J|A|C|S
COMMUNICATIONS

Published on Web 10/31/2007

Resolution-Enhanced 4D $^{15}N/^{13}C$ NOESY Protein NMR Spectroscopy by Application of the Covariance Transform

David A. Snyder,[†] Yingqi Xu,[‡] Daiwen Yang,[‡] and Rafael Brüschweiler*,[†]

Department of Chemistry and Biochemistry & National High Magnetic Field Laboratory, Florida State University, Tallahassee, Florida 32306, and Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543

Received July 24, 2007; E-mail: bruschweiler@magnet.fsu.edu

The application of NMR-based structure determination to increasingly larger and more complex systems presents many challenges, one of which is spectral crowding. As a remedy, the introduction of new dimensions over which signals are spread out renders them better interpretable. First 3D and more recently 4D NMR experiments, in particular, 4D NOESY experiments, have significantly extended the reach of NMR spectroscopy leading to high-resolution structures of ever more complex protein systems.[1] The introduction of the fourth dimension, however, comes at a cost of decreased resolution along one or more of the indirect dimensions, increased measurement time, or both.[2]

Covariance NMR[3−5] is a powerful method to endow the indirect dimension of a 2D NMR dataset with the same high resolution as the direct dimension. Covariance spectroscopy is particularly suitable for spectra that, under ideal sampling conditions, are symmetric with respect to the diagonal. Therefore, it was possible to apply the covariance method to a 4D ^{13}C-edited NOESY spectrum and convert it into a 4D spectrum that is fully symmetric with respect to $^{1}H-^{13}C$ donor and $^{1}H-^{13}C$ acceptor pairs.[6]

On the other hand, a 4D $^{13}C/^{15}N$ NOESY spectrum (e.g., a ^{13}C HSQC NOESY ^{15}N HSQC) contains structurally highly informative correlations between $^{1}H-^{15}N$ and $^{1}H-^{13}C$ moieties but cannot be covariance processed because it lacks the aforementioned symmetry property. Instead, four different 4D experiments that reflect all possible pathways among the $^{1}H-^{13}C$ and $^{1}H-^{15}N$ moieties are required: 4D $^{13}C/^{13}C$ NOESY, 4D $^{13}C/^{15}N$ NOESY, 4D $^{15}N/^{13}C$ NOESY, and 4D $^{15}N/^{15}N$ NOESY.

While recording these four 4D spectra by traditional means is unduly time-consuming and therefore unrealistic under most circumstances, the simultaneous recording of all four experiments in a single 4D NOESY has very recently been demonstrated[7] by employing a shared-evolution strategy[8] along the heteroatom dimensions. This experiment can be performed at the same resolution and without any substantial increase in measurement time over a "traditional" 4D NOESY.[7]

This communication reports the generalization of 4D covariance NMR to the extraction of NOE distance information between $^{1}H-^{15}N$ and $^{1}H-^{13}C$ groups from a shared-evolution $^{15}N/^{13}C$-edited NOESY spectrum of ubiquitin recorded in 96 h.[7] This results in a substantial resolution enhancement that allows for the extraction of additional peaks, some of which provide long-range structural restraints independent of any prior structural knowledge.

The covariance transform of a 4D spectrum is applied here after Fourier transform of both the direct proton dimension and the dimension of the heteroatoms ("acceptor heteroatom") attached to the protons of the direct dimension. Associated with each "acceptor pair" of chemical shifts is a "donor plane".

The latter contains cross-peaks for every covalently attached heteroatom/proton pair for which the proton provides an NOE to the given acceptor proton. Note that, as a consequence of Parseval's theorem, the covariance result is independent of whether the donor dimensions are in the time or frequency domain.[3,4] It is straightforward to reorder the data of each donor plane into a (column) vector, with one such vector associated with each acceptor pair. The covariance transform of the resulting matrix $\mathbf{M}$ is given by

$$\mathbf{C} = (\mathbf{M}^{\dagger}\mathbf{M})^{1/2} \tag{1}$$

where the dagger (†) indicates conjugate transposition if the donor planes are in the time domain and hyper-complex (or mere transposition when the donor planes are in the frequency domain and real-valued). A Matlab program implementing 4D covariance NMR, which is available from the authors, uses singular value decomposition (SVD) to calculate the matrix square-root operation in a computationally efficient manner in full analogy to 2D covariance NMR.[5]

Figure 1 demonstrates the resolution enhancement covariance NMR provides for the shared-evolution $^{15}N/^{13}C$-edited NOESY spectrum. Covariance NMR visibly sharpens the donor plane cross sections of NOESY cross-peaks and isolates additional peaks compared with the FT spectrum. Although it does not increase the digital resolution along the acceptor dimensions, covariance NMR nonetheless also improves their practical resolving ability. Due to the necessarily finite resolution of the acceptor heteroatom dimension of 4D NOESY spectra, NOESY cross-peaks often "leak" into adjacent donor planes. The sharpening effect of covariance NMR can resolve NOESY cross-peaks otherwise lying under the shoulders of intense cross-peaks in adjacent planes. For example, in Figure 1C, covariance NMR, by sharpening the V70HB/L71H NOESY cross-peak, isolates the Q2HG3/E16H signal as a distinct peak yielding a long-range constraint unobtainable from the 4D FT spectrum alone.

