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Clean absorption Mode NMR data acquisition based on time-proportional phase incrementation

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Abstract

Clean absorption mode NMR data acquisition is presented based on mirrored time domain sampling and widely used time-proportional phase incrementation (TPPI) for quadrature detection. The resulting NMR spectra are devoid of dispersive frequency domain peak components. Those peak components exacerbate peak identification and shift peak maxima, and thus impede automated spectral analysis. The new approach is also of unique value for obtaining clean absorption mode reduced-dimensionality projection NMR spectra, which can rapidly provide high-dimensional spectral information for high-throughput NMR structure determination.

Keywords

Clean Absorption Mode NMR; TPPI; GFT projection NMR; RD projection NMR

Introduction

It is by now widely accepted that NMR spectroscopy [1,2] plays a unique role for structural genomics (SG) [3,4]: many proteins do not form diffraction quality crystals, the success of structure determination by NMR and X-ray crystallography, *i.e.*, the quality of NMR spectra and crystallization success, are hardly correlated, the cost-effectiveness of NMR and X-ray structure production is nowadays comparable, and NMR is about equally successful for pro- and eukaryotic proteins. Currently, NMR structures represent ~10% of the output of the U.S. Protein Structure Initiative (PSI), and the Northeast Structural Genomics Consortium (NESG) and the Center for Eukaryotic Structural Genomics (CESG) contribute >90% of the PSI NMR structures.

In recent years, a large variety of new approaches were introduced to rapidly acquire high-quality NMR spectra encoding high-dimensional spectral information for high-throughput structure production [3,4]. High quality of the NMR spectra is desirable also because semi-automated data analysis is then greatly facilitated. Hence, one focus of NMR methodology development for SG is to improve the quality of the NMR spectra, that is, to minimize artifacts impeding automated analysis [5] and peak picking [6]. Very recently, we have introduced a protocol for ‘clean’ absorption mode NMR data acquisition [7] which can ensure that all peaks in a given NMR spectrum are detected in absorption mode [1,2] and are devoid of any residual dispersive signal components. These dispersive components are introduced by imperfections of the radio-frequency (r.f.) pulse sequences employed to acquire NMR spectra, and they

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effectively broaden peaks and shift their maxima. In turn, this reduces the accuracy with which the chemical shifts can be measured and impedes spectral analysis, in particular for systems exhibiting a high degree of chemical shift degeneracy.

Absorption mode NMR data acquisition can rely either on the ‘hypercomplex’ or the ‘time-proportional phase incrementation’ (TPPI) method [1,2], and clean absorption mode NMR was introduced using the hypercomplex method [7]. TPPI is based on co-incrementing an evolution period along with an r.f. pulse or receiver phase and relates to a concept introduced by Redfield [8] to obtain 1D absorption mode with a single analog-to-digital converter. For the sampling of indirect evolution periods of multiple-quantum NMR, TPPI was introduced for separating signals of different order [9,10] and then adapted for absorption mode signal detection in indirect evolution periods of multidimensional experiments [11]. Notably, frequency domain peaks located outside of the selected spectral range are ‘folded’ when employing TPPI, while they are ‘aliased’ when using the hypercomplex method [e.g., 2]. Hence, depending on the peak dispersion encountered for a given system, either TPPI or the hypercomplex method may be the preferred choice for obtaining absorption mode NMR spectra and methodology for clean absorption mode data acquisition is desirable to have for both approaches.

In reduced-dimensionality (RD) projection NMR [12,13], TPPI employed (along with appropriate scaling) to the projected chemical shifts enables one to place into separate spectral regions signals encoding different types of linear combinations of chemical shifts. This prevents an increase in spectral congestion when compared with the parent higher-dimensional NMR spectrum [13,15–18]. TPPI can thus play a role similar to the G-matrix transformation in GFT NMR [14], which generalizes the hypercomplex method for the quadrature detection of linear combinations of chemical shifts and allows one to edit the signals encoding different types of linear combinations into different sub-spectra. It is important to note that different phase corrections can be applied for different GFT NMR sub-spectra to reduce the intensity of residual dispersive peak components. In RD NMR spectra, all peaks encoding different linear combinations of shifts are located in the same spectra. Hence, only a single phase corrections can be applied, and new approaches for clean absorption mode RD NMR data acquisition meet with a high demand.

