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Determination of Long-Range Distances by Fast Magic-Angle-Spinning Radiofrequency-Driven $^{19}$F-$^{19}$F Dipolar Recoupling NMR

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Abstract

Nanometer-range distances are important for restraining the three-dimensional structure and oligomeric assembly of proteins and other biological molecules. Solid-state NMR determination of protein structures typically utilizes $^{13}$C-$^{13}$C and $^{13}$C-$^{15}$N distance restraints, which can only be measured up to ~7 Å due to the low gyromagnetic ratios of these nuclear spins. To extend the distance reach of NMR, one can harvest the power of $^{19}$F, whose large gyromagnetic ratio in principle allows distances up to 2 nm to be measured. However, $^{19}$F possesses large chemical shift anisotropies (CSAs) as well as large isotropic chemical shift dispersions, which pose challenges to dipolar coupling measurements. Here we demonstrate $^{19}$F-$^{19}$F distance measurements at high magnetic fields under fast magic-angle spinning (MAS) using radiofrequency-driven dipolar recoupling (RFDR). We show that $^{19}$F-$^{19}$F cross peaks for distances up to 1 nm can be readily observed in 2D $^{19}$F-$^{19}$F correlation spectra using less than 5 ms of RFDR mixing. This efficient $^{19}$F-$^{19}$F dipolar recoupling is achieved using practically accessible MAS frequencies of 15–55 kHz, moderate $^{19}$F rf field strengths, and no $^1$H decoupling. Experiments and simulations show that the fastest polarization transfer for aromatic fluorines with the highest distance accuracy is achieved using either fast MAS (e.g. 60 kHz) with large pulse duty cycles (> 50%) or slow MAS with strong $^{19}$F pulses. Fast MAS considerably reduces relaxation losses during the RFDR $\pi$-pulse train, making finite-pulse RFDR under fast-MAS the method of choice. Under intermediate MAS frequencies (25–40 kHz) and intermediate pulse duty cycles (15–30%), the $^{19}$F CSA tensor

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Supporting Information Available:
Additional NMR spectra and tables include:
- Tables of the $^{19}$F CSA and dipolar tensor parameters used in the simulations
- Tables of measured $^{19}$F RFDR buildup time constants;
- NMR pulse sequence;
- Additional experimental RFDR buildup curves;
- Additional simulated RDFR buildup curves at 60 kHz MAS;
- Effect of $^1$H decoupling on $^{19}$F RFDR transfer;
- Correlation of $^{19}$F RFDR exchange rates with $1/r^3$;
- Simulations of PNC buildup rates for different phenylene ring orientations.

This information is available free of charge via the Internet at http://pubs.acs.org.
orientation has a quantifiable effect on the polarization transfer rate, thus the RFDR buildup curves encode both distance and orientation information. At fast MAS, the impact of CSA orientation is minimized, allowing pure distance restraints to be extracted. We further investigate how relayed transfer and dipolar truncation in multi-fluorine environments affect polarization transfer. This fast-MAS $^{19}$F RFDR approach is complementary to $^{19}$F spin diffusion for distance measurements, and will be the method of choice under high-field fast-MAS conditions that are increasingly important for protein structure determination by solid-state NMR.

Graphical Abstract

Introduction

Determination of the three-dimensional structure of biological macromolecules by MAS NMR requires conformationally sensitive chemical shifts and inter-atomic distance restraints. The latter are commonly measured using dipolar couplings involving $^{13}$C and $^{15}$N spins $^1$. However, the low gyromagnetic ratios of $^{13}$C and $^{15}$N limit the measurable $^{13}$C–$^{13}$C and $^{13}$C–$^{15}$N distances to ~7 Å and ~5 Å, respectively $^2$–$^3$. Longer-distance restraints are crucial for determining the three-dimensional fold of large proteins and protein complexes and for elucidating the quaternary structures of oligomeric membrane proteins $^4$–$^6$. One strategy to lengthen the distance reach of solid-state NMR (SSNMR) is paramagnetic relaxation enhancement (PRE) $^7$–$^11$, which uses unpaired electrons with its 658-fold larger gyromagnetic ratio than $^1$H to enhance nuclear spin $T_1$ and $T_2$ relaxation in a distance-dependent fashion. However, introducing paramagnetic species into proteins can perturb protein structures and the flexible tag that is commonly used to link the paramagnetic center to the protein sidechain introduces positional uncertainty. The second strategy for measuring long distances is to exploit $^1$H and $^{19}$F spins, which have two of the largest gyromagnetic ratios among nuclear spins. The $10$-Å $^1$H–$^1$H and $^{19}$F–$^{19}$F dipolar couplings are 122 Hz and 108 Hz, respectively, which are 5 times stronger than the $5$-Å $^{13}$C–$^{15}$N dipolar couplings (24 Hz). However, $^1$H–$^1$H distance measurements are complicated by the dense proton network in protonated molecules, which homogeneously broadens the $^1$H NMR spectra and causes relayed transfer $^{12}$ and dipolar truncation $^{13}$. Protein perdeuteration followed by H/D back exchange $^{14}$–$^{16}$ and $^1$H-detected experiments under ultrafast MAS of ~100 kHz $^{17}$–$^{19}$ reduce these drawbacks. Indeed, a 3D HNH experiment with $^1$H–$^1$H radiofrequency-driven recoupling (RFDR) mixing has been previously proposed to measure $^1$H–$^1$H distances of ≤ 5 Å in perdeuterated and back-exchanged proteins $^{20}$. Nevertheless, such $^1$H–$^1$H distance measurement requires sensitivity-limited 3D and 4D correlation experiments and narrow $^1$H linewidths, which can be difficult to achieve in non-crystalline proteins.
Compared to $^1$H NMR, $^{19}$F NMR offers several advantages for long-range distance measurement. Fluorine is naturally absent in biological molecules but can be readily and sparsely incorporated into proteins through aromatic amino acids such as 5-$^{19}$F-Trp, 4-$^{19}$F-Phe and 3-$^{19}$F-Tyr. Comparisons of fluorinated and hydrogenated proteins have shown that sparse fluorination of proteins usually does not perturb protein structure. Because the number of introduced fluorines is small, $^{19}$F spectral assignment is straightforward based on characteristic chemical shifts, mutagenesis, and $^{19}$F dipolar coupling to neighboring residues.

