

Supplementary Information for “Solid-State NMR Spectroscopy of the HIV gp41 Membrane Fusion Protein Supports Intermolecular Antiparallel β Sheet Fusion Peptide Structure in the Final Six-Helix Bundle State”

Model of $(\Delta S/S_0)_f$ from couplings to na ^{13}C nuclei

Table III lists the $(S_1/S_0)_f^\alpha$ of FP-Hairpin samples which are used to estimate $\gamma^{na} \equiv (S_1/S_0)_f^\alpha$ due to couplings to na ^{13}C nuclei. An earlier model is applied to understand these experimental γ^{na} or equivalently the $(\Delta S/S_0)_f^\alpha = 1 - \gamma^{na}$, cf. Z. Zheng et. al. *Biochemistry*, (2006) **45**, 12960-12975 and Z. Zheng et. al. *Magn. Reson. Chem.* (2007) **45**, S247-S260. Consider a sphere of radius r around a ^{13}CO nucleus where $(S_1/S_0)_f \approx 0$ if there is a na ^{13}C nucleus within this sphere. The value of r that satisfies this criterion is estimated from density operator-based simulations of $(S_1/S_0)_f$ for a single $^{13}\text{CO}/^{13}\text{C}$ spin pair as a function of internuclear distance. The number of C nuclei within this sphere ($\equiv p$) is estimated from the geometric arrangement of C nuclei around ^{13}CO nucleus. Given the na ^{13}C probability = 0.011, the model estimates that $1 - \gamma^{na} \approx 0.011 \times p$. Both r and p will increase with dephasing time. Previous calculations are consistent with p values ranging between ~ 3 for $\tau = 8$ ms to ~ 7 for $\tau \geq 32$ ms ($r \approx 5$ Å). These values correspond to a $1 - \gamma^{na}$ range between 0.03 and 0.08 which matches well with the $(\Delta S/S_0)_f^\alpha$ listed in Table III.

Table S1. Values of $(S_1/S_0)_r^\beta \equiv A$ ^a

Sample (¹³ CO/ ¹⁵ N labels) ^b	A
FP-Hairpin (L7/F8)	0.28
FP-Hairpin (F8/L9)	0.24
FP-Hairpin (L12/G13)	0.32
FP-Hairpin (F11/L12)	0.24
FP-Hairpin (I4/G5)	0.36
FP34 (L7/F8)	0.27
N70 (L7/F8)	0.33

^a S_{0r} and S_{1r} intensities were determined using 1.0 integration windows centered at the peak β shift.

^b Black and red typefaces respectively refer to initial protein solubilization in Buffer and in Buffer + D-malt.

Table S2. Sample characteristics

Sample (¹³ CO/ ¹⁵ N labels) ^a	(L+C):P ^{b,c}	μ mole protein ^c
FP-Hairpin (L7/F8)	45:1	0.60
FP-Hairpin (L7/F8)	38:1	0.84
FP-Hairpin (F8/L9)	30:1	1.10
FP-Hairpin (F8/L9)	38:1	0.99
FP-Hairpin (L12/G13)	30:1	1.00
FP-Hairpin (L12/G13)	48:1	0.53
FP-Hairpin (F11/L12)	42:1	0.72
FP-Hairpin (I4/G5)	38:1	0.46
FP34 (L7/F8)	38:1	0.68
N70 (L7/F8)	45:1	0.53

^a Black and red typefaces respectively refer to initial protein solubilization in Buffer and in Buffer + D-malt.

^b L, C, and P respectively refer to lipid, cholesterol, and protein.

^c Quantities in initial protein solution and vesicle suspension.

