Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary Information

Post-assembly α-helix to β-sheet structural transformation within SAF-p1/p2a peptide nanofibers

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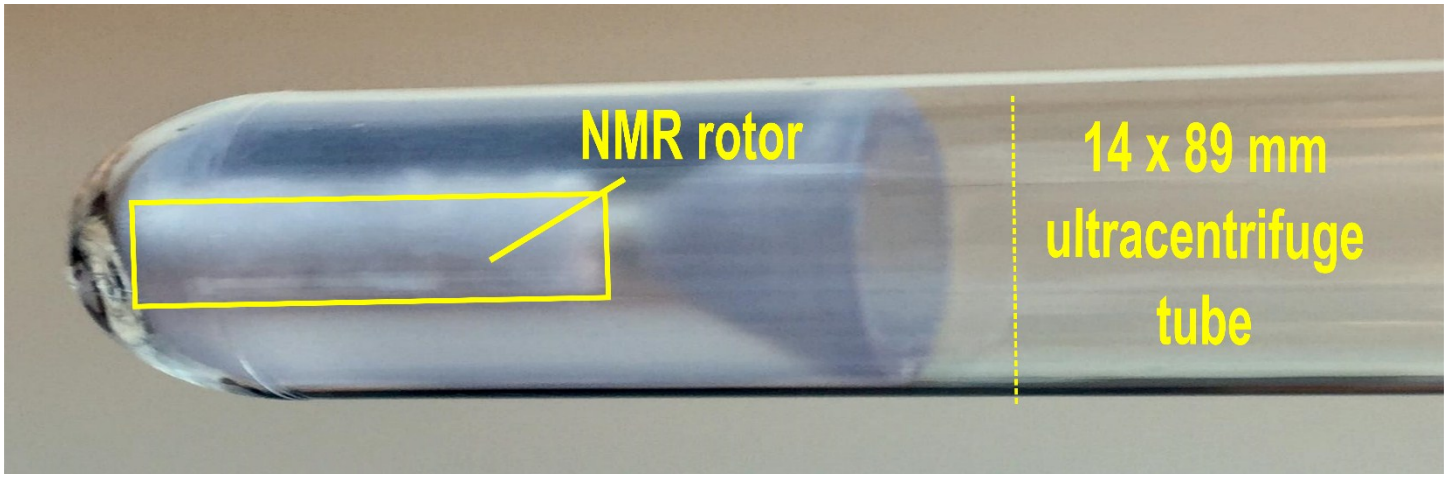

Fig. S1 | Funnel for concentration of nanofibers in solid-state NMR sample preparation by the direct centrifugation method.

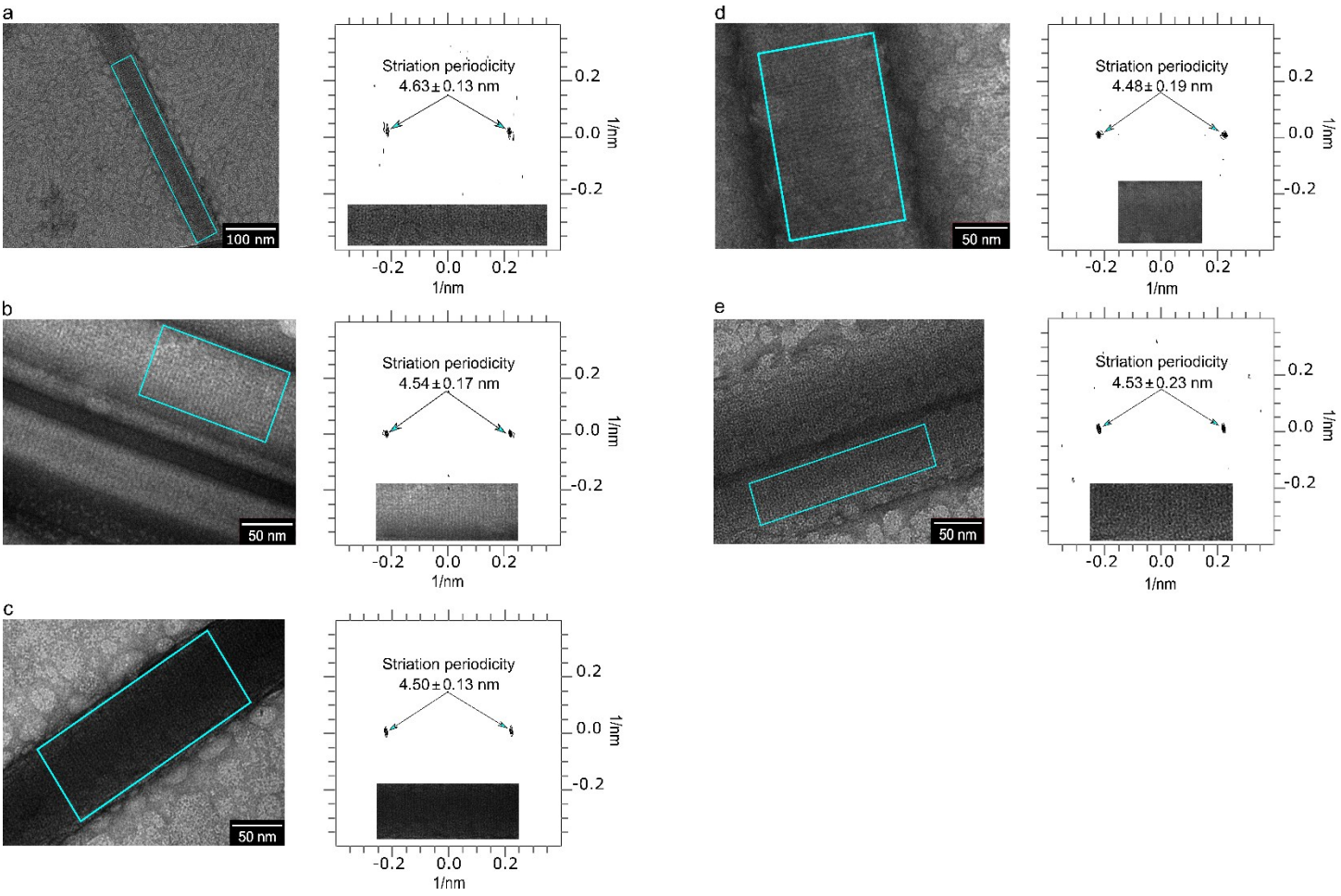

Fig. S2 | Assessment of striation periodicity by Fourier transform analysis of coiled-coil SAF-p1/p2a nanofiber images. (a-e) Fourier transform analysis of SAF-p1/p2a nanofibers imaged by TEM with background correction. The left half of each panel shows a negatively stained TEM image, while the right half of each panel shows a 2D Fourier transform of the region indicated by the blue rectangle. Each region analyzed by Fourier transform was rotated as shown in the Fourier transform plot inset. The indicated striation periodicity for each image was determined by Gaussian peak fitting of the Fourier transform.

