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Improving the quality of 2D solid-state NMR spectra of microcrystalline proteins by covariance analysis†

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Abstract

Gaining time, resolution, and sensitivity at the same time: covariance processing of two-dimensional NMR spectra of microcrystalline proteins improves spectral quality over conventional Fourier transformation despite a significant reduction of the experimental time.

Keywords

Covariance NMR; microcrystalline proteins; solid state NMR; catabolite repression phosphocarrier (Crh) protein; PARIS recoupling

Magic angle spinning (MAS) spectra of ¹³C-labeled microcrystalline proteins offer valuable insight into the structure and internal dynamics at atomic resolution.^{1–8} The standard method uses a two-dimensional Fourier transformation (2D FT). Since a high spectral resolution is mandatory in both dimensions of ¹³C-¹³C correlation experiments, a large number of time increments ($N_1 \geq 512$ for typical samples and experimental conditions) is required in the indirect t_1 dimension. This translates into protracted measurements that often require several days.

In this communication, we show that covariance processing^{9–11} of two-dimensional NMR spectra of microcrystalline proteins improves spectral resolution and sensitivity over conventional Fourier transformation despite a significant reduction of the experimental time. In covariance NMR, the covariance matrix **C** is obtained from the prescription $\mathbf{C} = (\mathbf{F}^T \cdot \mathbf{F})^{1/2}$, where **F** is the matrix of the 2D FT spectrum, **F**^T the transposed matrix, and 1/2 indicates the square root of the matrix. Spectrum **C** is thereby a symmetric matrix where the spectral resolution is the same along the indirect ω_1 dimension and direct ω_2 dimensions. Covariance processing has proven to be useful for resolution-enhancement of solution-state NMR spectra, for the reduction of NMR measurement time, or both.^{12,13} Similar benefits have been found for solid-state NMR spectra of small organic and inorganic crystalline and amorphous samples¹⁴ and small peptides.¹⁵

†Electronic Supplementary Information (ESI) available: [Covariance and 2D FT spectra using a small number of increments, covariance spectra with improved signal-to-noise ratio and long-range contacts, 1D cross-sections, experimental details].

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In solids, 2D FT ^{13}C - ^{13}C correlation spectra are inherently asymmetric, in particular when cross-polarization (CP) from protons to carbons is employed at the beginning of the evolution period.¹⁶ The asymmetry is most severe for cross-peaks between proton-carrying carbons and carbonyl, carboxyl, or quaternary carbons, since these suffer from a slow build-up during cross-polarization. Asymmetric cross-peak amplitudes can also arise because of local variations in internal mobility. Whatever their origin, asymmetries in ^{13}C - ^{13}C correlation spectra can be a challenge to covariance processing.

It is demonstrated here that covariance processing provides ^{13}C - ^{13}C correlation spectra with good resolution even if $N_1 \leq 256$ and even if the spectra are inherently asymmetric. Covariance and 2D FT processing are compared for ^{13}C - ^{13}C correlation spectra where the bandwidth of magnetization transfer is enhanced by PARIS recoupling.¹⁷ The method is applied to the ^{13}C - and ^{15}N -labelled 85 amino-acid microcrystalline domain-swapped homodimer of catabolite repression phosphocarrier protein (Crh) protein, which has a molecular mass of 10.4 kDa per monomer.¹⁸

The application of PARIS recoupling to this system has recently been shown to reveal a large number of sequential cross-peaks.¹⁹ We demonstrate here that covariance processing can increase both the resolution and the signal-to-noise ratio, revealing a number of additional sequential and long-range contacts, which were not accessible in an earlier study¹⁹ using 2D FT processing.

Figure 1 shows a comparison between 2D FT and covariance spectra obtained with different numbers N_1 of t_1 increments. Obviously, when reducing N_1 from 512 to 256, the resolution of the 2D FT spectra decreases significantly. On the other hand, the covariance spectra show hardly any difference when halving N_1 . The covariance spectrum in Fig. 1c has a better resolution than the 2D FT spectrum of Fig. 1b, although the data were recorded in half the time. The experimental time could even be reduced by a factor three ($N_1 = 171$) without losing much resolution (see ESI, Fig. S1).