Recently, we demonstrated that 2D $^{19}$F spin diffusion NMR experiments under 15–40 kHz MAS efficiently produce correlation peaks for $^{19}$F–$^{19}$F distances up to 1.6 nm. These experiments were carried out at a magnetic field of 14.1 Tesla with a $^{19}$F Larmor frequency of 564 MHz. Therefore, this MAS rate regime corresponds to a $^{19}$F chemical shift range of 25–70 ppm, which is necessary for reducing the number of spinning sidebands for aromatic fluorines, whose CSAs can be as large as 90 ppm. Under these magnetic field and MAS conditions, proton-driven $^{19}$F spin diffusion (PDS), without $^1$H irradiation, is efficient for transferring polarization between fluorines with the same isotropic chemical shift but different chemical shift tensor orientations, whereas dipolar-assisted polarization exchange (DARR), with $^1$H irradiation, is more efficient for transferring polarization between fluorines with different isotropic chemical shifts. Moreover, the study showed that the $^{19}$F spin diffusion rate constants have a simple dependence on inter-fluorine distances after adjusting for isotropic shift differences, thus quantitative $^{19}$F–$^{19}$F distances can be obtained from the measured spin diffusion rates. In addition to $^{19}$F–$^{19}$F distances, we also reported the measurement of $^{13}$C–$^{19}$F distances up to 10 Å with better than 1 Å accuracy using rotational-echo double-resonance (REDOR) experiments. This further broadens the utility of fluorines as molecular probes of protein structures.

Although $^{19}$F spin diffusion is still active under 40 kHz MAS, in higher magnetic fields where the MAS frequency needs to concomitantly increase to suppress the CSA sidebands, the spin diffusion mechanism will be progressively quenched both by faster MAS and by larger $^{19}$F chemical shift dispersions. Therefore, $^{19}$F–$^{19}$F dipolar recoupling will be necessary for distance measurements. A large number of homonuclear dipolar recoupling methods have been developed since the 1990’s. However, few of these pulse sequences work robustly for spin systems with both large resonance offsets and large chemical shift anisotropies, and dipolar recoupling sequences that require continuous-wave (CW) irradiation with rf fields that are integer multiples of the MAS frequency are impractical under fast MAS. Recently, a sandwiched pi-pulse (SPIP) technique was introduced to recouple homonuclear dipolar interactions between spins with large resonance offsets and CSAs. This sequence is a super-cycled and spin-locked version of the $R_2^1$ recoupling element, which requires a modest rf field of twice the MAS frequency. This sequence was demonstrated on unprotonated fluoroaluminates, which have a large $^{19}$F isotropic chemical shift range of 120 ppm and large CSAs of 100 ppm. At 18.8 T under 67 kHz MAS and with 100 kHz $^{19}$F rf pulses, the SPIP double-quantum experiment gave more broadband recoupling than seven other $R_{Nn}$ sequences. However, these inorganic fluorides have short F–F distances of only ~4 Å, which require very short mixing times of less than
300 μs for dipolar recoupling. Therefore, the applicability of the SPIP technique for measuring long-range $^{19}$F–$^{19}$F distances is unknown.

One of the simplest homonuclear dipolar recoupling sequences is the RFDR technique, which employs a single 180° pulse per rotor period to recouple the dipolar interaction \(42-43\). In the limit of short 180° pulses compared to the rotor period, RFDR relies on pulse-modulated chemical-shift difference to interfere with MAS averaging of the flip-flop part of the dipolar Hamiltonian. In contrast to spin diffusion, polarization transfer by δ-pulse RFDR is mediated by chemical-shift differences, and thus becomes more efficient at high magnetic fields. The average Hamiltonian for the δ-pulse RFDR with one π pulse per rotor period is

\[
\mathcal{H}_{\delta p} = -d_{\delta p}(\hat{T}_2^+ + \hat{T}_2^-) = -d_{\delta p} \frac{1}{2}(\hat{I}_1^+ \hat{I}_2^- + \hat{I}_1^- \hat{I}_2^+) \quad (1)
\]

where the raising \((\hat{I}_k^+)^{m}\) and lowering \((\hat{I}_k^-)^{m}\) operator of spin \(k\) comprise the zero-quantum spin Hamiltonian, also known as the “flip-flop term”, which drives spin diffusion. The effective dipolar coupling strength, \(d_{\delta p}\), scales with the full (isotropic and anisotropic) chemical shift difference \(\Delta \delta\) according to \(42-43\)

\[
d_{\delta p} = \frac{d_{12}^{(0)}}{\pi} \sum_{m=1,2} G_{\text{mfl}}(\theta) \cos m \phi \frac{\Delta \delta}{\omega_r} \left( \frac{\Delta \delta}{\omega_r} \right)^2 \sin \left( \frac{\pi \Delta \delta}{\omega_r} \right) \quad (2)
\]

where \(d_{12}^{(0)}\) is the dipolar coupling constant, \(\omega_r\) is the angular MAS frequency, \(\theta\) and \(\phi\) are the polar and azimuthal angle of the dipolar vector in the rotor frame, and \(G_{\text{mfl}}(\theta)\) is the polar-angle dependence of the time-dependent dipolar coupling.

In the absence of CSA, the effective dipolar coupling strength \(d_{\delta p}\) becomes zero for vanishing isotropic chemical shift difference, but is maximal under the \(n = 1\) and \(n = 2\) rotational-resonance conditions, \(\Delta \delta = n\omega_r\). In the presence of large CSA such as the case of $^{19}$F spins, rotational resonance can occur even when the isotropic shift difference is zero because of CSA-induced instantaneous chemical shift differences (i.e. \(n = 0\) rotational resonance) \(29\).

When the 180° pulse occupies a significant fraction (~30% or larger) of the rotor period, due to fast MAS and/or the use of weak rf pulses to avoid interference with $^1$H decoupling, then homonuclear dipolar recoupling occurs by a distinct mechanism that does not require chemical shift differences. The average Hamiltonian for this finite-pulse RFDR (fpRFDR) is \(44\)

\[
\mathcal{H}_{\text{fp}} = d_{\text{fp}} T_{20} = d_{\text{fp}} \frac{1}{\sqrt{6}}(3 \hat{I}_z \cdot \hat{I}_2 - \hat{I}_1 \cdot \hat{I}_2) \quad (3)
\]
which contains the full spin part of the dipolar Hamiltonian of a non-spinning sample. The effective dipolar coupling for this fpRFDR mechanism is

$$\tilde{d}_{fp} = \frac{3\sqrt{6} \pi}{2 \tau_{r}} \int_{\tau}^{\tau + \tau_{p}} d_{12}(t) \sin^{2} \beta_{fp}(t) dt$$  \hspace{1cm} (4)$$

where $d_{12}(t)$ is the time-dependent dipolar coupling under MAS, $\sin^{2} \beta_{fp}(t)$ is a scaling factor that results from a time-dependent phase $\beta_{fp}(t)$ that accounts for the finite pulse of duration $\tau_{p}$:

$$\beta_{fp}(t) = \int_{0}^{t} \omega_{1}(t') dt'$$.  \hspace{1cm} (5)$$

When the finite pulse is achieved using weak rf fields instead of fast MAS, then dipolar recoupling becomes frequency-selective. Under fast MAS, $^{13}$C RFDR without $^1$H decoupling yields more efficient polarization transfer than $^1$H-decoupled RFDR, by avoiding depolarization conditions between $^1$H decoupling fields, MAS, and $^{13}$C rf pulses. With weak $^{13}$C recoupling pulses, adiabatic inversion pulses have also been proposed to compensate for resonance offsets. Although $^{13}$C RFDR is well explored in terms of its dependence on pulse length, $^1$H decoupling, and MAS rates, the effect of CSA on RFDR has not been fully investigated. Moreover, while frequency-selective fpRFDR has been applied to $^{13}$CO-labeled peptides and proteins for distance measurements, RFDR in either the finite-pulse or $\delta$-pulse limits have not been used to quantify distances in multi-spin systems, spin systems with large isotropic chemical shift dispersion, and spin systems with large CSAs.