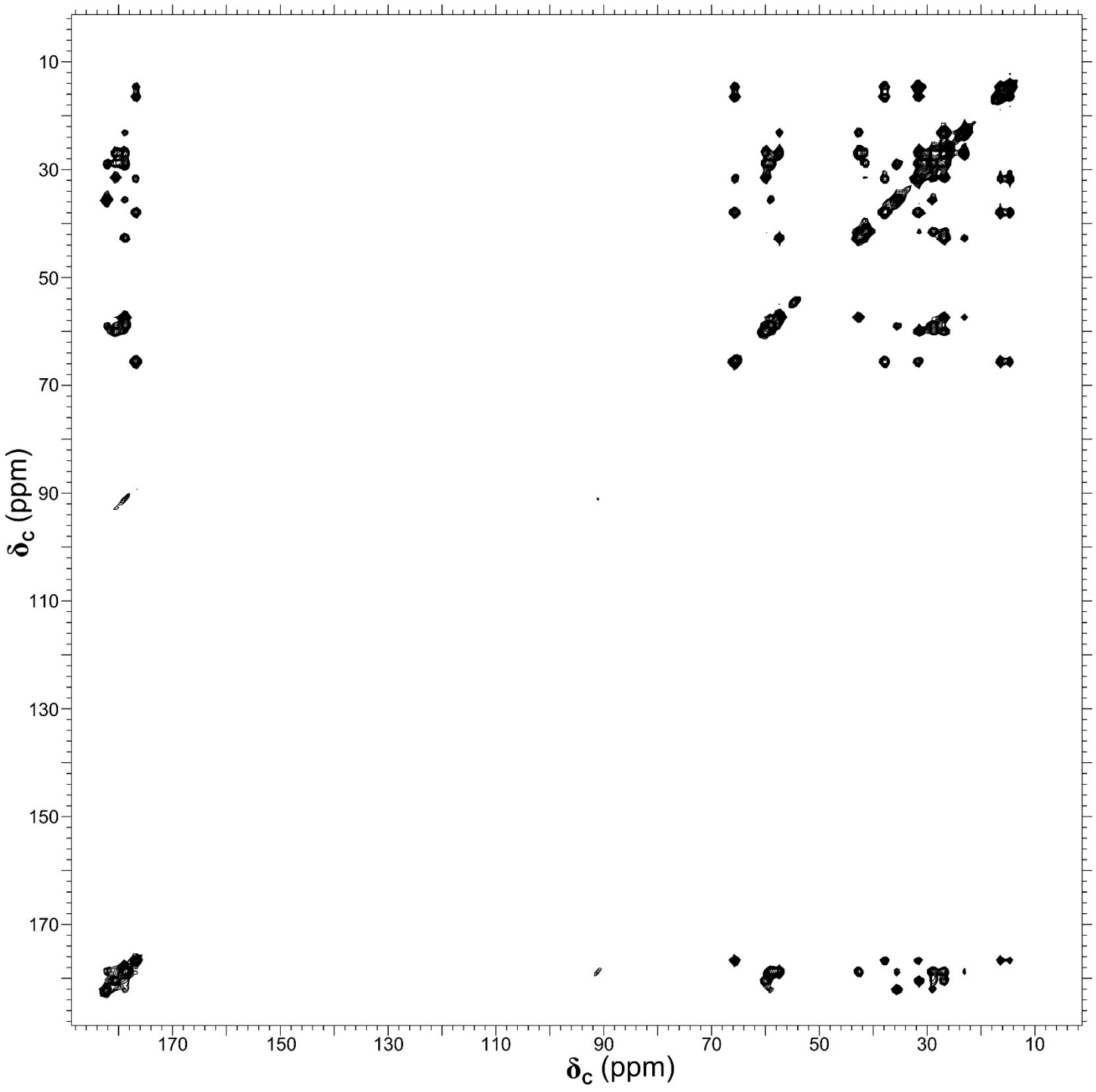

Fig. S3 Full 2D DARR spectrum (τ_m = 50 ms) from Fig. 2c.

Table S1 Chemical shifts (δ_C) and NMR peak widths of all labeled sites in coiled-coil nanofibers prepared by direct centrifugation. ^{13}C chemical shifts (in ppm) derived from 2D ^{13}C-^{13}C DARR data for labeled amino acids in α-helical SAF-p1/p2a nanofibers (Fig. 2c). Peak widths shown in parentheses correspond to FWHM measured in the direct dimension for each value of δ_C by fitting of 2D DARR crosspeaks to Gaussian functions.

Residue		CO	C_α	C_β	$C_{\gamma1}$	$C_{\gamma2}$	$C_{\delta1}$	$C_{\delta2}$	C_ϵ
p1-K6		180.5 (0.67)	60.3 (0.59)	31.9 (0.48)	27.2 (0.63)	-	29.3 (0.57)	-	41.9 (0.42)
p1-L12		178.8 (0.69)	57.7 (0.56)	43.1 (0.58)	27.3 (0.87)	-	23.5 (0.41)	23.4 (0.44)	-
p2a-E15		178.9 (0.73)	59.4 (0.62)	29.5 (0.66)	36.0 (0.64)	-	182.2 (0.55)	-	-
p2a-I23		176.8 (0.74)	66.0 (0.64)	38.3 (0.54)	32.1 (0.55)	16.8 (0.38)	15.2 (0.38)	-	-

Table S2 Chemical shifts (δ_C) and peak widths of all labeled sites in nanofibers following the helix-to-sheet transition. ^{13}C chemical shifts (in ppm) derived from 2D ^{13}C-^{13}C DARR data for labeled amino acids in the β-sheet structure formed by heating SAF-p1/p2a nanofibers to 40 °C (Fig. 3b). Peak widths shown in parentheses correspond to FWHM measured in the direct dimension for each value of δ_C by fitting of 2D DARR crosspeaks to Gaussian functions.

