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Higher-Rank Correlation NMR Spectra with Spectral Moment Filtering

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Abstract

Higher-rank correlation spectroscopy is introduced as an alternative to 3D Fourier-transform (FT) NMR spectroscopy for resonance assignment and molecular structure determination. The method combines standard 2D FT spectra that share a common frequency dimension, such as a 2D ^{13}C-^{1}H HSQC and a 2D ^{1}H-^{1}H TOCSY spectrum, and constructs higher-rank correlation spectra with ultra-high spectral resolution. Spectral overlap along a common dimension, in particular the ^{1}H dimension, is addressed by a spectral filtering method, which identifies mismatches between the 1st and 2nd moments of cross-peak profiles. The method, which provides a substantial speed-up over traditional 3D FT spectroscopy while effectively suppressing false peaks, is demonstrated for the triple-rank ^{13}C-^{1}H HSQC-TOCSY spectrum of a cyclic decapeptide with different mixing times. Higher-rank correlation spectroscopy is usefully applicable to the analysis of a wide range of NMR spectra of synthetic and natural products.

Keywords

Multidimensional NMR spectroscopy; 3D spectral reconstruction; resolution enhancement; covariance NMR; spectral moment filtering; HSQC; TOCSY; antamanide

3D and higher dimensional NMR spectroscopy has become a standard tool for the interpretation of NMR spectra.[1] For example, a 3D Fourier transform (FT) NMR spectrum has distinct advantages over a 2D spectrum, especially in the case of cross-peak overlap. However, the collection of a 3D or higher dimensional FT NMR spectrum with good spectral resolution along all dimensions is typically very time-consuming, which is why 3D NMR has not been widely applied to common chemical systems, such as natural products, synthetic organic molecules, and complex mixtures. Over the past years, a number of methods have been introduced to address this limitation, as reviewed recently.[2]

Here, we present an approach for the construction of higher-rank correlation NMR spectra for such systems from two or more 2D spectra that share common frequency dimensions. These spectra display ultra-high resolution along all dimensions requiring modest measurement times, which makes them particularly suitable for resonance assignment and structural analysis. The occurrence of false peaks is largely suppressed by using a spectral filter based on moment analysis of cross-peaks. The method is demonstrated for a pair of TOCSY[3] and HSQC[4] spectra of the cyclic decapeptide antamanide.

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Supporting Information Available: Detailed description of moment filtering method; additional 2D cross sections analogous to Figs. 1 and 2 for different TOCSY mixing times (2 figures); selected 1D traces through 3D and 3R spectra before and after filtering (2 figures). This material is available free of charge via the Internet at http://pubs.acs.org.

A higher-rank correlation spectrum **H** is constructed from a set of standard 2D FT NMR spectra **D**, **E**, **F**, **G**,..., each consisting of $N_1 \times N_2$ data points:

$$H_{ijklm\ldots} = D_{ij} E_{kj} F_{kl} G_{ml} \ldots \quad (1)$$

where indices i,k,m = 1,...,N_1 and j,l = 1,...,N_2. Note that indices that appear twice on the right-hand side run over common frequency dimensions of the pair of spectra involved. Generally, a spectrum **H** of rank N combines (N-1) 2D parent spectra. **H** spreads spin connectivities over multiple orthogonal dimensions and thereby facilitates analysis, in full analogy to high dimensional FT spectroscopy.

There are important differences, however, between the higher-rank correlation spectrum **H** of Eq. (1) and the corresponding N-dimensional (ND) FT spectrum. First, **H** has a spectral resolution along each dimension that is identical to the resolution of its 2D parent spectra. Hence for typical 2D spectra, **H** has ultra-high resolution at a measurement time that is only a small fraction of a ND FT spectrum with the same resolution. Second, in the presence of overlap between resonances along a shared dimension, **H** contains extraneous cross-peaks that do not occur in the corresponding ND FT spectrum. In order to suppress such spurious correlations, we use a spectral filtering process, which compares the mean positions (1st moments) and linewidths (2nd moments) of pairs of peaks to be multiplied with each other. For N = 3, Eq. (1) can be modified to

$$H_{ijk} = D_{ij} E_{kj} f\left(\mu_{D,ij} - \mu_{E,kj}, \sigma^2_{D,ij} - \sigma^2_{E,kj}\right) \quad (2)$$

where $\mu_{D,ij}$ and $\sigma_{D,ij}$ are the mean and the standard deviation, respectively, along ω_2 of a cross-peak (or noise) at position (i,j) in spectrum **D**. The moments are determined locally over a limited frequency interval that exceeds the typical linewidth of the spectrum. This avoids divergence issues, e.g. of the 2nd moment of a Lorentzian lineshape, over large frequency ranges and it permits the meaningful comparison of peak lineshapes of the parent spectra **D** and **E**. The filter function *f* decays smoothly from 1 to 0 if the absolute value of one or both of its arguments exceeds a given threshold (Supporting Information). Construction of a higher-rank correlation spectrum using Eqs. (1),(2) permits considerable resolution enhancement while false peaks are effectively suppressed as is demonstrated here.