Overall, covariance NMR results in the resolution of 102 additional, true peaks, which are not distinct local peak maxima in the 4D FT spectrum. The enhanced resolution of covariance NMR spectra also leads to more precisely determined peak locations, resulting in an increased number of peaks uniquely assignable even in the absence of prior structural information. Even with the ability of covariance NMR to extract additional peaks from unresolved features in the FT spectrum, covariance NMR does not result in an appreciable false-positive rate (Table 1). The 4D covariance approach applied to the 23.8 kDa protein DdCAD-1[7] has a similar relative performance compared to the 4D FT spectrum (see Table 1 and Supporting Information).

The resolution enhancement provided by covariance NMR is substantial: compared to the fully processed FT spectrum (including linear prediction and zero filling), covariance NMR increases the number of donor points in each plane by a factor of approximately five. An FT spectrum with this resolution would require a measurement time of ~20 days. Covariance NMR, by obviating the requirement for a Fourier transform in the donor dimensions,

[†] Florida State University.
[‡] National University of Singapore.

10.1021/ja075533n CCC: $37.00 © 2007 American Chemical Society

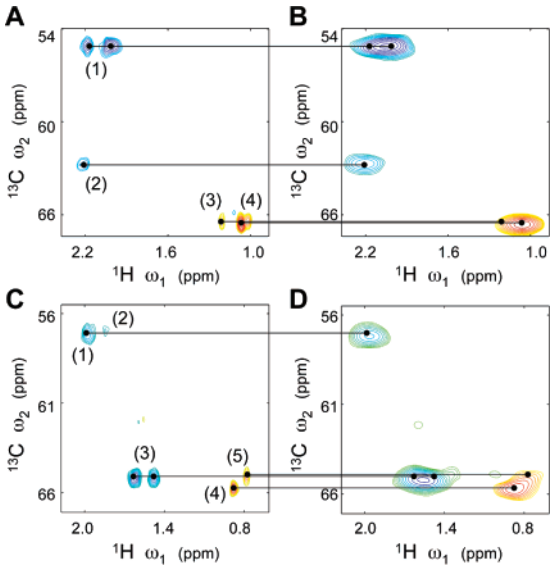

Figure 1. Comparison between shared-evolution 4D ^{13}C/^{15}N-edited NOESY donor planes of ubiquitin before (B,D) and after (A,C) covariance processing. (A, B) A region of the donor plane associated with the T55-NH acceptor pair ($\omega_4 = 8.832$, $\omega_3 = 108.6$ ppm). The covariance spectrum distinguishes (1) between the R54HB2/T55H and R54HB3/T55H signals, sharpens (2) the D58HB3/T55H peak, and resolves the long-range (3) T22HG2/T55H peak from the intraresidue (4) T55HG2/H peak. (C, D) A region of the L71-NH donor plane ($\omega_4 = 8.104$, $\omega_3 = 123.3$ ppm). Covariance NMR sharpens the (1) V70HB/L71H signal isolating the small long-range (2) Q2HG3/E16H signal as a distinct peak, centered in a nearby plane. Covariance NMR also resolves (3) NOESY cross-peaks between each of the geminal HB protons of L71 and L71H as well as the (4) V70HG1/L71H and (5) V70HG2/L71H NOESY cross-peaks. The 4D dataset was recorded at $T = 298$ K with 18 complex points in each heteroatom dimension and 26 complex points in the indirect (donor) proton dimension, which took 96 h to record on a 500 MHz Bruker spectrometer equipped with a cryoprobe. Measuring the acceptor heteroatom dimension, as well as the donor carbon chemical shifts, with a sweep width of 22 ppm ensured adequate resolution along the heteroatom dimensions, with the sign (indicated by yellow-red vs green-blue contouring) alternating with the N-fold aliasing of the signal.[7]

Table 1. Comparative Evaluation of Peaks between the FT and Covariance Spectra of Ubiquitin and DdCAD-1[a]

		FT	covariance
peaks in ubiquitin[b]	true[c]	459	561
	false[c]	9	37
	false positive (FP) rate (%)	1.9	6.2
unambiguous assignments[b,d]	total	223	309
	long range[e]	26	44
DdCAD-1[b]	FP rate (%)[f]	4.0	10.4

[a] Picked and assigned without the use of a 3D structural model. [b] Only those peaks extracted from features common to both the FT and covariance spectra are considered. [c] True if chemical shifts of peak correspond to an effective interproton distance of <5Å in PDB[9] entries 1G6J[10] or 1D3Z[11] and false otherwise. [d] Number of peaks that can be assigned to a unique valid structural constraint and for which no other assignments are possible at reasonable match tolerances (0.035 ppm for the direct ^{1}H dimension in the FT and both ^{1}H dimensions in the covariance spectrum, 0.05 ppm for the indirect ^{1}H dimension in the FT spectrum, and 0.5 ppm for heteroatoms). [e] A peak between residues i and j with $|i - j| \geq 5$. [f] Percentage of peaks not corresponding to an effective interproton distance of <5Å in the PDB[9] entry 1YHP.[12]

also potentially allows for the use of nonuniform sampling schemes that can further decrease the measurement time required to obtain a high-resolution spectrum.[13]

