

Elimination of $^{13}\text{C}\alpha$ Splitting in Protein NMR Spectra by Deconvolution with Maximum Entropy Reconstruction

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NMR studies of protein structure, dynamics, or intermolecular interactions require the assignment of the polypeptide backbone resonances. Scalar couplings between backbone nuclei provide the information necessary for sequential assignment, but they also present experimental difficulties by splitting resonances, decreasing both resolution and sensitivity. A number of methods, including composite pulse decoupling^{1,2} and adiabatic decoupling,^{3,4} can be used to eliminate this splitting, while retaining the scalar coupling when it is needed to effect coherence transfer. However, decoupling resonances spanning a broad frequency range can be difficult, especially for homonuclear decoupling: Bloch–Siegert effects can perturb resonance frequencies (rendering frequency correlations ambiguous), and the additional RF radiation can lead to unwanted sample heating.

Attempts to use postacquisition data processing to simplify multiplets in NMR spectra began nearly contemporaneously with the first Fourier transform (FT) NMR experiments.⁵ The application of nonlinear methods⁶ beginning in the 1980s provided the basis for robust deconvolution without noise amplification. Nevertheless, these methods are not widely used, and continued development and application of experimental approaches attest to a general lack of knowledge concerning the capabilities of nonlinear deconvolution, the dearth of appropriate software, or both. The aim of this communication is to demonstrate that postacquisition deconvolution is a viable and sometimes preferable alternative.

Sequential backbone assignments of ^{13}C - and ^{15}N -enriched proteins are routinely obtained by recording a set of triple-resonance experiments, such as HNCA and HN(CO)CA. These are standard tools for correlating the amide proton and nitrogen frequencies with those of the intraresidue $^{13}\text{C}\alpha$ and sequentially adjacent $^{13}\text{C}\alpha$ nuclei.^{7,8} Unfortunately, the $\text{C}\alpha$ peaks (except for Gly residues) are split into doublets because of the $^{13}\text{C}\alpha$ – $^{13}\text{C}\beta$ coupling. These splittings can make it difficult to resolve separate $^{13}\text{C}\alpha$ resonances and at the same time reduce sensitivity.

Carbon–carbon scalar couplings can be refocused and effectively removed using constant-time experiments.⁹ While this eliminates the $^{13}\text{C}\alpha$ – $^{13}\text{C}\beta$ splittings in HNCA and HN(CO)CA spectra, it requires a long constant-time evolution period during which the $^{13}\text{C}\alpha$ magnetization is transverse. This results in significant loss of signal due to the short transverse relaxation times of the $^{13}\text{C}\alpha$ nuclei. Homonuclear decoupling using adiabatic rapid passage has also been applied to eliminate the $^{13}\text{C}\alpha$ – $^{13}\text{C}\beta$ couplings in HNCA and HN(CO)CA experiments.¹⁰ However, this does not remove all $^{13}\text{C}\alpha$ – $^{13}\text{C}\beta$ splittings, since the spectral regions of some $^{13}\text{C}\beta$ signals (e.g., serine and some threonine residues) overlap with those of the $^{13}\text{C}\alpha$ resonances, so they cannot be decoupled. Moreover, homonuclear decoupling causes significant Bloch–Siegert shifts

of the $^{13}\text{C}\alpha$ resonances. Bloch–Siegert shifts and the problem with decoupling serine $^{13}\text{C}\beta$ signals can be avoided if proteins are labeled with ^{13}C only on backbone but not on side-chain atoms.¹¹ However, this method requires the chemical synthesis of specifically labeled amino acids and overexpression in mammalian cell lines to avoid isotopic scrambling.

In this work we present an efficient and robust way to eliminate $^{13}\text{C}\alpha$ – $^{13}\text{C}\beta$ splittings in multidimensional spectra by deconvolution using maximum entropy (MaxEnt) reconstruction.^{12–14} Deconvolution circumvents the need for backbone-labeled proteins or calibration of multiple-band decoupling schemes and, therefore, does not introduce shifts in the resonance positions. The theory and algorithm behind MaxEnt reconstruction are explained elsewhere.¹² The function we deconvolve is a modulation in the $^{13}\text{C}\alpha$ dimension by $\cos(\pi Jt)$, where J is an approximation to the $^{13}\text{C}\alpha$ – $^{13}\text{C}\beta$ coupling constant. The resulting spectra are free of splittings.