In this study, we present experimental results and numerical simulations of $^{19}$F RFDR for distance measurements under 15–55 kHz MAS at 14.1 Tesla. In this magnetic field, the aromatic $^{19}$F chemical shift anisotropies are about 50 kHz (~90 ppm), while isotropic chemical shift differences can be as large as about 69 kHz (~100 ppm). We show that $^{19}$F RFDR gives two-orders-of-magnitude faster polarization transfer rates compared to $^{19}$F spin diffusion for spins with different isotropic chemical shifts; 2D cross peaks for distances of 2.8–9.5 Å are observed with time constants of 0.3–4 ms at 25 kHz MAS. With $^{19}$F rf duty cycles of 9–33% for the recoupling period, the buildup rates depend on the $^{19}$F CSA tensor orientation in a quantifiable manner. Thus, $^{19}$F RFDR buildup rates encode both distance and orientational information, making the technique extremely sensitive to three-dimensional structure. Numerical simulations show that pure distance constraints with minimal dependence on the CSA tensor orientation can be obtained using fpRFDR under 60 kHz or faster MAS, with an expected accuracy of better than 0.5 Å. At intermediate MAS frequencies of 25–40 kHz, windowed $^1$H decoupling during the recoupling period does not increase the $^{19}$F sensitivity or change the polarization transfer rate, consistent with the
behavior of $^{13}$C RFDR at fast MAS $^{46}$. Finally, we explore multi-fluorine relayed transfer. The presence of a third fluorine affects the buildup amplitudes but has only minimal impact on the buildup rates of the spin pair of interest, provided that relayed transfer through the additional spin is not faster than direct spin-to-spin polarization transfer. Otherwise, relayed transfer interferes with the primary transfer, where the CSA tensor orientation of the third spin impacts the observed buildup curve. These results extend a previous study of $^{19}$F RFDR at a lower magnetic field under slow MAS (12–13 kHz) $^{49}$, by showing that fast-MAS $^{19}$F RFDR in high fields have simplifying features that facilitate distance quantification, namely reduced sensitivity to CSA, considerably slower relaxation that reduces signal loss during RFDR mixing, and the elimination of $^1$H decoupling.

Materials and Methods

Fluorinated compounds

Three fluorinated compounds with a range of chemical shifts and distances are studied: sitagliptin phosphate (C16H15F6N5O·H3PO4·H2O), 7-chloro-1-(2,4-difluorophenyl)-6-fluoro-4-oxo-1,4-dihydro[1,8]naphthyridine-3-carboxylic acid (PNC), and formyl-Met-Leu-Phe (f-MLF) in which the Met CH3 is substituted with CF3 and Phe is tagged with 4-$^{19}$F. Sitagliptin is an FDA-approved anti-diabetic compound comprising a trifluoro-substituted phenylene ring linked to a trifluoromethyl-containing triazolopyrazine group. The fluorinated phenylene ring enhances the interaction to a hydrophobic pocket of the target protein while the CF3 group interacts electrostatically with sidechains of arginine and serine residues, thus increasing reactivity. PNC consists of a para- and ortho-fluorinated phenylene ring linked to a fluorinated naphthyridine ring by a C–C bond. The relative orientation of the naphthyridine and phenylene rings was not known for this research compound, and the present data allow partial determination of this structure. The distance between the two fluorines of the phenylene ring and the distance between the para-fluorine and the naphthyridine fluorine are both invariant to the relative orientation of the two rings. To avoid intermolecular $^{19}$F–$^{19}$F dipolar couplings, sitagliptin and f-MLF were diluted at a 1 : 6 mass ratio with hydrogenated Trp and f-MLF, respectively, while PNC was diluted at a 1 : 5 mass ratio with hydrogenated Trp $^{29}$. To minimize clustering and self-association of the fluorinated molecules, we dissolved the fluorinated compound and the unfluorinated matrix in a heated protic solvent, then freeze-dried the mixture. The solvents and dissolution temperatures were 1 : 3 (v/v) 2-propanol : water mixture at 60°C for PNC diluted in Trp, water at 80°C for sitagliptin diluted in Trp, and acetic acid for f-MLF.

Solid-State NMR Experiments

All SSNMR experiments were conducted on a Bruker Avance III HD spectrometer operating at a magnetic field of 14.1 T with a $^{19}$F Larmor frequency of 564.66 MHz, using a 1.9 mm HFX MAS probe and a 1.3 mm HXY probe modified to FXY. For the 1.3 mm probe, the $^1$H resonance frequency was changed to $^{19}$F by increasing the length of the $\lambda/4$ transmission line by ~6%. The $^{19}$F RFDR pulse sequence is shown in Fig. S1, where the $^{19}$F 180° pulses were phase-cycled according to the xy-32 scheme $^{50}$. The experiments were conducted under MAS frequencies ($\nu_r$) of 14.9 kHz, 25 kHz, 38 kHz, and 55 kHz. The RFDR mixing times ($\tau_{\text{mix}}$) ranged from 0.32 ms to 15.36 ms. In most experiments the $^{19}$F 180° pulse
length was 6 μs, corresponding to an rf field strength (ν1) of 83 kHz. These MAS frequencies and rf fields translate to rf duty cycles, f = ν1/2νr, of 9–33% in each rotor period (τR), and were chosen because the 19F pulse length was sufficiently short to excite the full 19F chemical shift range while the MAS frequencies, with the exception of 14.9 kHz, were sufficiently fast to suppress most CSA sidebands. A few experiments were conducted with a weaker 19F rf field of 62.5 kHz to investigate the polarization transfer in the fpRFDR limit. A much longer 19F pulse length of 12 μs, for an rf field of 41.7 kHz, distorted the spectra (data not shown) and is thus not tenable for typical 19F chemical shift dispersions in organic and biological compounds.

