Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Fast Magic-Angle Spinning ^{19}F NMR Spectroscopy of HIV-1 Capsid Protein Assemblies

Mingzhang Wang[+], Manman Lu[+], Matthew P. Fritz, Caitlin M. Quinn, In-Ja L. Byeon, Chang-Hyeock Byeon, Jochem Struppe, Werner Maas, Angela M. Gronenborn, and Tatyana Polenova**

anie_201809060_sm_miscellaneous_information.pdf

Author Contributions

A.M.G. and T.P. conceived and directed the project. M.W. and M.L. prepared the samples, performed NMR experiments and analyzed the data. M.P.F., C.M.Q., and J.S. contributed to the MAS NMR experiments and data analysis and/or simulations. I.-J.L.B. performed solution NMR experiments. C.H.B. prepared mutant CA proteins. T.P. and A.M.G. took the lead in writing the manuscript. All authors discussed the results and contributed to the manuscript preparation. M.W. and M.L. contributed equally.

EXPERIMENTAL

Sample preparation

5-Fuoroindole (Sigma Aldrich) was used as a precursor to uniformly incorporate fluorine at position 5 into all Trp residues of the HIV-1 CA protein.[1] 5-^{19}F-Trp,U-^{13}C,^{15}N-labeled CA, 5-^{19}F-Trp,U-^{15}N-labeled CA, 5-^{19}F-Trp,U-^{13}C,^{15}N-labeled A14C/E45C/W184A/M185A CA, and 5-^{19}F-Trp,^{15}N-labeled CA proteins were expressed and purified as reported previously with modifications.[2,3] In brief, 5-^{19}F-Trp,U-^{13}C,^{15}N-enriched CA was expressed in modified M9 medium, containing $^{15}NH_4Cl$, U-$^{13}C_6$-glucose and 20 mg of 5-fuoroindole per 1 L medium, using 0.8 mM IPTG for induction. Cells were grown at 18 °C and harvested after 16 h by centrifugation. Cell pellets were suspended in 25 mM sodium phosphate buffer (pH 7.0), ruptured by sonication on ice, and the cell debris was removed by centrifugation at 27,000 g at 4 °C for 1 h. The pH of the supernatant was adjusted to 5.8 with acetic acid, and the conductivity was adjusted to below 2.5 ms/cm by dilution, followed by an additional centrifugation at 27,000 g at 4 °C for 1 h. The final supernatant was loaded onto a cation exchange column (HiTrap SP HP 5 mL, GE Healthcare), and the protein was eluted with a 0-1 M NaCl gradient in 25 mM sodium phosphate buffer (pH 5.8), 1 mM DTT, 0.02% NaN_3. Fractions containing CA protein were pooled and further purified by gel filtration using a size-exclusion column (HiLoad 26/600 Superdex 75 prep grade, GE Healthcare), equilibrated with 25 mM sodium phosphate buffer (pH 5.5), 1 mM DTT, 0.02% NaN_3. Fractions containing CA protein were combined and concentrated to 20-30 mg/mL.

Fractions containing cross-linked hexamer protein were collected and concentrated to 28 mg/mL, buffer exchanged into 50 mM Tris, 1.0 M NaCl (pH 8.0) for 2.5 days, for assembly into tubes. To prepare 5-^{19}F-Trp CA cross-linked hexamer polyethylene glycol (PEG) precipitate, 30% PEG 4000 solution was added to an equal volume of the solution of 28 mg/mL CA cross-linked hexamer, followed by incubation at 37 °C for 1 h and then 4 °C overnight. By dialyzing these tubular assemblies against 25 mM sodium phosphate (pH 6.5), 1.5 mg (60 μM) soluble clean hexamer protein solution, containing 7 % D_2O were prepared for solution NMR experiments.

