

Residual water suppression by indirect covariance NMR

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Residual water solvent signals in 2D NMR experiments adversely affect appearance and subsequent analysis of spectra. A method for water suppression that is based on indirect covariance processing is described. It produces a symmetric spectrum with a water signal that is substantially decreased or completely absent. The method, which can be combined with other water suppression schemes, is demonstrated for 2D TOCSY, NOESY, and ROESY spectra of the protein, ubiquitin in aqueous solution. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: water suppression; 2D NMR; indirect covariance NMR

INTRODUCTION

Multidimensional ¹H NMR spectra of molecules in aqueous solution require efficient suppression of the water signal. Since the full water signal is typically of the order of 10³ to 10⁵ times larger than the solute signal, it is reduced by one or the combination of several different solvent suppression schemes. Residual water is manifested in 2D FT spectra in the form of a vertical strip along ω_1 indicative of t_1 -noise along the water resonance (Fig. 1(a)) that may obscure cross peaks that are close to the water signal and thereby impede spectral analysis. By contrast, the horizontal waterline along ω_2 is typically much less intense and does not cause major problems for data analysis.

Some water suppression methods such as solvent pre-saturation, the WATERGATE sequence,¹ and excitation sculpting² are an integral part of the applied NMR pulse sequence, while others reduce the water signal at the data processing stage. Processing methods include low-pass filter deconvolution,³ symmetrization, principal component analysis,⁴ singular value decomposition,⁵ congruent matrix pencils,⁶ and wavelet transform.⁷ It is demonstrated here how water t_1 -noise signals of common 2D NMR spectra can be easily and efficiently removed by indirect covariance transform.

METHOD

Indirect covariance spectroscopy is a variant of covariance spectroscopy^{8–10} and was originally introduced for establishing ¹³C–¹³C connectivities from 2D heteronuclear

HSQC–TOCSY experiments.¹¹ Blinov *et al.* introduced asymmetrical processing of indirect covariance spectra to eliminate potential artifacts.^{12,13} The basic idea behind indirect covariance is the following. A 2D FT spectrum, represented by the real $N_1 \times N_2$ matrix **F**, is converted to a symmetric $N_1 \times N_1$ matrix

$$\mathbf{C} = (\mathbf{F} \cdot \mathbf{F}^T)^{1/2} \quad (1)$$

where superscript T refers to the matrix transpose and the square-root denotes the matrix square-root (for nondiagonal matrices, the matrix square-root generally differs from the matrix that contains the square-root of the individual matrix elements). Note that matrix $\mathbf{F} \cdot \mathbf{F}^T$ has as its elements the dot products between rows of **F** and the indirect covariance transform of Eqn (1) has the effect that both frequency axes of spectrum **C** correspond to the indirect frequency axis ω_1 of spectrum **F**.¹¹ Therefore, the spectral resolution of **C** along both dimensions is determined by the spectral resolution along the indirect (ω_1) dimension of **F**. By contrast, the direct covariance spectrum^{8–10} $\mathbf{C}_{direct} = (\mathbf{F}^T \cdot \mathbf{F})^{1/2}$ has its resolution along both dimensions determined by the resolution of **F** along the direct dimension ω_2 .

Figure 1 compares a 2D FT NOESY spectrum of ubiquitin (Fig. 1(a)) with the direct covariance (Fig. 1(b)) and indirect covariance spectrum (Fig. 1(c)). The peaks in the boxed area illustrate the intrinsic power of indirect covariance spectroscopy to reconstruct cross peaks in the vicinity of the waterline. To further reduce the water signal, matrix **F** can be preprocessed by setting the columns of **F** that are strongly affected by the water signal to zero. The selected columns constitute a vertical strip, which is centered at the water signal and which covers the full spectral width along ω_1 and width Δ_{H_2O} along ω_2 , whereby Δ_{H_2O} can be adjusted if necessary. This modified 2D FT spectrum is then subjected to indirect covariance processing according to Eqn (1) resulting

