

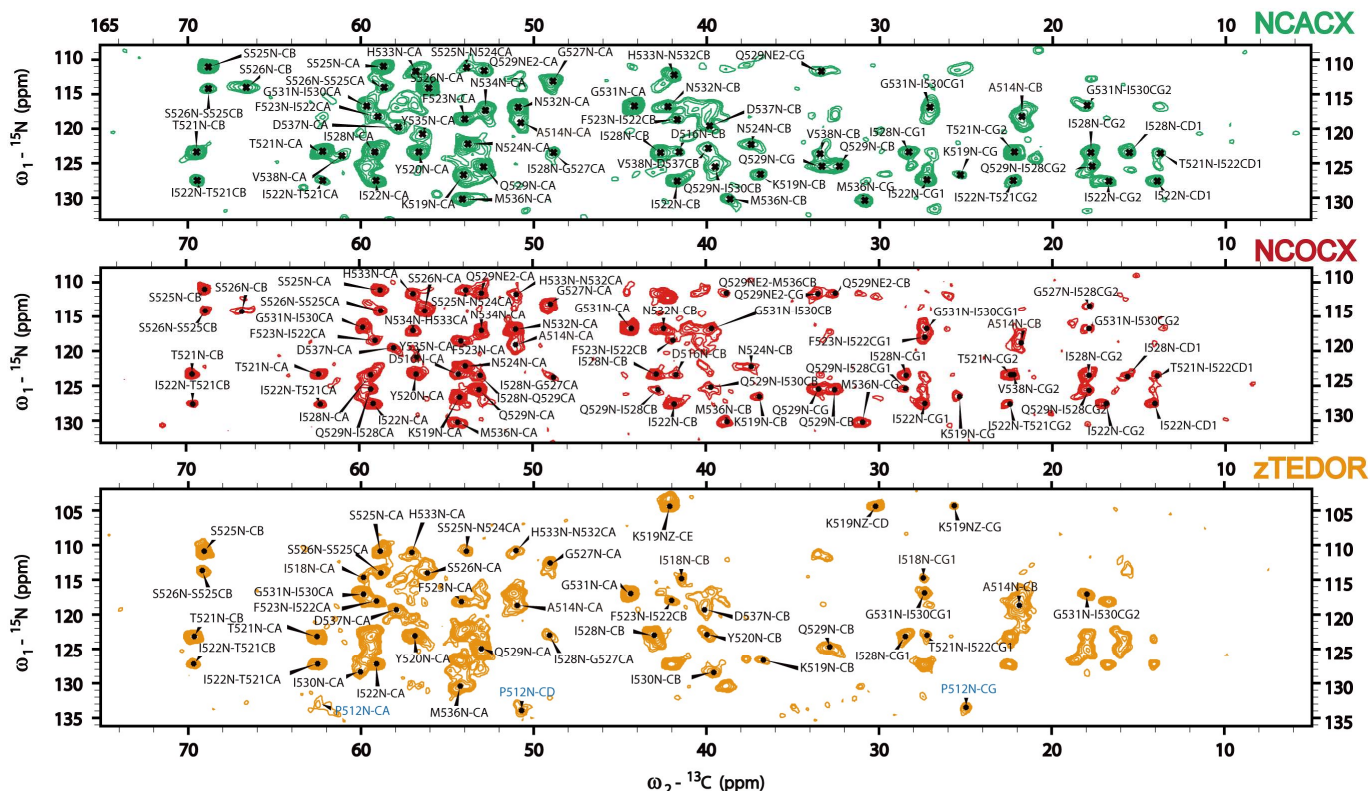
Supplementary Information

The Structure of Mouse RIPK1 RHIM-containing Domain as a Homology Model and in RIPK1/RIPK3 Complex

