

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

Markus Weingarth^a, Piotr Tekely^{a*}, Rafael Brüschweiler^{b*}, and Geoffrey Bodenhausen^a

^aDépartement de Chimie, associé au CNRS, Ecole Normale Supérieure, 24 rue Lhomond, 75231 Paris, Cedex 05, France. ^bDepartment of Chemistry and Biochemistry, and National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL USA,

Electronic Supplementary Information

Fig. S1: ^{13}C - ^{13}C broad-band correlation spectrum (using PARIS recoupling) of microcrystalline Crh processed by: a) the covariance method using a small number of increments ($N_1 = 171$) in the indirect t_1 dimension; b) using the standard 2D FT NMR method with $N_1 = 171$. Despite a reduction of the experimental time by a factor of about three, corresponding to a total acquisition time of ~ 4.5 h, the covariance spectrum shows only a minor loss in quality compared to Fig. 1b ($N_1 = 512$) (see main text). This allows a rapid verification of structural homogeneity. The resolution is sufficient to resolve many intra-residue C^α - C^β cross-peaks. In the spectral region revealing cross-peaks between carbonyl and aliphatic carbons, the resolution is best in the covariance spectrum. The lowest contour levels are drawn five times above the noise level

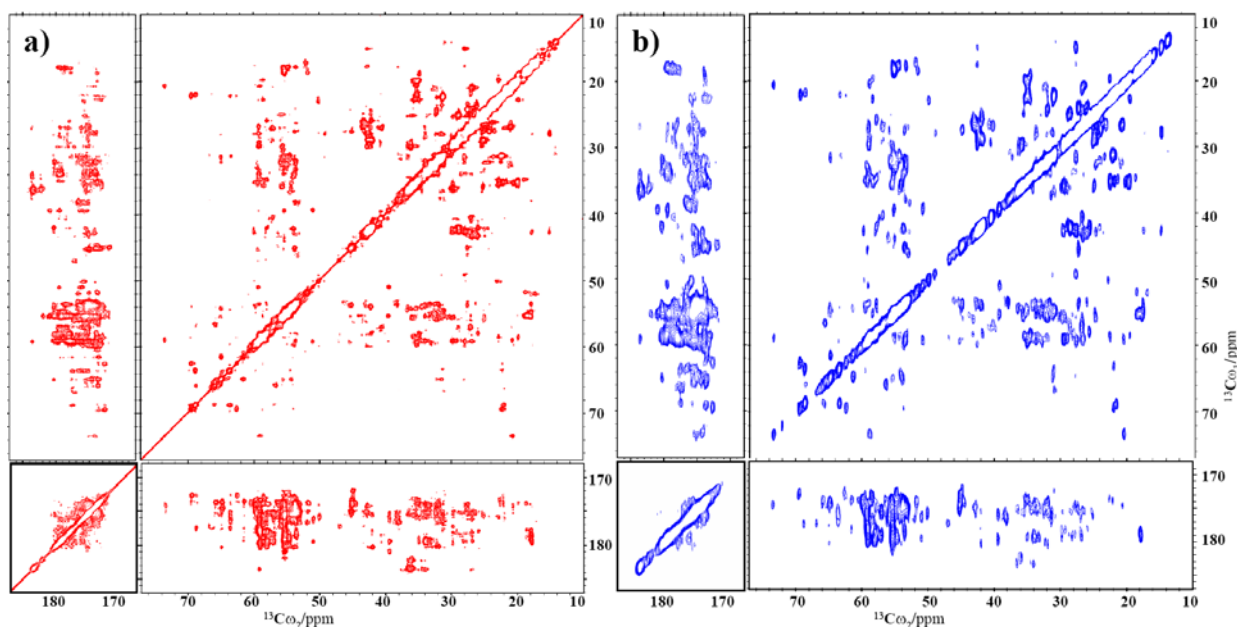


Fig. S2: Stacked plot of the 2D FT spectrum of Fig 1b. The spectrum is clearly not symmetric with respect to the main diagonal, particularly for cross-peaks due to a transfer from aliphatic to carbonyl carbons and vice-versa (regions A and B). This reflects unequal amplitudes of the magnetization after the initial cross-polarization (CP).

