Covariance Enhancement of 4D $^{15}N/^{13}C$ NOESY Data

D.A. Snyder (FSU, Chemistry and Biochemistry), F. Zhang (NHMFL), Y. Xu (National University of Singapore), D. Yang (National University of Singapore), R. Brüschweiler (FSU, Chemistry and Biochemistry, NHMFL)

Introduction

Covariance NMR [1] is a technique which, either following or in lieu of a Fourier Transform of a multi-dimensional NMR spectrum, allows for the indirect dimension to achieve the same nominal resolution and spectral width as the direct dimension. Recent work has demonstrated the applicability of Covariance NMR to four dimensional (4D) NOESY spectra [2]. Shared-evolution NOESY experiments allow ^{15}N-edited and ^{13}C-edited NOESY data to be collected in a single experiment [3]. The application of Covariance NMR to a shared evolution NOESY experiment yields a 4D spectrum displaying all the correlations available from a 2D experiment and with resolution surpassing that typically obtained in 4D experiments [4].

Experimental

4D NOESY data, as well as other NMR spectra to verify ubiquitin resonance assignments, were collected on a 800 MHz Bruker magnet with a cryogenically cooled TCI probe at the NHMFL facility. Shared evolution data were collected off-site using a 500 MHz Bruker spectrometer equipped with a cryoprobe.

Results and Discussion

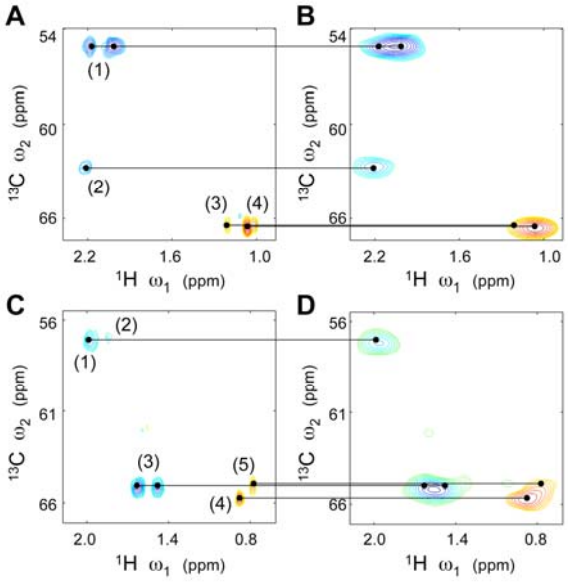

Figure 1. Comparison between shared evolution 4D $^{13}C/^{15}N$-edited NOESY donor planes of ubiquitin before (B,D) and after (A,C) covariance processing. **A,B**. A region of the donor plane associated with the T55-NH acceptor pair (ω_4 = 8.832 ω_3 = 108.6 ppm). The covariance spectrum distinguishes (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 intra-residue (4) T55HG2/H peak. **C,D**. A region of the L71-NH donor plane (ω_4 = 8.104, ω_3 = 123.3 ppm). Covariance NMR considerably 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 resolution enhancement afforded by 4D-covariance NMR allows identification and assignment of more peaks, including ones reporting on long-range protein distances.

Acknowledgements
This work has been supported by NIH grant R01-GM066041.

References

[1] Brüschweiler, R., *et al*. Journal of Chemical Physics, **120**, 5253-5260 (2004).
[2] Snyder, D.A., Zhang, F. and Brüschweiler, R. Journal of Biomolecular NMR. **39**, 165-175 (2007).
[3] Xu, Y.; Long, D.; Yang, D. Journal of the American Chemical Society, **129**, 7722-7723 (2007).
[4] Snyder, D.A., *et al.* Journal of the American Chemical Society. **129**, 14126-14127 (2007).