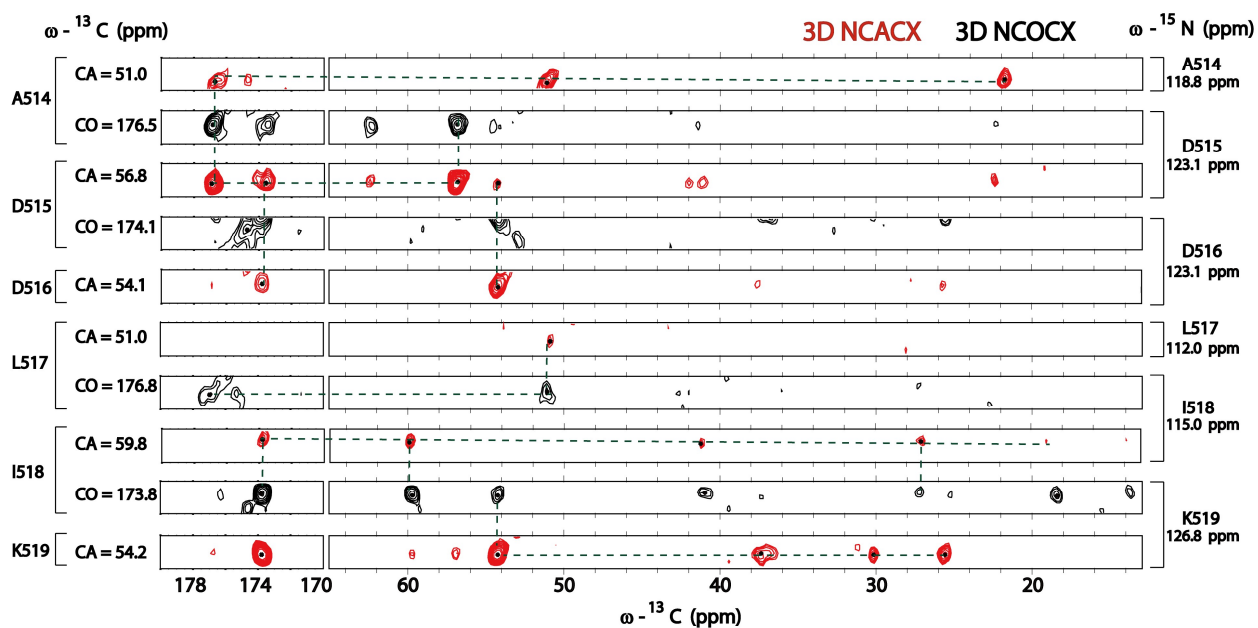
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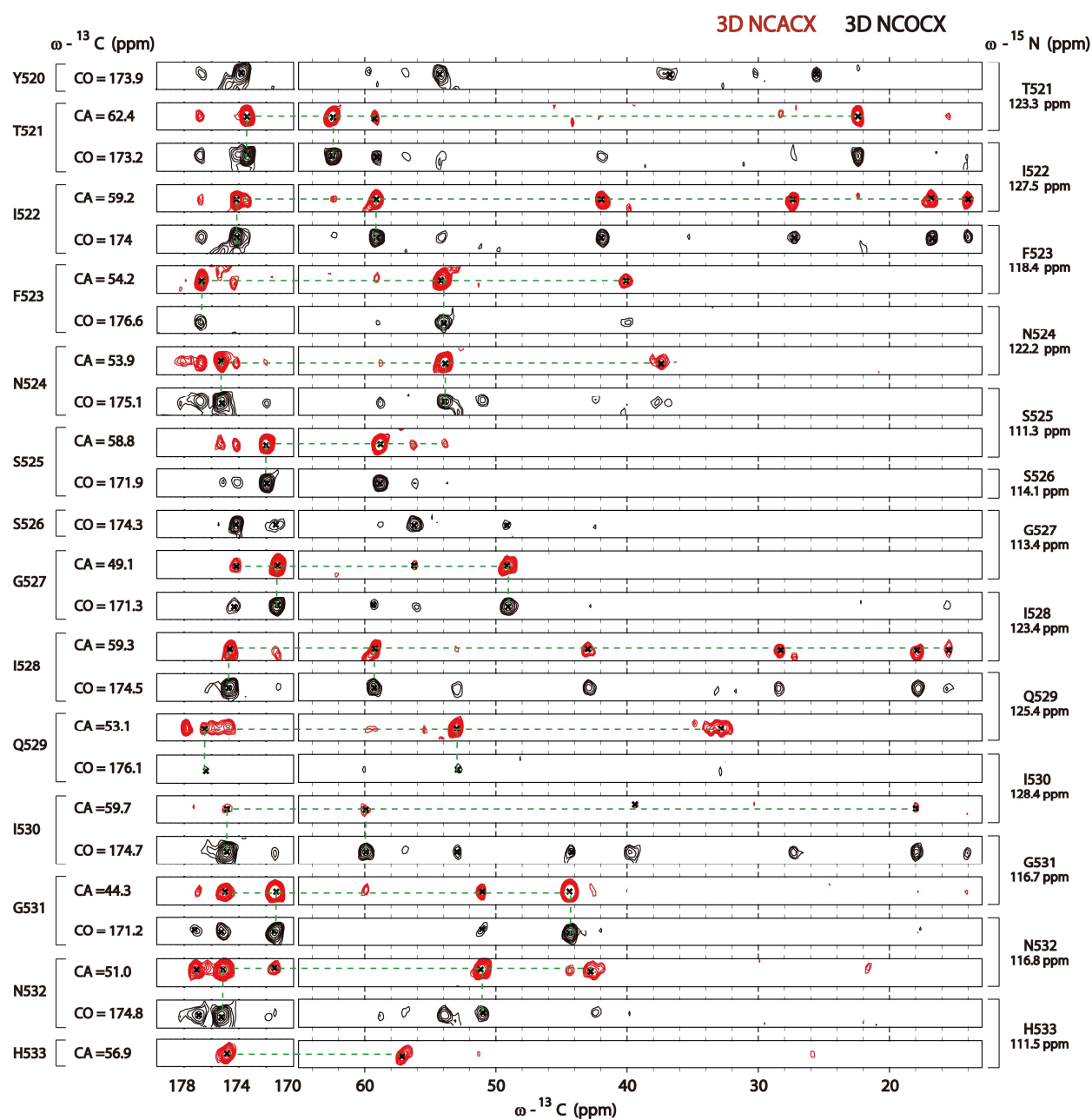
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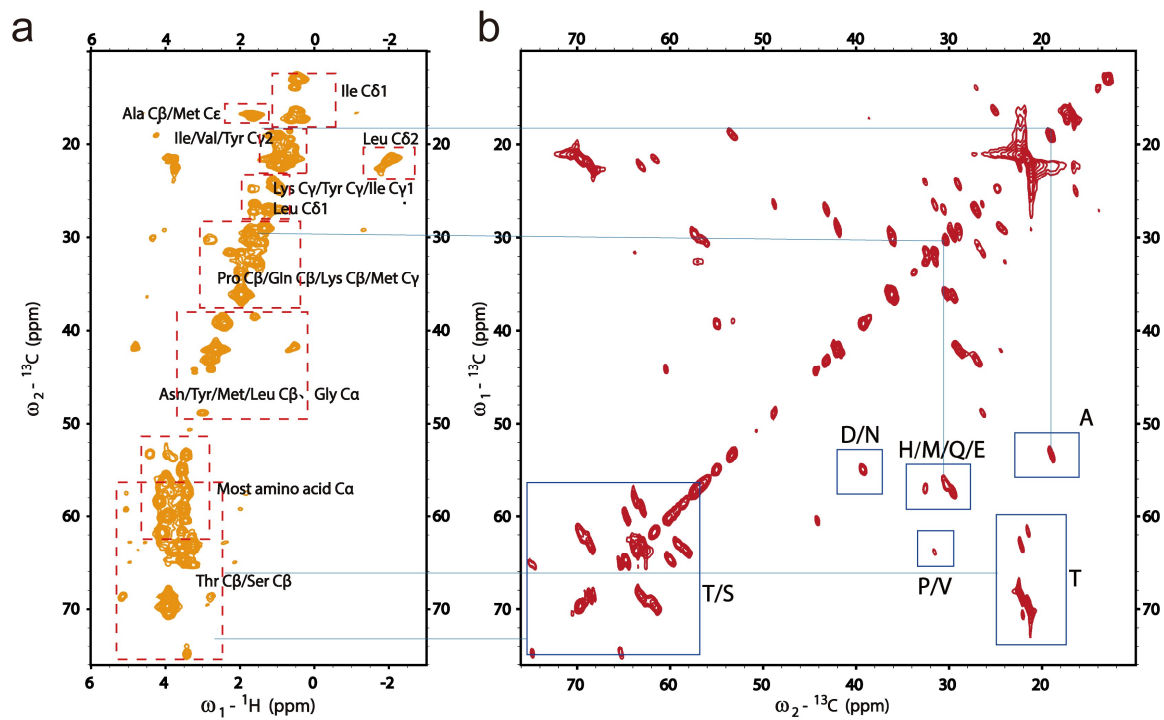
Supplementary Figure 2. ^{15}N - ^{13}C 2D ssNMR spectra of uniformly ^{15}N , ^{13}C -labeled mouse RIPK1 fibrils. 2D ^{15}N - ^{13}C NCACX (green), NCOCX (red) and TEDOR (orange) experiments. The ambiguous assignments were shown in blue. All spectra were obtained at 700 MHz field strength, 15 kHz MAS and 293 K.