HNCA experiments were recorded on a 0.8 mM sample of a 14-kDa fragment of the transcription factor Cdc5.¹⁵ The experiments were performed on a Bruker DMX500 spectrometer equipped with a TXI-Cryoprobe. The Rowland NMR Toolkit (RNMRTK) was used for data processing. (RNMRTK is available via the Internet at <http://www.rowland.org/rnmrtk>.)

Figure 1a shows a typical ^1H – $^{13}\text{C}\alpha$ plane from the HNCA spectrum using standard FT processing. Each peak is split into a doublet by the $^{13}\text{C}\alpha$ – $^{13}\text{C}\beta$ coupling during the t_1 period. Figure 1b shows the same spectrum with the $^{13}\text{C}\alpha$ – $^{13}\text{C}\beta$ splitting removed by deconvolution using MaxEnt reconstruction. The 1D cross-sections show that the signal-to-noise ratio is significantly increased by the elimination of the $^{13}\text{C}\alpha$ – $^{13}\text{C}\beta$ splitting. Note that overlapped peaks are also correctly deconvolved (peaks indicated by the three arrows).

Figure 1c shows the corresponding ^1H – $^{13}\text{C}\alpha$ plane of a $\text{C}\beta$ -decoupled HNCA (Cbd-HNCA)¹⁰ spectrum. Although it has higher sensitivity than the HNCA spectrum, the $^{13}\text{C}\beta$ decoupling causes significant Bloch–Siegert shifts of the $^{13}\text{C}\alpha$ resonances. In addition, the signal observed at 9.1 ppm in the ^1H dimension (serine 64) remains split by the $^{13}\text{C}\alpha$ – $^{13}\text{C}\beta$ coupling because several $^{13}\text{C}\beta$ resonances were not decoupled due to overlap with the $\text{C}\alpha$ region (serine 64 $^{13}\text{C}\beta$ chemical shift: 63.5 ppm). The expected intensity increase was not obtained for several peaks in the Cbd-HNCA spectrum, especially those close to the edge of the region covered by the decoupling RF field. The $^{13}\text{C}\beta$ decoupling may attenuate their magnetization.

Figure 2 shows the dependence of the line shape of peak A in Figure 1b on the J value used for deconvolution. The signal intensities and the line shapes of the peaks deconvolved with $J = 31$, 36, and 41 Hz are quite similar to one another, indicating that the small natural variations of the $^{13}\text{C}\alpha$ – $^{13}\text{C}\beta$ coupling constants in a protein do not prevent effective deconvolution using MaxEnt

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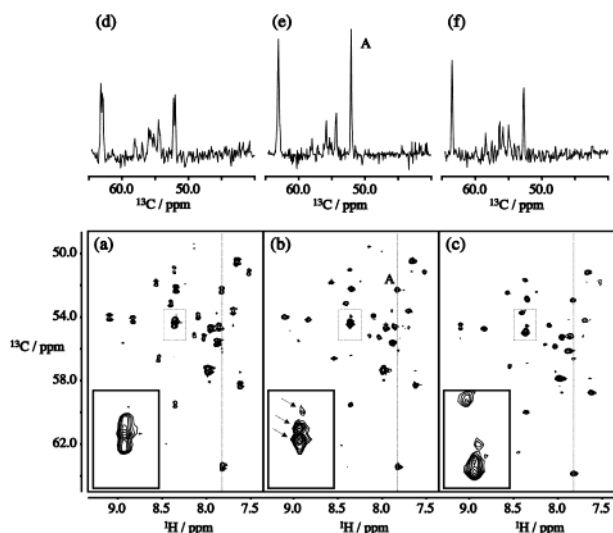