The expansions shown in Fig. 2a–d reveal cross-peaks between aliphatic and carbonyl carbons. This region is highly asymmetric in 2D FT spectra (see ESI Fig. S2). Even when comparing spectra with $N_1 = 512$, covariance offers better resolution than 2D FT. This allows one to separate the intra-residue $\text{C}'_i\text{-C}^\alpha_i$ contacts in Val23 and Ile47, in a region where the 2D FT spectrum only shows a poorly resolved cross-peaks. One can also clearly distinguish the sequential $\text{C}'_i\text{-C}^\beta_{i-1}$ and $\text{C}'_i\text{-C}^\beta_{i+1}$ contacts Thr30-Ser31 and Ser56-Thr57. In the 2D FT spectrum of Fig. 2b, the intra-residue $\text{C}'_i\text{-C}^\beta_i$ contact within Thr59 overlaps with two other cross-peaks, but it is well resolved in the covariance spectrum. Furthermore, the intra-residue $\text{C}'_i\text{-C}^\alpha_i$ contact within Thr57 is only resolved in the covariance spectrum, but not in the 2D FT. Somewhat surprisingly, this short-range contact gives rise to weaker signals than the longer-range $\text{C}'_i\text{-C}^\beta_i$ contact of Thr57, a trend that could also be observed for Thr62 and Thr30 in both 2D FT and covariance spectra. This is due to the fact that the C^β resonances are less shielded than the C^α carbons in these three threonine residues so that the $\text{C}^\beta\text{-C}'$ chemical shift difference is closer to the rotational resonance (R^2) condition than for the $\text{C}^\beta\text{-C}'$ pair.

Thus covariance processing renders a number of assignments more reliable by disentangling overlapping signals. A truncation to $N_1 = 256$ hardly affects the resolution in covariance spectra (Fig. 2c), while the resolution of the 2D FT spectrum is severely deteriorated. Moreover, some new peaks are showing up in covariance spectra (marked by green asterisks). These cross peaks are mostly buried in the noise in the 2D FT spectra. Not all of these peaks could be assigned since many of the C' resonances cannot be resolved due to spectral crowding. Fig. 3 shows some examples of the improved signal-to-noise ratio obtained with covariance processing ($N_1 = 512$, panels a, d) in comparison with 2D FT processing ($N_1 = 512$, panels b, e). In

particular, sequential $C^{\alpha}_i-C^{\beta}_{i+1}$ and $C^{\alpha}_i-C^{\beta}_{i-1}$ contacts such as for the pair Ile64 $^{\beta}$ – Ala65 $^{\alpha}$ and long-range contacts as for the pair Gly13 $^{\alpha}$ – Lys53 $^{\beta}$ (6.4 Å) are only visible in covariance spectra. Spectrum (d) shows another example where the long-range contact Thr62 CO – Gln3 $^{\beta}$ (5.84 Å) appears in the covariance spectrum, but not in the 2D FT spectrum. The latter long-range contact also illustrates the virtues of the broad-band PARIS recoupling sequence at very high magnetic fields.¹⁹ The improved signal-to-noise helps identifying intra-residue contacts. For example, the Lys50 CO resonance cannot be identified unambiguously in the 2D FT spectrum but the covariance spectrum (see ESI Fig. S3) revealed this resonance via an intra-residue $C' - C^{\beta}$ cross-peak.

Fig. 4 shows another spectral region where structurally relevant sequential and long-range contacts could be identified. The 2D FT spectrum with $N_1 = 512$ increments is poorly resolved and highly asymmetric. The covariance spectra provide substantial improvements in resolution and, in some cases, in sensitivity, which allowed us to assign a number of peaks for the first time, including structurally important long-range contacts. Thus we could unambiguously assign the $C^{\alpha}-C^{\alpha}$ long-range contacts Val61–Val6 (5.59 Å) and Val61–Lys37 (5.36 Å), which lead to clearly separated cross-peaks in the covariance spectra while the 2D FT spectra show only a featureless blob.