Residue		CO	C_α	C_β	$C_{\gamma1}$	$C_{\gamma2}$	$C_{\delta1}$	$C_{\delta2}$	C_ϵ
p1-K6		174.8 (1.11)	55.1 (1.12)	36.0 (1.32)	24.9 (1.23)	-	29.4 (0.82)	-	41.6 (0.76)
p1-L12		174.8 (1.19)	54.0 (1.18)	46.0 (1.18)	27.7 (1.60)	-	25.8 (1.50)	24.1 (1.61)	-
p2a-E15		174.4 (1.14)	55.0 (1.24)	33.5 (1.21)	36.7 (1.23)	-	182.7 (1.25)	-	-
p2a-I23		173.7 (1.98)	59.3 (1.62)	41.3 (1.75)	27.8 (1.90)	17.2 (1.36)	13.9 (1.06)	-	-

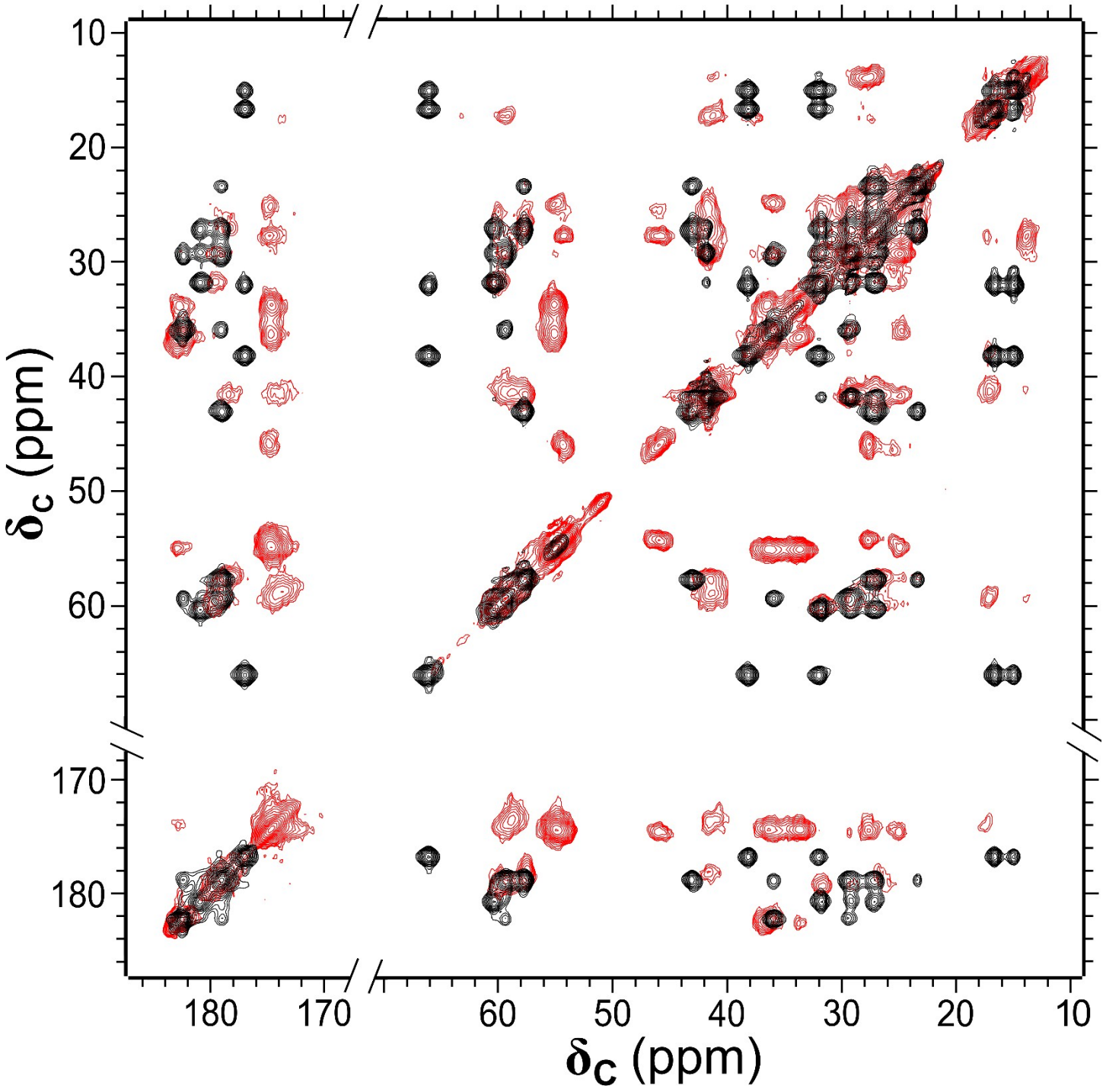

Fig. S4 Overlay of 2D DARR spectra (τ_m = 50 ms) from Figs. 2c (black, α-helical structure) and 3b (red, β-sheet structure).

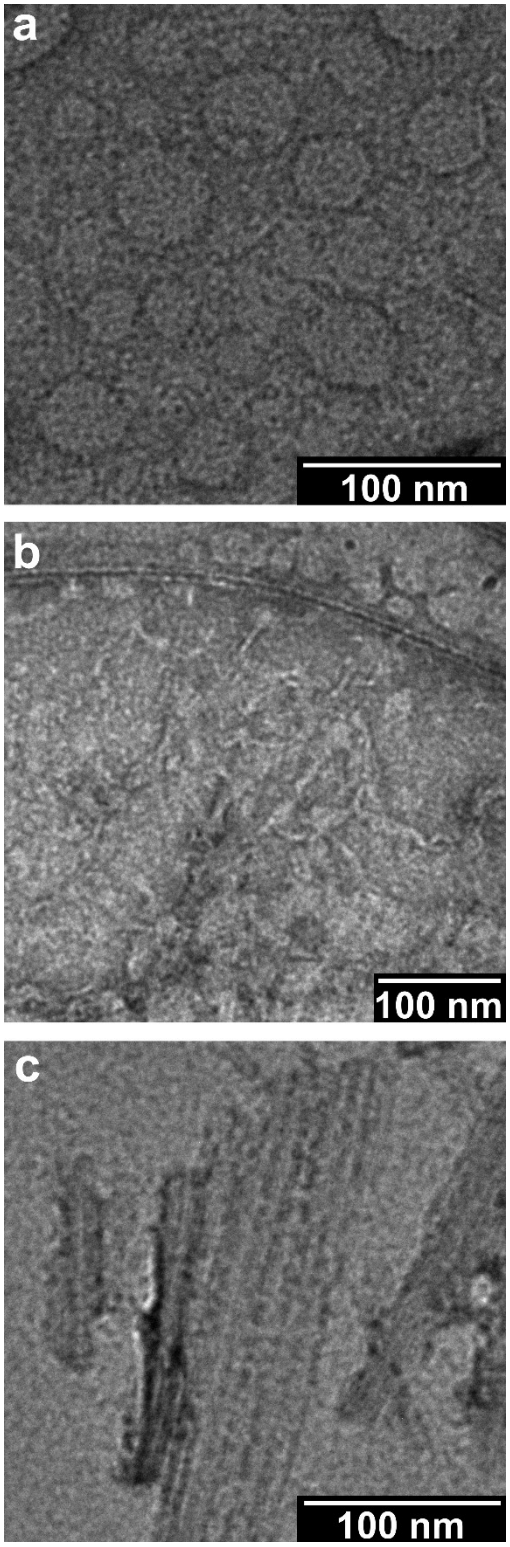

Fig. S5 High-magnification TEM images from SAF-p1/p2a β-sheet samples, chosen to exemplify the different nanostructure morphologies observed: (a) globular aggregates, (b) worm-like fibrils, (c) and straight fibers with longitudinal striations.