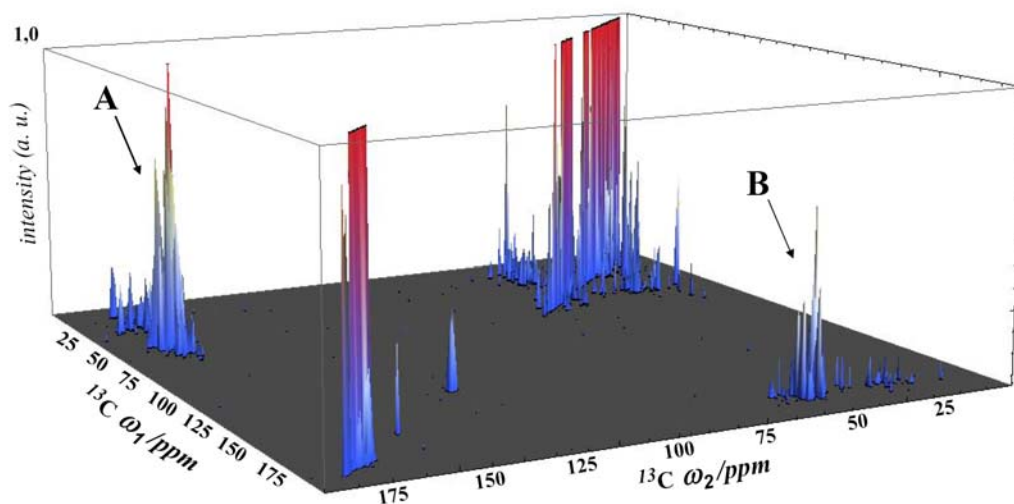


Fig. S3: Example of the improved signal-to-noise ratio obtained with covariance processing ($N_1 = 512$, panel a) in comparison with 2D FT processing ($N_1 = 512$, panel b) which helps identifying intra-residue contacts. The Lys50^{CO} resonance cannot be identified unambiguously in the 2D FT spectrum (b) but the covariance spectrum (a) reveals this resonance via an intra-residue C^β-CO cross-peak.

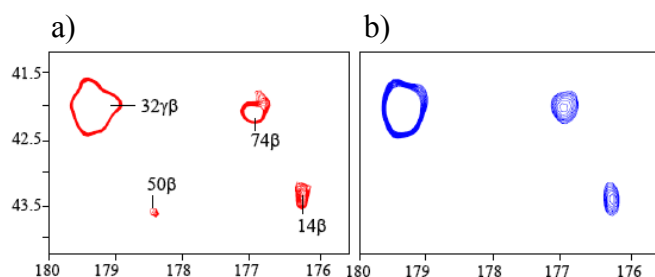


Fig. S4: Covariance processing gives access to long-range contacts, which could not be assigned in 2D FT spectra due to a lack of resolution. Examples are the long-range contact Val61^α-Val6^α (5.59 Å), which connects the domain-swapped β1 and β4 strands, and the contact Val61^α-Lys37^α (5.36 Å) within the monomer, connecting strand β4 with the small loop between strands β2 and β3.

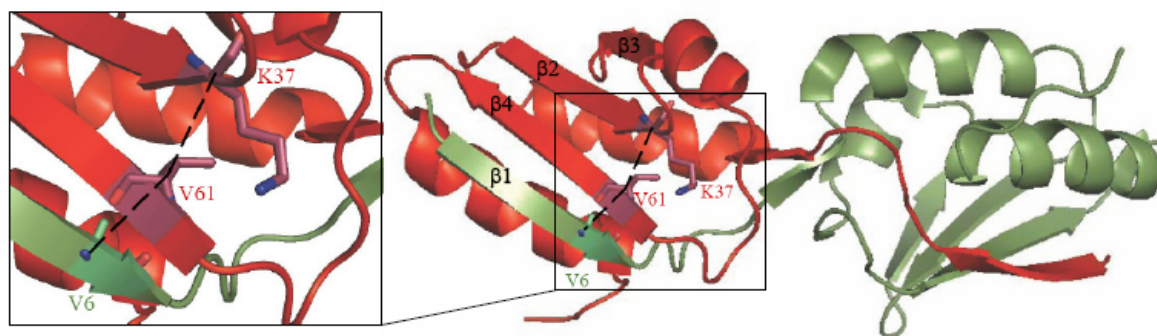


Fig. S5a: Cross-sections along ω_2 at $\omega_1 = 62.5$ ppm of the 2D FT spectrum (Fig. 1b, $N_1 = 512$), and the covariance-processed spectra (Fig. 1a with $N_1 = 512$ and Fig. 1c with $N_1 = 171$). The covariance cross-sections show the same S/N ratio as the 2D FT spectrum. For lower N_1 , slight deviations could be observed for two C^α-C' cross-peaks, presumably due to decreased resolution. The covariance spectra are free of artefacts, except for the diagonal (highlighted by a green asterisks), showing typical autocorrelation peaks which become broader when N_1 decreases.

