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Filtered Backprojection for the Reconstruction of a High-Resolution (4,2)D CH_3-NH NOESY Spectrum on a 29 kDa Protein

Brian E. Coggins,[†] Ronald A. Venters,[‡] and Pei Zhou*,[†]

Department of Biochemistry and Duke University NMR Center, Duke University Medical Center, Durham, North Carolina 27710

Received May 12, 2005; E-mail: peizhou@biochem.duke.edu

Projection−reconstruction (PR) NMR has recently been introduced as a method for collecting multidimensional NMR data, requiring significantly less measurement time than the conventional methodology.[1−6] Projections of a spectrum are collected at various angles by simultaneously evolving indirect dimensions, and the full spectrum is then reconstructed mathematically. PR−NMR demonstrations have focused on the sequential assignment of proteins using up to 5D experiments.[1−4] We recently developed a suite of (4,2)D PR sequential assignment pulse sequences and applied them to two proteins of 29 and 30 kDa.[6] With these experiments, we can obtain very high resolution 4D spectra of medium- to large-sized proteins in the time conventionally needed for 3D spectra. We also extended PR−NMR to side chain experiments based on scalar couplings.[7]

Higher-dimensional NOESY is central to the structural analysis of large proteins and would be an excellent application for PR−NMR. Three-dimensional NOESY spectra contain many overlapping peaks, and assignment ambiguities make interpretation difficult. Yet due to restrictions on measurement time, 4D NOESY experiments can only be collected if resolution is severely curtailed. PR−NMR could facilitate collecting 4D NOESY spectra at a much higher resolution than is otherwise feasible. Naturally, the measurement time would have to be long enough to detect weak cross-peaks, but a suitable approach would collect data at a much higher resolution in that time than with conventional sampling, without sacrificing sensitivity.

The PR−NMR procedures described to date would not be successful for NOESY, as they have difficulties reconstructing arbitrary numbers of peaks, reproducing the correct peak shapes, or retaining full sensitivity.[6] To facilitate the application of PR−NMR to NOESY, we have utilized a new approach, centered around the filtered backprojection (FBP) reconstruction algorithm, developed in the radioastronomy and medical imaging fields.[8−10] FBP is a *quantitatively accurate* reconstruction technique, provided that a sufficient number of projections are available. In addition, it accumulates intensity from all projections to realize the full sensitivity of the projection data.

Mathematically, the process of measuring projections is the Radon transform (operator **R**), an integral transform; FBP is the analytical inversion of this transform ($\mathbf{R}^{-1}$).[8−11] For frequency domain projection data, *P*, the reconstruction of a spectrum, *S*, can be written, using operator notation,[9] as:

$$S = \mathbf{R}^{-1}P = \mathbf{BF}w(t)\mathbf{F}^{-1}P \qquad (1)$$

where **F** is the Fourier transform with respect to the radial dimension, $\mathbf{F}^{-1}$ is its inverse, $w(t)$ is a filter or window function, and **B** is the backprojection operation. Thus, reconstruction requires a filtering step ($\mathbf{F}w(t)\mathbf{F}^{-1}$) before backprojection (**B**) (Figure 1). Using the theoretically most accurate filtering function for a (4,2)D PR−NMR experiment—a quadratic function $w(t) = t^2$—the reconstruction equation for (4,2)D PR−NMR CH_3-NH NOESY can be written explicitly as:

$$S(\omega_{HM},\omega_{CM},\omega_N;\omega_{HN}) = \int_0^{\pi}\int_0^{\pi}\int_{-\infty}^{\infty}\left(\int_{-\infty}^{\infty} P(\omega_{tilt'},\theta,\phi;\omega_{HN})e^{i2\pi t_{tilt}\omega_{tilt'}}\,d\omega_{tilt'}\right) t_{tilt}{}^2 e^{-i2\pi\omega_{tilt}t_{tilt}}dt_{tilt}\,d\theta d\phi \qquad (2)$$

where the ω values are frequency coordinates, ω_{tilt} is the point of projection for the coordinates in *S*, θ and ϕ are projection angles, and t_{tilt} and $\omega_{tilt'}$ are variables of integration in the Fourier transforms. (For full derivations and definitions pertaining to both equations, see the Appendix in the Supporting Information.)