Figure 1. ^1H – ^{13}C α planes from 3D HNCA experiments with an 0.8 mM sample of a 14-kDa fragment of the transcription factor Cdc5. Data sets of 170 (^{13}C) \times 50 (^{15}N) \times 512 (^1H) complex points were recorded for spectral widths of 3125 (^{13}C), 1370 (^{15}N), and 6410 Hz (^1H) in each experimental time of 21 h. The regions enclosed in dashed boxes are expanded below left. (a) ^1H – ^{13}C α plane, taken from a standard HNCA experiment. The ^{13}C dimension was processed using Fourier transformation, showing the ~ 35 Hz coupling between $^{13}\text{C}\alpha$ and $^{13}\text{C}\beta$. (b) $^{13}\text{C}\alpha$ dimension was transformed, and the coupling was deconvolved with MaxEnt reconstruction. The J value for deconvolution was 36 Hz. (c) ^1H – ^{13}C α plane, taken from a Cbd-HNCA experiment. The $^{13}\text{C}\beta$ decoupling was achieved by using a WURST profile. (d–f) 1D cross-sections on top of each section are taken along the ^{13}C dimension at the position indicated by the dashed lines.

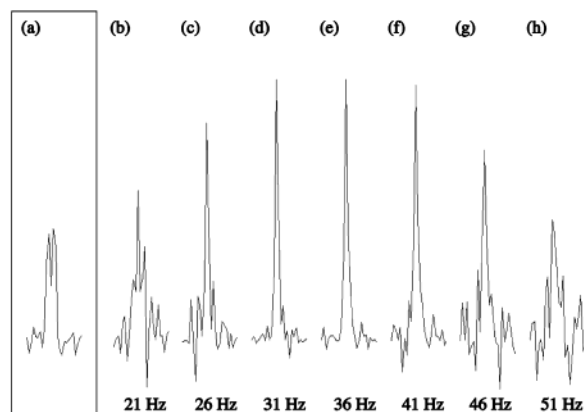


Figure 2. 1D slices of the HNCA are shown, taken along the ^{13}C dimension at the position of signal A in Figure 1. (a) $^{13}\text{C}\alpha$ dimension was processed using Fourier transformation. (b–h) $^{13}\text{C}\alpha$ dimension was transformed and the coupling was deconvolved with MaxEnt reconstruction. The J values used are shown below each spectrum.

reconstruction with a single fixed value for J . Stoven et al. have described a scheme for using MaxEnt to deconvolve multiple unknown J modulations.¹⁶ Such an approach is not necessary when (as here) the couplings are known in advance. Furthermore, when

the pattern and magnitude of the couplings are unknown, there are multiple solutions that cannot be resolved without prior knowledge.

Signals arising from glycine $^{13}\text{C}\alpha$ nuclei are not modulated by $^{13}\text{C}\alpha$ – $^{13}\text{C}\beta$ coupling and thus will have their intensity diminished as a result of MaxEnt deconvolution. Since glycine $^{13}\text{C}\alpha$ signals are observed in the high-field region quite apart from other $^{13}\text{C}\alpha$ resonances, standard FT processing can be used to compute the spectrum for this region, and the absence of $^{13}\text{C}\alpha$ – $^{13}\text{C}\beta$ splitting provides an additional tool for unambiguously identifying glycine residues.

Postacquisition deconvolution of ^{13}C – ^{13}C coupling simplifies the design of pulse sequences, since it is not necessary to implement complicated band-selective decoupling sequences. Using multiple-band decoupling methods requires that the bandwidth of the decoupling field be checked prior to the experiment; decoupling via deconvolution omits this time-consuming procedure. Moreover, Bloch–Siegert shifts and unwanted heating due to the RF fields used for composite-pulse or adiabatic decoupling are avoided.

In conclusion, deconvolution with MaxEnt reconstruction is a versatile and robust method to eliminate the splitting caused by scalar couplings, especially homonuclear one-bond couplings such as $^{13}\text{C}\alpha$ – $^{13}\text{C}\beta$, which are normally eliminated by band-selective decoupling. In this example we used MaxEnt to eliminate the splitting caused by $^{13}\text{C}\alpha$ – $^{13}\text{C}\beta$ coupling in an HNCA spectrum. The same technique can be applied to other triple-resonance experiments to increase both resolution and sensitivity.

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