$^{13}C/^{15}N$-^{19}F Intermolecular REDOR NMR Study of the Interaction of TAR RNA with Tat Peptides

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Sample Preparation

The TAR RNA 29mer 5'-GGCAGA-U[5F]-CUGAGCCUGGGAGCU(pS)CUC-U[5F]-GCC-3' (U[5F] indicates a 5-F base-labeled Uridine, A indicates a 1' deuterium substitution, and pS indicates a phosphorothioate label) was purchased from Dharmacon. The RNA oligonucleotide has been converted to the 2'-hydroxyl form and desalted by the manufacturer. The homogeneity of the sample was checked by analytical denaturing polyacrylamide gel electrophoresis, and no further purification was performed. A 4.04 μmol sample was dissolved in buffer (50 mM NaCl, 10 mM sodium cacodylate, pH 5.5), then frozen by liquid nitrogen and lyophilized. There are 10% NaCl and 4.7% cacodylate respectively in the final composition, as measured by weight upon lyophilization

Peptide Synthesis

The 11-mer peptide 47YGRKKKRRQRRR57 was synthesized on a third generation Rainin PS3 solid phase peptide synthesizer using standard FMOC chemistry. The amino acid corresponding to Arg52 in Tat was uniformly ^{13}C and ^{15}N labeled using arginine obtained in protected form from Isotec (Sigma-Aldrich). After cleavage, the crude peptide was purified by HPLC. The purity was confirmed with mass spectrometry and no further purification was required.

Complex Formation

The lyophilized TAR RNA sample was re-dissolved in 1530 μl sterile H_2O. 4.85 μmol (1.2 equivalents) of purified 11-mer peptide was dissolved in 1530 μl of the same buffer used earlier. The peptide solution was added to the TAR RNA solution drop by drop avoiding the formation of any precipitation. The final mixed solution was kept at 37 °C for 50 min with continued gentle vortexing. After cooling to room temperature, the sample was frozen by liquid nitrogen and lyophilized, then transferred to the MAS rotor.

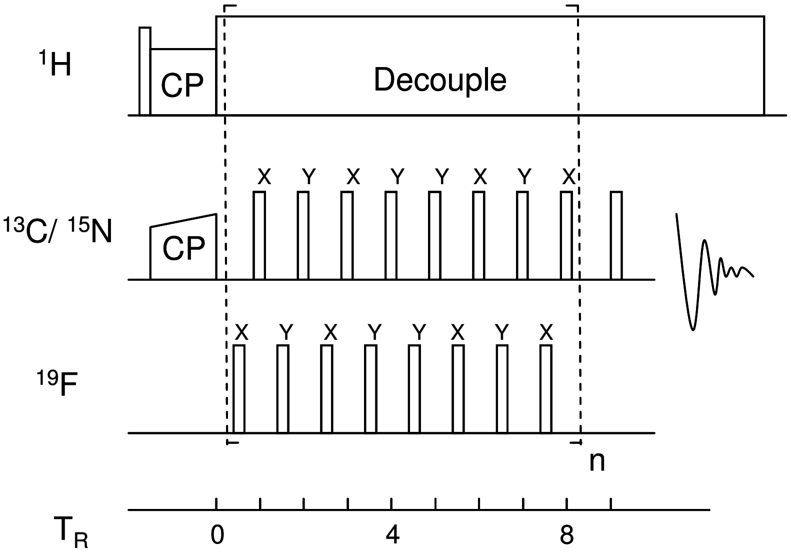

Figure S1. XY-8 phase cycling REDOR pulse sequence with alternating π-pulses on both the $^{13}C/^{15}N$-observed and ^{31}P-dephasing channels.