In most experiments no 1H decoupling was applied during the RFDR period. In a few experiments, windowed or continuous-wave 1H decoupling was applied during the RFDR period. For the t1 evolution period and t2 acquisition period, 71.4 kHz TPPM 1H decoupling was used for experiments at 14.9 and 25 kHz MAS, 10 kHz WALTZ-16 decoupling was used for 38 kHz MAS, and no 1H decoupling was applied under 55 kHz MAS. Even without 1H decoupling, the 19F linewidths of sitagliptin were only ~0.6 ppm at 55 kHz, similar to those at slower MAS with 1H decoupling. This observation is consistent with a recent study showing 19F line narrowing by MAS rates above 50 kHz 22. The initial 19F magnetization was generated by 1H-19F cross polarization (CP) for PNC and direct polarization (DP) for sitagliptin and f-MLF. The former increases the 19F spectral sensitivity and avoids the long 19F T1 relaxation times (> 30 s) in PNC 51, while the latter takes advantage of the fast 19F T1 relaxation in sitagliptin and f-MLF due to trifluoromethyl rotations, which enhances both the CF3 signals and the aromatic 19F signals due to 19F spin diffusion during the recycle delay. As a result, short recycle delays of 1.5 s to 3.0 s were used for most experiments, with dummy scans used for sitagliptin and f-MLF. The indirect chemical shift dimension was recorded using TD1 = 400, 450 and 530 for PNC, sitagliptin and f-MLF, respectively, and the number of scans for each t1 point was 16. All experiments were performed at actual temperatures of 300±5 K, by choosing set temperatures of 260 K for the 55 kHz MAS experiments, 280 K for the 38 kHz MAS experiments and 290 K for 14.9 and 25 kHz MAS experiments to compensate for frictional heating 52.

Polarization transfer buildup curves were obtained by normalizing the 19F–19F centerband cross peak intensities with respect to the integrated intensities of all centerband peaks in the ω1 cross section. Buildup time constants (τRFDR) were obtained from exponential fits of mixing-time dependent intensities using the equation I(τRFDR) = (I0−I∞)e−τmix/τRFDR + I∞, where I0 and I∞ indicate the initial and equilibrium intensities, respectively. 19F relaxation during the RFDR period was measured from the first slice of each 2D spectrum as a function of the mixing time, normalized by the intensity of the shortest mixing time spectrum.

**Spin Dynamics Simulations**

We simulated the measured 19F RFDR buildup curves using the SIMPSON software 53. The initial z-polarization of spin 1 was set to 1 while the polarization of all other fluorines was 0. Polarization transfer was evaluated as the time-dependent buildup of the initial unpolarized spins. The simulated buildup intensities were rescaled to match the experimentally measured plateau intensities, which cannot be easily predicted because they depend on the number of
spins, multi-spin interactions, and interference of the $^{19}$F CSA with $^{19}$F-$^{19}$F dipolar polarization transfer. Tables S1 and S2 summarize the orientations and magnitudes of the $^{19}$F CSA tensors and dipolar tensors used in the simulation. Resonance offsets were chosen such that the sum of all $^{19}$F isotropic chemical shifts is zero. The Euler angles of the aromatic $^{19}$F CSA tensor were chosen to put the $\delta_{zz}$ axis perpendicular to the ring plane, the $\delta_{yy}$ principal axis along the C–F bond, and the $\delta_{xx}$ axis in the ring plane, perpendicular to the $\delta_{yy}$ and $\delta_{zz}$ axes. Powder averaging used 10,240 orientations generated by the REPULSION scheme, with 320 crystal orientations and 32 $\gamma$-angles.

To speed up the calculation of the dipolar coupling between a fast-rotating CF$_3$ group and an aromatic fluorine, we represent the three methyl fluorines by a single effective spin. Since all model compounds have much longer CF$_3$–F distances (6–10 Å) than intra-methyl F–F distances, the effective CF$_3$–F dipolar coupling can be approximated as $\omega_{\text{eff}} \approx P_2(\cos 10^\circ) \sqrt{3} \omega_a \approx 1.65 \omega_a$, where $\omega_a$ is the actual dipolar coupling to one of the three CF$_3$ fluorines and $P_2(\cos 10^\circ)$ is the approximate orientational factor due to the ~10° angle between each CF$_3$–F vector and the average vector from the aromatic F to the center of the methyl group. Fig. S2 verifies that a single spin with a $\sqrt{3}$ fold stronger dipolar coupling than $\omega_a$ has the same buildup intensities up to 15 ms mixing as three spins at the center of the methyl group.

**Results and Discussion**

**$^{19}$F RFDR polarization transfer rates and amplitudes**

Fig. 1 shows the $^{19}$F RFDR spectra and buildup curves of sitagliptin at 38 kHz MAS. This anti-diabetic compound contains three aromatic fluorines and a CF$_3$ group, with F–F distances of 2.8–9.5 Å. The 2D correlation spectra were measured using mixing times of 0.42 ms to 15.16 ms, in the absence of $^1$H decoupling. Cross peaks are readily observed within 5 ms of mixing, even for the longest-distance spin pair of F$_O$ and CF$_3$. The cross peak buildup curves (Fig. 1c) show time constants ($\tau_{\text{RFDR}}$) of 0.27 ms to 9.89 ms, which are 10-fold shorter than the spin diffusion time constants for this compound and three orders of magnitude shorter than $^{13}$C–$^{13}$C spin diffusion time constants for ~7 Å distances. For the two directions of polarization transfer within each spin pair, the buildup curves are approximately symmetric, in contrast to spin diffusion, whose exchange rates are asymmetric due to multi-spin effects and inhomogeneous proton distribution. Each aromatic fluorine equilibrates to a normalized cross-peak intensity of ~0.17 whereas the CF$_3$ intensity plateaus to ~0.5, indicating full exchange of the six-spin system.

f-MLF contains only two fluorine centers: a CF$_3$ group and an aromatic fluorine (Fig. 2). The two directions of polarization exchange gave similar values of 2.8 and 3.9 ms for the 8.9 Å distance, measured at 25 kHz MAS. However, the equilibrium intensities differ significantly: the CF$_3$ → FP cross peak equilibrated to 2% of the total CF$_3$ intensity whereas the reverse FP → CF$_3$ cross peak equilibrated to 75% of the total FP intensity. This drastic difference can be attributed to truncation of the weak CF$_3$–F dipolar coupling (~250 Hz) by the intra-methyl $^{19}$F–$^{19}$F dipolar coupling of 9.1 kHz. In comparison, FP does not experience strong F–F dipolar couplings and thus exhibits higher cross peak intensities.
The PNC 38 kHz RFDR data show the polarization transfer dynamics among exclusively aromatic fluorines (Fig. 3). The transfer rate within each $^{19}$F spin pair is symmetric, with average buildup time constants of 0.63 ms, 2.17 ms, and 5.32 ms for the shortest ($F_O\rightarrow F_P$), intermediate ($F_O\rightarrow F_N$) and longest ($F_N\rightarrow F_P$) distances, respectively. Interestingly, data measured at 25 kHz MAS showed average buildup time constants of 0.44 ms, 2.15 ms, and 2.05 ms for the shortest ($F_O\rightarrow F_P$), intermediate ($F_O\rightarrow F_N$) and longest ($F_N\rightarrow F_P$) distances, respectively (Fig. S3). The similar buildup time constants for the 6.1 and 9.5 Å distances at 25 kHz MAS reflects uncertainties in the orientation of the naphthyridine ring relative to the phenylene ring (vide infra), which impacts CSA tensor orientations relative to the dipolar vector and distances to the $F_O$ spin. The cross peaks show different equilibrium intensities, with the $F_N$–$F_P$ pair exhibiting the lowest intensities.