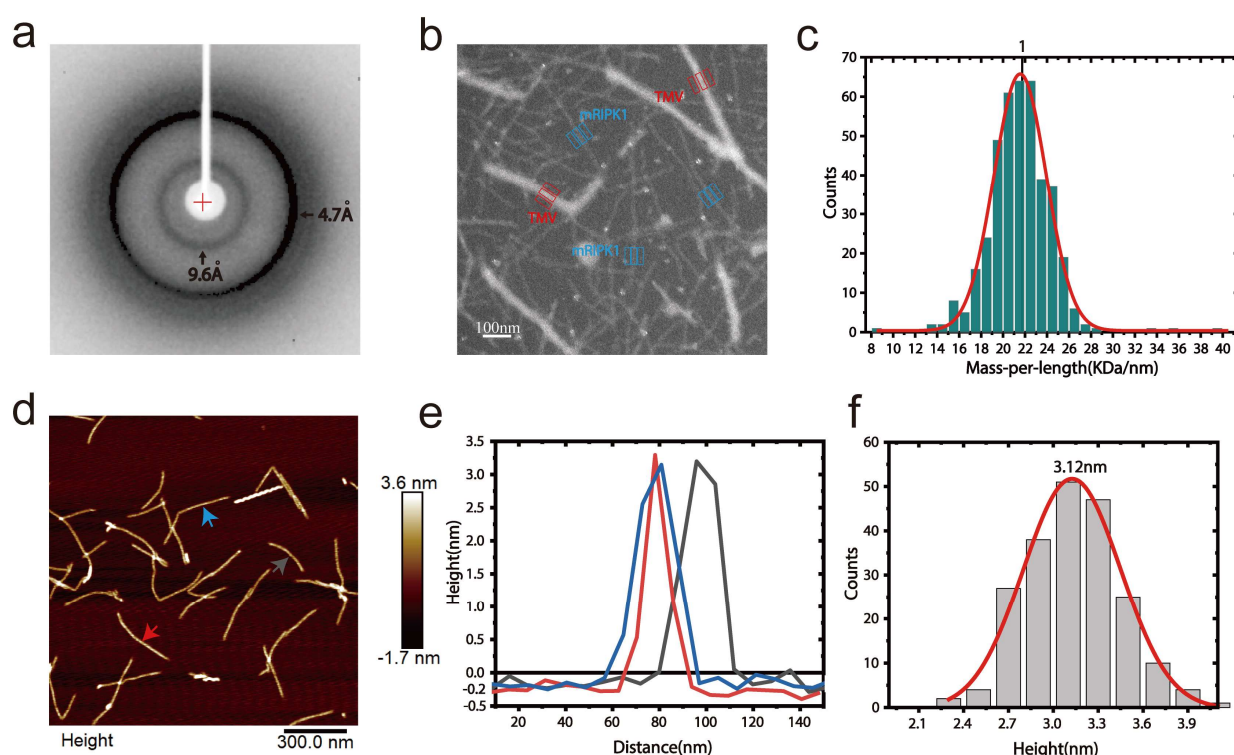
Supplementary Figure 3. 3D NCACX (red) and NCOCX (black) spectra of uniformly ^{15}N , ^{13}C -labeled mouse RIPK1 fibrils (residues A514-K519), collected at 700 MHz field strength, 15 kHz MAS and 273 K.



Supplementary Figure 4. 3D NCACX (red) and NCOCX (black) spectra of uniformly ^{15}N , ^{13}C -labeled mouse RIPK1 fibrils (residues T521-H533), collected at 700 MHz field strength, 15 kHz MAS and 273 K.

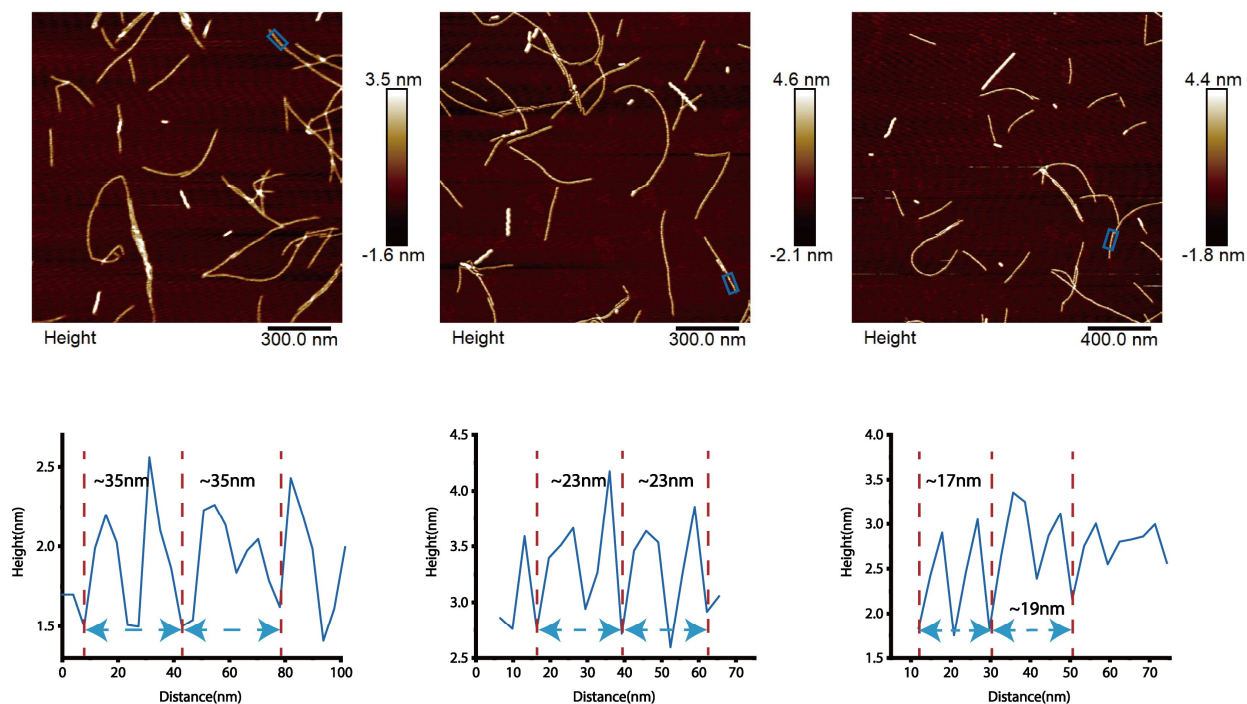


Supplementary Figure 5. *J*-coupling based ssNMR detecting flexible regions of mRIPK1 fibril.
2D ^1H - ^{13}C INEPT (a) and INEPT-TOBSY (b).