FP-Hairpin ΔS_r spectra with deconvolution

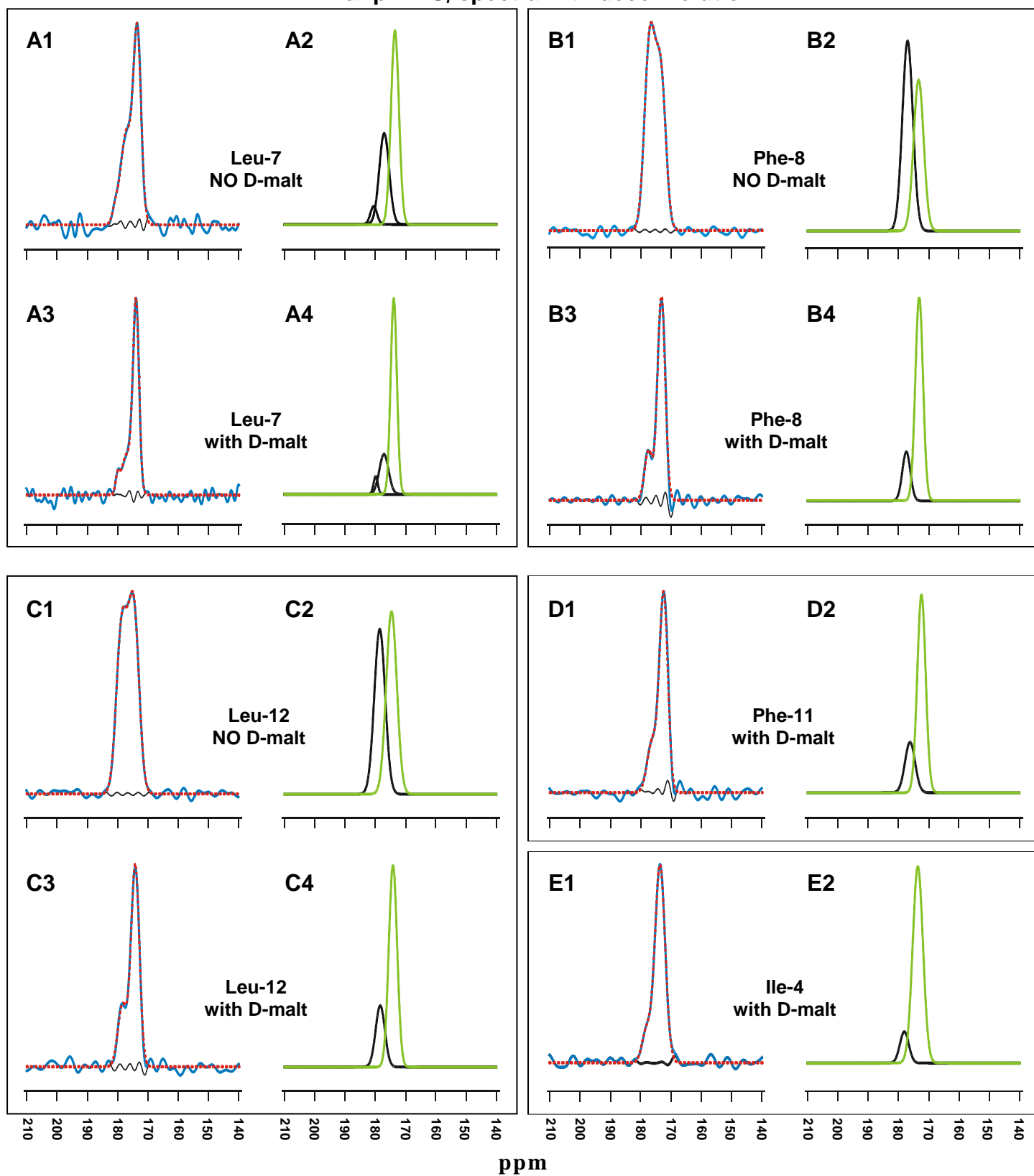
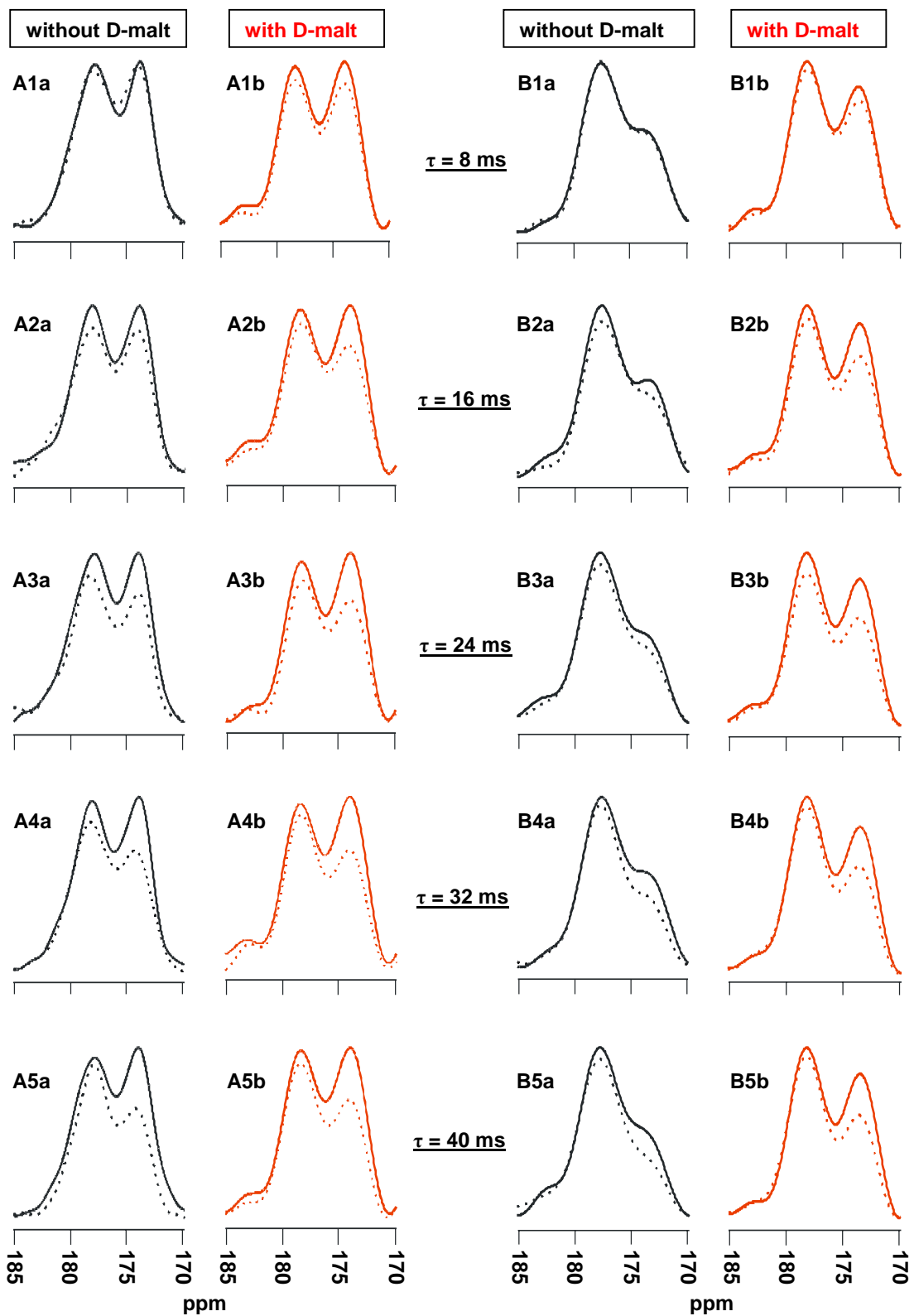