Two homonuclear ^{1}H-^{1}H 2D TOCSY spectra and a ^{13}C-^{1}H 2D HSQC spectrum were recorded at 700 MHz field strength and 316 K for 0.7 mM uniformly ^{13}C-labeled cyclic decapeptide antamanide (-Val-Pro-Pro-Ala-Phe-Phe-Pro-Pro-Phe-Phe-)[5] dissolved in $CDCl_3$. TOCSY mixing used DIPSI-2[6] with mixing times of 25 and 90 ms. For comparison, standard 3D FT HSQC-TOCSY spectra[7] were collected with the same mixing times. For clarity, we term the spectrum that is reconstructed via Eq. (2) triple-rank (3R) HSQC-TOCSY.

Figure 1 shows a region of the 90 ms 2D TOCSY spectrum together with the ^{1}H-^{1}H planes of the 3D HSQC-TOCSY and the 3R HSQC-TOCSY constructed using Eq. (1) (with **D** = HSQC and **E** = TOCSY) at fixed ^{13}C frequency. The plane for the 3R spectrum is equivalent to the one of the 3D spectrum, except that it has higher spectral resolution stemming from the 2D TOCSY and HSQC parent spectra. Hence the 3R spectrum is composed of 1024 ^{13}C-edited TOCSY planes, which permit straightforward identification of proton spin systems because they lie in different ^{13}C planes. 3R HSQC-TOCSY spectra with variable mixing times further help resonance assignment (see Supporting Information).

In the event of overlap between 2 proton resonances that belong to 2 different spin systems, the 3R spectrum calculated according Eq. (1) displays extraneous cross-peaks that correlate the 2 spin systems with each other. To suppress such artifacts, the filter function *f* in Eq. (2) is applied, which identifies a potential mismatch between the 1st or 2nd moments of the proton lineshapes as is exemplified in Figure 2 (see also Supporting Information). It shows the 2 HSQC Cα–Hα cross-peaks of Pro3 and Phe5 whose proton frequencies at 4.1 ppm do overlap (Fig. 2c) and the 2D TOCSY strip (Fig. 2d) that belongs to the same proton frequency range. Cross sections through the 3D HSQC-TOCSY spectrum belonging to the ^{13}C spins attached to the 2 overlapping protons allow disambiguation of the 2 spin systems (Fig. 2a,b), although the low ^{13}C resolution leads to strong leakage from neighboring spin systems. The cross sections through the filtered 3R HSQC-TOCSY spectrum (Fig. 2g,h) unravel the spin-spin connectivities unequivocally removing any false positive correlations visible in the same cross sections of the unfiltered spectrum (Fig. 2e,f). The filter function *f* effectively discriminates between the differential line positions and linewidths of the overlapping proton resonances as is visible from the horizontal traces depicted in Fig. 2c,d. Comparison of the full 3R HSQC-TOCSY spectrum of antamanide with the 3D FT spectrum shows that out of 25 overlapping aliphatic protons 22 can be disambiguated using the filtering approach underlying Eq. (2). Overlaps that go undetected have nearly identical 1st and 2nd moments (Fig. S2) illustrating the present limitation of this approach. In the absence of molecular symmetry such cases are however notably rare. The filter function (Eq. (S3)) corresponds to a smoothed step function that is maximal when the differences between the means and variances is zero and goes to zero when the differences substantially exceed the predefined threshold values. The smoothing has the effect that the edges of the cross-peaks are not abruptly cut off so that their appearance is not distorted. The performance of the filter is notably robust with respect to the threshold parameters as they can be varied by 30%-50% without a decline of filter performance.