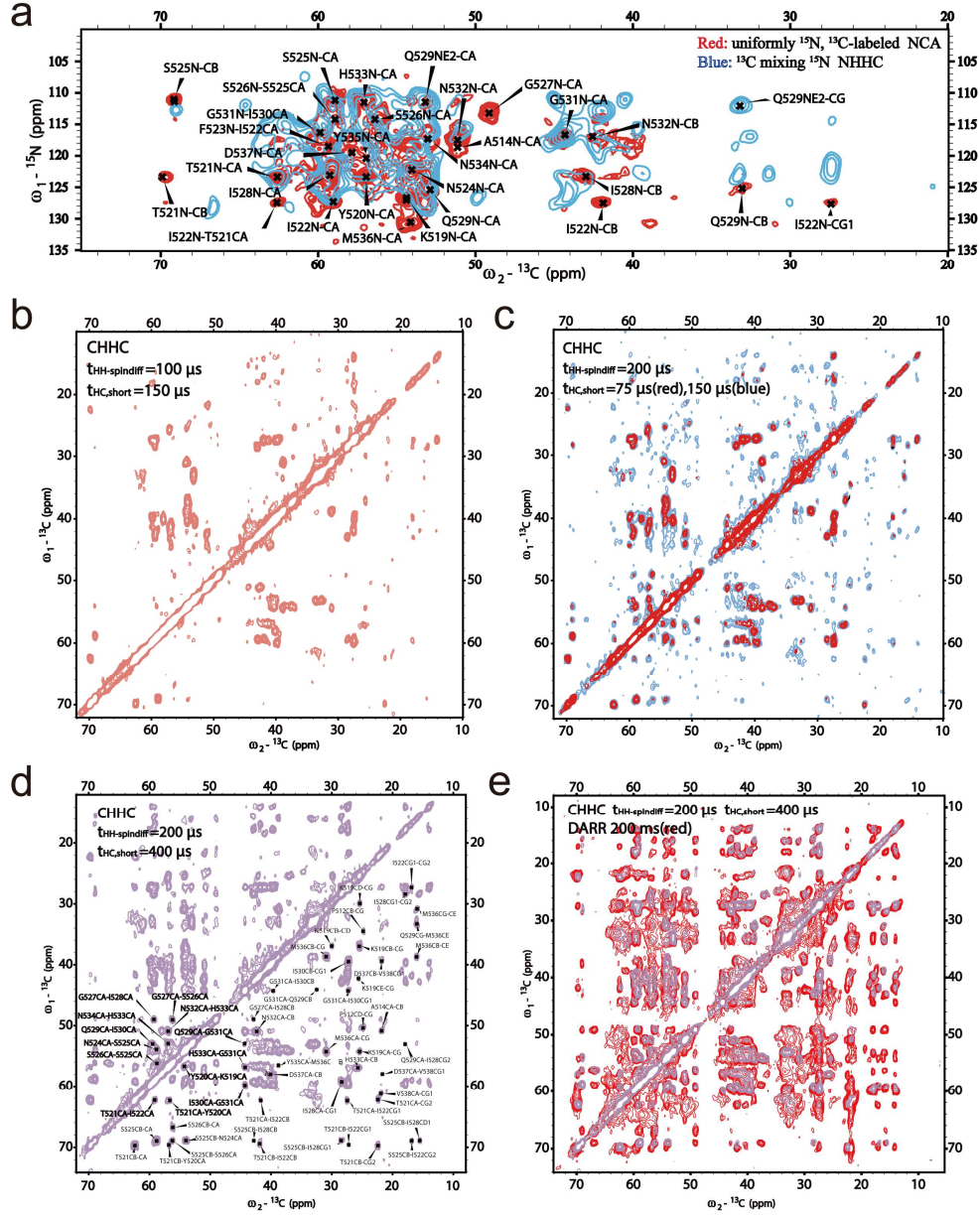
Supplementary Figure 6. Validation of mRIPK1 fibril structure using X-ray, BT-TEM and AFM.

(a) X-ray fiber diffraction of the mRIPK1 fibrils. The diffractions at approximately 4.7 Å and 9.6 Å indicating a typical cross- β sheet structure. (b) One representative BT-TEM image of mRIPK1 fibrils with the blue boxes showing the reading areas for individual fibrils, and the red boxes representing the reading areas for tobacco mosaic virus (TMV) rods. TMV was used as internal control for mass-per-length (MPL) ratio determination. (c) A histogram showing the distribution of MPL values of mRIPK1 fibrils, indicating an MPL value of 21.6 kDa/nm at the maximum height. (d) The AFM image of mRIPK1 fibrils on mica surface, the black scale bar representing 300 nm. (e) AFM cross-section height profile at three positions of mRIPK1 fibrils indicated by the three arrows in (d), showing the fibril height about 3.1 nm. (f) Plots of the AFM height distribution, showing fibril height about 3.1 nm with the distribution width ± 0.4 nm. Source data are provided as a Source Data file.

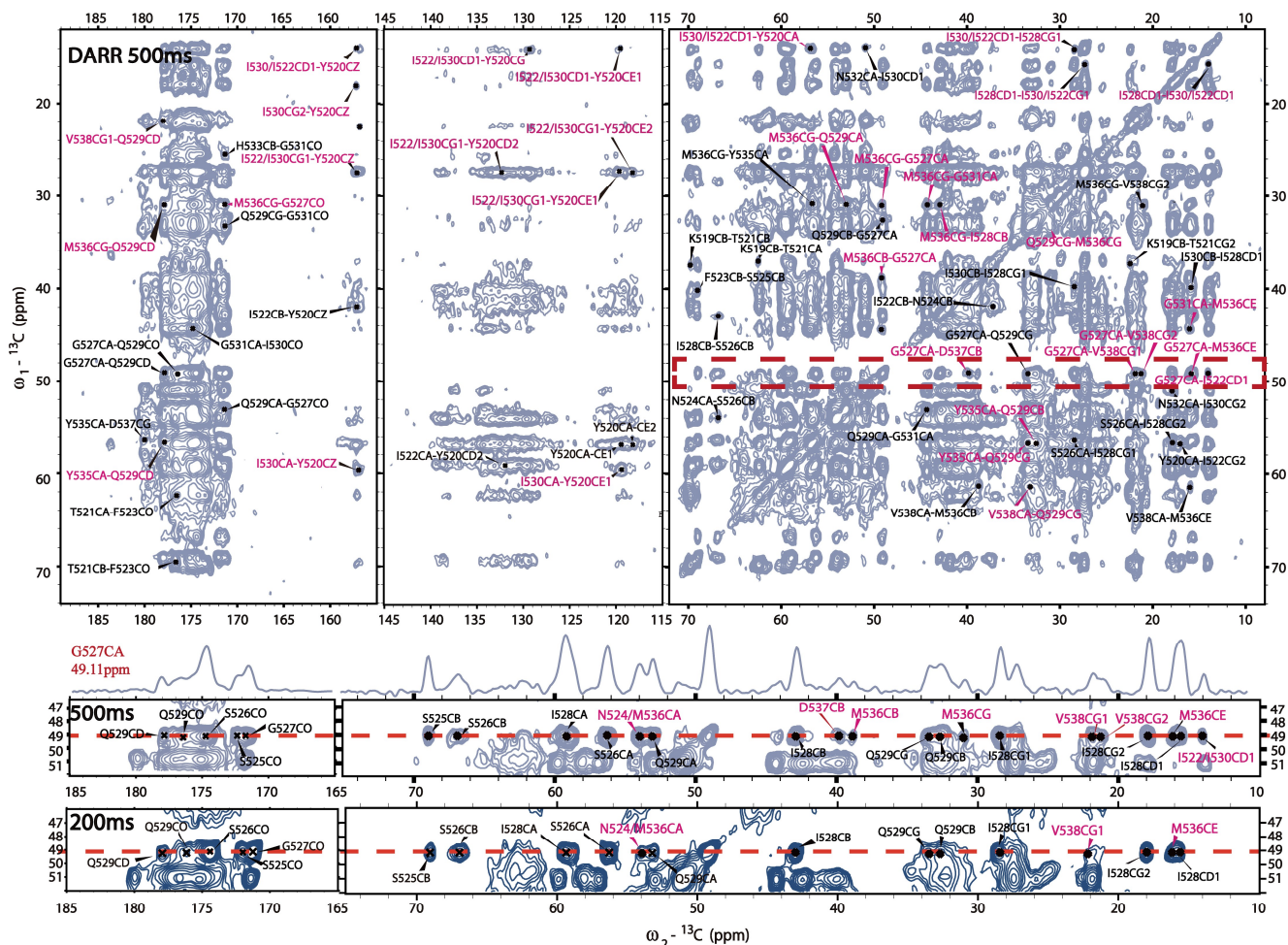


Supplementary Figure 7. AFM measurement showing mRIPK1 fibrils with multiple twist states.