For solution NMR experiments, the concentrated 5-^{19}F-Trp,^{15}N-labeled CA wild type (WT) and Trp-substituted mutant proteins were buffer exchanged into 25 mM sodium phosphate (pH 6.5) and diluted to 1.2-1.5 mg/ml (47-60 μM) protein, containing 7 % D_2O. 5-^{19}F-Trp,U-^{13}C,^{15}N-labeled and 5-^{19}F-Trp,^{15}N-labeled CA wild type (WT) and 5-^{19}F-Trp,^{15}N-labeled CA W80Y assemblies were prepared from 20-26 mg/mL protein solutions in 25 mM phosphate buffer, 1.0 M NaCl (pH 6.5), followed by incubation at 37°C for 1 h and 4°C overnight. 5-^{19}F-Trp,^{15}N-labeled CA W23I assemblies were prepared from 22 mg/mL protein solution in 25 mM phosphate buffer, 2.4 M NaCl (pH 6.5), followed by incubation at 37°C for 1 h and 4°C overnight. The 5-^{19}F-Trp CA

WT and mutant assemblies were pelleted at 10,000 g and packed into Bruker MAS NMR rotors of appropriate sizes. The morphology was characterized by transmission electron microscopy (TEM), using a Zeiss Libra 120 transmission electron microscope operating at 120 kV. Assemblies were stained with uranyl acetate (0.5-1% w/v), deposited onto 400 mesh, formval/carbon-coated copper grids, and dried for 45 min in the air. The copper grids were glow discharged prior to staining, so that the tubular assemblies are uniformly spread on the grid surface and adhere to it.

MAS NMR spectroscopy

^{19}F MAS NMR experiments were performed on a Bruker 19.96 T Bruker AVANCE III spectrometer, outfitted with 1.9 mm HX and 1.3 mm HCN MAS probes. The Larmor frequencies were 850.4 MHz (^{1}H), 213.8 MHz (^{13}C), 86.2 MHz (^{15}N), and 800.1 MHz (^{19}F). The sample temperature was calibrated using KBr as a temperature sensor and was maintained at 15±1°C throughout the experiments using a Bruker temperature controller.

14.1 mg of 5-^{19}F-Trp,U-^{15}N-labeled CA (1 M NaCl) assemblies, 13.8 mg of 5-^{19}F-Trp,U-^{15}N-labeled CA W80Y (1 M NaCl) assemblies, 12.1 mg of 5-^{19}F-Trp,U-^{15}N-labeled CA W23I (2.4 M NaCl) assemblies, 15.9 mg of 5-^{19}F-Trp,U-^{15}N-labeled CA A204C (1 M NaCl) assemblies, 13.1 mg of 5-^{19}F-Trp,U-^{13}C,^{15}N-labeled CA cross-linked hexamer (1 M NaCl) assemblies, and 12.9 mg of 5-^{19}F-Trp,U-^{13}C, ^{15}N-labeled CA A14C/E45C/W184A/M185A cross-linked hexamer (PEG4000) precipitates were packed into 1.9 mm thin-wall Bruker rotors. ^{19}F MAS NMR spectra were collected at MAS frequencies of 15 kHz, 35 kHz and 40 kHz, and the frequencies were controlled to within ± 5 Hz by a Bruker MAS controller. The typical 90° pulse length for ^{19}F was 1.9-3.2 µs. The recycle delay was 3 s for all experiments. For 2D ^{19}F-^{19}F PDSD spectra, the mixing time was 1 s. For 2D ^{19}F-^{19}F RFDR spectra, the RFDR mixing time was 8 ms. A series of 2D ^{19}F-^{19}F RFDR experiments with different mixing times, ranging from 0.8 ms to 100 ms were performed on CA A14C/E45C/W184A/M185A cross-linked hexamer (1M NaCl) assemblies for the polarization transfer experiments.

^{1}H-detected (H)NH and (H)CH HETCOR and ^{19}F MAS NMR spectra were acquired on tubular assemblies of 5-^{19}F-Trp,U-^{13}C,^{15}N-labeled CA and U-^{13}C,^{15}N-labeled CA. Experiments were carried out at 19.96 T, using a 1.3 mm HCN probe, and a MAS frequency of 60 kHz, controlled to within ± 5 Hz by a Bruker MAS controller. The sample temperature was at 15±1 °C. 3.7 mg of 5-^{19}F-Trp,U-^{13}C, ^{15}N-labeled CA (1 M NaCl), and 4.4 mg of U-^{13}C,^{15}N-labeled CA (1 M NaCl) tubular assemblies were packed into 1.3 mm Bruker rotors. The typical 90° pulse lengths were 2.1 µs (^{1}H), 3.1-3.5 µs (^{13}C), 3.25-3.35 µs (^{15}N), and 3.35 µs (^{19}F). For 2D ^{13}C-^{13}C RFDR spectra, the RFDR mixing time was 3.2 ms, and ^{1}H swfTPPM decoupling[4] (10 kHz) during RFDR

was used. For 2D (H)NH and (H)CH HETCOR, the WALTZ-16 broadband decoupling[5] (10 kHz) was used during the FID acquisition periods.