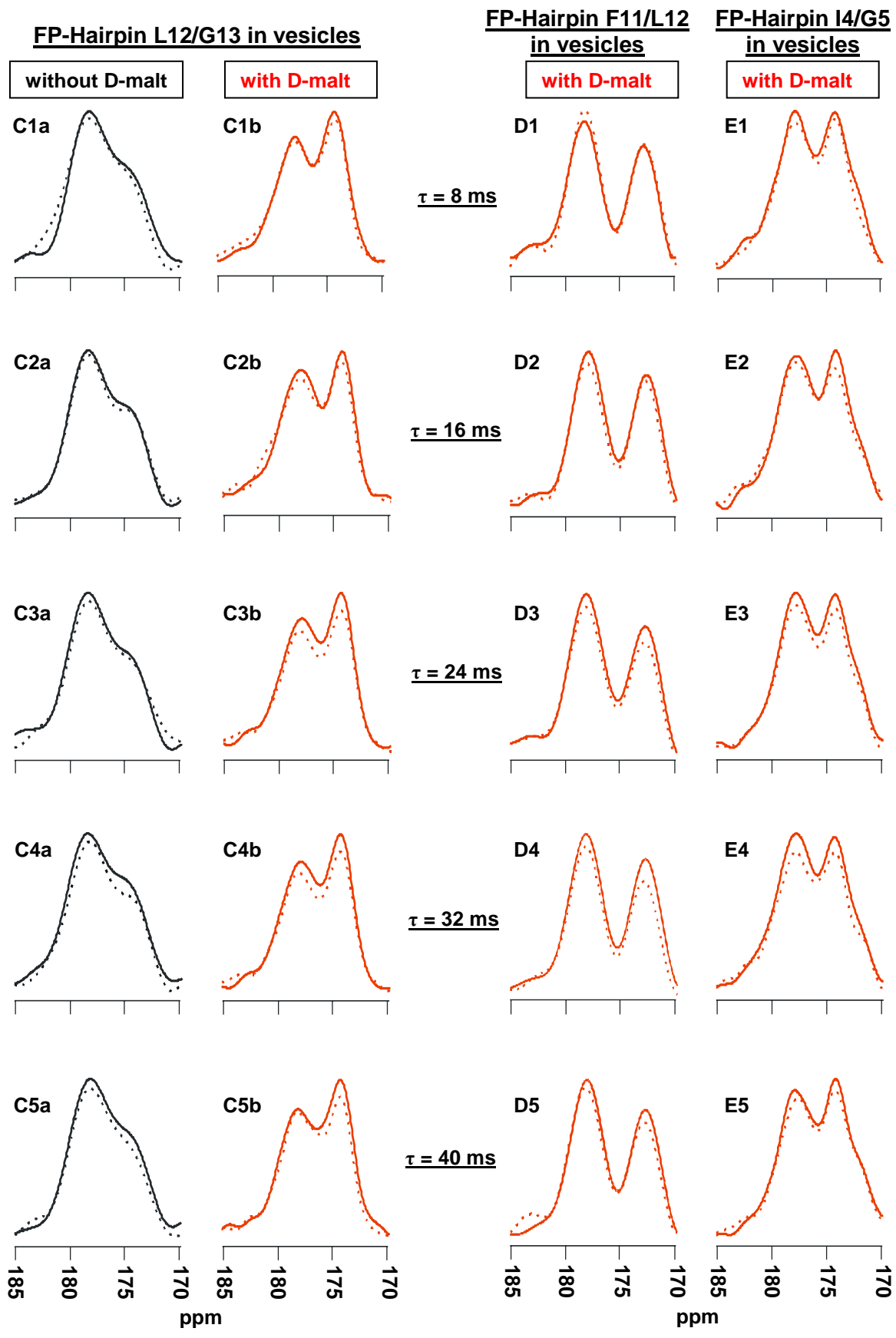