The resolution enhancement imparted by covariance NMR provides several improvements over the FT spectrum. First, the visibly sharper spectral features potentially allow for the analysis of spectra at tighter chemical shift tolerances for assignment: the median deviation of the donor ^{1}H shifts from their average chemical shift values is reduced from 0.02 to 0.01 ppm. Larger proteins having more complicated spectra benefit from the ability to make assignments using tighter tolerances as these result in a greater yield of unambiguously assigned NOESY cross-peaks (even prior to initial structural analysis) leading to more straightforward structure calculations. Second, as shown in Figure 1, covariance NMR resolves peaks leading to additional long-range constraints which are unidentifiable in the lower (donor) resolution FT spectrum. Although a 4D FT signal unresolved in its donor (e.g., ^{1}H$-^{13}$C) cross section may have its symmetric signal resolved in the acceptor (^{1}H$-^{13}$C) cross section, the symmetric signal can have an unresolved donor cross section too, which makes identification of the signal as well as its symmetric counterpart difficult. Covariance NMR, on the other hand, resolves both cross sections simultaneously.[6] Third, covariance NMR resolves many pairs of peaks associated with geminal proton pairs nearby a common acceptor proton, facilitating stereospecific assignments.

The shared-evolution method embeds, with little or no additional measurement time, an asymmetric 4D ^{15}N/^{13}C-edited NOESY experiment, in a symmetric experiment amenable to covariance transformation. The substantial resolution enhancement achieved in this way allows for the extraction of a large number of additional NOESY cross-peaks. The results demonstrate the ability of covariance NMR to substantially enhance spectral information and speed up the collection of 4D NOEs involving amide and carbon protons, thus indicating the power of shared-evolution covariance spectroscopy as a tool for efficient, high-resolution NMR-based studies of protein structure.

Acknowledgment. The authors acknowledge the support of BMRC Grant R154000272305 (Singapore) to D.W.Y. and of NIH Grant GM066041 to R.B.

Supporting Information Available: Figure comparing 4D covariance and 4D FT NOESY spectra of DdCAD-1. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Clore, G. M.; Gronenborn, A. M. *Science* **1991**, *252*, 1390−1399. (b) Xu, Y.; Zheng, Y.; Fan, J. S.; Yang, D. W. *Nat. Methods* **2006**, *3*, 931−937.
(2) Kim, S.; Szyperski, T. *J. Am. Chem. Soc.* **2003**, *125*, 1385−1393.
(3) Brüschweiler, R. *J. Chem. Phys.* **2004**, *121*, 409−414.
(4) Brüschweiler, R.; Zhang, F. *J. Chem. Phys.* **2004**, *120*, 5253−5260.
(5) Trbovic, N.; Smirnov, S.; Zhang, F.; Brüschweiler, R. *J. Magn. Reson.* **2004**, *171*, 277−283.
(6) Snyder, D. A.; Zhang, F.; Brüschweiler, R. *J. Biomol. NMR* **2007**, *39*, 165−175.
(7) Xu, Y.; Long, D.; Yang, D. *J. Am. Chem. Soc.* **2007**, *129*, 7722−7723.
(8) (a) Farmer, B. T.; Mueller, L. *J. Biomol. NMR* **1994**, *4*, 673−687. (b) Frueh, D. P.; Vosburg, D. A.; Walsh, C. T.; Wagner, G. *J. Biomol. NMR* **2006**, *34*, 31−40. (c) Kay, L. E.; Wittekind, M.; Mccoy, M. A.; Friedrichs, M. S.; Mueller, L. *J. Magn. Reson.* **1992**, *98*, 443−450. (d) Lin, Z.; Xu, Y. Q.; Yang, S.; Yang, D. W. *Angew. Chem., Int. Ed.* **2006**, *45*, 1960−1963. (e) Xia, Y. L.; Yee, A.; Arrowsmith, C. H.; Gao, X. L. *J. Biomol. NMR* **2003**, *27*, 193−203.
(9) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. *Nucleic Acids Res.* **2000**, *28*, 235−242.
(10) Babu, C. R.; Flynn, P. F.; Wand, A. J. *J. Am. Chem. Soc.* **2001**, *123*, 2691−2692.
(11) Cornilescu, G.; Marquardt, J. L.; Ottiger, M.; Bax, A. *J. Am. Chem. Soc.* **1998**, *120*, 6836−6837.
(12) Lin, Z.; Sriskanthadevan, S.; Huang, H.; Siu, C. H.; Yang, D. *Nat. Struct. Mol. Biol.* **2006**, *13*, 1016−1022.
(13) Chen, Y.; Zhang, F.; Bermel, W.; Brüschweiler, R. *J. Am. Chem. Soc.* **2006**, *128*, 15564−15565.

JA075533N