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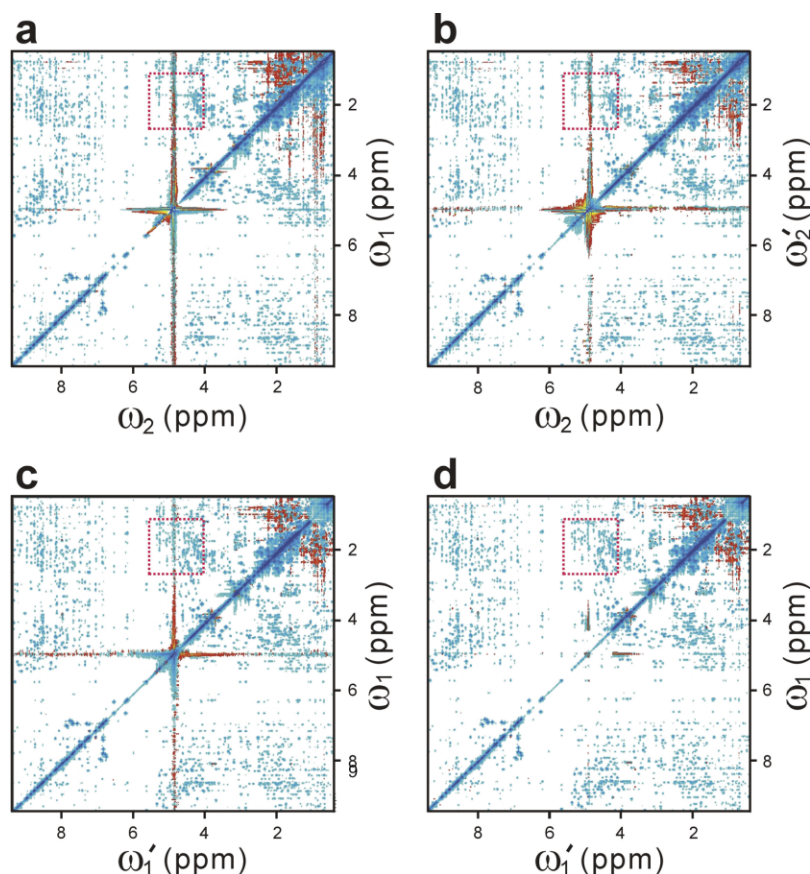


Figure 1. 2D NOESY spectrum of ubiquitin in 95% H₂O and 5% D₂O using WATERGATE¹ for solvent suppression: (a) 2D FT spectrum; (b) direct covariance spectrum; (c) indirect covariance spectrum; (d) indirect covariance spectrum with water strip removed prior to indirect covariance processing.

in the indirect covariance spectrum C_0 , which is essentially free of the water signal (Fig. 1(d)).

The water streak in F along ω_1 leads to an unwanted contribution to the dot product of any pair of rows in $F \cdot F^T$. This is the reason why removal of the water streak prior to application of Eqn (1) improves the quality of the spectrum. As noted above, the indirect covariance NMR spectrum depicts only frequencies along both axes sampled during the t_1 evolution period; therefore, removal of proton signals in F under the water signal does not cause a major loss of spin correlation information in the indirect covariance spectrum C_0 .

MATERIALS AND EXPERIMENTS

All NMR experiments were performed on a 0.7 mm sample of (unlabeled) ubiquitin in 95% H₂O and 5% D₂O at pH 5.0 and carried out at 800 MHz NMR field strength at room temperature. 2D TOCSY, NOESY, and ROESY NMR experiments were conducted with spectral widths of 14.0 ppm, 11.0 ppm, and 10.2 ppm, respectively, along both dimensions. The 2D TOCSY experiment with 1024 t_2 and 256 t_1 (complex) data points was collected using the MLEV-17 mixing sequence¹⁴ with a 60 ms mixing time using excitation sculpting for water suppression.² The 2D NOESY experiment with 1024 t_2 and 512 t_1 (complex) data points had a 120 ms mixing time and it used the WATERGATE 3-9-19 pulse sequence element with gradients for water suppression.¹

The 2D ROESY experiment with 1024 t_2 and 512 t_1 (complex) data points was collected with a 125 ms cw-spinlock pulse for mixing and with presaturation for water suppression. States-TPPI was used for quadrature detection along the indirect dimension for all the three experiments. The time-domain data were apodized using a cosine function, zero-filled to final size of 2048 × 2048 data points, 2D Fourier transformed, and polynomially baseline corrected along both dimensions using NMRPipe.¹⁵ All covariance processing was performed using in-house Matlab programs. For indirect covariance processing Eqn (1) was either applied directly to the 2D FT spectrum, or after a strip along ω_1 around the water resonance of width $\Delta_{H_2O} = 0.69$ ppm (for TOCSY), 1.45 ppm (for NOESY), or 1.77 ppm (for ROESY) was set to zero.