In this publication we show that clean absorption mode NMR can be based on the use of TPPI. As for clean absorption mode NMR based on the hypercomplex method [7], TPPI-based clean absorption mode NMR requires ‘mirrored sampling’ the time domain, that is, the time domain is sampled both in a forward and a backward manner. Since TPPI is widely used, the approach presented here enables one to readily adapt TPPI-based implementations for clean absorption mode data acquisition. Furthermore, the required ‘dual TPPI’ can be used for an arbitrary number of indirect dimensions of a multidimensional NMR experiment, as well as for RD projection NMR.

Theory

The theory of TPPI-based clean absorption mode NMR, which requires ‘mirrored time domain sampling’ (MS) [7,19], is described in detail in the Supporting information. For MS, the time domain is sampled twice, that is, once in a forward manner from time 0 to $+t_{\max}$, and once backward from time 0 to $-t_{\max}$, where $t_{\max}$ represents the maximal evolution time (Fig. 1).

Forward TPPI sampling (Fig. 1a) yields a time domain signal $S_+(t) \propto \cos \Phi e^{i(\alpha+\Omega)t} + \sin \Phi e^{i\pi/2} e^{i(\alpha+\Omega')t}$, where α and Φ represent, respectively, a chemical shift and a phase shift (e.g., a phase error which can not be remove by a zero- or first-order phase correction [2]), and Ω' denotes the TPPI frequency shift [2]. Corresponding backward TPPI sampling (Fig. 1b) yields $S_-(t) \propto \cos \Phi e^{i(\alpha+\Omega)t} - \sin \Phi e^{i\pi/2} e^{i(\alpha+\Omega')t}$, so that addition of the two spectra (Fig. 1c) yields

‘dual TPPI sampled’ clean absorption mode signals $S^{ADD}(t) \propto \cos\Phi e^{i(\alpha+\Omega')t}$. On the other hand, the subtraction of forward and backward TPPI sampled data sets yields $S^{SUB}(t) \propto \sin\Phi e^{i\pi/2} e^{i(\alpha+\Omega')t}$, that is, purely dispersive signals are obtained (Fig. 1d). Such ‘difference dual TPPI spectra’ can be made absorptive by applying a zero-order phase correction of 90° after complex FT, or after performing a Hilbert transformation [1]. Subsequently, integration of the two corresponding peaks manifested in the added and subtracted spectra can be integrated, so that Φ (or an NMR parameter encoded in Φ) can be measured with high accuracy (see legend of Fig. 1).

As discussed previously [7], clean absorption mode data acquisition leads to a reduction of the signal maximum [and therefore the signal-to-noise ratio (S/N)] relative to a hypothetical absorptive signal by a factor of $\cos\Phi$. Hence, it is advantageous to employ commonly used techniques [1,2,7] to avoid phase corrections prior to dual TPPI sampling, so that only the remaining residual dispersive components are removed by the sampling approach warranting clean absorption mode data acquisition.

Using a formalism similar to the one that was introduced for clean absorption mode NMR based on the hypercomplex method [7], it is straightforward to show that dual TPPI can be applied to an arbitrary number of indirect dimensions of a multidimensional NMR experiment (see Supporting information Section IV), and equally well to an arbitrary number of jointly sampled indirect dimensions in an RD NMR experiment.

In RD NMR [12,13,15–18], one chemical shift α_0 is detected in quadrature (using either TPPI or the hypercomplex method), while the jointly sampled projected chemical shift α_1 yields solely a cosine modulation (or alternatively solely a sine modulation) of the transfer amplitude (Fig. 2). As a result, two RD NMR spectra in which both α_0 and α_1 are either forward (Fig. 2a) or backward sampled (Fig. 2b) can be added up to yield a clean absorption mode spectrum devoid of dispersive peak components (Fig. 2c). However, the corresponding phase shifts Φ_0 and Φ_1 are manifested as differential intensity reductions of the two components of the chemical shift doublet (Fig. 2c). When recording two RD NMR spectra in which α_0 and α_1 are, respectively, backward and forward sampled (Fig. 2d), or forward and backward sampled (Fig. 2e), the resulting sum yields a clean absorption mode spectrum in which the intensity reduction is ‘swapped’ between the two components of the doublet (Fig. 2f). Hence, when adding the two thus obtained clean absorption mode spectra (Figs. 2c, f), the resulting spectrum exhibits peak components of equal intensity (Fig. 2g). These findings can be generalized for RD NMR with more than one projected chemical shift: clean absorption mode RD NMR spectra can be obtained by adding two spectra in which, for example, all jointly sampled chemical shifts are either forward or backward sampled. Elimination of intensity variations within the shift multiplets then requires that all possible combinations of forward and backward sampled evolution periods are recorded and added up. However, since phase shifts are quite generally small in practice, variations of intensities within the shift multiplets remain moderate. Hence, clean absorption mode RD NMR spectra can be obtained by recording only two RD NMR spectra, *independent* of the number of jointly sampled chemical shifts.