As a demonstration of the potential of this approach, we have used PR−NMR, with FBP reconstruction, to determine the 4D methyl/amide NOESY spectrum of the 29 kDa human carbonic anhydrase II (HCA II) from 2D projections. For a (4,2)D experiment, simulations indicate that 400 projections measured using 100 intermodulated experiments, with angles chosen so that the projections are distributed evenly in angle space (Table S1), would be sufficient. The simulation shows the expected peak shape and a nearly flat baseline centered at zero (Figure 1). In contrast, reconstruction using backprojection alone[5] would lead to peak broadening as well as an uneven, elevated baseline.[6]

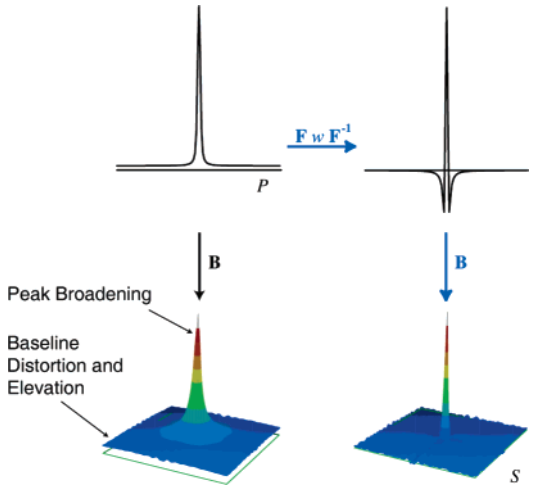

Figure 1. Filtered backprojection reconstruction (blue operations). A simulation is shown for the 100 projection angles used for the NOESY experiment, reconstructing a Lorentzian peak of finite line width. Top left, projection data; top right, after filtering; bottom right, the FBP reconstruction. The reconstructed peak shows the correct line shape. Bottom left, the result is compared to backprojection alone.

[†] Department of Biochemistry.
[‡] Duke University NMR Center.

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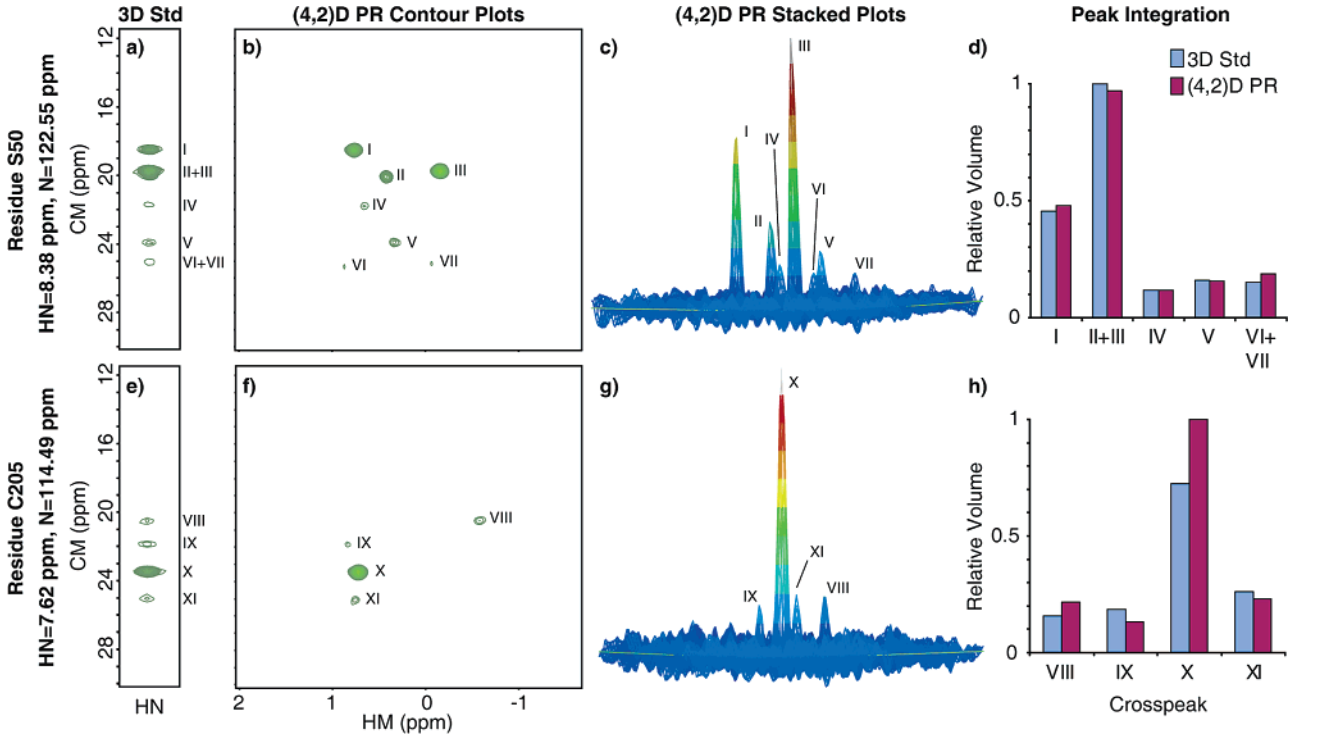