^{19}F MAS NMR experiments on 5-^{19}F-Trp,^{15}N-labeled CA tubular assemblies were carried out on an 11.7 T wide-bore Bruker AVANCE III spectrometer outfitted with a 2.5 mm MAS HFX probe. The Larmor frequencies were 500.1 MHz (^{1}H) and 470.6 MHz (^{19}F). 19.6 mg of 5-^{19}F-Trp,^{15}N-labeled CA (2.4 M NaCl) tubular assemblies were packed into a 2.5 mm thin-wall Bruker rotor. ^{19}F MAS NMR spectra were collected at MAS frequencies of 4 kHz and 30 kHz, controlled to within ± 5 Hz by a Bruker MAS controller. The typical 90° pulse lengths were 2.5 μs (^{1}H), and 3.2 μs (^{19}F). The recycle delay was 3 s for all experiments. At MAS of 30 kHz and 4 kHz, ^{1}H swfTPPM decoupling strength of 17 kHz and 100 kHz, respectively, were used during the acquisition period.

^{19}F chemical shifts were indirectly referenced to the adamantane-referenced ^{13}C chemical shifts.[6] 5-^{19}F-DL-Trp powder was used as a secondary reference standard, -44.6 ppm at 290 K. The ^{1}H, ^{13}C and ^{15}N chemical shifts were referenced with respect to DSS, adamantane and ammonium chloride used as external referencing standards.

All ^{19}F MAS spectra were processed using TopSpin. Baseline correction was applied using manually defined baseline points for 1D spectra. 2D ^{13}C-^{13}C RFDR, (H)NH, and (H)CH HETCOR spectra were processed in TopSpin and with NMRpipe,[7] and analyzed using SPARKY.[8] For 2D data sets, 30° or 60° shifted sine bell apodization, followed by a Lorentzian-to Gaussian transformation was applied in both dimensions. Forward linear prediction to twice the number of the original data points was used in the indirect dimension in some data sets followed by zero filling to twice the total number of points.

Determination of ^{19}F chemical shift tensors

The principal components of ^{19}F chemical shift tensors were determined by fitting the spinning sideband intensities according to the Herzfeld-Berger protocol.[9] The peak intensities were obtained from TopSpin and were input into the HBA program, version 1.7.5.[10]

Simulations of RFDR buildup curves

The SIMPSON software package (versions 1.1.2 and 2)[11] was used to perform the numerical simulations of ^{19}F-^{19}F RFDR buildup curves, under the NMRBox environment.[12] All experimental and processing parameters (i.e., Larmor frequency, MAS frequency, RF field strength, number of t_1 points, finite pulse lengths, zero-filling, line broadening, etc.) were taken into account in the simulation. The F-F spin pair model was employed with an isotropic chemical

shift difference of 5.0 ppm. For both spins the reduced anisotropy was 40 ppm and the asymmetry parameter values were 0.2 and 0.1. 320 REPULSION angles {α,β} and 5 γ angles (100 crystallites) were used to calculate a powder average for all simulations.