While reconstruction of 3D spectra from 2D projections has attracted significant attention over the recent past,[2,8] the present method is unique in that it uses standard 2D TOCSY and HSQC spectra as input, which are routinely collected in NMR studies. Without moment filtering, higher-rank correlation NMR is closely related to covariance spectroscopy[9,10] and its generalizations, including unsymmetric covariance and hyperdimensional NMR.[11-15] In fact, after summation over all indices that occur twice in Eqs. (1),(2), one obtains the unsymmetric indirect 2D covariance spectrum **C**:

$$\mathbf{C}=\mathbf{D}\cdot\mathbf{E}^{T}\cdot\mathbf{F}\cdot\mathbf{G}^{T}\ldots \quad (3)$$

While spectra **C** and **H** have in common that peak picking is postponed until peak assignment is self-evident, the 3R spectrum **H** spreads spin connectivities along the ultra-high resolution ^{13}C dimension, which conveniently sorts individual proton spin systems into different planes. The full 3R spectrum has a total size of 8.1 GBytes, which can be easily handled by computer memory available on modern desktop computers. This makes 3R spectroscopy usefully applicable to the analysis of a wide range of NMR spectra of synthetic and natural products.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Cavanagh, J.; Fairbrother, WJ.; Palmer, AG.; Skelton, NJ.; Rance, M. Protein NMR Spectroscopy: Principles and Practice. Academic Press; 2006.
2. Felli IC, Brutscher B. Recent Advances in Solution NMR: Fast Methods and Heteronuclear Direct Detection. Chemphyschem 2009;10:1356–1368. [PubMed: 19462391]
3. Braunschweiler L, Ernst RR. Coherence Transfer by Isotropic Mixing - Application to Proton Correlation Spectroscopy. J. Magn. Reson 1983;53:521–528.
4. Bodenhausen G, Ruben DG. Natural Abundance Nitrogen-15 NMR by Enhanced Heteronuclear Spectroscopy. Chem. Phys. Lett 1980;69:185–189.
5. Kessler H, Müller A, Pook KH. Assignment of All Proton, Carbon, and Nitrogen NMR Signals of Antamanide in Chloroform Solution. Liebigs Annalen 1989:903–912.
6. Shaka AJ, Lee CJ, Pines A. Iterative Schemes for Bilinear Operators; Application to Spin Decoupling. J. Magn. Reson 1988;77:274–293.
7. Bax A, Clore GM, Gronenborn AM. H-1-H-1 Correlation Via Isotropic Mixing of C-13 Magnetization, a New 3-Dimensional Approach for Assigning H-1 and C-13 Spectra of C-13-Enriched Proteins. J. Magn. Reson 1990;88:425–431.
8. Kupce E, Freeman R. Natural-Abundance 15N-13C Correlation Spectra of Vitamin B-12. Magn. Reson. Chem 2007;45:103–105. [PubMed: 17201010]
9. Brüschweiler R, Zhang F. Covariance Nuclear Magnetic Resonance Spectroscopy. J. Chem. Phys 2004;120:5253–5260. [PubMed: 15267396]
10. Zhang F, Brüschweiler R. Indirect Covariance NMR Spectroscopy. J. Am. Chem. Soc 2004;126:13180–13181. [PubMed: 15479045]
11. Blinov KA, Larin NI, Williams AJ, Mills KA, Martin GE. Unsymmetrical Covariance Processing of COSY or TOCSY and HSQC NMR Data to Obtain the Equivalent of HSQC-COSY or HSQC-TOCSY Spectra. Journal of Heterocyclic Chemistry 2006;43:163–166.
12. Kupce E, Freeman R. Hyperdimensional NMR Spectroscopy. J. Am. Chem. Soc 2006;128:6020–6021. [PubMed: 16669655]
13. Lescop E, Brutscher B. Hyperdimensional Protein NMR Spectroscopy in Peptide-Sequence Space. J. Am. Chem. Soc 2007;129:11916–11917. [PubMed: 17845051]
14. Benison G, Berkholz DS, Barbar E. Protein Assignments Without Peak Lists Using Higher-Order Spectra. J. Magn. Reson 2007;189:173–181. [PubMed: 17919953]
15. Snyder DA, Ghosh A, Zhang F, Szyperski T, Brüschweiler R. Z-matrix Formalism for Quantitative Noise Assessment of Covariance Nuclear Magnetic Resonance Spectra. J. Chem. Phys 2008;129:104511. [PubMed: 19044928]