**Dependence of $^{19}$F RFDR on MAS frequency and $^1$H decoupling**

To investigate how $^{19}$F RFDR polarization transfer depends on the MAS frequency, we measured the sitagliptin 2D spectra under 14.9 kHz, 25 kHz, 38, and 55 kHz MAS (Fig. S3). We observed clear cross peaks at short mixing times of 2.3–2.7 ms at all MAS frequencies (Fig. 4a), but the total spectral intensities differ. Faster MAS reduced the number of sidebands (Fig. 4b) and dramatically slowed down $^{19}$F relaxation during the recoupling period (Fig. 4e), thus enhancing the total spectral sensitivity. The total 2D intensities, which is equal to the first slice of the 2D spectrum, showed a relaxation time constant of 3.6 ms at 14.9 kHz MAS, which increased to 27.0 ms at 55 kHz MAS. No significant relaxation differences were observed between CF$_3$ and aromatic fluorines. Relaxation during the RFDR period reflects zero-quantum coherence decays that mainly arise from residual $^1$H–$^{19}$F dipolar couplings, which are better suppressed by fast MAS.

The MAS frequency ($\nu_r$) also affects the $^{19}$F rf duty cycle or pulse fraction ($f$) in the rotor period: at a constant rf field ($\nu_1$) of 83 kHz, the $^{19}$F pulse fraction increases from 9% to 33% as the MAS frequency increases from 14.9 kHz to 55 kHz. Among the three MAS frequencies, 25 kHz MAS ($f = 15\%$) gave the fastest RFDR polarization transfer (Fig. 4f) from $F_3\rightarrow F_O$, whereas 38 kHz and 55 kHz MAS yielded faster $F_M\rightarrow F_P$ polarization transfer. This trend can be rationalized by the fact that the $\delta$-pulse RFDR that operates at low rf duty cycles is optimal with large chemical shift differences whereas the fpRFDR that dominates at high rf duty cycles is insensitive to isotropic chemical shift differences (vide infra). At even faster MAS and with correspondingly stronger $^{19}$F rf fields, the impact of $^{19}$F CSA on the RFDR build up curve is expected to be smaller, but multi-spin relayed transfer remains. Indeed, simulated build up curves for 60 kHz MAS using an rf field of 125 kHz ($f = 24\%$) show similar build up rates as those measured under 38 kHz MAS with an rf field of 83.3 kHz ($f = 23\%$) (Fig. S4), indicating that relayed transfer and overall transfer efficiencies are similar for the same pulse fraction $f$, irrespective of the MAS frequency.

To further investigate how the rf duty cycle affects $^{19}$F relaxation and polarization transfer, we measured the 38 kHz RFDR build up curves using a weak $^{19}$F rf field of 62.5 kHz ($f = 30\%$). Under this condition, polarization transfer speeded up moderately compared to 83 kHz $^{19}$F pulses (Fig. 4d). However, the $^{19}$F relaxation time also shortened from 22.6 down to 14.0 ms (Fig. 4c), consistent with the notion that residual $^1$H–$^{19}$F dipolar coupling is the
main cause of relaxation during the recoupling period. Signal sensitivity is thus reduced despite faster polarization transfer.

These 2D $^{19}$F-$^{19}$F correlation spectra were measured without $^1$H decoupling during the RFDR recoupling period, to avoid simultaneous rf irradiation on the shared $^1$H and $^{19}$F channel of the probe. To investigate if $^1$H decoupling significantly changes the polarization transfer rate or $^{19}$F spectral sensitivity, we measured the 2D spectra of sitagliptin with $^1$H decoupling either between $^{19}$F π pulses or during the entire mixing time (Fig. S5). Windowed $^1$H decoupling did not change the spectral intensities nor the buildup rates, as shown by 1D $^{19}$F selective excitation spectra measured at 25 kHz MAS. On the other hand, full CW $^1$H decoupling at an rf field of 83–100 kHz for a $^{19}$F rf field of 62.5 kHz caused three-fold faster signal loss compared to the undecoupled spectrum (Fig. S5e), indicating severe relaxation loss due to $^1$H-$^{19}$F cross polarization.

**Correlation of RFDR buildup rates with internuclear distances**

Fig. 5 summarizes the measured $^{19}$F-$^{19}$F RFDR buildup rates, $k_{RFDR}$ (Tables S4, S5) as a function of internuclear distances on a logarithmic scale. As a dipolar-driven polarization transfer process, the RFDR buildup rates are expected to scale with distances as $k_{RFDR} = c \cdot r^{-3}$, in contrast to spin diffusion, which scales as $r^{-6}$. Assuming an $r^{-3}$ dependence, a log-log plot displays a linear relationship and reports on the exponent according to

$$\log(\tau_{RFDR}^{-1}) = \log(c) - 3\log(r) \quad (6)$$

Fig. 5a displays a clear correlation of exchange rates with internuclear distances, but with significant scatter at 25 kHz and 38 kHz MAS. At 14.9 kHz MAS, however, the scatter is reduced and the data agree well with an $r^{-3}$ dependence (Fig. 5b). An alternative linearized representation of $\tau_{RFDR}^{-1}$ as a function of $1/r^3$ is shown in Fig. S6.

Although the rf duty cycles vary in our experiments and $^{19}$F CSAs are significant, the data from the three model compounds at 14.9, 25 kHz and 38 kHz MAS fall on the $r^{-3}$ curve with a remarkably consistent slope of 150±30 ms$^{-1}$ Å$^3$ (Fig. 5a). This coefficient predicts RFDR buildup time constants of 0.8, 6.7 and 22.5 ms for F–F distances of 5, 10 and 15 Å. Thus, a $^{19}$F-$^{19}$F distance of 1.5 nm should be readily measurable under 38 kHz spinning, where the $^{19}$F relaxation time is ~23 ms. At even faster MAS, the distance reach should improve further. The combined data show a distance accuracy of $2\sigma = 3.0$ Å, estimated based on the difference between the predicted distance for each experimental and the actual distance. We attribute this deviation, which is outside the random noise of the spectra, to relayed transfer and the influence of the $^{19}$F chemical shift tensor orientation relative to the dipolar vector. In proteins that contain only a small number of fluorinated residues, the spin system will be much more dilute than the small-molecule compounds studied here, therefore the distance accuracy is expected to improve well below 3.0 Å. Interestingly, the 14.9 kHz MAS dataset shows the least deviation between the predicted and measured distances (Fig. 5b), with an accuracy of $2\sigma = 1.0$ Å, suggesting that δ-pulse RFDR is less sensitive to CSA than
fpRFDR at intermediate MAS. To further determine the influence of CSA on distance extraction, we next carried out spin dynamics simulations.