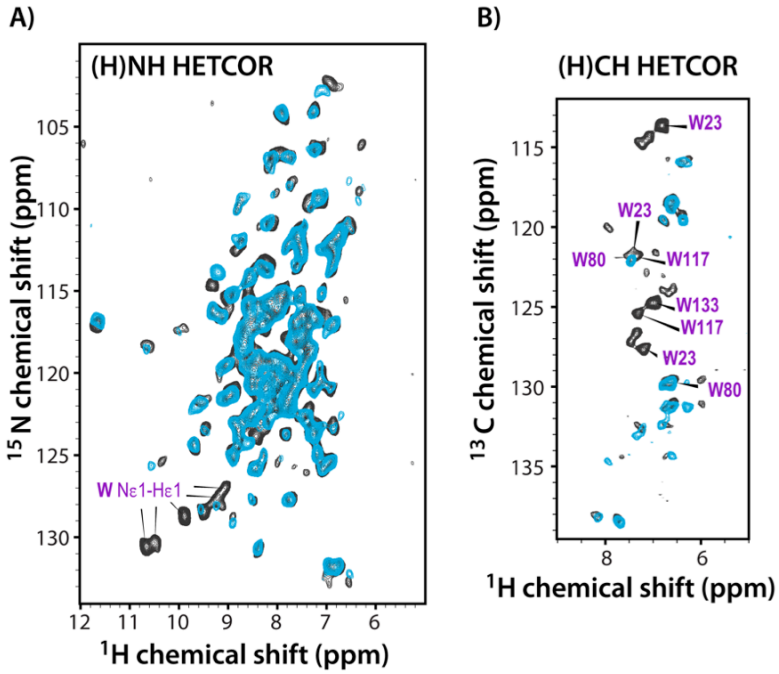

Figure S1. A) (H)NH HETCOR and B) (H)CH HETCOR spectra of tubular assemblies of U-^{13}C,^{15}N-CA (black), and 5-^{19}F-Trp, U-^{13}C,^{15}N CA (blue), acquired at 19.96 T (850 MHz) and a MAS frequency of 60 kHz. Note the disappearance of resonances corresponding to the indole ring of Trp residues in the fluorinated sample.

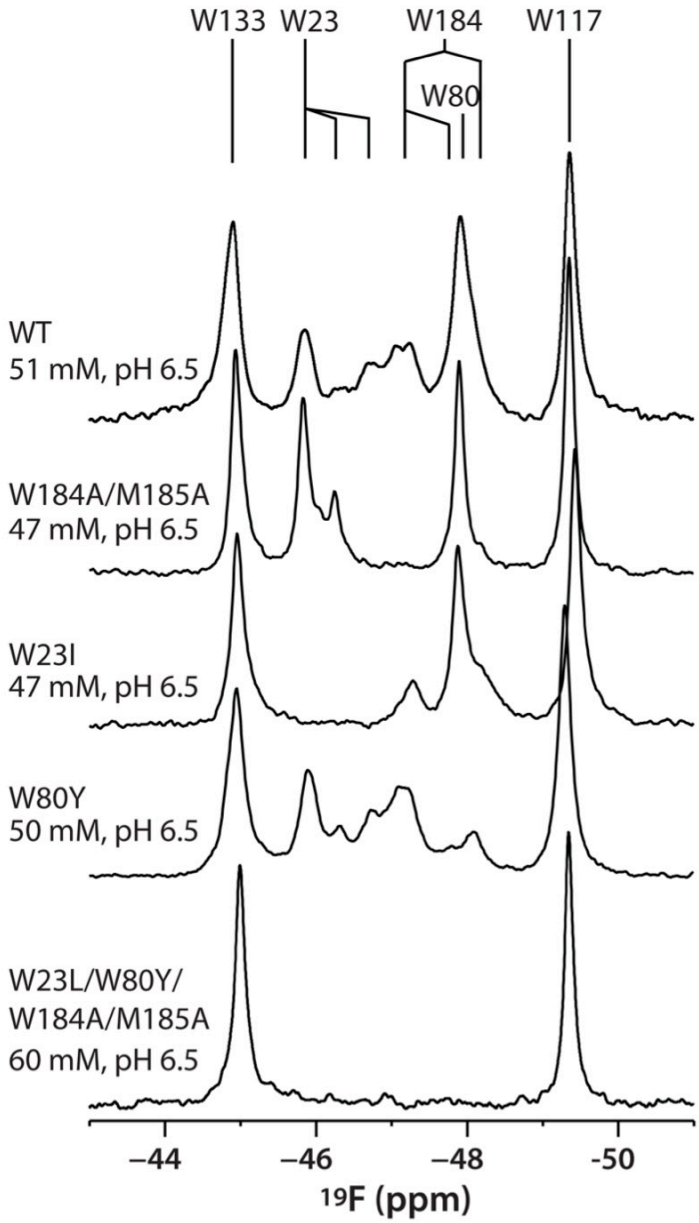

Figure S2. ^{19}F solution NMR spectra of (top to bottom) 5-^{19}F-Trp, U-^{15}N CA (top) and 5-^{19}F-Trp,U-^{15}N CA mutants W184A/M185A, W23I, W80Y and W23L/W80Y/W184A/M185A. The spectra were recorded at 14.1 T. The sample conditions are listed next to each spectrum. Assignments are shown on top.