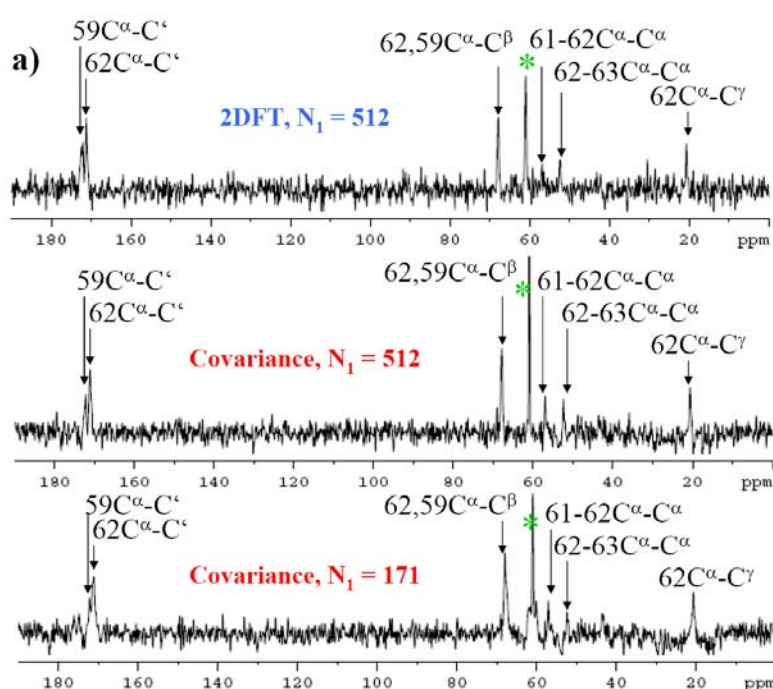


Fig. S5b Cross-sections along ω_2 at $\omega_1 = 53.6$ ppm of the 2D FT spectrum (Fig. 1b, $N_1 = 512$), and covariance-processed spectra (Fig. 1a with $N_1 = 512$ and Fig. 1c with $N_1 = 171$). Like in Fig. S5a, no artefacts were observed in the covariance-processed spectra and the S/N ratio was conserved with respect to the 2D FT spectra. For very weak cross-peaks, the intensity pattern of the covariance-processed spectra can differ, presumably because of different noise levels in 2D FT and covariance spectra.