Results and Discussion

To exemplify clean absorption mode NMR data acquisition based on dual TPPI, we recorded for ^{13}C , ^{15}N -labeled 8 kDa NESG protein target CaR178 a simultaneous constant-time 2D [$^{13}\text{C}_{\text{aliphatic}}/^{13}\text{C}_{\text{aromatic}}$, ^1H]-HSQC spectrum [7], in which aromatic signals are folded along the indirect dimension. The spectrum was phased so that the aliphatic peaks are absorptive. Hence, the folded aromatic peaks (Figs. 3a–d) exhibit a dispersive component which cannot be removed by conventional data processing [2]. These dispersive components are cancelled in the dual TPPI spectrum (Fig. 3c). To exemplify clean absorption mode data acquisition for

RD NMR, we recorded 2D $\zeta^{\alpha\beta}\zeta^{\alpha}$ (CON)HN spectra (Figs. 3e,f; for contour plots see Fig. S2, S3 in the supporting information). As predicted by theory, dispersive peak components and imbalances of intensities of shift multiplet components can be readily eliminated.

As was shown for clean absorption mode NMR data acquisition based on the hypercomplex method [7], TPPI-based clean absorption mode data acquisition thus enables one to also remove dispersive components arising from phase errors which cannot be removed by a zero- or first-order phase correction. Since the peak folding characteristics are different for the hypercomplex and TPPI method [2], one of the two methods might be preferable when considering the peak dispersion encountered for a particular system. In contrast to $\pi/4$ -phase shifted MS [7], dual TPPI, like ‘dual States’ [7] acquisition, requires that twice the measurement time is invested to cancel the dispersive components. However, along the lines described in [7], dual TPPI can likewise be concatenated with a 2-step phase cycle employed, for example, to suppress axial peaks and/or the residual solvent resonance line. Clean absorption mode NMR spectra are then obtained without investment of additional spectrometer time.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

FT	Fourier transformation
GFT	G-matrix FT
NESG	Northeast Structural Genomics Consortium
NMR	nuclear magnetic resonance
PSI	protein structure initiative
RD	reduced-dimensionality
SG	structural genomics
TPPI	time-proportional phase incrementation

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Fig. 1.

Clean absorption mode NMR data acquisition based on TPPI (see text). The phase shift Φ is assumed to be 15° (for comparison, the dashed grey lines represent time domain modulations of unit amplitude and $\Phi = 0$). Forward time domain sampling and the corresponding frequency domain spectrum shown in **a** are in blue, backward time domain sampling and the corresponding frequency domain spectrum shown in **b** are in red, and linear combinations shown in **c** and **d** are depicted in green. The quadrature image peaks, which are discarded after FT, are depicted by dashed lines. The intensities of frequency domain peaks in (a) and (b) were calculated using Eq. S68 in [7]. The absorption mode c^{SUB} -dual TPPI spectrum is shown in grey and can either be obtained after Hilbert transform [1] following real FT (as indicated) or by zero order phase correction by 90° after complex FT [1,2].

Fig. 2.

Clean absorption mode RD NMR data acquisition. Quadrature detection of chemical shift α_0 is assumed to be accomplished by use of TPPI, but the hypercomplex method could be used equally well (see supporting information). α_1 represents the jointly sampled chemical shift resulting in a cosine modulation of the transfer amplitude, so that the peak components of the chemical shift doublet are located at $\alpha_0 - \alpha_1$ and $\alpha_0 + \alpha_1$. The phase shifts associated with α_0 and α_1 are assumed to be $\Phi_0 = 20^\circ$ and $\Phi_1 = 30^\circ$, respectively, and the intensities of frequency domain peaks were calculated using Eq. S68 in [7]. For **a**, **b**, **d**, and **e** it is assumed that data are acquired with, respectively, forward/forward, backward/backward, backward/forward and forward/backward sampling of α_0/α_1 . Summation of (a) and (b), or (d) and (e), yields clean absorption mode spectra with intensity imbalances of the doublet components, as is shown **c** and **f**. Those can be added up to eliminate the imbalances, as is shown in **g**.