Table S1. ^{19}F chemical shifts of F-Trp HIV-1 CA protein in solution.

	^{19}F chemical shift (ppm)*
W23	-45.9, -46.3, -46.7
W80	-47.9
W117	-49.4
W133	-44.9
W184	-48.1, -47.2, -47.8

*Buffer: 25 mM Na phosphate, pH 6.5,
5 mM DTT, 0.02% NaN_3, 7% D_2O;T = 298 K

Table S2. MAS NMR experimental ^{19}F isotropic chemical shifts for 5F-Trp CA capsid assemblies and mutants. All spectra were recorded at 19.96 T.

Sample	Residue	δ_{iso} (ppm)
CA W23I NaCl precipiate	W80/W184	-47.2
	W117	-48.9
	W133	-44.8
CA W80Y tubes	W23	-46.3
	W117	-49.1
	W133	-44.7
	W184	-47.2
CA A14C/E45C/W184A/M185A hexamer tubes	W23	-45.3
	W80	-47.7
	W23a*	-46.8
	W117	-49.1
	W133	-44.6
CA A14C/E45C/W184A/M185A hexamer PEG precipitate	W23	-45.4
	W80	-47.8
	W23a*	-46.5
	W117	-49.2
	W133	-44.6

*The second, minor conformer of W23

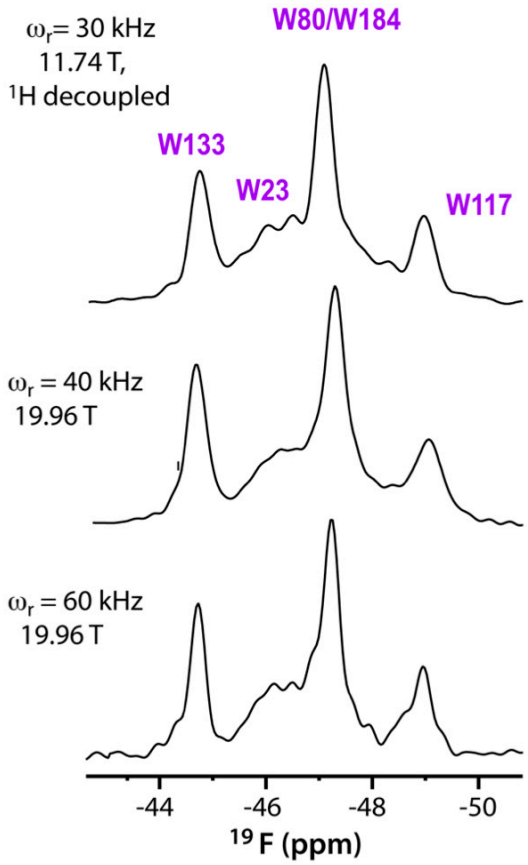

Figure S3. ^{19}F MAS NMR spectra of 5-^{19}F-Trp, U-^{15}N CA tubular assemblies.

Table S2. ^{19}F-^{19}F distances in HIV-1 CA and A14C/E45C/W184A/M185A proteins.

PDB 4XFX* CA NL4-3

5-F-Trp-CA	W23	W80	W117	W133	W184
	Intramolecular Distance (Å)				
W23	20.0	23.1	23.4	19.1	28.6
W80	33.3	34.1	12.6	8.8	33.1
W117	25.1	21.7	28.5	9.8	42.5
W133	26.2	28.6	27.2	33.0	33.8
W184	33.0	35.8	32.2	27.6	2.1-9.4
F-F	Intermolecular Distance (Å)				

PDB 3H4E*

5-F-Trp-CA A14C/E45C/W184A/M185A	W23	W80	W117	W133
	Intramolecular Distance (Å)			
W23	19.9	22.9	23.8	19.3
W80	33.2	34.3	12.5	8.8
W117	25.1	22.1	29.3	9.9
W133	26.1	28.5	27.9	33.2
F-F	Intermolecular Distance (Å)			

*Coordinates were modified and include F in the 5 position of all tryptophans.