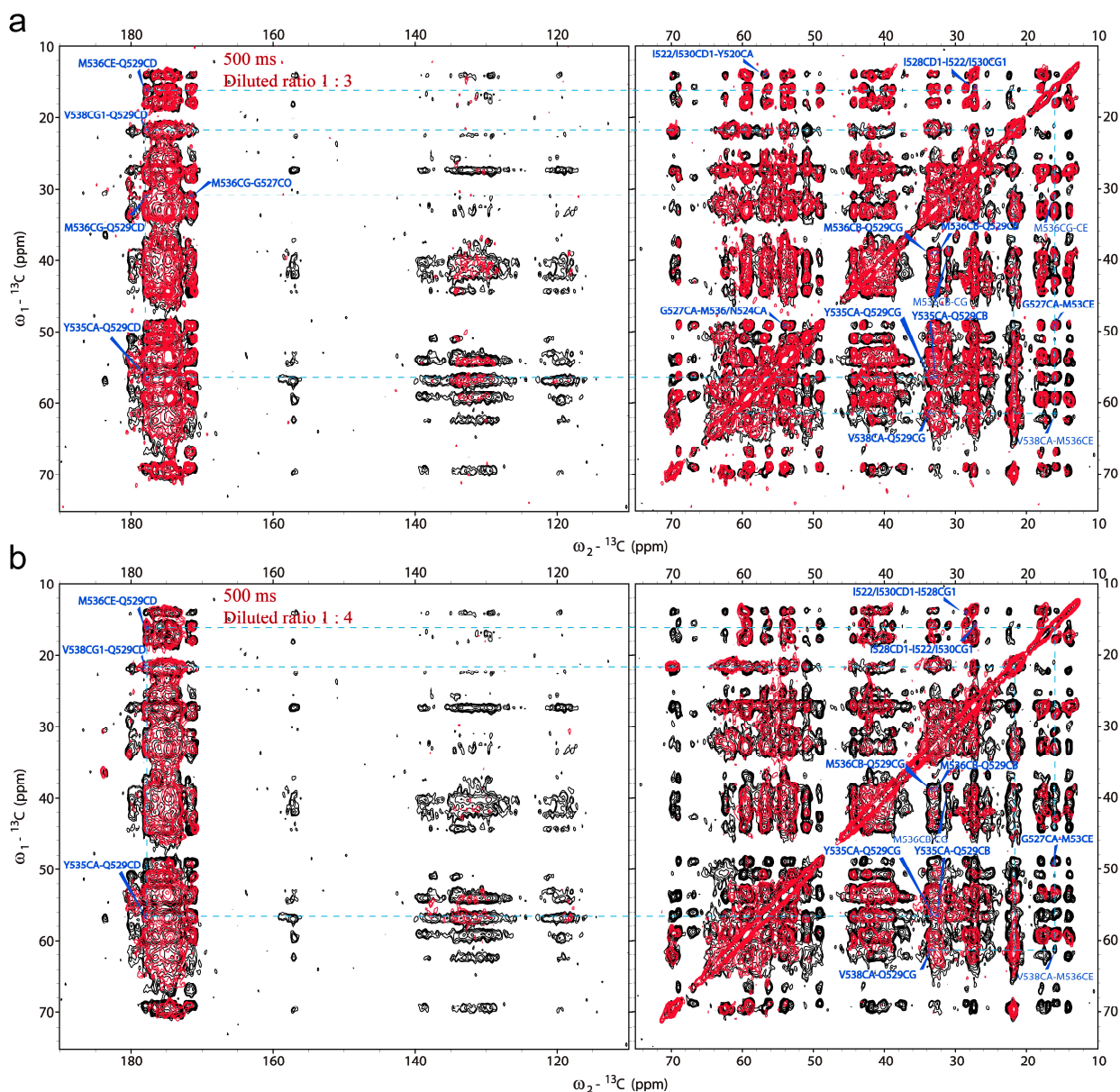
Only one fibril with a twist of 35 nm, 12 fibrils with a twist of 16-19 nm, 16 fibrils with a twist range of 21-24 nm, and most of the remaining fibers showing no clear twist pattern indicating the heterogeneous twist state. Source data are provided as a Source Data file.



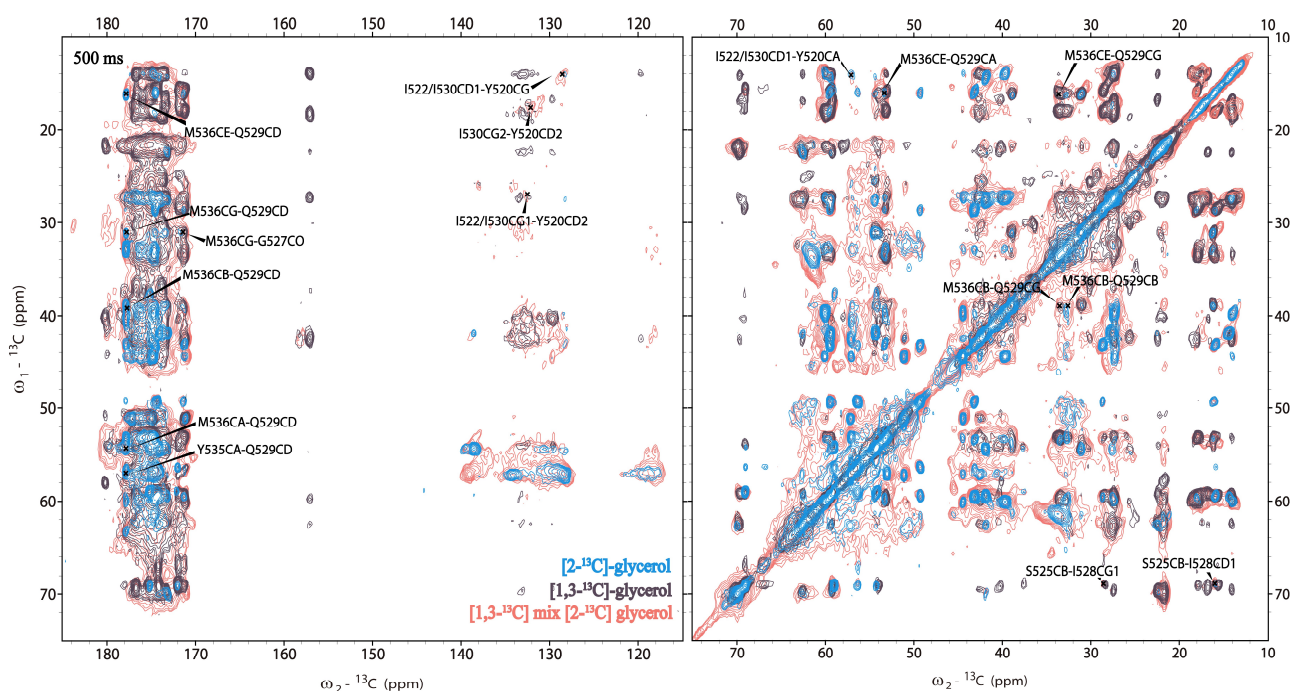
Supplementary Figure 8. 2D ssNMR spectra showing no evidence of the antiparallel β -sheet conformation. (a) Superposition of the NCA spectrum of uniformly ^{15}N , ^{13}C -labeled fibrils (red) at 255 K with NHHC spectrum of fibrils prepared using ^{15}N and ^{13}C -labeled peptide in 1:1 ratio (blue) at the same temperature, showing T521N-C α , S525N-C α , I528N-C α , Q529N-C α , G531N-C α , H533N-C α and other intermolecular correlations. (b, c, d) 2D ^{13}C - ^{13}C correlation spectra obtained with ^1H - ^1H spin-diffusion (CHHC) using a uniformly ^{15}N , ^{13}C -labeled sample at different conditions showed cross-peaks without indications of an antiparallel conformation. (e) Comparison of the 2D NMR spectra of mRIPK1 CHHC and DARR. These experiments were carried on a 16.4 T NMR spectrometer (700 MHz ^1H frequency, $\omega_r = 15 \text{ kHz}$, $T = 293 \text{ K}$).