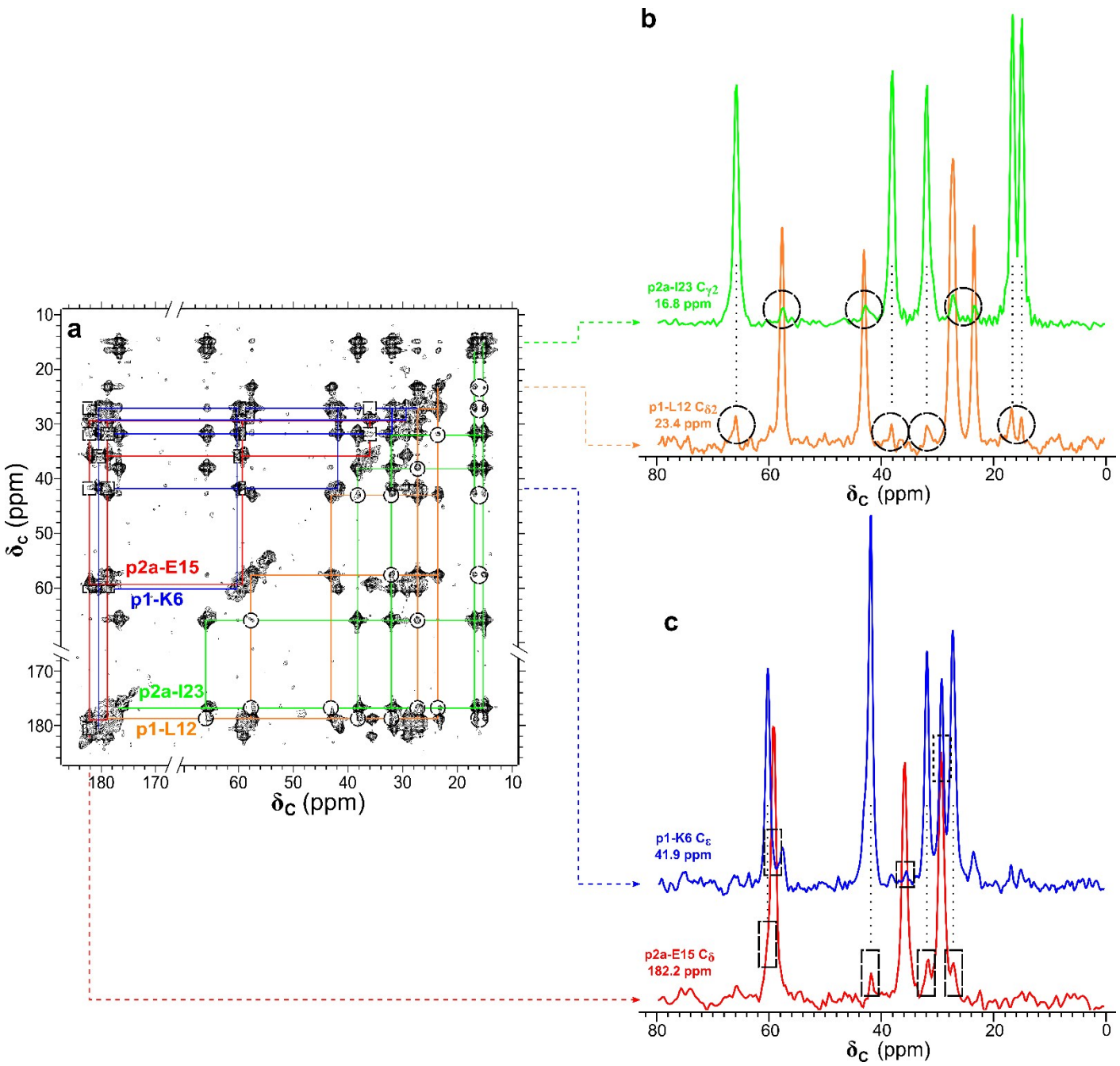

Fig. S6 Evidence of co-assembly in coiled-coil nanofibers prepared by direct centrifugation. (a) The 2D DARR spectrum shown in Fig. 5a, along with selected horizontal and (for p2a E15 C_δ) vertical 1D slices from this spectrum showing (b) inter-residue dipolar couplings (circled crosspeaks) between SAF-p1 L12 and SAF-p2a I23, and (c) inter-residue dipolar couplings (boxed crosspeaks) between SAF-p1 K6 and SAF-p2a E15. The dotted box in Panel (b) indicates the position of an expected crosspeak that cannot be distinguished due to spectral overlap of on-diagonal peaks between SAF-p1 K6 C_δ and SAF-p2a E15 C_β.

