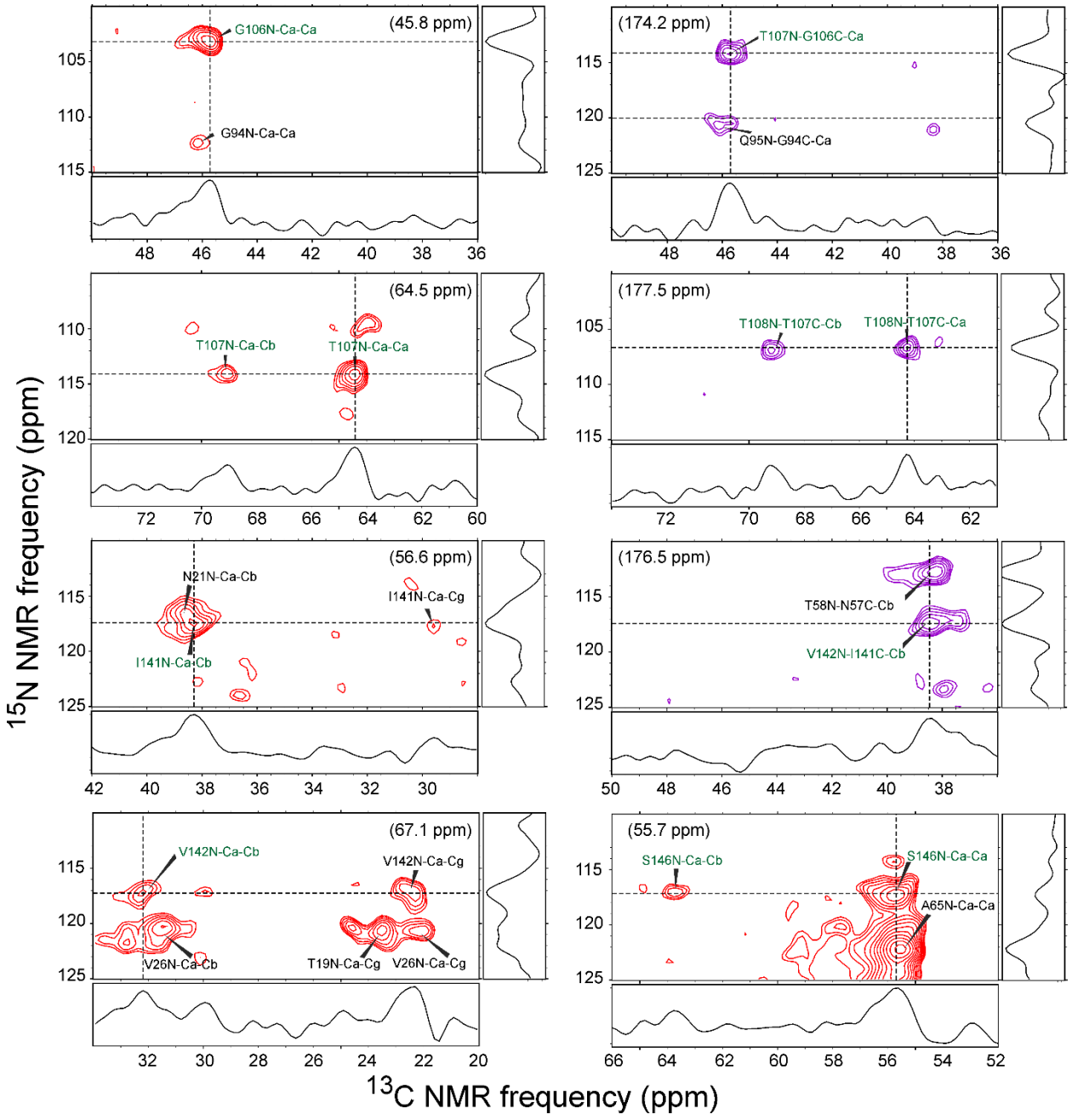

Figure S4: 2D planes and 1D slices from 3D NCACX (red contours) and 3D NCOCX (violet contours) spectra of NTD-labeled CA assemblies. Crosspeak assignments for residues listed in Table S2 are shown in green. Planes are labeled with their ^{13}C chemical shifts in the CA or CO dimension (*i.e.*, the second dimension of the 3D spectra). 1D slices at the positions of horizontal and vertical dashed lines indicate the signal-to-noise ratios. Contour levels increase by successive factors of 1.2. Spectra for G106 and T107 assignments were obtained at 14.1 T. Other spectra were obtained at 17.5 T. Experimental conditions are given in Table S1.