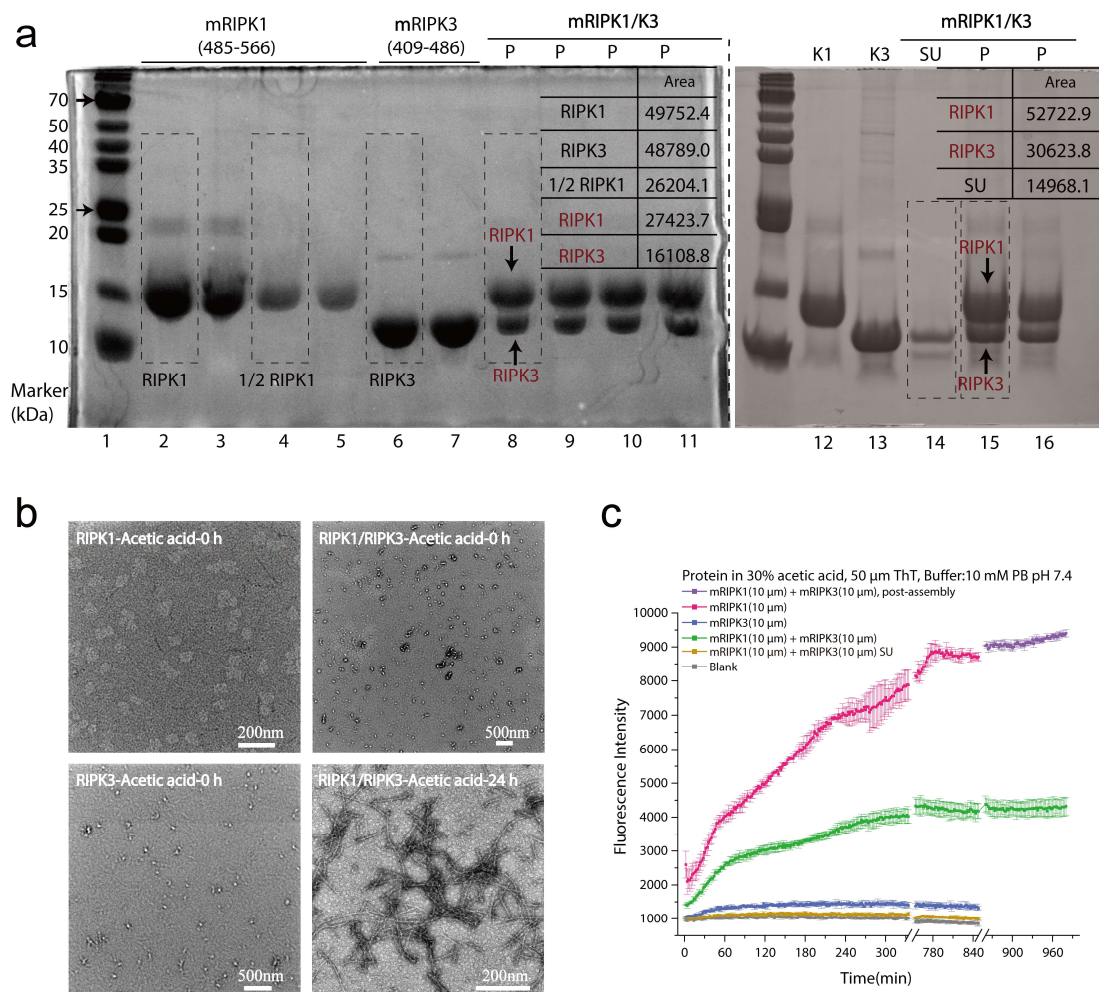
Supplementary Figure 9. 2D ^{13}C - ^{13}C DARR spectra of uniformly ^{15}N , ^{13}C -labelled mRIPK1 fibrils with long mixing times (200ms, 500ms DARR), showing long-range correlation peaks for mRIPK1 fibril. Correlation peaks separated by three or more residues on the protein sequence were marked in magenta, and spaced within two residues apart were marked in black. G527C α contacts with M536, V538 and other residues were shown at the bottom with two mixing times indicated.



Supplementary Figure 10. Comparison of 2D ^{13}C - ^{13}C spectra of mRIPK1 fibrils with 500 ms DARR mixing. Spectra in black were from uniformly ^{15}N , ^{13}C -labelled mRIPK1 fibrils, and spectra in red were from samples mixing uniformly ^{15}N , ^{13}C -labeled peptide with non-isotopically labeled mRIPK1 at a dilution ratio of 1:3 (a) and 1:4 (b), respectively. The experiments were performed at 293 K.