Solid-State NMR Experiments

Rotational Echo Double Resonance (REDOR) experiments were performed on a home-built spectrometer at 11.74 T magnet field operating at Larmor frequencies of 500.00 MHz for ^1H, 470.47 MHz for ^{19}F, 125.76 MHz for ^{13}C and 50.98 MHz for ^{15}N, using a 4 mm HFX triple-resonance Varian/Chemagnetics magic angle spinning probe. All measurements were performed using XY-8 phase cycling with alternating π-pulses on both the $^{13}C/^{15}N$-observed and ^{19}F-dephasing channels (Figure S1) with a 2 s relaxation delay. ^{13}C-^{19}F REDOR was performed at -32 °C under a sample spinning speed of 6,000 Hz which was regulated to ±2 Hz. The ^{13}C NMR signal was enhanced using ramped cross-polarization with a ^{1}H-^{13}C contact time of 2.6 ms. Pulse lengths were 3.6 μs for ^{1}H π/2 pulses, 6.0 μs for ^{13}C π pulses, and 8.2 μs for ^{19}F π pulses. REDOR points were collected at the dephasing time 1.33 ms with 20k scans (experiment time 22.2h), 2.67 ms with 30k scans (experiment time 33.3h), 4.00 ms with 36k scans (experiment time 40.0h), and 5.33 ms with 40k scans (experiment time 44.4h). ^{15}N-^{19}F REDOR was also performed at -32 °C under a sample spinning speed of 8,000 Hz which was regulated to ±2 Hz. The ^{15}N NMR signal was enhanced using ramped cross-polarization with a ^{1}H-^{15}N contact time of 2.6 ms. Pulse lengths were 3.4 μs for ^{1}H π/2, 6.0 μs for ^{15}N π and 6.5 μs for ^{19}F π. REDOR points were collected at dephasing times of 1 ms with 22k scans (experiment time 24.4h), 3 ms with 26k scans (experiment time 28.9h), 5 ms with 50k scans (experiment time 55.6h) and 6 ms with 56k scans (experiment time 62.2h). The corresponding REDOR MAS spectrum (S) and reference spectrum (S_0) are shown in Figure S2 and Figure S3.

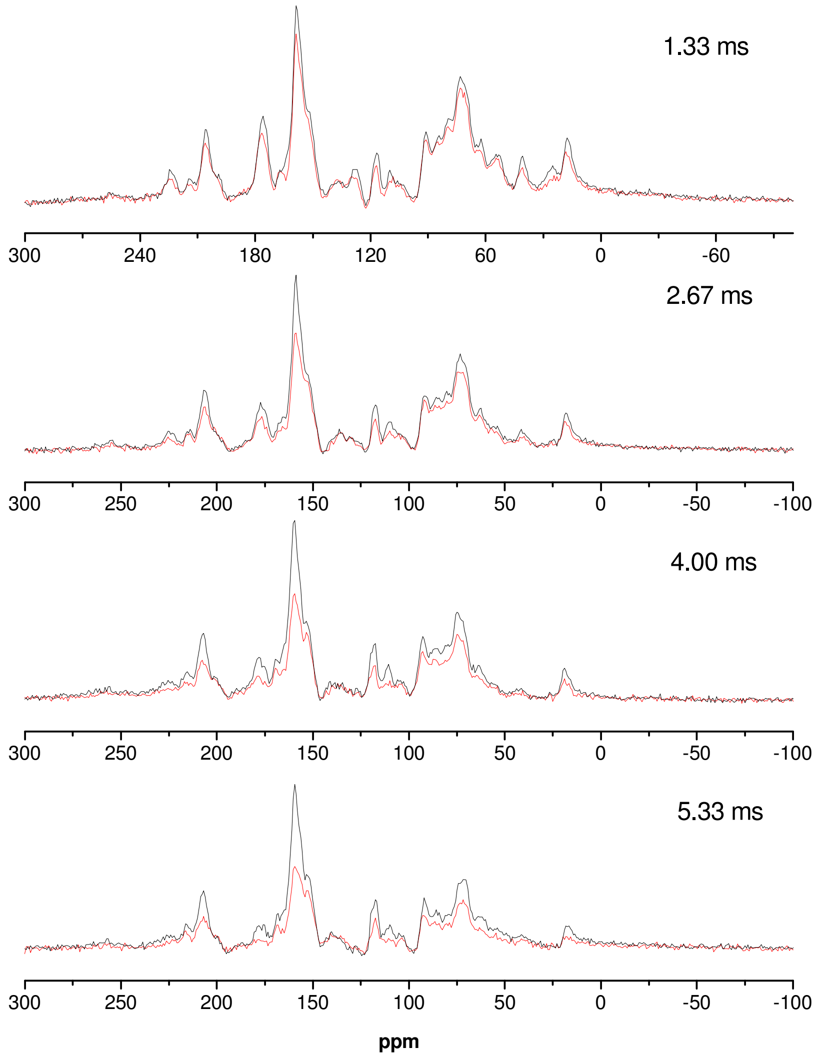

Figure S2. ^{13}C-observed REDOR MAS spectra (S, red line) and reference spectra (S_0, black line) recorded at the dephasing time 1.33 ms (20k scans), 2.67 ms (30k scans), 4.00 ms (36k scans) and 5.33 ms (40k scans).

