Communication

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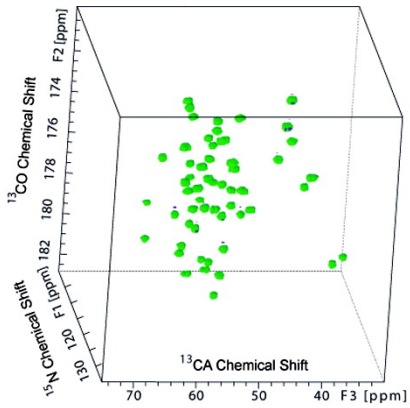

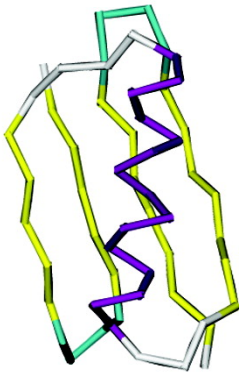

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Backbone Assignments in Solid-State Proteins Using *J*-Based 3D Heteronuclear Correlation Spectroscopy

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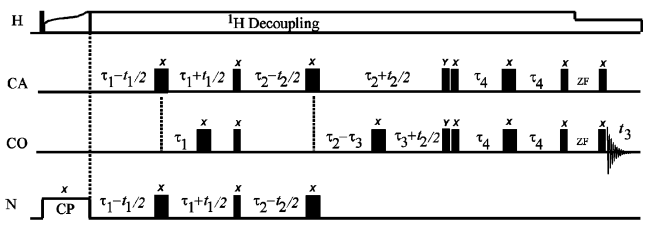
Solid-state NMR spectroscopy is emerging as a mainstream tool in structural biology due to its unique capability to yield atomic-level information in macroscopically disordered systems, such as fibrils,[1] macromolecular assemblies,[2] and membrane proteins.[3] Resonance assignments are an essential first step of structural studies, and in all studies to date, these have employed through-space, dipolar-driven correlation spectroscopy.[4] Scalar-coupling driven correlation in solids has been reported by several groups,[5,6] but so far heteronuclear (^{13}C, ^{15}N) scalar-based spectroscopy has not been demonstrated, despite its potential for improving backbone chemical shift assignment procedures.

Recently we reported that through-bond homonuclear ^{13}C correlation spectroscopy can be implemented in a highly efficient manner for the assignment of protein side-chain resonances with solid-state NMR.[6] Here we show that heteronuclear 3D correlation experiments can similarly be implemented using purely scalar-based transfers for the assignment of backbone resonances. Again we find substantially increased spectral resolution without compromising sensitivity, which we find to be comparable to, or better than, that of dipolar methods. We illustrate this on two proteins, the β1 immunoglobulin binding domain of protein G (GB1) and reassembled thioredoxin (TRX).

Our approach makes use of the 3D *J*-based MAS experiments shown in Figure 1. These experiments are inspired by their solution-state counterparts, particularly as implemented by Bertini and coworkers.[7] In the MAS versions, the indirect evolution periods are rotor synchronized along with the τ refocusing periods. As in liquid-state, the combined constant-time evolution and mixing period improves resolution through homonuclear and heteronucler decoupling in the indirect dimension, giving increased sensitivity when the scalar couplings are partially or fully resolved.

Figure 2 shows the application of the *J*-MAS NCOCA, NCACO, and CANCO to uniformly-^{13}C,^{15}N-enriched GB1. This combination of experiments allows an unambiguous assignment of the full protein backbone, one part of which is traced out in the figure. As in solution, both one- and two-bond correlations are observed in the NCA transfers, with the one-bond transfer being more intense. Under 25 kHz magic angle spinning (MAS) and 150 kHz proton decoupling (conditions where T_2' is maximized[8] and residual dipolar couplings suppressed[9]), we find strong backbone correlations consistently throughout the spectra, with sensitivity comparable to dipolar-driven correlation using SPECIFIC CP (for NC transfers) and DARR (for CC spin diffusion transfers), albeit at necessarily lower MAS rates. Most dramatically, we find significantly improved resolution, with a reduction in line widths in the indirect dimension