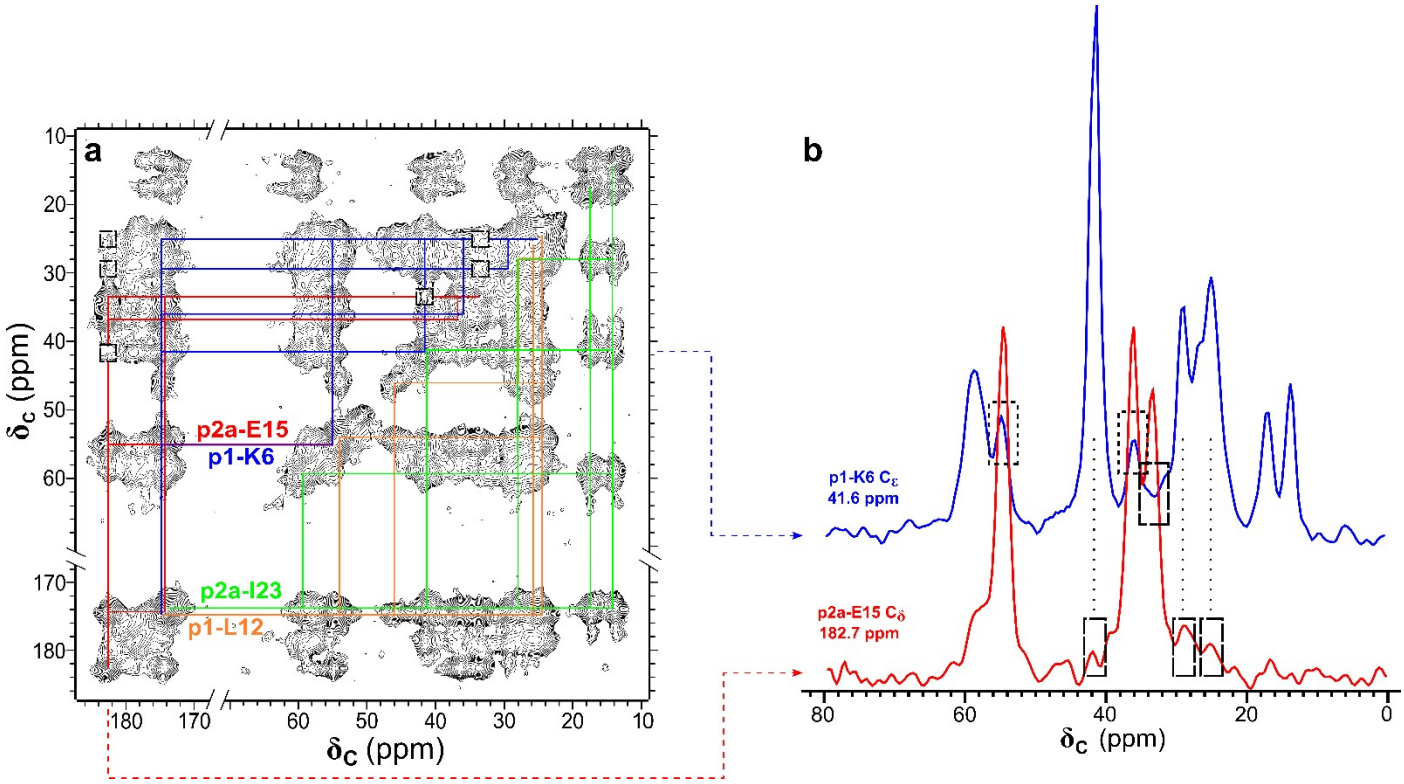

Fig. S7 Evidence of co-assembly following the helix-to-sheet transition in nanofibers heated to 40 °C. (a) The 2D DARR spectrum shown in Fig. 5b, along with (b) the horizontal 1D slice taken from this spectrum at the SAF-p1 K6 C_ϵ peak position and the vertical 1D slice taken from this spectrum at the SAF-p2a E15 C_δ peak position. Boxed crosspeaks indicate inter-residue dipolar couplings between SAF-p1 K6 and SAF-p2a E15, with dotted boxes marking positions where crosspeaks would be expected but cannot be distinguished due to spectral overlap of on-diagonal peaks between the two residues.

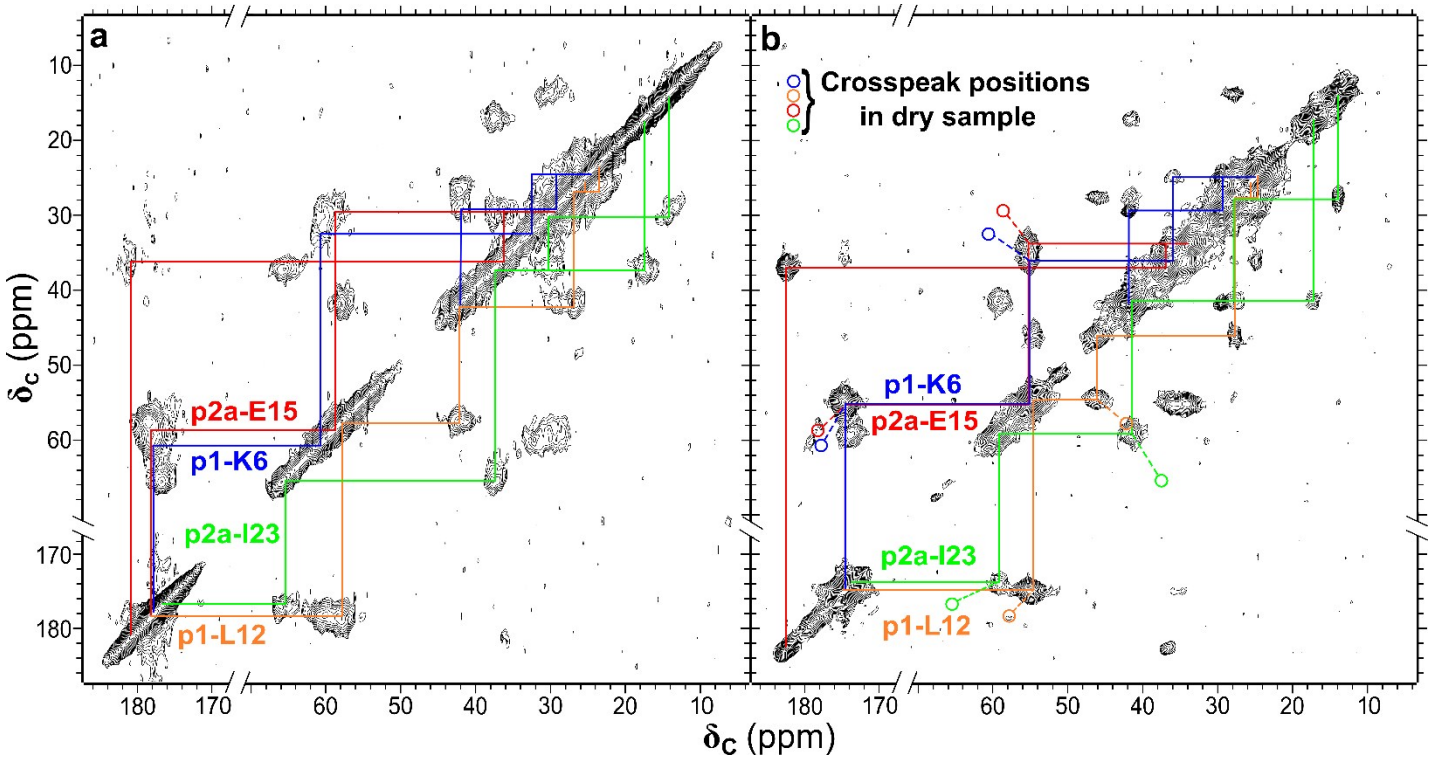

Fig. S8 2D fpRFDR spectra of lyophilized and rehydrated SAF-p1/p2a nanofibers. (a) 2D ^{13}C-^{13}C fpRFDR spectrum of the lyophilized (dry) SAF-p1/p2a nanofibers. Colored lines denote chemical shift assignment pathways for the ^{13}C-labeled residues (indicated by matching colored text), and only connect diagonal peak and crosspeak positions of directly bonded carbons. (b) 2D ^{13}C-^{13}C fpRFDR spectrum collected following rehydration (~1 μL/mg sample) of the lyophilized nanofibers. Colored dots with dashed lines indicate the changes in crosspeak positions observed following rehydration for near-backbone ^{13}C pairs that are sensitive to secondary structure.