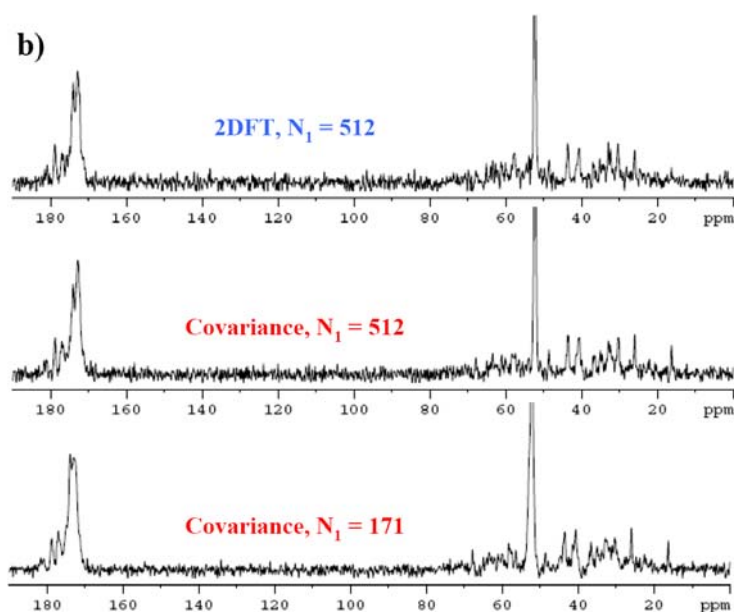
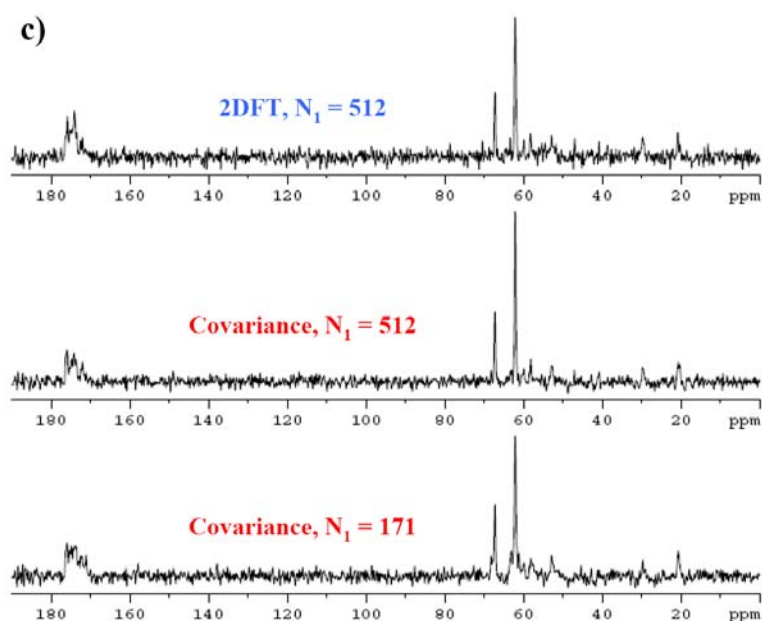


Fig. S5c Cross sections along ω_2 at $\omega_1 = 61.5$ ppm of the 2D FT spectrum (Fig. 1b, $N_1 = 512$), and covariance-processed spectra (Fig. 1a with $N_1 = 512$ and Fig. 1c with $N_1 = 171$). Although, once again, no artefacts could be observed in the covariance-processed spectra, the S/N ratio of the aliphatic peak at $\omega_2 = 68$ ppm and the carbonyl region at $\omega_2 \sim 175$ ppm differs in the 2D FT and the covariance-processed spectra. These results show that a spectrum suitable for analysis by 2D FT is also suitable for covariance processing, with the additional advantages described in the main text. Hence a high S/N is not a prerequisite for the application of this method.



Experimental Section

NMR experiments: The data were recorded at 21.2 T (900 MHz for protons) using a 2.5 mm rotor spinning at 23 kHz. Broadband ^{13}C - ^{13}C magnetization transfer in microcrystalline Crh was achieved using PARIS recoupling¹⁴ with a proton radio-frequency (rf) amplitude $\nu_1^{\text{H}} = 23$ kHz, a mixing time $\tau_m = 85$ ms, and reversing the rf phase after intervals $\tau_p = \tau_{\text{rot}}/2$, i.e., every half-rotor period. PISSARRO decoupling¹⁶ with a proton rf field $\nu_1^{\text{H}} = 100$ kHz was applied during both evolution and acquisition times. The spectrum was acquired using States-TPPI acquisition with $N_2 = 2492$ and $N_1 = 512$ complex points with 16 scans. The spectral widths were of 62.5 and 15.87 kHz in ω_2 and ω_1 respectively. The cross-polarization (CP) contact time was 2 ms and the delay between scans 3 s. The total experimental time was around 13 h.

Data processing: 2D FT processing was performed with Topspin 2.0. The matrix was zero-filled from 2492 to 4096 points in t_2 ; in the t_1 domain, $N_1 = 512$ complex points were recorded, and the matrix was deliberately cut down to $N_1 = 256$ or 171 complex points, followed by zero-filling to 1024 real points in all cases. Shifted square-cosine apodization functions were applied in both dimensions prior to 2D FT. After phase correction, the real part of the 2D FT spectrum was used for covariance processing, which was carried out with Mathematica in about 5 min for a 1024x4096 matrix on an Intel Quad Core computer running at 2.83 GHz with 3.8 GB RAM. The spectra were analyzed and assigned with the help of the Sparky package (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco). The covariance processing step could be accelerated by singular value decomposition⁸.