**Simulations of the $^{19}$F RFDR buildup curves**

To understand the $^{19}$F RFDR polarization transfer process more quantitatively, we simulated RFDR buildup curves by taking into account the $^{19}$F chemical shift tensor principal values (Tables S1, S2) and principal axes orientations (Fig. S7) \(^{54-55}\). For aromatic-aromatic $^{19}$F RFDR in sitagliptin, we obtained excellent agreement between the simulated and experimental curves at 25 and 38 kHz MAS (Fig. 6a, c). The initial fast oscillation in the $F_M$–$F_P$ polarization transfer is reproduced in the simulations and can be attributed to the fact that the dipolar coupling (4.8 kHz) between these two spins is much stronger than the dipolar couplings to the remote spins (670 Hz to 1025 Hz for $F_M$). To simulate the $CF_3$–aromatic $^{19}$F polarization transfer, we used not only the original phenylene ring orientation (Fig. 1a) but also an 180°-flipped ring orientation (Fig. 7a), weighted by a 2 : 1 ratio to give the best agreement with the experimental data. The latter conformation significantly shortens the $CF_3$–$F_O$ distances while increasing the $CF_3$–$F_M$ distance (Table S2), thus a mixture of the two conformations is required. Two conformers with different phenylene ring orientations are physically reasonable since sitagliptin was diluted in hydrogenated tryptophan and was lyophilized from solution. An asymmetric population for the two conformations may arise from the fact that fluorine tends to cluster with other fluorine atoms, which should favor conformer 1 (Fig. 1a), the structure found by X-ray diffraction \(^{59}\) of sitagliptin phosphate. The altered distances between aromatic fluorines and the trifluoromethyl group also affect polarization transfer among the aromatic $^{19}$F spins (see below), which provide additional information about the sitagliptin conformation besides the $CF_3$ buildup curves. Using this dual conformational model (with a 2 : 1 ratio of the two conformers), the simulated sitagliptin buildup curves show reasonable agreement with the experimental data.

We next investigated the effects of the $^{19}$F CSA tensor orientation and relayed transfer by simulating the $F_O$–$F_P$ polarization transfer in sitagliptin (Fig. 7). When the $F_O$ CSA tensor orientation angle $\beta$ is arbitrarily changed by 90°, the equilibrium intensity increased by 20% and the buildup rate also increased (Fig. 7b), indicating that certain CSA orientations facilitate polarization transfer \(^{48}\). Changing the $F_O$–$F_P$ dipolar vector orientation by 90° while holding the CSA tensor orientation constant also affected the buildup curves. To investigate how relayed transfer and dipolar truncation affects polarization transfer, we compared the $F_O$–$F_P$ buildup curves between conformer 1 and conformer 2 (Fig. 7a), which differ by a phenylene ring flip. The $F_O$–$F_P$ distance is unchanged by the ring flip while the $F_O$–$CF_3$ distance is significantly shorter in conformer 2 (6.8 Å) than in conformer 1 (9.5 Å). Fig. 7c shows that the $F_O$–$F_P$ buildup equilibrates to a lower intensity in conformer 2 than in conformer 1, suggesting that dipolar truncation by $CF_3$ reduces the magnetization transfer from $F_O$ to $F_P$. When the $CF_3$ group is removed in conformer 1, the buildup curve equilibrated to a lower intensity than in the $CF_3$–containing case, suggesting that relayed transfer from $F_P$ to $CF_3$ facilitates $F_O$–$F_P$ polarization transfer by depleting the $F_P$ magnetization. Therefore, the position of the $CF_3$ group relative to $F_P$ and $F_O$ influences whether polarization transfer equilibrates to a higher intensity by relayed effects (conformer...
1) or to a lower intensity due to dipolar truncation (conformer 2). Although the buildup intensities are impacted by the third spin, Fig. 7c shows that the initial buildup rates are mostly unaffected by the multi-spin effects, suggesting that one can extract the primary distance in the presence of additional fluorines. This holds true as long as relayed transfer is slower than the primary transfer mechanism, i.e. the relay spin should not be very close to one of the two spins of interest.

To investigate how δ-pulse RFDR and fpRFDR differ in their distance resolution and sensitivity to the 19F CSA tensor orientation, we simulated the buildup curves of a two-spin system, using the F_O and F_M spins in sitagliptin as a proxy but varying the F_O CSA tensor orientation angle β from 0° to 90° (Fig. 8). The buildup curves were simulated for internuclear distances of 5.4 Å to 9 Å under different MAS frequencies and 19F rf field strengths. Fig. 8a shows buildup curves for 15 kHz MAS and 83 kHz 19F rf fields (f = 9%), representing the δ-pulse RFDR limit. It can be seen that the buildup curves are easily distinguishable with better than 0.5 Å resolution, and the distinction is much larger than the spread caused by the CSA tensor orientation, consistent with the experimentally measured distance accuracy for 14.9 kHz MAS (Fig. 5). However, the low spectral sensitivity at 14.9 kHz MAS due to CSA sidebands and fast relaxation makes this condition untenable for distance measurements. At 25 and 38 kHz MAS for pulse fractions of 15% and 30%, simulations indicate larger sensitivity to the 19F CSA, with a distance resolution of 1 Å when the tensor orientation is unknown (Fig. 8b, c). This distance accuracy is still better than seen in the experimental data (Fig. 5) due to the absence of relayed transfer in these two-spin simulations. Further increasing the MAS rate to 60 kHz and comparing two rf fields of 50 kHz (f = 60%) (Fig. 8d) and 125 kHz (f = 24%) (Fig. 8e), we found reduced dependence on the CSA tensor orientation and an improved distance resolution of better than 0.5 Å. The polarization transfer rates are faster with weak rf pulses than with strong and short pulses, which can be understood as follows: under fast MAS, the CSA is increasingly averaged out and δ-pulse RFDR polarization transfer can no longer occur by the n = 0 rotational resonance mechanism 29,48. Moreover, transfer via the n = 1 and n = 2 rotational resonance conditions is inefficient for the aromatic fluorines F_O and F_M given their relatively small isotropic chemical shift difference of ~12 kHz, compared to the MAS frequency of 60 kHz (Fig. 8d, e). In contrast, fpRFDR is unaffected by isotropic shift differences 43–44 and provides an efficient transfer mechanism for aromatic fluorines. Thus, using weaker rf pulses is expected to benefit RFDR transfer between aromatic fluorines at fast MAS.