(a) *J* – MAS NCACO (and NCOCA)



(b) *J* – MAS CANCO

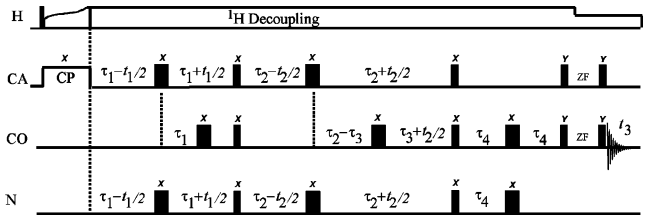


Figure 1. Constant-time *J*-MAS NCACO, NCOCA, and CANCO 3D heteronuclear correlation experiments. In these pulse sequences, thin vertical lines indicate $\pi/2$ pulses and wide vertical lines indicate π pulses that are either selective (single carbon channel) or broadbanded (shown as simultaneous CA and CO pulses). The NCOCA experiment is obtained by flipping the CA and CO channels shown in part a. The indirect evolution increments, τ refocusing periods, and z-filter (zf) are all rotor synchronized.

to 30−40 Hz for both carbon and nitrogen. Even under more routine experimental conditions, such as 14.7 kHz MAS and 90 kHz decoupling used for TRX and GB1 in Figures S1 and S2 of the Supporting Information, we find that sensitivity of the 3D experiments is nevertheless adequate for acquisition of high-quality spectra. More importantly, even under these moderate decoupling conditions the resolution is significantly improved compared with the dipolar experiments by the elimination of the heteronuclear and homonuclear couplings.

On the basis of the experimentally measured T_2' relaxation rates of 104 ms for backbone ^{15}N, 96 ms for ^{13}CO, and 36 ms for ^{13}CA, we estimate theoretically that over half of the intensity loss in these experiments is still due to relaxation at our experimental conditions of 25 kHz MAS and 150 kHz ^{1}H decoupling. Yet these experiments are competitive with dipolar methods in terms of overall sensitivity, and at even faster MAS rates and/or with deuteration[10] (where more efficient decoupling becomes possible at lower power), we anticipate further improvements in both sensitivity and resolution. We note that dipolar and scalar experiments offer complementary information; homonuclear dipolar mixing conditions can be adjusted to yield additional sequential correlations to validate the resonance assignments, while certain cross-peaks that are attenuated in the

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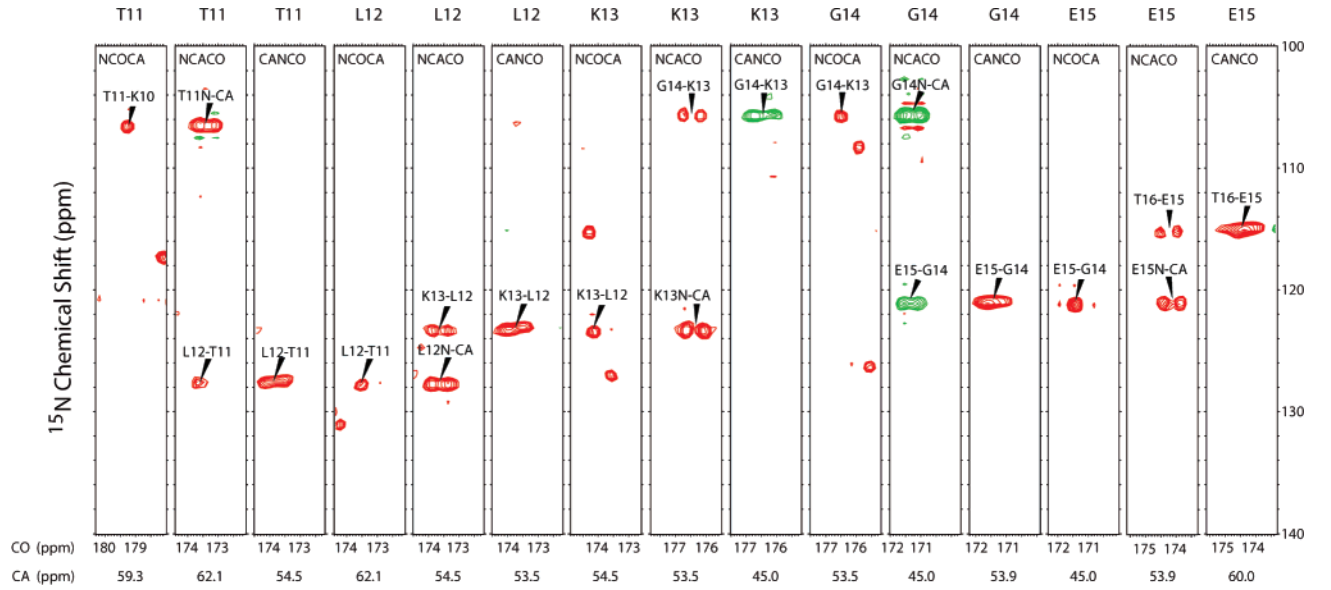