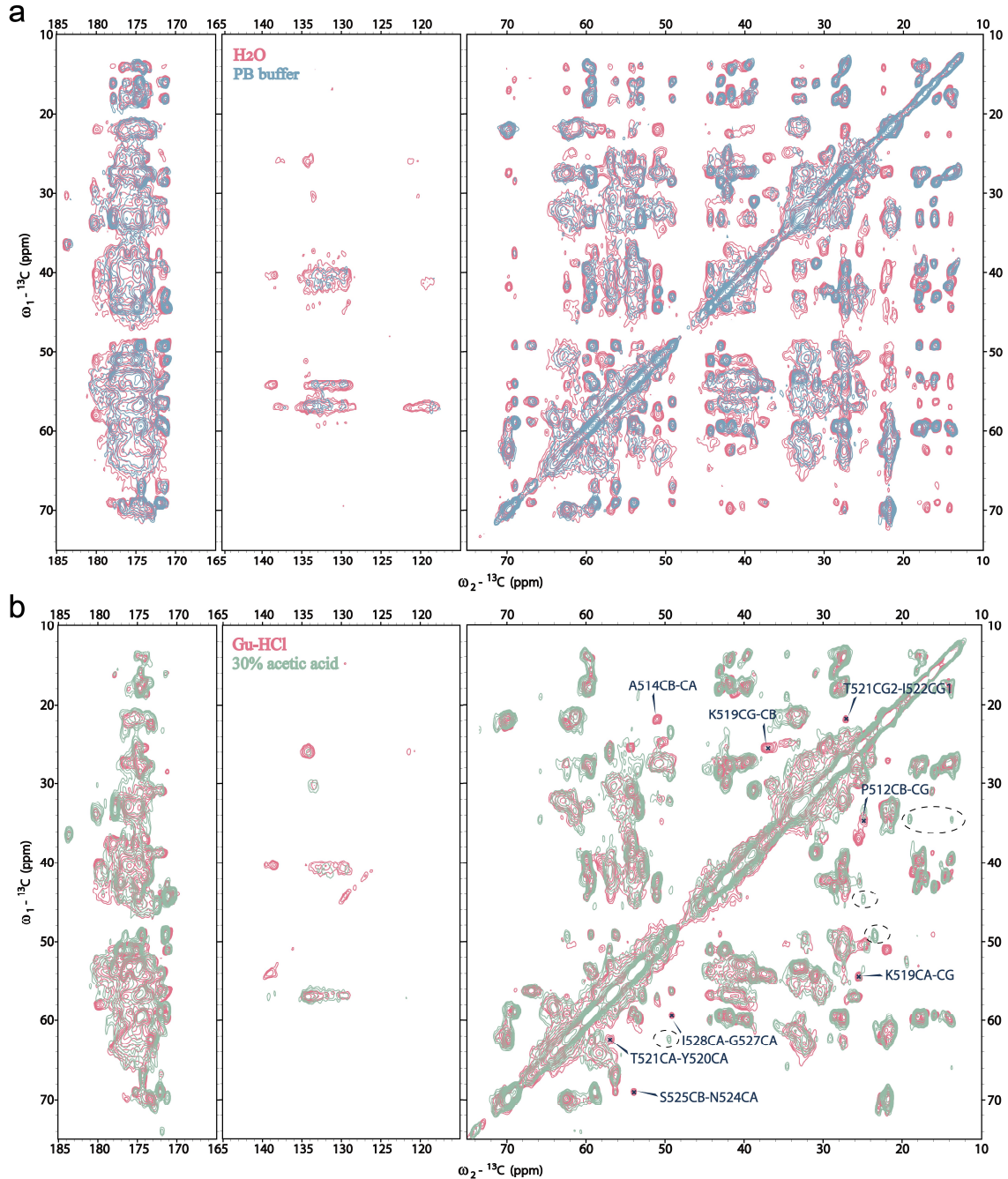


Supplementary Figure 11. Comparison of 2D ^{13}C - ^{13}C spectra of mRIPK1 fibrils with 500 ms DARR mixing for long-range inter-residue correlations. The fibril was prepared from mRIPK1 peptides using $[2\text{-}^{13}\text{C}]$ -glycerol (blue), $[1,3\text{-}^{13}\text{C}]$ -glycerol (black) labeling method, or peptides obtained from both labeling schemes in 1:1 ratio (red). Black labels indicate intramolecular correlations since they were kept unchanged after mixing two types of mRIPK1 peptides.



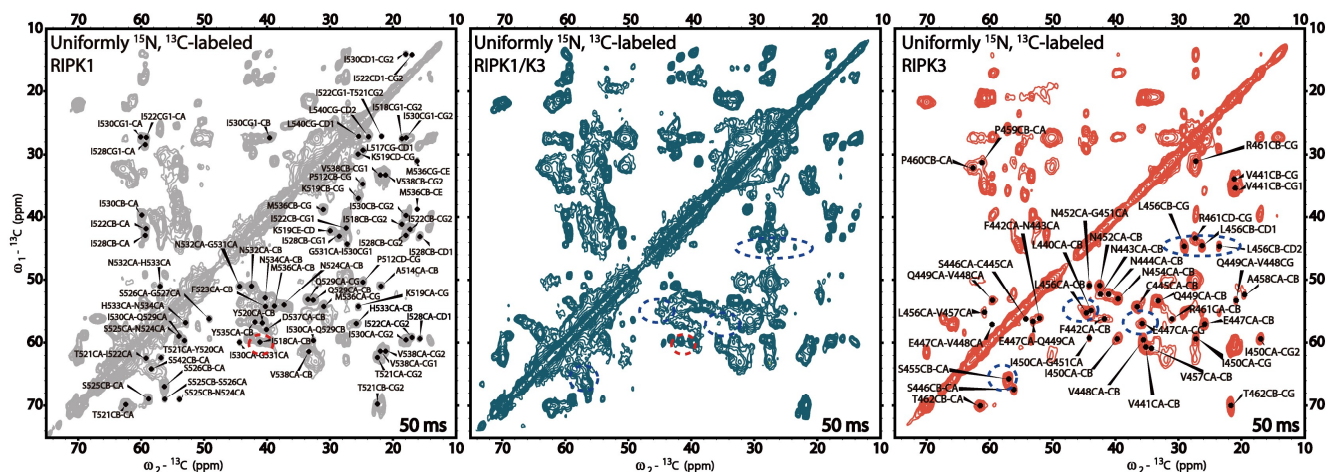
Supplementary Figure 12. The preparation of mRIPK1/mRIPK3 fibril. (a) SDS-PAGE analysis revealed a slightly less than 2:1 ratio of mRIPK1 to mRIPK3 in the hetero-amyloid. Both protein solutions were first adjusted to the same concentration using UV absorption reading. The solution of mRIPK1 was shown in lane 2-5 and 12 for different repeats while mRIPK3 was observed in lane 6, 7 and 13. The starting mRIPK1:mRIPK3 protein concentration ratio indicated by the intensity reading of the band was about 1:1. The loading of lane 4, 5 for mRIPK1 was adjusted to half of lane 2 and 3, and the reading of the intensity was also about half, confirming the linear relationship between the intensity reading and the protein concentration. It can also be noticed that a weak oligomer band was shown for both mRIPK1 and mRIPK3. However, the oligomer band disappeared or got too weak to see when the loading was reduced (lane 4 and 5). The two protein solutions were mixed with equal volume, and the hetero-amyloid was formed after dialysis. The fibril suspension solution was centrifuged and the pellets were dissolved in 8 M urea for further gel analysis (lane 8-11, 15-16). Lane 8-11, lane 15-16 were two different batches of samples. The supernatant after collecting the hetero-

amyloid was also obtained and concentrated in urea, showing dominant mRIPK3 band (lane 14). (b) TEM images of the mRIPK1, mRIPK3 and mRIPK1/ mRIPK3 solution in 30% acetic acid before and after dialysis for 24 h. (c) The time course of amyloid fibril formation monitored using a Thioflavin-T (ThT) binding assay for mRIPK1 (red), mRIPK3 (blue), mRIPK1/RIPK3 (green), and post-assembly mixture of mRIPK1 fibril and mRIPK3 fibril in 1:1 ratio (purple). In this experiment, 20 μ M protein sample was mixed with 50 μ M ThT and the mixture was incubated for 10 hours in a total volume of 200 μ L. The mixture was prepared using 10 mM PB buffer (pH 7.4). A supernatant sample after collecting the hetero-amyloid mRIPK1/RIPK3 was also kept (similar to lane 14 without urea) and incubated for ThT experiment (brown). The data presented included the mean values with the standard deviation shown in the curves from three repeats. Source data are provided as a Source Data file.