Table S3 Chemical shifts in lyophilized and rehydrated nanofibers. ^{13}C chemical shift assignments (δ_C) derived from 2D ^{13}C-^{13}C fpRFDR data for labeled amino acids in SAF-p1/p2a prepared via lyophilization and subsequent rehydration. Values in unshaded rows correspond to the lyophilized (dry) SAF-p1/p2a nanofibers (Fig. S8a), and values in shaded rows correspond to the rehydrated sample (Fig. S8b).

Residue	CO	C_α	C_β	$C_{\gamma1}$	$C_{\gamma2}$	$C_{\delta1}$	$C_{\delta2}$	C_ϵ
	δ_C, in ppm							
p1-K6	177.9	60.6	32.4	24.6	-	29.2	-	42.0
	174.4	55.2	36.0	24.9	-	29.3	-	41.8
p1-L12	178.2	57.9	42.1	26.8	-	25.3	23.6	-
	174.8	54.6	46.1	27.7	-	25.6	24.7	-
p2a-E15	178.1	58.6	29.5	36.1	-	180.9	-	-
	174.5	55.3	33.9	36.9	-	182.4	-	-
p2a-I23	176.8	65.6	37.5	30.1	17.5	14.2	-	-
	173.8	59.2	41.4	27.8	17.2	13.9	-	-

Table S4 Secondary chemical shifts in lyophilized and rehydrated nanofibers. Secondary ^{13}C chemical shifts ($\Delta\delta_C$) at near-backbone positions of labeled residues derived from 2D ^{13}C-^{13}C fpRFDR data.§ Values in unshaded rows correspond to the lyophilized (dry) SAF-p1/p2a nanofibers (Fig. S8a), and values in shaded rows correspond to the rehydrated SAF-p1/p2a sample (Fig. S8b).

	$\Delta\delta_C$ (ppm)		
	CO	C_α	C_β
p1-K6	1.3	4.4	-0.7
	-2.2	-1.0	2.9
p1-L12	0.6	2.8	-0.3
	-2.8	-0.5	3.7
p2a-E15	1.5	2.0	-0.4
	-2.1	-1.3	4.0
p2a-I23	0.4	4.5	-1.3
	-2.6	-1.9	2.6

§ Calculation of $\Delta\delta_C$ values performed using random-coil chemical shifts from Wishart *et al.* (Reference 30 in main text)

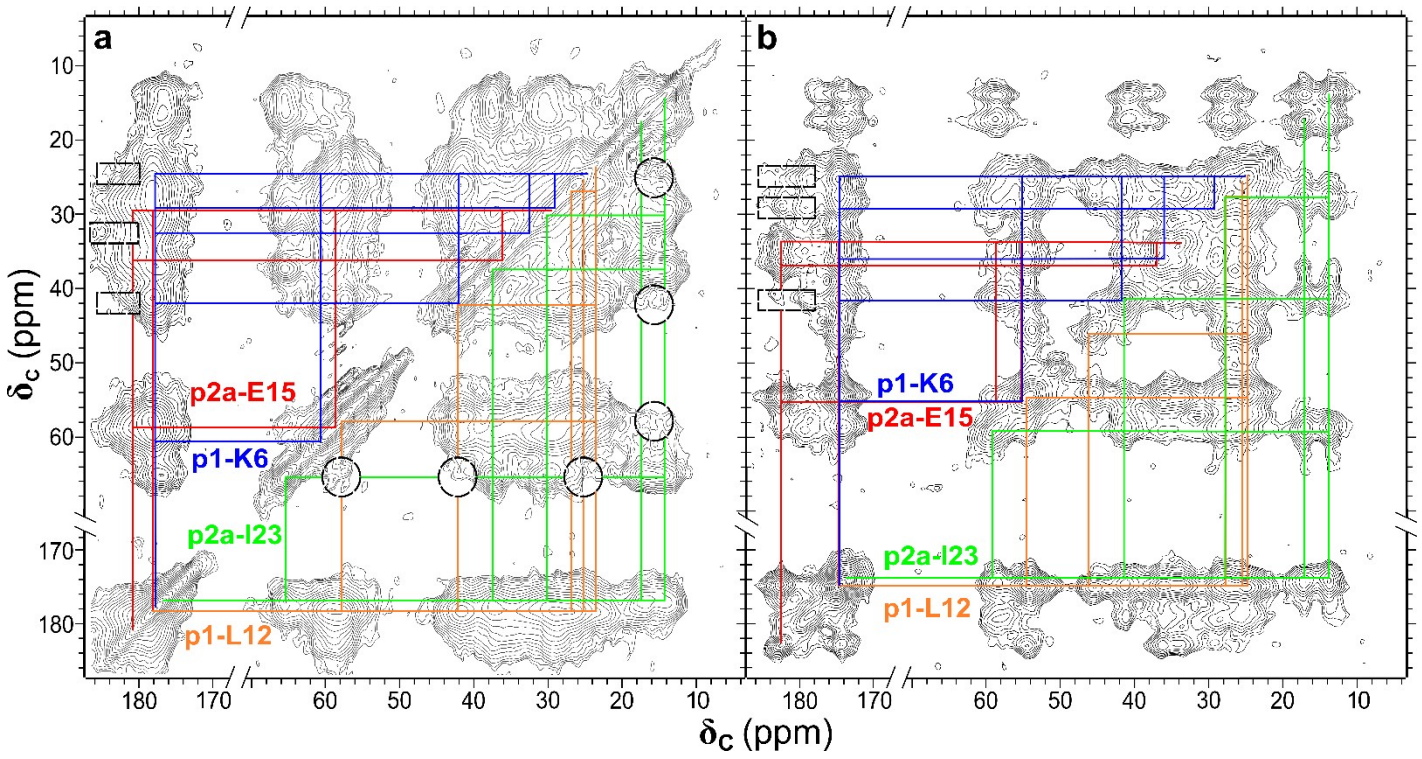

Fig. S9 2D DARR spectra of lyophilized and rehydrated SAF-p1/p2a nanofibers. (a) 2D ^{13}C-^{13}C DARR spectrum collected with τ_m = 500 ms on lyophilized SAF-p1/p2a nanofibers. Selected crosspeaks resulting from inter-residue dipolar couplings between SAF-p1 L12 and SAF-p2a I23 or between SAF-p1 K6 and SAF-p2a E15 are circled below the diagonal or boxed above the diagonal, respectively. (b) 2D ^{13}C-^{13}C DARR spectrum collected with τ_m = 500 ms following rehydration of lyophilized SAF-p1/p2a nanofibers. Selected crosspeaks resulting from inter-residue dipolar couplings between SAF-p1 K6 and SAF-p2a E15 are boxed above the diagonal.