RESULTS AND DISCUSSION

The method is demonstrated in Fig. 2 for all the three experiments. All panels show the region of the spectrum indicated by the dashed box in Fig. 1. The first column from the left shows the spectral region obtained by 2D FT processing, the 2nd column shows the region calculated by direct covariance processing, the 3rd column shows the spectral region obtained by indirect covariance processing, and the 4th column shows the spectral region obtained by indirect covariance processing after removal of the water strip.

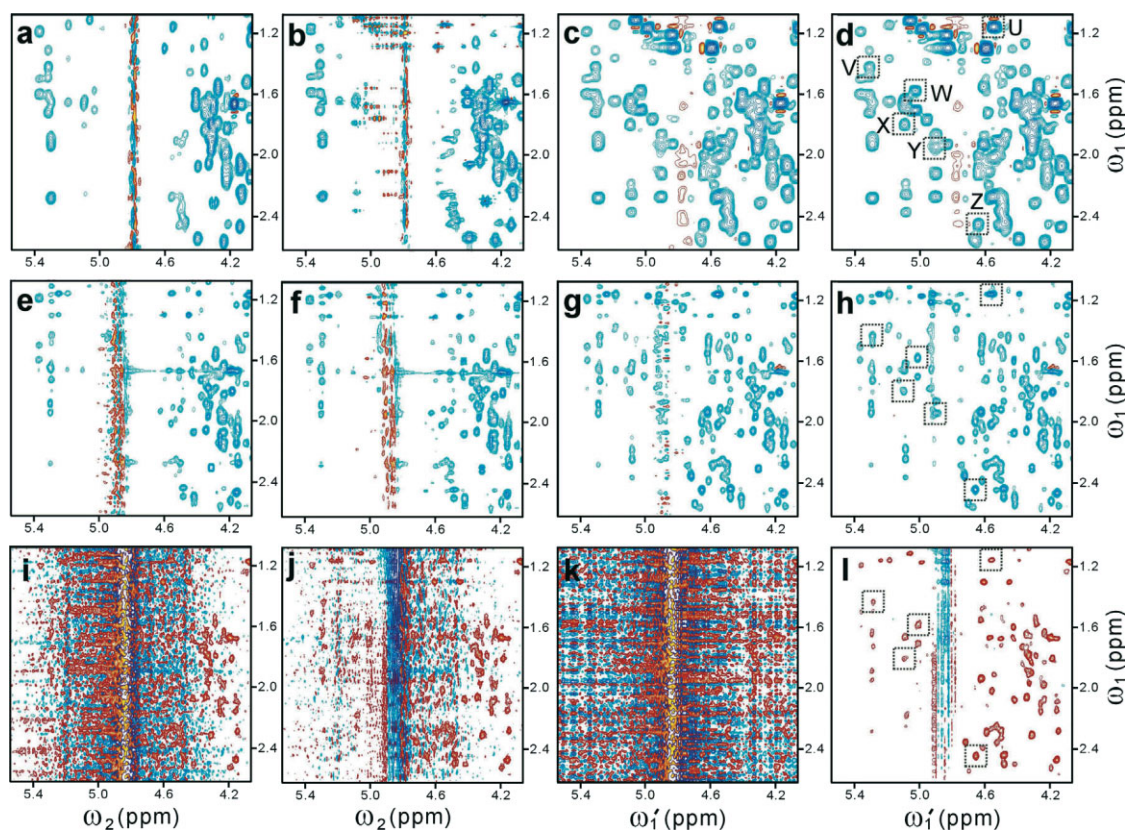


Figure 2. 2D spectral region, indicated by the boxed area in Fig. 1, for different types of experiments and different processing schemes. Comparison of the same spectral region of 2D FT spectrum (1st column from left), direct covariance spectrum (2nd column), indirect covariance spectrum using Eqn (1) (3rd column), and indirect covariance spectrum with water strip removed prior to application of Eqn (1) (4th column) of TOCSY (1st row), NOESY (2nd row) and ROESY (3rd row) experiments of ubiquitin. Positive contours are in blue and negative ones in red. The widths $\Delta_{\text{H}_2\text{O}}$ of the water strips that are zeroed prior to indirect covariance processing are 0.69 ppm (for TOCSY), 1.45 ppm (for NOESY), and 1.77 ppm (for ROESY). Selected cross peaks indicated by squares and letters belong to the following resonances (see BMRB entry 68¹⁶): (U) T55HB-HG2, (V) K6HA-HB2, (W) L71HA-HB2, (X) L67HA-HG, (Y) E16HA-HB3, (Z) P37HA-HB3.