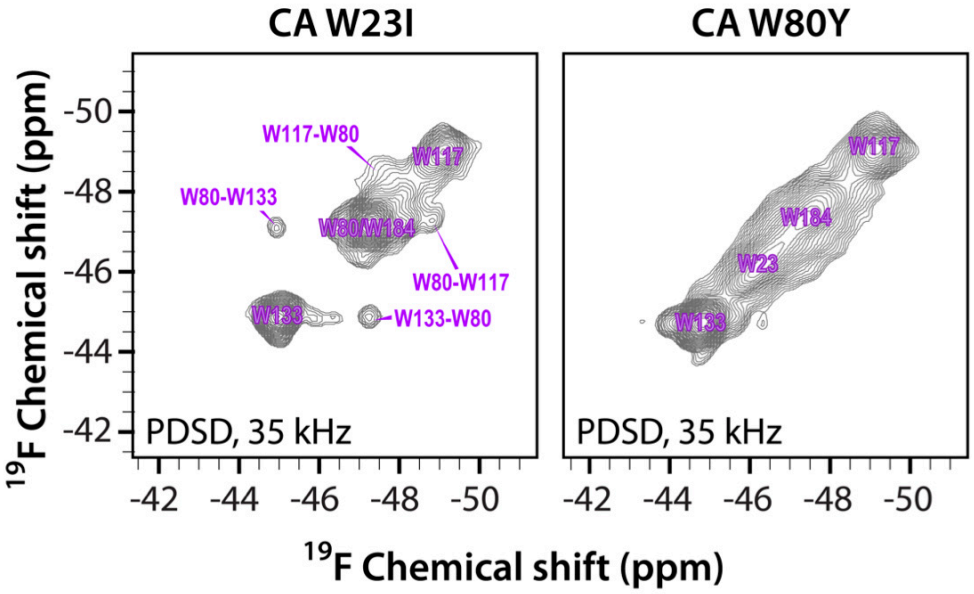

Figure S4. 2D ^{19}F-^{19}F correlation spectra of 5-^{19}F-Trp CA mutants.