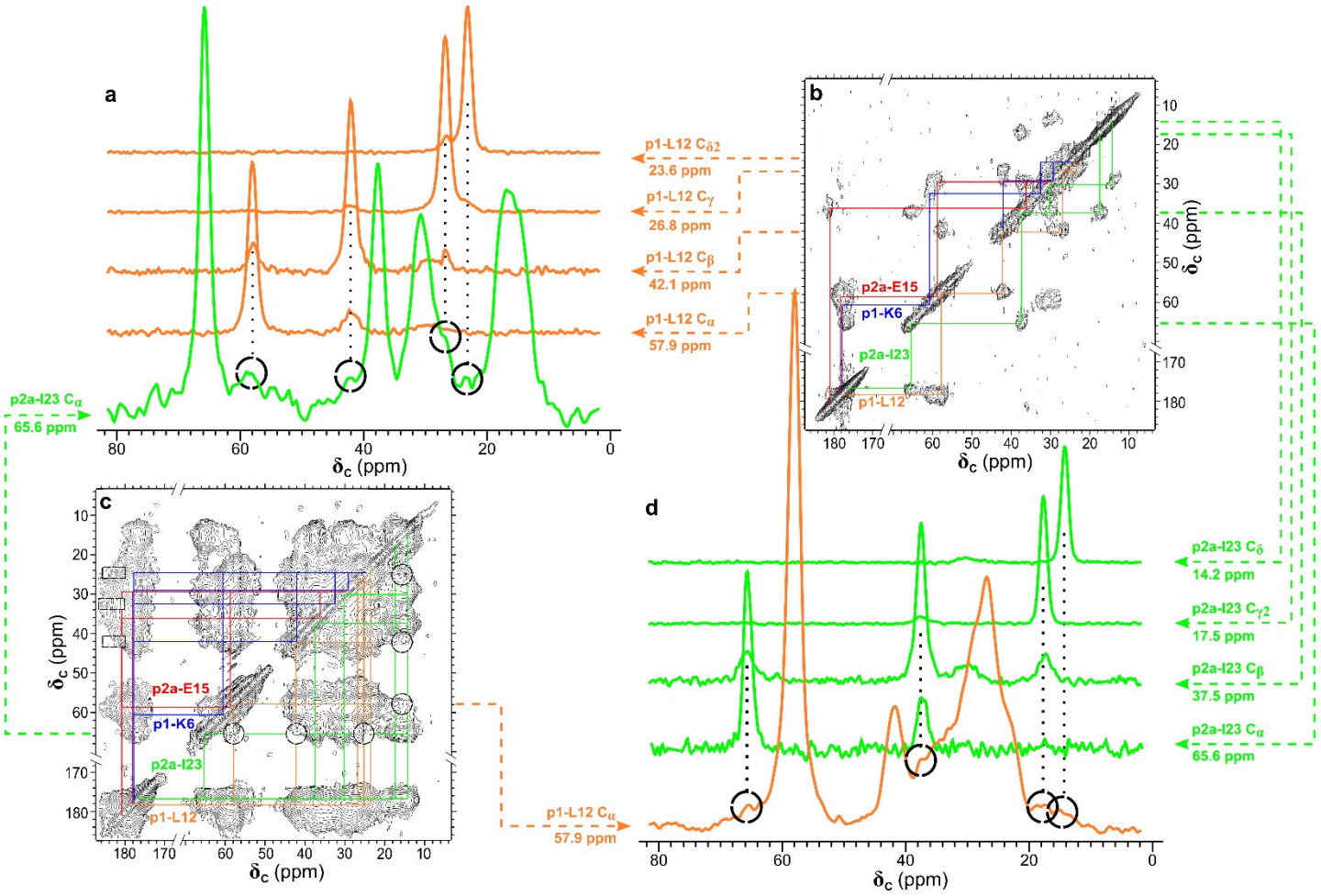

Fig. S10 NMR evidence of inter-peptide interactions between hydrophobic sidechains in lyophilized coiled-coil nanofibers. (a) and (d) Overlaid 1D slices corresponding to SAF-p1 L12 and SAF-p2a I23 from (b) 2D fpRFDR and (c) 2D DARR (τ_m = 500 ms) spectra of lyophilized α-helical nanofibers, with slice positions and horizontal or vertical orientation relative to the 2D spectra indicated by colored arrows. Crosspeaks arising from inter-residue dipolar couplings are circled on 1D DARR slices.