Figure 2. Representative data from the (4,2)D PR NOESY experiment and the conventional 3D control. The residues chosen show complicated patterns, with closely clustered peaks, large dynamic range, very weak peaks, and peaks that overlap in the 3D control spectrum. For all panels, the cross-peaks are: I, V78 Hγ2; II, V49 Hγ2; III, V49 Hγ1; IV, V78 Hγ1; V, L79 Hδ1; VI, L44 Hδ1→A257 HN, from an adjacent plane; VII, L79 Hδ2; VIII, V206 Hγ2; IX, L203 Hδ2; X, V206 Hγ1; XI, L140 Hδ1. (a and e) Strips from the 3D control experiment. (b and f) Contour plots of the corresponding planes from the (4,2)D PR experiment. (c and g) Stacked plots of the PR experiment, showing that the reconstruction is free of artifacts. (d and h) Comparison of integrated peak volumes in the 3D and PR experiments.

A 0.9 mM sample of ILV methyl-protonated, $^{2}H/^{13}C/^{15}N$-labeled HCA II was used. Spectra were measured using a Varian INOVA 800 MHz spectrometer equipped with a cryoprobe.

A 4D $^{13}C/^{15}N$-separated NOESY pulse sequence[12,13] was adapted for PR and used to collect 100 2D projection experiments at the angles described above. A mixing time of 200 ms was used. For each projection, 48 complex points were recorded in the tilted dimension, with four transients per FID. The total measurement time was 88 h. Tilted axes were extended by linear prediction to 64 complex points and zero-filled to 128 points. Reconstructions were calculated at 128 point resolution in each indirect dimension using a custom C++ program, PR-CALC, to be described elsewhere. Filtering was accomplished within this program by applying the quadratic filter to the real and imaginary components in the time domain before backprojection.

As a control to assess sensitivity, a conventional 3D (H)CNH $^{13}C/^{15}N$-separated NOESY experiment was recorded in one-half of the measurement time on the same spectrometer. Taking into account the loss of sensitivity (50%) in the 4D pulse sequence due to quadrature detection in the additional dimension, this 3D spectrum should have equal sensitivity to that of the (4,2)D PR experiment. For each indirect axis, 48 complex points were recorded and extended to 64 points by linear prediction and to 128 points by zero-filling, before a conventional 3D Fourier transform (full parameters given in Table S3).

Representative planes from the PR−NMR and control experiments are shown in Figure 2. All cross-peaks are correctly reproduced, and integrations show approximately the same relative peak volumes. Stacked plots in Figure 2c,g show that the reconstruction is free from artifacts and of good sensitivity. Peak positions and the absence of artifacts were confirmed by comparison with a 4D $^{13}C/^{15}N$-separated spectrum collected in 10 days at 50 × 16 × 16 complex point resolution (Figure S3).

This demonstration shows that PR−NMR can be used successfully for the quantitative determination of NOESY spectra. Using this new approach of measuring many projections, each of relatively weak sensitivity, and reconstructing with FBP, a high-resolution 4D NOESY spectrum was measured in only 88 h, representing less than 5% of the measurement time that would be needed for a conventional 4D spectrum of equal resolution.

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Supporting Information Available: Mathematical details of FBP; experimental parameters; comparison to conventional 4D data. This material is available free of charge via the Internet at http://pubs.acs.org.

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