Fig. 8f summarizes the RFDR polarization transfer efficiency, in the absence of relaxation, as a function of MAS rate and 19F rf field strength. The contour levels show the intensities at 20 ms RFDR mixing for a 19F–19F distance of 10 Å. Note that the contour map reflects the fraction of the transferred polarization, not the actual intensity of the spectrum since relaxation is not taken into account. It can be seen that high transfer is achieved with δ-pulse slow-MAS RFDR and with fpRFDR at 60 kHz MAS. To represent the experimental sensitivity, we next included relaxation effects by interpolating the 19F relaxation times from the experimental data acquired at 14.9, 25, 38 and 55 kHz MAS using 83 kHz 19F pulses. A stretched sigmoid function, $T_{RFDR} = a + b(1 + \exp(-a(\nu_{MAS} - \nu_0)/c))$ was used to interpolate the relaxation times, and the simulated polarization transfer efficiency at $\nu_1 = 83$ kHz was...
corrected by the relaxation factor according to $c_{rel} = \exp(-t_{mix}/T_{RFDR})$. These intensities are shown as an orange line in Fig. 8f. It can be seen that increasing the MAS frequency from 40 to 60 kHz increases the spectral sensitivity of the transferred polarization by up to two-fold.

Taken together, these simulations indicate that fast-MAS fpRFDR is the regime of choice for $^{19}$F spins with small isotropic shift differences, as it allows both efficient polarization transfer with little signal loss and high distance accuracy, without significant sensitivity to $^{19}$F CSA tensor orientations. At intermediate MAS rates of 25–40 kHz and using strong rf pulses, the polarization transfer rates decrease for aromatic F–F polarization transfer, and the CSA tensor orientations should be included to obtain accurate distances. Slow MAS RFDR measurements are not desirable as they suffer from considerable signal loss. For spins with large isotropic shift differences such as CF$_3$–aromatic pairs, δ-pulse RFDR can work well even under fast MAS, with potentially faster polarization transfer than fpRFDR. For sitagliptin at 55 kHz MAS, we observed faster aromatic-CF$_3$ transfer using 83 kHz pulses compared to 62.5 kHz pulses (data not shown).

Using the sensitivity of $^{19}$F RFDR on CSA tensor orientation, we investigated the relative orientation of the phenylene and naphthyridine rings in PNC (Fig. S7a, c). Changing the phenylene ring orientation changed the F$_N$–F$_O$ distance from 4.8 Å to 8.2 Å, the relative orientation of the CSA and dipolar tensors, and the contribution of relayed transfer to polarization exchange. Planar conformations with $\epsilon = 0^\circ$ and $\epsilon = 180^\circ$ can be ruled out due to steric clashes (Fig. S7a). Notably, the simulated curves contradict the measured curves for $\epsilon = 0^\circ$ (Fig. S8). Instead, good agreement with experimental data was obtained using a superposition of $\epsilon = 30^\circ$ and $150^\circ$ buildup curves, suggesting that the phenylene ring is moderately out of, but still close to, the plane from the naphthyridine ring.

**Comparison of RFDR and spin diffusion for $^{19}$F–$^{19}$F distance measurements**

It is useful to compare the relative merits of spin diffusion and RFDR for measuring nanometer $^{19}$F–$^{19}$F distances. $^1$H-driven $^{19}$F spin diffusion has the advantages of simplicity, low rf power, and insensitivity to the $^{19}$F CSA tensor orientation. $^{19}$F polarization transfer using the CORD sequence gave exchange rates that correlate well with internuclear distances once isotropic chemical shifts are taken into account. However, for distances above 1.5 nm, spin diffusion mixing times of ~500 ms are required under moderate MAS. This not only increases the experimental time but also reduces the spectral sensitivity due to $^{19}$F $T_1$ relaxation, which is usually faster than $^{13}$C $T_1$ relaxation. Moreover, at 60 kHz or faster MAS, the spin diffusion mechanism is progressively quenched. In comparison, RFDR has the key advantage that it gives MAS-independent polarization transfer. For $^{19}$F RFDR at 20–40 kHz MAS, the $^{19}$F chemical shift tensor orientation should be taken into account in simulations if the goal is to extract accurate distances from the buildup curves. This CSA tensor orientation dependence makes $^{19}$F RFDR a potential tool for structural refinement through both distance and orientational restraints. At 60 kHz or faster MAS, the CSA orientation dependence decreases, and accurate distances can be measured in both the fpRFDR regime and the intermediate duty cycle regime. Taken together, $^{19}$F spin diffusion is well suited for pure distance extraction at MAS rates below ~40 kHz, while RFDR is advantageous under fast MAS and with additional information about molecular orientation.
Conclusion