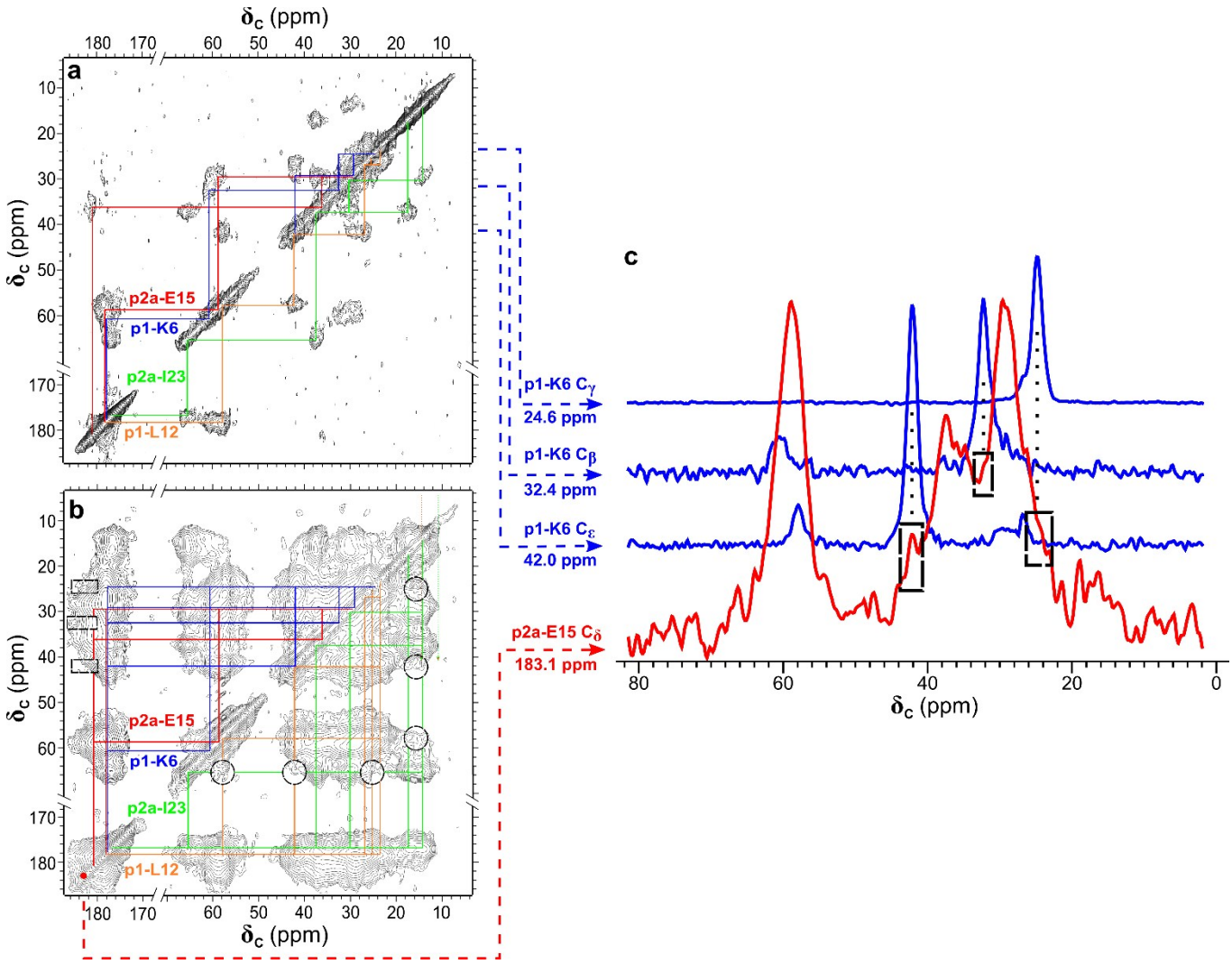

Fig. S11 NMR evidence of inter-peptide interactions between charged sidechains in lyophilized coiled-coil nanofibers. (a) 2D fpRFDR and (b) 2D DARR (τ_m = 500 ms) spectra of lyophilized SAF-p1/p2a nanofibers. (c) Overlaid 1D fpRFDR and 1D DARR slices corresponding to signals from SAF-p1 K6 and SAF-p2a E15 (respectively), with slice positions and horizontal or vertical orientation relative to the 2D spectra indicated by colored arrows. Crosspeaks resulting from inter-residue dipolar couplings are boxed on the 1D DARR slice corresponding to SAF-p2a E15 C_δ, which was taken slightly downfield of its chemical shift assignment (Table S3) in order to avoid spectral overlap with the backbone CO signals of other residues.

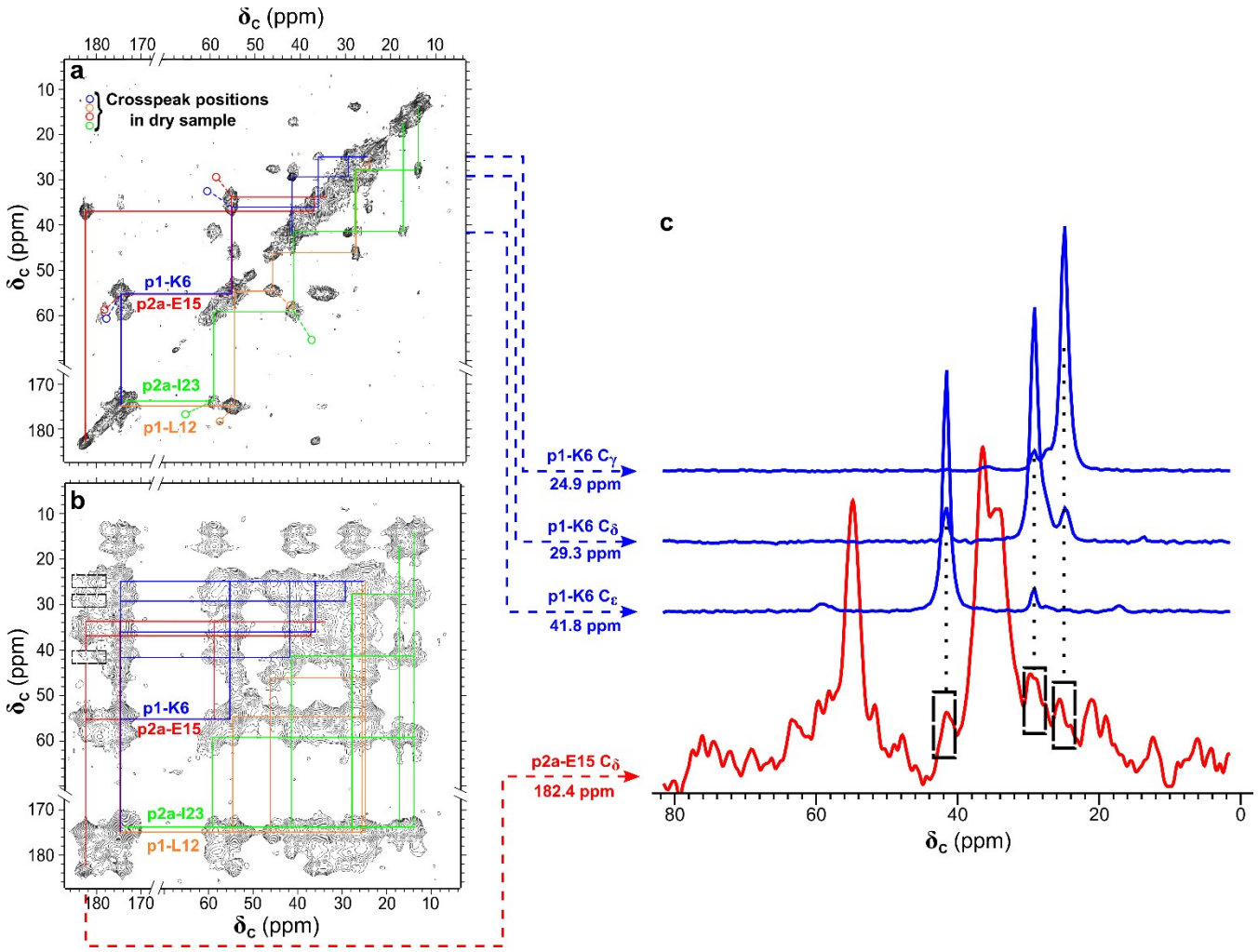

Fig. S12 NMR evidence of co-assembly following the helix-to-sheet transition in rehydrated nanofibers. (a) 2D fpRFDR and (b) 2D DARR (τ_m = 500 ms) spectra collected following rehydration of lyophilized SAF-p1/p2a nanofibers. (c) Overlaid 1D fpRFDR and 1D DARR slices corresponding to signals from SAF-p1 K6 and SAF-p2a E15 (respectively), with slice positions and horizontal or vertical orientation relative to the 2D spectra indicated by colored arrows. Crosspeaks arising from inter-residue dipolar couplings are boxed on the 1D DARR slice corresponding to SAF-p2a E15 C_δ.