Figure 2. The backbone walk in uniformly-^{13}C,^{15}N-enriched GB1[13] using the *J*-MAS NCACO, NCOCA, and CANCO experiments (Figure 1). Data were acquired on a 9.4 T Bruker DSX spectrometer (^1H frequency 400.13 MHz) equipped with a triple resonance 2.5 mm MAS probe spinning at a MAS rate of 25 kHz. 80 kHz ^{13}C and 50 kHz ^{15}N pulses were used throughout for the hard pulses along with 150 kHz SPINAL64 ^1H decoupling[11] during the constant-time intervals and 100 kHz decupling during the 16 ms z-filter and acquisition (t_3). r-SNOB pulses of 180 and 420 μs were used for the selective α- and carbonyl-π pulses, respectively, and were rotor synchronized as described in ref 12. In all experiments, the carbon carrier was centered in the α region and the selective carbonyl pulses were implemented using a phase modulation. Experiment specific parameters for NCACO: $\tau_1 = 9.6$ ms, $\tau_2 = 12.8$ ms, $\tau_3 = \tau_4 = 4.4$ ms; 4 scans, 1024 complex points in t_3 (total acquisition time 34.1 ms), 128 complex points in t_2 (total acquisition time 20.48 ms), and 38 complex points in t_1 (total acquisition time 18.24 ms); recycle delay of 3 s for a total experiment time of 65 h. Acquisition parameters for NCOCA: $\tau_1 = \tau_2 = 11.2$ ms, $\tau_3 = 5.2$ ms, and $\tau_4 = 3.2$ ms; 4 scans, 512 complex points in t_3 (total acquisition time 20.5 ms), 45 complex points in t_2 (total acquisition time 21.6 ms), and 45 complex points in t_1 (total acquisition time 21.6 ms). Recycle delay, 4 s; total experiment time, 36 h. Acquisition parameters for CANCO: $\tau_1 = 12.8$ ms, $\tau_2 = \tau_3 = 13.2$ ms, and $\tau_4 = 11.6$ ms; 2 scans, 1024 complex points in t_3 (total acquisition time 17.1 ms), 38 complex points in t_2 (total acquisition time 18.24 ms), and 128 complex points in t_1 (total acquisition time 20.48 ms). Recycle delay. 4 s; total experiment time. 45 h. All three spectra were processed with 10 Hz line broadening in the directly detected t_3 dimension and no apodization in the indirect t_1 and t_2 dimensions.

dipolar experiments because of motion are present in the scalar correlations. Our results demonstrate that scalar-based methods are sufficiently well-developed to serve as a complementary tool to dipolar methods, which will be especially useful for assignment of large proteins, where resonance overlap presents a major challenge to solid-state NMR. On the basis of our sensitivity, we anticipate that assignment of proteins in the range of 30−40 kDa should be feasible with current technology, even at 9.4 T, while moving to higher fields will extend this range even further.

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Supporting Information Available: Pulse sequences with full phase cycles and optional off-resonance pulse compensation; experimental T_2' vs MAS rate and decoupling power; 1D traces from 3D spectra and anticipated upper size limit for protein correlation; 14.1 T spectra of thioredoxin and GB1. This material is available free of charge via the Internet at http://pubs.acs.org.

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