The current experiments and simulations demonstrate that $^{19}$F RFDR is highly efficient in producing cross peaks for $^{19}$F–$^{19}$F distances of ~10 Å under fast MAS at high magnetic fields. Most cross peaks measured in this study reach equilibrium intensities well within 5 ms under 15–55 kHz MAS, in the absence of $^1$H decoupling. Therefore, $^{19}$F RFDR provides an efficient and high-sensitivity method to measure $^{19}$F–$^{19}$F distances of 1–2 nm. The polarization transfer efficiency of δ-pulse RFDR decreases with increasing MAS rate, whereas the transfer efficiency of fpRFDR increases with the MAS rate. Moreover, fast MAS slows down $^{19}$F relaxation, which increases the spectral sensitivity, thus fpRFDR under fast MAS is the method of choice for aromatic fluorines. The large $^{19}$F CSAs affect the RFDR polarization transfer rates in a MAS- and pulse-length dependent manner, exerting the strongest influence for rf duty cycles of 15–25%. Multi-spin effects can either speed up or slow down polarization transfer by relayed transfer or dipolar truncation. Distance accuracy of better than 0.5 Å can be achieved for isolated spin pairs under either δ-pulse RFDR at slow MAS or fpRFDR under fast MAS, where the CSA tensor orientation dependence is the smallest. These features make $^{19}$F RFDR a promising tool for measuring nanometer-range $^{19}$F–$^{19}$F distances to constrain protein structures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
2D $^{19}$F RFDR data of sitagliptin. (a) Molecular structure and F–F distances in sitagliptin. The CF$_3$ distances to the aromatic fluorines are considered from the center of gravity of the three methyl fluorines. (b) Representative 2D RFDR spectrum and 1D cross sections, measured at 38 kHz MAS with a mixing time of 2.53 ms. Spinning sidebands (ssb) are indicated by asterisks in the CF$_3$ cross section. (c) Buildup curves for the four fluorines. Best-fit single-exponential curves and time constants are indicated along with the distances.
Figure 2. 2D $^{19}$F RFDR data of f-MLF. (a) Molecular structure. An effective fluorine atom (black) is shown at the center of mass of CF$_3$ to indicate the CF$_3$–F distance. (b) Representative 2D spectrum and 1D cross sections, measured at 25 kHz MAS with a mixing time of 5.12 ms. (c) Buildup curves. The CF$_3$ → F transfer has small amplitude due to dipolar truncation by the strongly coupled $^{19}$F spins within the methyl group.
Figure 3.
2D RFDR data of PNC. (a) Molecular structure and F–F distances in PNC. (b) Representative 2D RFDR spectrum, measured at 38 kHz MAS with a mixing time of 3.37 ms. (c) Buildup curves for the three fluorines. Best-fit single-exponential curves and buildup time constants are indicated along with the inter-fluorine distances.
Figure 4.
Dependence of $^{19}$F RFDR polarization transfer on MAS rate and $^{19}$F pulse strength. (a) Representative 2D spectra of sitagliptin under 14.9, 25, 38 and 55 kHz MAS at mixing times of 2.33–2.68 ms. All spectra were measured with a $^{19}$F rf field of 83 kHz without $^1$H decoupling during the mixing period. The extra diagonal peaks in the 55 kHz spectrum results from a dynamic conformation of sitagliptin due to excess solvent in the sample. (b) CF$_3$ cross sections of the 2D spectra at the four MAS frequencies, illustrating the dramatic differences in the number of spinning sidebands and hence spectral sensitivities. (c) F$_M$→F$_O$ buildup curves at 38 kHz MAS measured with $^{19}$F rf fields of 83 kHz and 63 kHz. The weaker rf field slightly increased the polarization transfer rate. (d) Total 2D spectral intensity as a function of mixing time for two $^{19}$F rf fields under 38 kHz MAS. The weaker rf field
accelerated relaxation. (e) Total 2D spectral intensity as a function of mixing time under different MAS rates. Fast MAS slows down relaxation. (f) $\text{CF}_3 \rightarrow \text{FO}$ and $\text{FM} \rightarrow \text{FP}$ RFDR buildup curves at different MAS rates.
Figure 5.
Correlation of measured $^{19}$F RFDR buildup rates with distances. (a) Buildup rates of sitagliptin, f-MLF, and PNC at 38 and 25 kHz MAS, measured with a $^{19}$F rf field strength of 83.3 kHz. The rates show an $r^{-3}$ dependence and a distance accuracy of $2\sigma = 3.0$ Å. Larger deviations occur due to CSA and multi-spin effects but are comparable to $2\sigma = 3$ Å. Buildup rates involving CF$_3$ groups were divided by a factor of 1.65 to account for the three spins in CF$_3$. Data points with distance error bars result from conformational distribution in the model compound. (b) Buildup rates of sitagliptin at 14.9 kHz MAS. There is less scatter in $k_{RFDR}$ but the random-noise error bars are larger compared to the 38 kHz and 25 kHz data due to lower sensitivity at slower MAS. For clarity, the error bar for the data point marked with an asterisk ($^*$; $\sigma = 45$ ms$^{-1}$) is not shown.
Figure 6.
Comparison of experimental and simulated $^{19}$F RFDR buildup curves for sitagliptin at 25 kHz MAS (a, b) and 38 kHz MAS (c, d). The $^{19}$F rf field strength was 83 kHz. (a, c) Aromatic-to-aromatic transfer. (b, d) CF$_3$-to-aromatic transfer. Simulations took into account the $^{19}$F CSA tensor and used a single effective fluorine with 1.65-fold dipolar coupling to represent the CF$_3$ group. Open and closed symbols represent two directions of polarization transfer, measured from two sides of the spectral diagonal. Simulations yielded symmetric
transfer rates, which is attributed to the use of a single effective CF$_3$ spin. To account for this, simulated intensities were rescaled to the plateau value of the experimental curves.
Figure 7.
Influence of $^{19}$F CSA tensor orientation and relayed transfer on $^{19}$F→$^{19}$F RFDR polarization transfer. The sitagliptin F<sub>O</sub>→F<sub>P</sub> data is simulated. (a) Schematics of fluorine positions for two conformers and for conformer 1 without the CF<sub>3</sub> group. (b) Effect of the $^{19}$F CSA and dipolar tensor orientations on RFDR at 25 and 38 kHz MAS. The F<sub>O</sub> CSA tensor polar angle $\beta$ and the F<sub>O</sub>→F<sub>P</sub> dipolar tensor orientation were arbitrarily varied by 90°. Both affected the F<sub>O</sub>→F<sub>P</sub> polarization transfer in the conformer 1 structure. (c) Effect of relayed transfer through CF<sub>3</sub> on F<sub>O</sub>→F<sub>P</sub> RFDR (4.7 Å). Conformers 1 and 2 results in different buildup
curves despite the same $F_O-F_P$ distance. The presence of the CF$_3$ group increases the equilibrium intensity of RFDR transfer.
Figure 8.
Impact of the MAS frequency and $^{19}$F rf field strength on RFDR polarization transfer. (a–e) Simulated buildup curves for 5.4 Å, 6 Å, 7 Å, 8 Å, and 9 Å distances in a two-spin system. The CSA and dipolar tensor orientations of the $F_O$–$F_M$ pair in sitagliptin were used. The $F_O$ CSA tensor angle $\beta$ was varied from $0^\circ$ to $90^\circ$ in $30^\circ$ steps to examine the effect of CSA tensor orientation on RFDR buildup. (a) Buildup curves for 15 kHz MAS and a $^{19}$F rf field of 83 kHz, corresponding to an rf duty cycle of 9%. (b) Buildup curves for 25 kHz MAS with a $^{19}$F rf field of 83 kHz ($f = 15\%$). (c) Buildup curves for 38 kHz MAS with a $^{19}$F rf field of 62.5 kHz ($f = 30\%$). (d) Buildup curves for 60 kHz MAS with a $^{19}$F rf field of 50 kHz ($f = 60\%$). (e) Buildup curves for 60 kHz MAS with a $^{19}$F rf field of 125 kHz ($f = 24\%$). The 20 ms intensity is indicated as a dashed line in each panel to guide the eye for the relative transfer rates. (f) Fraction of transferred magnetization at 20 ms mixing for a 10 Å distance as a function of MAS frequency and rf field strength (contour map). The experimentally accessible cross peak intensity with 83 kHz $^{19}$F pulses is shown as an orange line. The contour map reports the relaxation-free transfer efficiency, while the intensity curve includes relaxation during RFDR mixing. The fastest polarization transfer occurs under the slow-MAS $\delta$-pulse RFDR limit and the fast-MAS fpRFDR limit, but the highest sensitivity, particular for polarization transfer between similar isotropic chemical shifts, is found using fpRFDR fast-MAS.