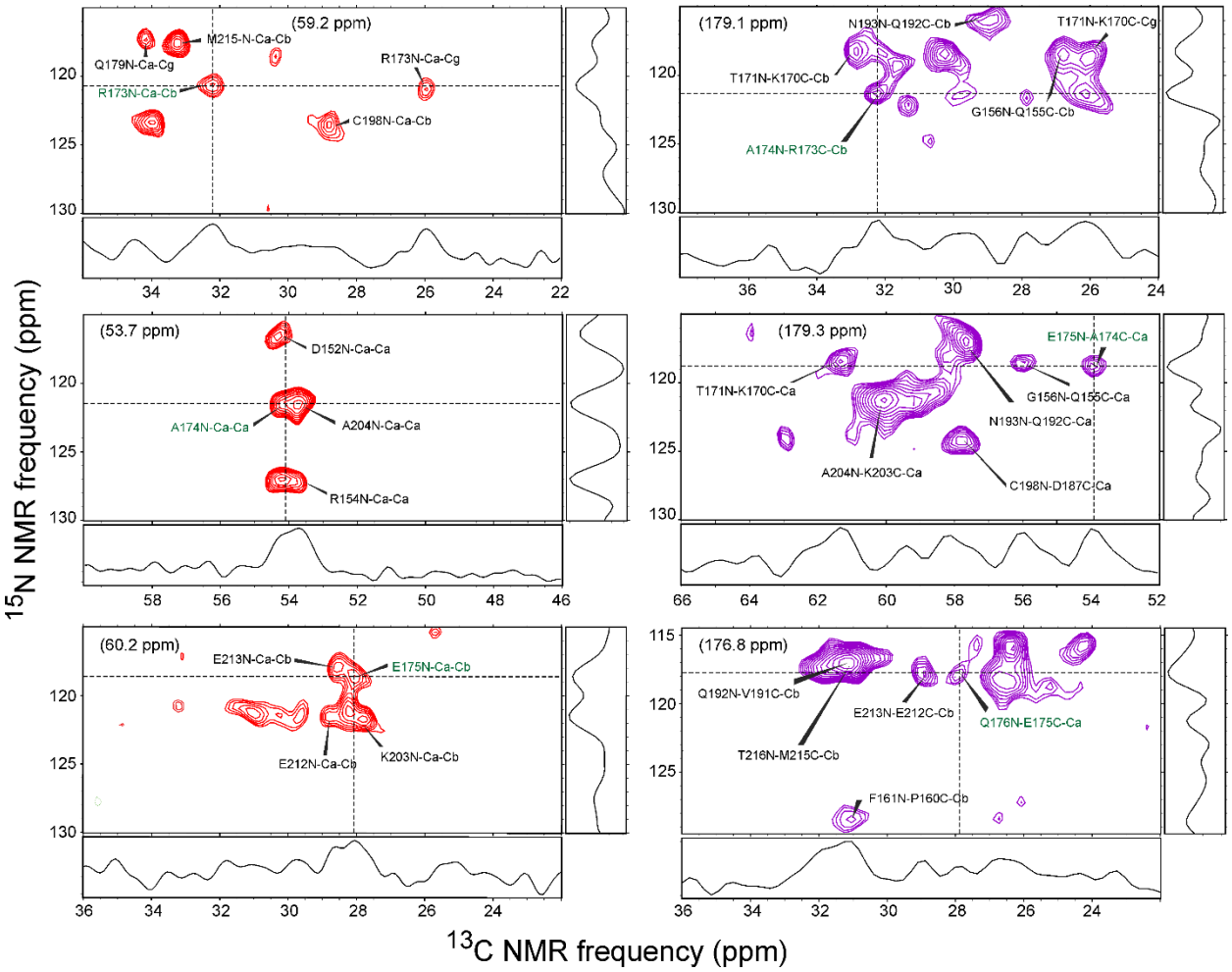

Figure S5: Same as Fig. S4, but for CTD-labeled CA assemblies. Spectra were obtained at 14.1 T with experimental conditions in Table S1.