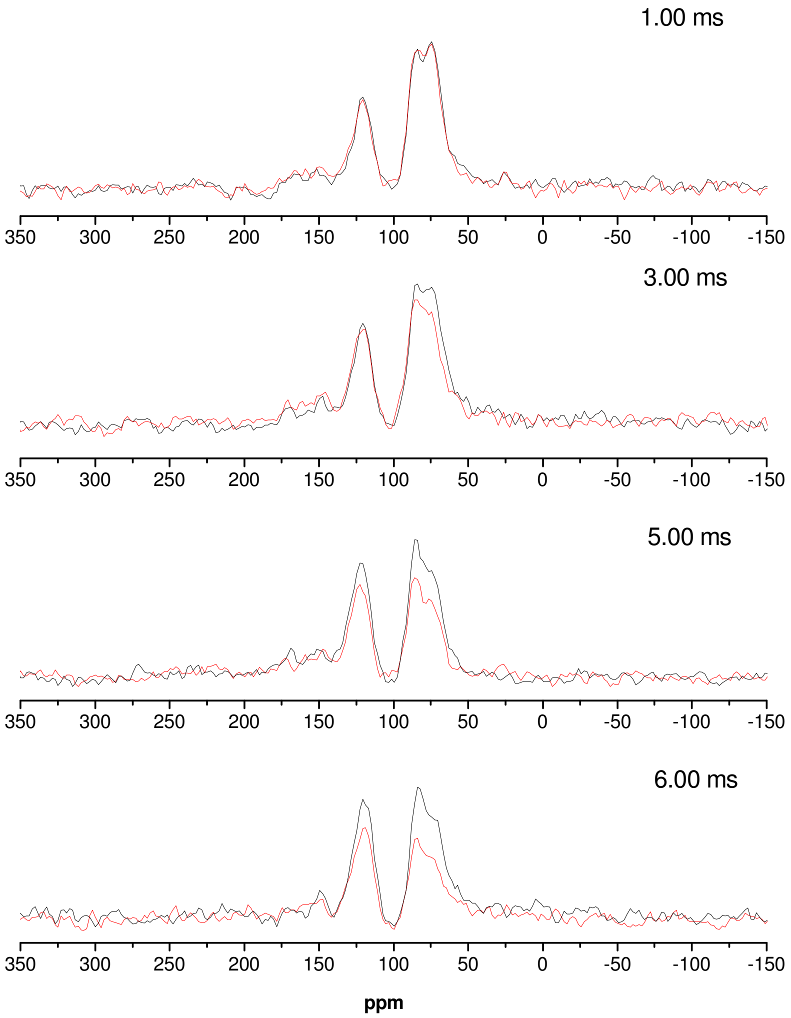

Figure S3. ^{15}N-observed REDOR MAS spectrum (S, red line) and reference spectrum (S_0, black line) recorded at the dephasing time 1.00 ms (20k scans), 3.00 ms (26k scans), 5.00 ms (50k scans) and 6.00 ms (56k scans).

Data Processing

Data processing was performed with *in-house* NMR processing software. S and S0 were calculated from the peak area of C_ζ (150 ppm - 162 ppm), CO (172 ppm - 180 ppm), NH (109 ppm - 134 ppm), and Nε and Nη1, η2 (60 ppm -93 ppm). Parts of Nε and Nη1, η2 signals are overlapped, but they can be separated into two Gaussian-shaped peaks. All simulations of the experimental REDOR data were performed using the simulation software SIMPSON[1], with the assumption of ideal cross polarization and proton decoupling, and without consideration of relaxation. A minimal set of 232 $\{\alpha, \beta\}$ Euler angles defined by Zaremba-Conroy-Wolfsberg scheme[2,3] with 10 γ angles was used to obtain Powder averages. The corresponding experimental parameters are described above.

Labeled C_ζ and CO are well resolved, and the natural abundance ^{13}Cs at the same chemical shift can be ignored. In the simulation of C_ζ, all homonuclear ^{13}C-^{13}C couplings are neglected since C_ζ is not directly connected to any other carbon. The system is simplified to a single C-F pair. In the simulation of CO, the homonuclear ^{13}C-^{13}C coupling is only considered from the connected Cα, which is assumed to be 2080.45 Hz (1.54 Å). The system is simplified to two C and one F atoms. CO-F and Cα-F are assumed to have similar distances since they are so close to each other. The assumptions in C-F fittings are supported by test simulations.

Labeled nitrogens are also well resolved except Nη1 and Nη2, which results in the multiple solutions of F - Nη1 distance and F – Nη2 distance. In the simulation of NH, all homonuclear ^{15}N-^{15}N couplings are neglected since NH is not directly connected to any other nitrogen. The system is simplified to a single N-F pair. In the simulation of Nε and Nη1,η2, the homonuclear ^{15}N-^{15}N couplings are only considered within the guanidinium group, and are assumed to be 111 Hz (2.23 Å). The system is simplified to three N and one F atoms. Similar distances of N-F pairs are assumed in the simulation since they are in the same guanidinium group. The assumptions in N-F fittings are also supported by test simulations.