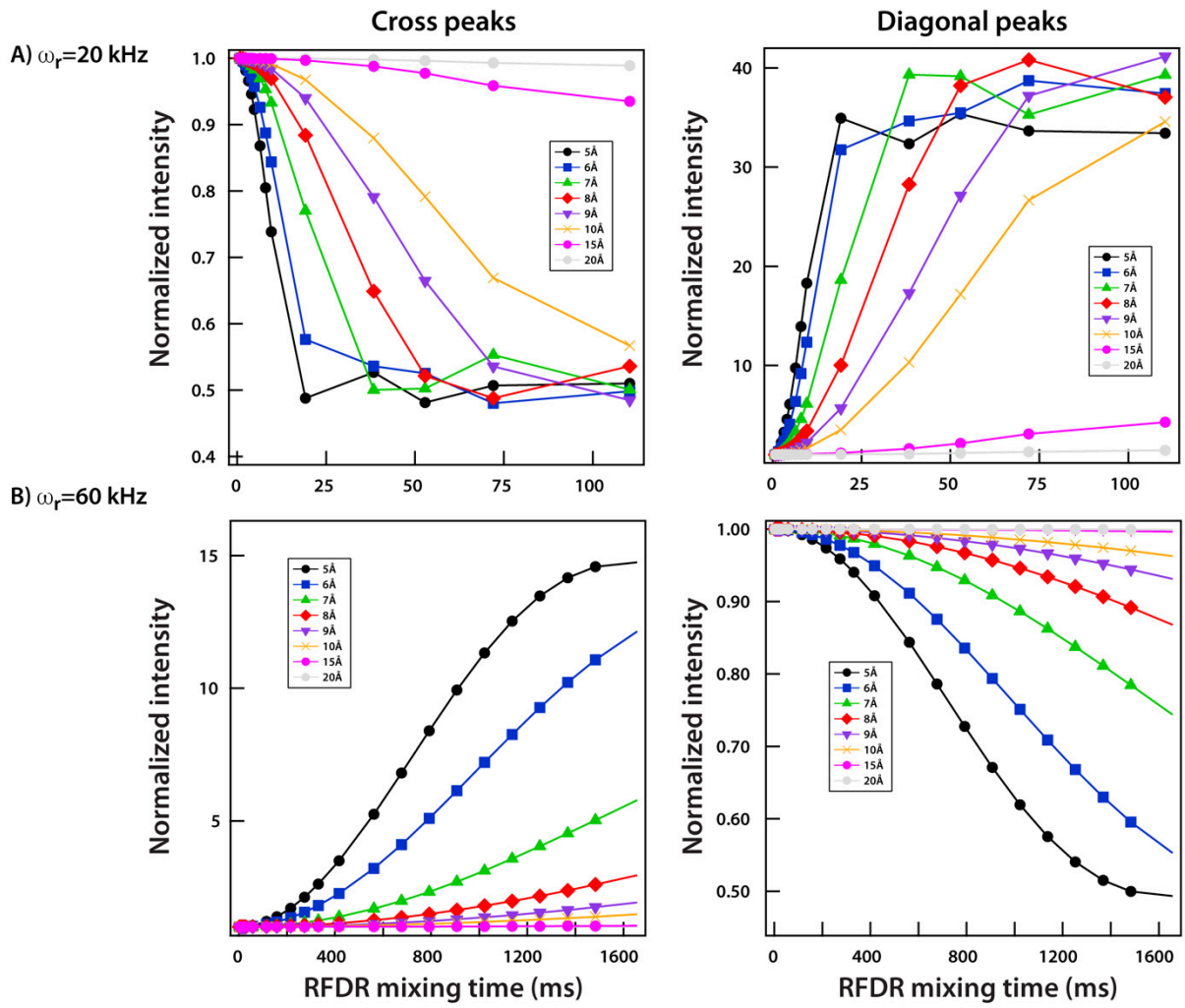

Figure S5. Simulated ^{19}F-^{19}F RFDR buildup curves for cross peaks (left) and diagonal peaks (right) corresponding to ^{19}F-^{19}F distances of 5 – 20 Å. The magnetic field strength was 19.96 T. Other simulation parameters are provided in the Experimental section.

References

(1) Crowley, P. B.; Kyne, C.; Monteith, W. B. *Chem. Commun.* **2012**, *48*, 10681.
(2) Han, Y.; Hou, G. J.; Suiter, C. L.; Ahn, J.; Byeon, I. J. L.; Lipton, A. S.; Burton, S.; Hung, I.; Gor'kov, P. L.; Gan, Z. H.; Brey, W.; Rice, D.; Gronenborn, A. M.; Polenova, T. *J. Am. Chem. Soc.* **2013**, *135*, 17793.
(3) Sun, S. J.; Han, Y.; Paramasivam, S.; Yan, S.; Siglin, A. E.; Williams, J. C.; Byeon, I. J. L.; Ahn, J.; Gronenborn, A. M.; Polenova, T. *Methods. Mol. Biol.* **2012**, *831*, 303.
(4) Chandran, V. C.; Madhu, P. K.; Kurur, N. D.; Brauniger, T. *Magn. Reson. Chem.* **2008**, *46*, 943.
(5) Shaka, A. J.; Keeler, J.; Frenkiel, T.; Freeman, R. *J. Magn. Reson.* **1983**, *52*, 335.
(6) Maurer, T.; Kalbitzer, H. R. *J. Magn. Reson. Ser. B* **1996**, *113*, 177.
(7) Delaglio, F.; Grzesiek, S.; Vuister, G. W.; Zhu, G.; Pfeifer, J.; Bax, A. *J. Biomol. NMR* **1995**, *6*, 277.
(8) Goddard, T. D.; Kneller, D. G. *SPARKY 3*, University of California, San Francisco, 2004.
(9) Herzfeld, J.; Berger, A. E. *J. Chem. Phys.* **1980**, *73*, 6021.
(10) Eichele, K. *HBA 1.7.5*, Universität Tübingen, 2015.
(11) Bak, M.; Rasmussen, J. T.; Nielsen, N. C. *J. Magn. Reson.* **2000**, *147*, 296.
(12) Maciejewski, M. W.; Schuyler, A. D.; Gryk, M. R.; Moraru, I. I.; Romero, P. R.; Ulrich, E. L.; Eghbalnia, H. R.; Livny, M.; Delaglio, F.; Hoch, J. C. *Biophys. J.* **2017**, *112*, 1529.