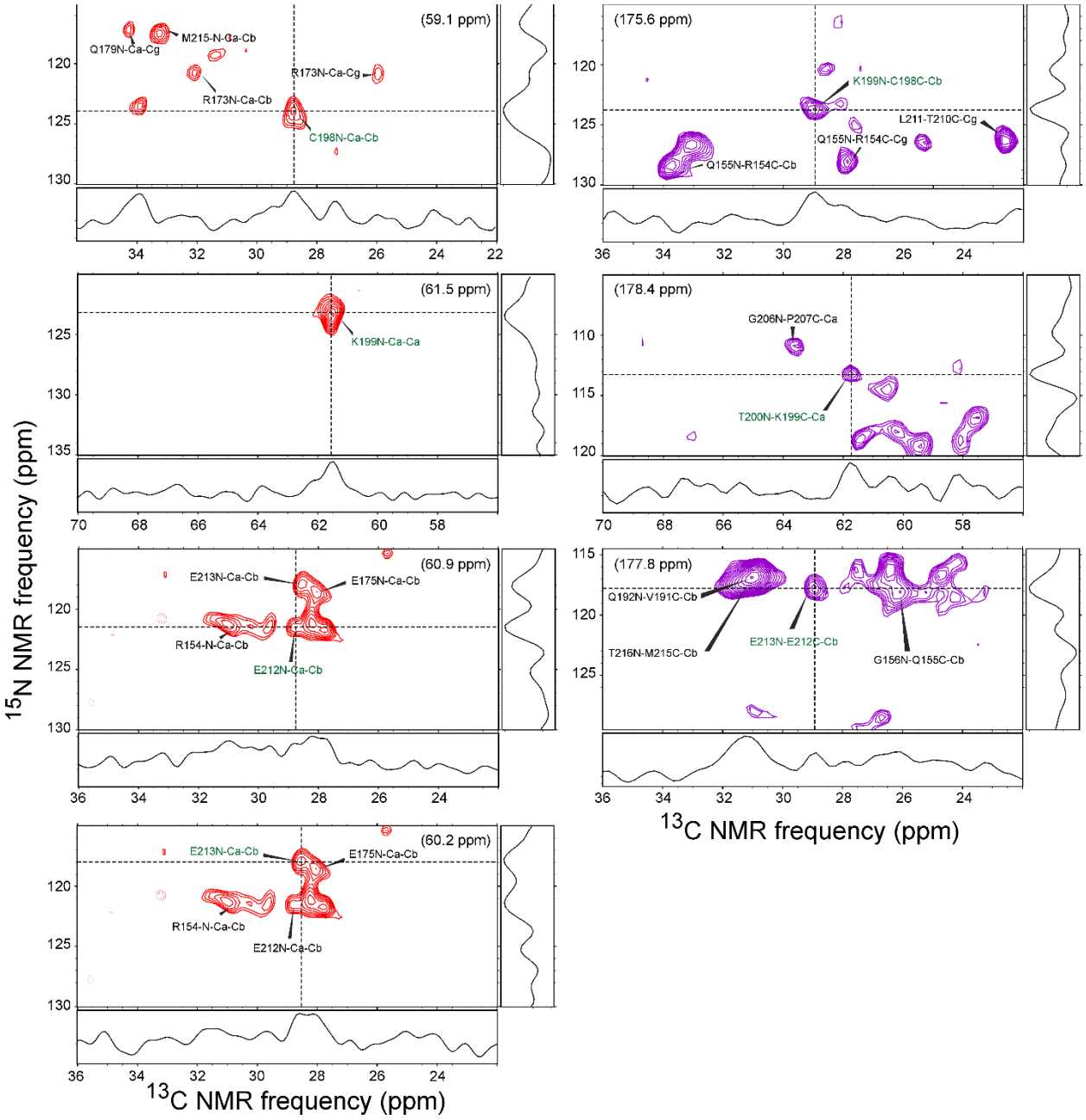

Figure S6: Same as Fig. S5.