The latter contact occurs within the monomer while the former concerns the domain-swapped $\beta 1$ strand (see ESI Fig. S4). In addition, some sequential $C^{\alpha}_i-C^{\alpha}_{i+1}$ contacts emerge, such as Val2–Gln3 or Ser52–Leu53, which are poorly resolved in the 2D FT spectrum or hidden under ridges associated with the nearby $C^{\alpha}_i-C^{\alpha}_{i+1}$ cross-peaks Val33–Phe34, Leu63–Ile64 and Glu72–Ala73. Moreover some new cross-peaks (green asterisks) appear, but could not be assigned because of spectral crowding. This suggests that even for spectra collected with a large number of increments N_1 , covariance processing may provide advantages over standard 2D FT.

For datasets with $N_1 < N_2$, as commonly obtained for biomolecules, covariance processing offers a valuable alternative to 2D FT. A further benefit is that neither baseline correction nor apodization are required in the indirect ω_1 dimension.¹¹ It might be useful to combine the two processing strategies in a complementary fashion. Indeed, we found a small number of sequential peaks which could be observed either in the 2D FT or in the covariance spectrum but not in both. Covariance processing could be used for resolution enhancement in the indirect dimension as an alternative to forward linear prediction, which has met some success in solid state NMR of biosolids^{18b, 21}. While the latter method is known to produce artefacts when applied to data with a low signal-to-noise ratio,²² covariance processing turned out to be remarkably robust under our experimental conditions and is not susceptible to artefacts, except for $N_1 \leq 128$ (see ESI Fig. S5). In practice, covariance analysis can be performed ‘on the fly’, i.e., while the experiment is running, and the experiment can be interrupted once target thresholds for the digital resolution and signal-to-noise ratio have been reached.²³

In conclusion, we have shown that covariance processing is beneficial for ^{13}C - ^{13}C correlation spectra of microcrystalline proteins, despite the inherent asymmetry of the corresponding 2D FT spectra. We were able to reduce the number of increments in t_1 by a factor of three without sacrificing any resolution in the indirect dimension. This affords additional assignments of sequential and long-range cross peaks, which are essential for structure determination of proteins in the solid state. Covariance NMR has been generalized to 4D spectra for resolution enhancement.²⁴ As solid state NMR is applied to biomolecules of ever-increasing size,²⁵ such advances might become vital to analyze higher-dimensional NMR spectra that are not necessarily symmetric.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Two-dimensional correlation spectra of microcrystalline Crh obtained with PARIS recoupling¹⁷ to induce a broad-band ^{13}C - ^{13}C transfer of magnetization, processed by (a) covariance with $N_1 = 512$, (b) 2D FT with $N_1 = 512$, (c) covariance with $N_1 = 256$ and (d) 2D FT with $N_1 = 256$. The lowest contour levels lie five times above the noise level. PISSARRO decoupling²⁰ with a proton rf field $\nu_1^{\text{H}} = 100$ kHz was applied during both evolution and acquisition times.

Fig. 2.

Enlargement of the region of Fig. 1 containing cross-peaks between carbonyl and aliphatic carbons processed by (a) covariance with $N_1 = 512$, (b) 2D FT with $N_1 = 512$, (c) covariance with $N_1 = 256$, and (d) 2D FT with $N_1 = 256$. Green asterisks indicate weak but significant cross-peaks that can only be resolved in covariance spectra.

Fig. 3. Increase of signal-to-noise ratio obtained with covariance processing ($N_1 = 512$, panels a, d) in comparison with 2D FT processing ($N_1 = 512$, panels b, e). Weak but unambiguous correlations appear in the covariance spectra that are hidden in the noise of 2D FT spectra.

Fig. 4.

Expansions of Fig. 1, showing the same spectral region above (a, c) and below (b, d) the main diagonal: (a), (b) covariance spectrum with $N_1 = 512$ and (c), (d) the corresponding spectrum processed with 2D FT. The green asterisks indicate unassigned cross-peaks. The lowest contour levels are drawn at four times the noise level.