Depending on the experimental water suppression scheme, in both the 2D FT and the direct covariance spectra the vicinity of the waterline is either largely void of cross peaks or contaminated by strong t_1 -noise. Indirect covariance processing (3rd column of Fig. 2) largely eliminates the water signal in the TOCSY and NOESY spectra, but fails to do so in the case of the solvent presaturated ROESY spectrum whose waterline is significantly stronger (and broader) than for the other two spectra. Elimination of the vertical water strip in the 2D FT spectrum prior to indirect covariance processing (4th column) improves the situation in all three spectra and provides reconstruction of a large number of cross peaks (assignments for selected cross peaks, labeled by letters, are given in the caption to Fig. 2).

The spectral regions further apart from the waterline are remarkably insensitive to the width of the zeroed water strip, $\Delta_{\text{H}_2\text{O}}$. Since peak reconstruction in the strip area is based at least in part on the spectral information contained on the other side of the diagonal, $\Delta_{\text{H}_2\text{O}}$ should be kept reasonably small. Generally, no useful spectral information can be gained within the square of width $\Delta_{\text{H}_2\text{O}}$ around the water diagonal peak of the indirect covariance spectrum. For the above examples, $\Delta_{\text{H}_2\text{O}}$ values between 0.6 and 1.8 ppm provide good water suppression results. However, other

choices for $\Delta_{\text{H}_2\text{O}}$ provide equivalent results: the TOCSY spectrum (1st row) spectrum is essentially unaffected when $\Delta_{\text{H}_2\text{O}}$ is varied between 0 and 0.69 ppm; the NOESY spectrum (2nd row) does hardly change when $\Delta_{\text{H}_2\text{O}}$ varies between 0.1 and 1.45 ppm; the ROESY spectrum (3rd row) is virtually the same when $\Delta_{\text{H}_2\text{O}}$ is varied between 1.16 and 1.77 ppm. This illustrates the robustness of the water suppression scheme with respect to the particular choice of the $\Delta_{\text{H}_2\text{O}}$ parameter.

While 'vertical' cutting (along ω_1) prior to indirect covariance processing improves the spectral quality, 'horizontal' cutting (along ω_2) will only result in a cross-shaped zero-intensity region centered on the diagonal peak of the water resonance. Conversely, direct covariance spectra gain only little when the horizontal water strip is removed prior to covariance processing. Vertical cutting can not only be applied to the water strip, but also to other resonances that show strong t_1 noise, such as methyl resonances, or regions that display severe baseline distortions in the 2D FT spectrum along ω_1 . This will reduce such artifacts in the associated indirect covariance spectrum analogous to the suppression of solvent artifacts.

The main drawback of indirect covariance is that its digital spectral resolution $\Delta\nu_1$ is restricted by the number

of t_1 increments N_1 , which is $\Delta\nu_1 = 1/(N_1 \cdot \Delta t_1)$ where Δt_1 is the time-increment in the evolution period t_1 . Because the total measurement time is inversely proportional to the spectral resolution $\Delta\nu_1$, one typically sets $N_1 < N_2$ and therefore the spectral resolution of the indirect covariance spectrum is lower than the one of the standard covariance spectrum, which maintains high-resolution with as little as $N_1 = 48$ for a TOCSY experiment of a decapeptide.¹⁷ Nonetheless, the ubiquitin TOCSY with only 256 t_1 increments (Fig. 2 (1st row)) produces an indirect covariance spectrum that has reasonably good quality to allow spectral analysis including peak assignments. Like direct covariance NMR, the indirect covariance method presented here can be applied to most homonuclear 2D experiments, including 2QF-COSY, but not to COSY itself because the dispersive diagonal peaks adversely affect the matrix product in Eqn (1).

CONCLUSION

Indirect covariance spectroscopy is remarkably resilient to water artifacts. In addition, after deletion of the characteristic water strip of 2D FT spectra prior to indirect covariance processing, the water signal is essentially entirely removed. The procedure, which does not require iterative fitting or elaborate parameter adjustments, produces symmetric 2D spectra whose spectral resolution is determined by the sampling scheme during the evolution period. Indirect covariance spectra are best suited for cross-peak analysis close to the water line, whereas direct covariance spectra are preferable for all other spectral regions. Fully symmetric 2D spectra that are free of solvent artifacts are particularly

suitable for automatic spectral analysis encountered in high-throughput applications, including applications in proteomics and metabolomics.

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