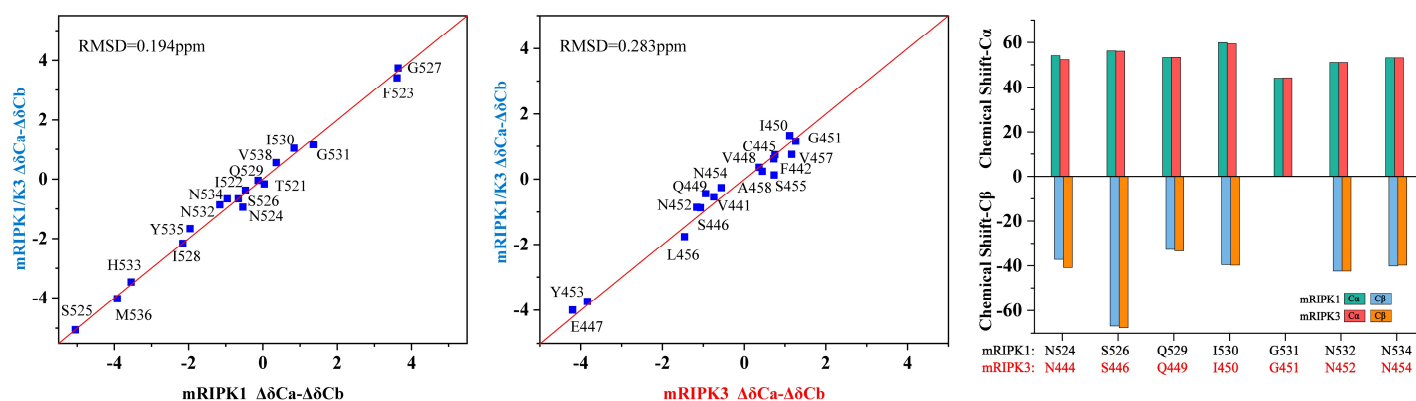


Supplementary Figure 13. 2D ^{13}C - ^{13}C spectra of mRIPK1 protein under different assembly conditions. (a) 2D ^{13}C - ^{13}C spectra of mRIPK1 homo-amyloid with 500 ms DARR mixing under two different assembly conditions. mRIPK1 solution dialyzed against H₂O (pink) or PB at pH 7.4 (light blue) conditions. Light blue represented mRIPK1 fibrils prepared using ^{13}C -labeled protein mixed with ^{15}N -labeled protein in 30% acetic acid, followed by dialysis against 10 mM PB buffer at pH 7.4. Pink represented a fully ^{13}C -labeled mRIPK1 sample in Gu-HCl/Tris/NaCl/imidazole, pH 8.0 prepared by dialysis against Milli-Q water. The ^{13}C -labeled sample in pink was twice as much as in light blue, thus showing a higher number of relevant peaks and higher intensities than the light blue spectrum. (b) 2D

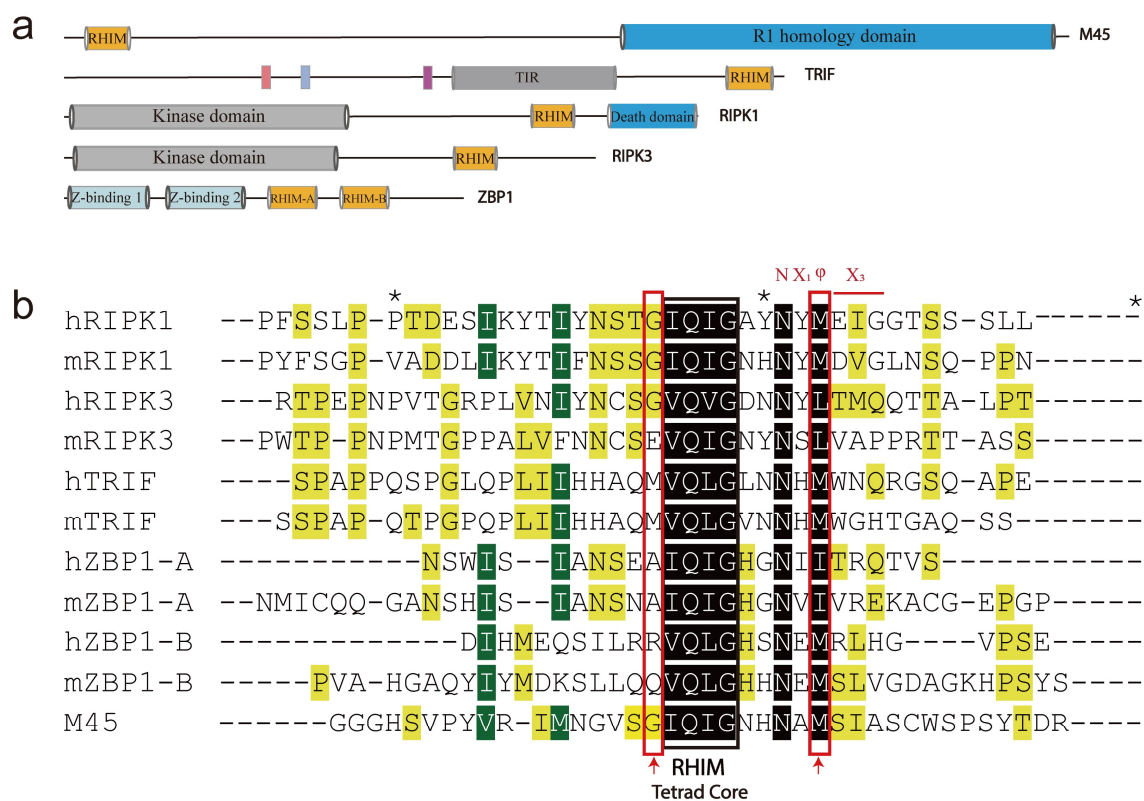
^{13}C - ^{13}C spectra of mRIPK1 homo-amyloid with 50 ms DARR mixing under two different assembly conditions (30% acetic acid dissolving the protein (light green) and Gu-HCl/Tris/NaCl/imidazole, pH 8.0 condition (pink)). Both were followed by water dialysis. There are intensity differences at several peaks, labeled in the figure with assignments for the pink spectrum and dashed circles for the green spectrum. All NMR spectra were collected at 293 K and $\omega_r = 15$ kHz.



Supplementary Figure 14. The comparison of 2D ^{13}C - ^{13}C spectra of uniformly ^{15}N , ^{13}C -labeled protein fibrils using 50 ms DARR mixing. (left) 2D ^{13}C - ^{13}C spectrum of uniformly ^{15}N , ^{13}C -labeled mRIPK1 fibrils (grey), (middle) mRIPK1/mRIPK3 hetero-amyloid fibrils (dark green) and (right) mRIPK3 fibrils (red). The red dotted line indicates a peak of mRIPK1 (I518C α -C β) with attenuated intensity in the complex. The blue dotted line indicates mRIPK3 peaks (E447C α -C γ , S455C β -C α , L456C α -C β , and L456C β -C γ , L456C β -C δ 1, L456C β -C δ 2) displaying a significant decrease in the intensity in the complex.



Supplementary Figure 16. ^{13}C chemical shift changes for mRIPK1, mRIPK3 when forming mRIPK1/mRIPK3 hetero-amyloid. The chemical shift changes were compared using the secondary chemical shifts. (left) The comparison between mRIPK1 homo- and hetero-amyloids with the x axis displaying the secondary chemical shifts for mRIPK1 homo-amyloid and y axis for mRIPK1 in hetero-amyloids. (middle) The comparison between mRIPK3 homo- and hetero-amyloids with the x axis displaying the secondary chemical shifts for mRIPK3 homo-amyloid and y axis for mRIPK3 in hetero-amyloids. (right) The chemical shifts comparison between mRIPK1 and mRIPK3 homo-amyloids for the sites with the same type of residues. Source data are provided as a Source Data file.



Supplementary Figure 17. (a) Schematic representation and (b) Sequence alignment of RHIMs for human and murine RIPK1, RIPK3, TRIF (TIR domain-containing adapter-inducing IFN- β), ZBP1 and M45.