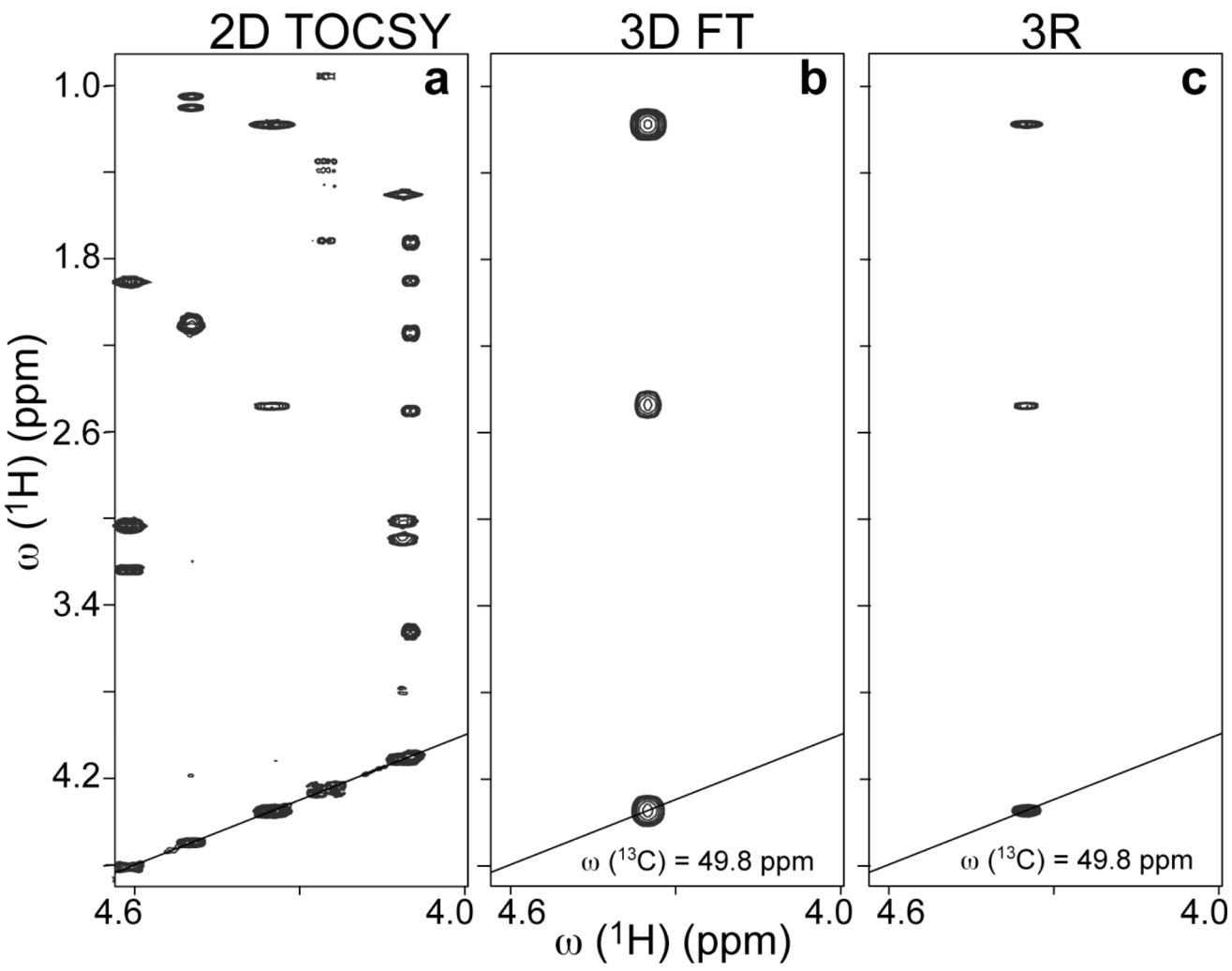

Figure 1.

Comparison between corresponding sections of (a) 2D TOCSY, (b) 3D ^{13}C-^{1}H HSQC-TOCSY and (c) triple-rank (3R) HSQC-TOCSY spectrum of antamanide that contain the Ala4 spin system. The plane of (c), which is taken at the ^{13}Cα frequency of Ala4 of 49.8 ppm, permits the same clean isolation of the Ala4 spin system as the corresponding plane (b) of the 3D FT experiment. A TOCSY mixing time τ_m=90 ms was used. The HSQC experiment was collected with 900(^{13}C)×1536(^{1}H) data points and zero-filled to 1K×2K points; the TOCSY experiment was collected with 900×1536 points and zero-filled to 1K×2K points; the 3D HSQC-TOCSY was collected with 64(^{13}C)×110(^{1}H)×1K(^{1}H) points and zero-filled to 128(^{13}C)×256(^{1}H)×2K (^{1}H) points. The 3R spectrum has 1K(^{13}C)×1K(^{1}H)×2K(^{1}H) data points.