A χ^2 analysis (Figure S4) was performed to find the best parameters for simulating the data. The red lines in Figure S4 are polynomial fittings which are used to calculate the inverse of the second derivative of χ^2 at the minimum[4]. The errors of the distance estimates are calculated as $\sigma^2 = 2\left(\frac{\partial^2 \chi^2}{\partial r^2}\right)^{-1}_{r=r\,\mathrm{min}}$.

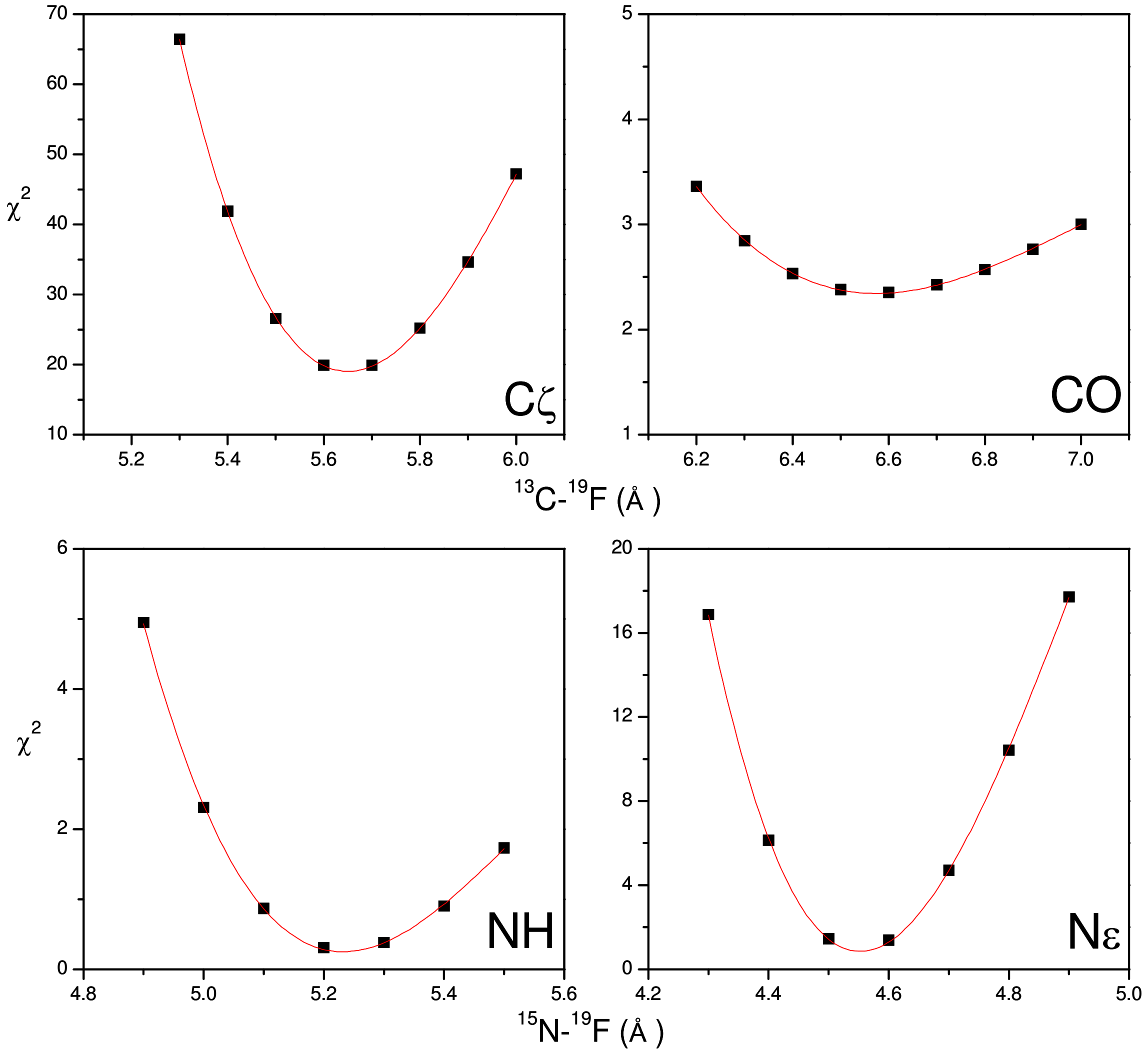

Figure S4. The graphs of the χ^2 function identify values for which the REDOR data are best fit by the simulations.

References

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