Fig. 3.

a–d: Cross sections containing a signal arising from an aromatic CH-moiety, which were taken at $\omega_2(^1\text{H}) = 8.13$ ppm along $\omega_1(^{13}\text{C})$ from simultaneous constant time 2D [$^{13}\text{C}_{\text{aliphatic}}/^{13}\text{C}_{\text{aromatic}}, ^1\text{H}$]-HSQC spectra [7]. The spectra were acquired for 8 kDa NESG target protein CaR178 at 25 °C on a Varian INOVA 500 MHz spectrometer equipped with a conventional $^1\text{H}\{^{13}\text{C}, ^{15}\text{N}\}$ -triple resonance probe. The spectrum was phased to obtain absorption mode signals for the aliphatic moieties so that the folded aromatic signals exhibit a dispersive peak component. The ppm-scale represents the calibration of the aliphatic part of the spectrum. **a** Forward sampled TPPI, **b** backward sampled TPPI, **c** dual TPPI resulting from the addition of (a) and (b), and **d** dual TPPI resulting from forming the difference of (a) and

(b). In (c), the dispersive peak components visible in (a) and (b) are cancelled. The vertical dashed lines indicate the peak maximum in (c), and the actual aromatic ^{13}C chemical shift of the folded signal is given. In (d), the dotted line represents the absorptive peak obtained after Hilbert transformation, or application of a 90° phase correction after complex FT (see text and Supporting Information). The integration of the peaks in (c) and (d) enables one to accurately measure the phase shift Φ (Eq S15 in the Supporting Information). The ^{13}C carrier frequency was set to 43.0 ppm, the spectral width of the indirect ^{13}C -dimension was set to 68 ppm (i.e., aromatic signals are folded), and $t_{1,\text{max}}(^{13}\text{C}) = 21$ ms. Each spectrum shown (a) and (b) was recorded in 28 min, so that the total measurement time of the dual TPPI spectra was 56 min.

e–f: Cross sections taken along the RD-dimension ω_1 from RD 2D $\underline{\text{C}}^{\alpha\beta}\underline{\text{C}}^{\alpha}(\text{CON})\text{HN}$ spectra at $\omega_2(^1\text{HN}) = 8.14$ ppm (for contour plots of the 2D spectra, see Figs. S2 and S3 in the supporting information). The spectra were acquired for 8 kDa NESG target protein CaR178 at 25 °C on a Varian INOVA 600 spectrometer equipped with a cryogenic $^1\text{H}\{^{13}\text{C},^{15}\text{N}\}$ -triple resonance probe using the radio-frequency (r.f.) pulse scheme of $(4,3)\text{D } \underline{\text{C}}^{\alpha\beta}\underline{\text{C}}^{\alpha}(\text{CO})\text{NHN}$ [20]. Quadrature detection of $\Omega(^{13}\text{C}^{\alpha})$ was accomplished using TPPI with the carrier position set to 56 ppm. $\Omega(^{13}\text{C}^{\alpha\beta})$ was encoded in an in-phase splitting yielding shift doublets with peak components located at $\Omega(^{13}\text{C}^{\alpha}) \pm \Omega(^{13}\text{C}^{\alpha\beta})$. TPPI was employed also to the 90° r.f. pulse generating transverse $^{13}\text{C}^{\alpha\beta}$ magnetization, so that the carrier position was shifted from 43 ppm to an apparent position at 3 ppm. The resulting spectral width in the RD dimension was 320 ppm and $t_{1,\text{max}} = 6.7$ ms. **e** Spectrum acquired within 53 min using RD NMR data acquisition with forward/forward sampling of $\Omega(^{13}\text{C}^{\alpha})/\Omega(^{13}\text{C}^{\alpha\beta})$ (see also Fig. 2a). **f** Sum (see also Fig. 2g) of four spectra acquired within 53 min each using forward/forward, backward/backward, backward/forward and forward/backward sampling (see also Figs. 2a, b, d, e) of $\Omega(^{13}\text{C}^{\alpha})/\Omega(^{13}\text{C}^{\alpha\beta})$. Dispersive peak components which are visible in (e), as well as doublet component imbalances visible in the sums corresponding to Figs. 2c and 2d (cross sections not shown), are eliminated.