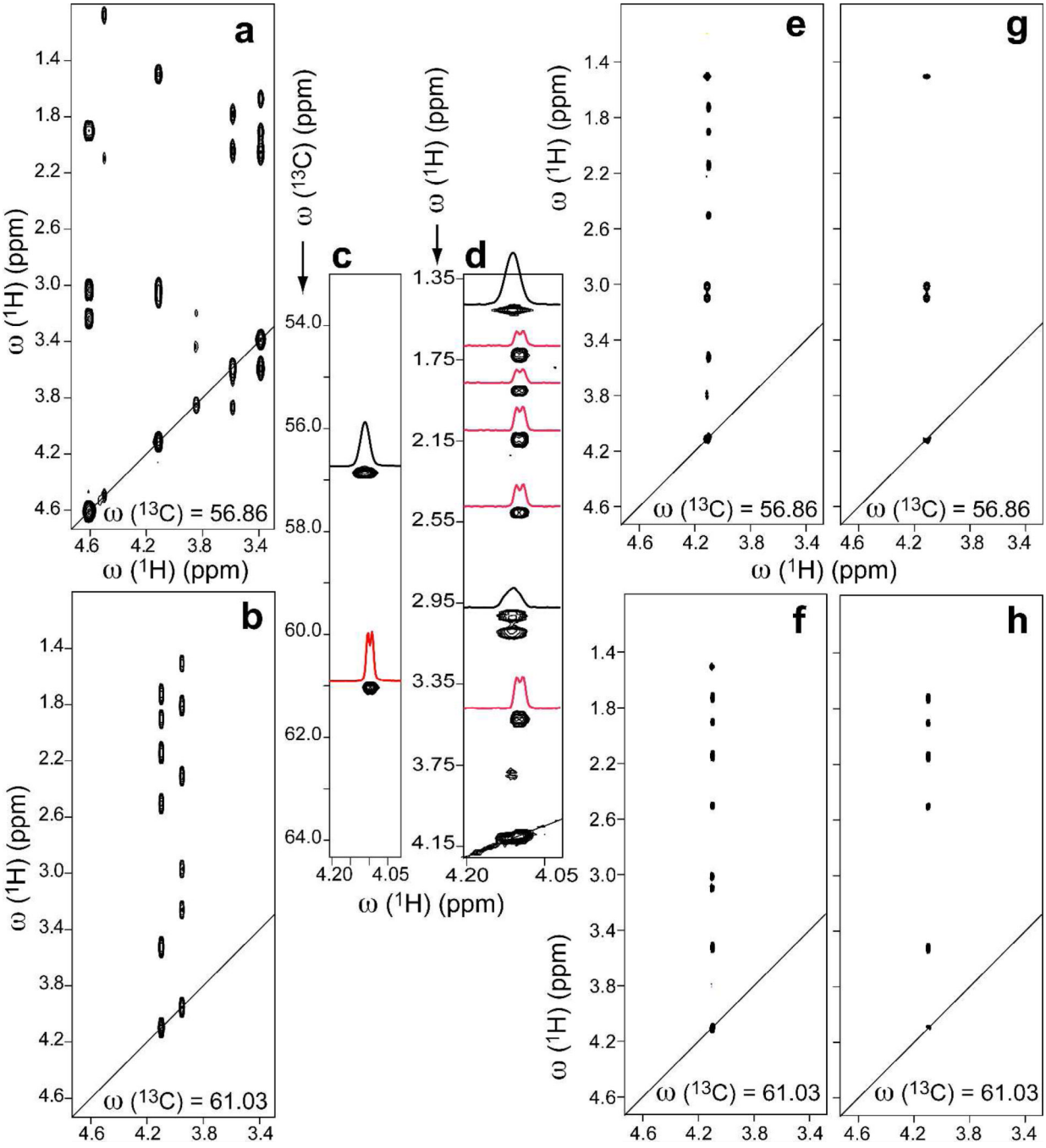

Figure 2.

Performance of triple-rank correlation method with moment filtering for antamanide (Eq. 2). For the 2 overlapping Hα resonances of Pro3 (red) and Phe5 (black) (c,d) show 2D HSQC and TOCSY (τ_m=90 ms) strip plots, (a,b) show 3D FT strip plots at constant ^{13}C frequency, (e,f) show 3R planes before and (g,h) after filtering. Peak cross sections in (c,d) demonstrate differential ^{1}H line positions and linewidths that permit identification of false peaks in (e,f) and their successful suppression in (g,h) based on moment filtering.