Fig. S1. Deconvolution of REDOR ΔS_r spectra of FP-Hairpin samples in membranes (fig. 3) with a single backbone $^{13}\text{CO}/^{15}\text{N}$ -amide labeled spin pair at (A) L7/F8, (B) F8/L9, (C) L12/G13, (D), F11/L12, and (E) I4/G5. Panels A1, B1, and C1 display spectra of samples with initial FP-Hairpin solubilization in Buffer, and panels A3, B3, C3, D1 and E1 display spectra of samples with initial FP-Hairpin solubilization in Buffer + D-malt. For each of these panels, the experimental ΔS_r , deconvolution sum, and difference spectra are respectively displayed as solid blue, broken red, and black lines. Panels A2, A4, B2, B4, C2, C4, D2, and E2 respectively display the individual deconvolution Gaussian lineshapes of the A1, A3, B1, B3, C1, C3, D1, and E1 spectra. The green and black lineshapes are respectively assigned to β sheet and α helical conformation and Table I lists the peak shift, linewidth, and fractional integrated intensity of each of the lineshapes.⁴⁴ Good fitting of the L7/F8 spectra required two α helical lineshapes.

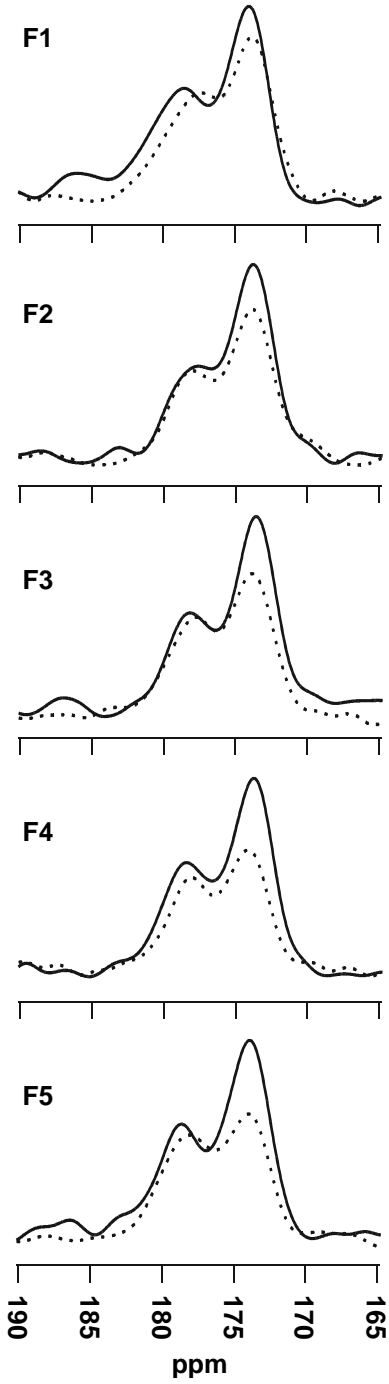
FP-Hairpin L7/F8 in vesicles

FP-Hairpin F8/L9 in vesicles





N70 L7/F8 in vesicles



$\tau = 6.7$ ms

$\tau = 14.7$ ms

$\tau = 22.7$ ms

$\tau = 30.7$ ms

$\tau = 38.7$ ms

$\tau = 46.7$ ms

FP34 L7/F8 in vesicles

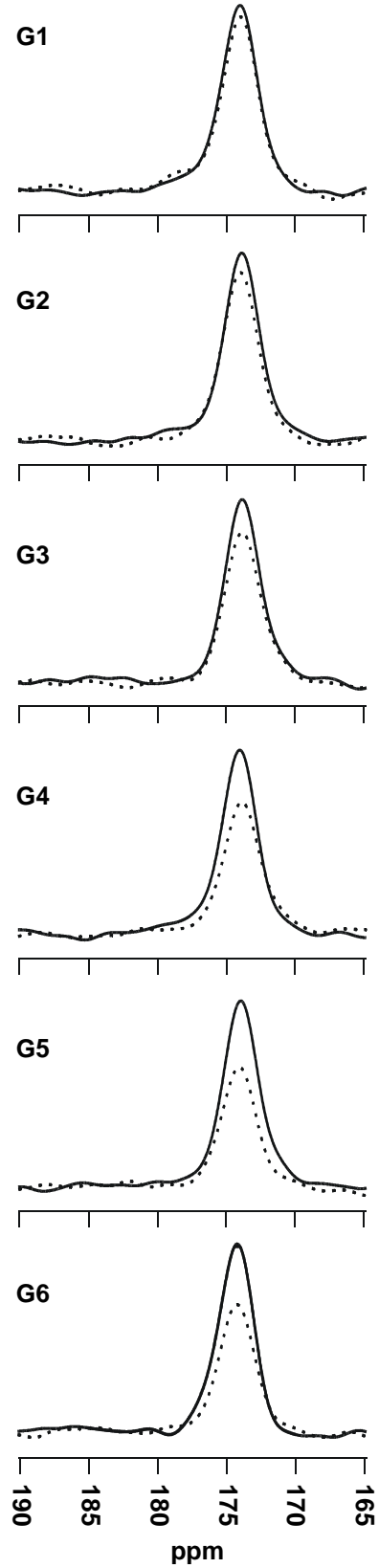


Fig. S2. fpCTDQBU ^{13}C O spectra for (A-E) FP-Hairpin, (F) N70, and (G) FP34 samples. Solid and dashed traces are respectively S_{0f} and S_{1f} spectra. The proteins had $^{13}\text{C}/^{15}\text{N}$ labels at (A, F, G) L7/F8, (B) F8/L9, (C) L12/G13, (D) F11/L12, and (E) I4/G5. Black and red spectra respectively correspond to samples with initial protein solubilization in Buffer or Buffer + D-malt. Processing of spectra Aa, Cb, and F + G included Gaussian line broadening of 150, 100, and 200 Hz. Processing of all spectra included polynomial baseline correction.