GFT NMR Spectroscopy: Rethinking Multidimensional Data Acquisition
Drawbacks of Multidimensional FT NMR

- Measurement times scale with $\Pi n_j$
3D and 4D:

Sampling versus Sensitivity Limitation

Time Domain

Frequency Domain

5D +:

Sampling Limitation
Drawbacks of Multidimensional FT NMR

- Measurement times scale with $\Pi n_j$
- Low precision of chemical shift measurement in indirect dimensions
Structural Genomics


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IMNMR 6/30/03
A pilot project in structural genomics focused on proteins from eukaryotic model organisms and human. The project targets representative proteins to provide "coverage" of fold space, and those that are interesting from a functional genomics perspective. It also explores the complementary aspects of X-ray crystallography and NMR spectroscopy.

**TARGET PROGRESS**
selected: 7886, cloned: 2162,
expressed: 1531, purified: 949,
structures (nmr 99, x-ray 75): 76,
structures submitted to pdb: 72.

**PARTICIPATING INSTITUTIONS**
Rutgers University
Columbia University
Cornell University
Hippman-Woodward Research Institute
Pacific Northwest National Laboratories
The State University of New York at Buffalo
UMDNJ - Robert Wood Johnson Medical School
University of Toronto
Yale University

**SUPPORTED BY**
the Protein Structure Initiative of the
NIH National Institutes of General Medical Sciences

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Comments to: Kat Edwards
Objectives:
HTP NMR Structure Determination

- **Minimize NMR Measurement Time** (cryogenic probes)
  - Reduce costs per structure
  - Reduce demand for long-term sample stability

- **Automated Assignment:**
  - **High Dimensionality and Spectral Resolution**
    - Redundancy of spectral data
    - Small number of spectra
3-dimensional

2-dimensional

Challenge: keep information upon projection
Challenge: keep information upon projection
Two-Spin Coherence Spectroscopy
(J. Biomol. NMR 3, 1993)

\[ K_x S_y e^{i\Omega K t} \cos(\Omega_s t) \]
K-S
Spin System

Two-Spin Coherence Spectroscopy
(J. Biomol. NMR 3, 1993)

\[ K_x S_y e^{i \Omega t} \cos(\Omega_s t) \]

\[ S: ^{13}C \]
\[ K: ^{15}N \]
magnetization. During $t_2$ the two-spin coherence evolves with both chemical shifts, $\Omega(\text{${}^{15}\text{N}$})$ and $\Omega(\text{${}^{13}\text{C}$})$, while $\text{${}^1\text{H}$}$ and $\text{${}^{13}\text{C}$} = \text{O}$ are decoupled by 180°-pulses, yielding

$$\sigma(d) = A_y N_y \cos[\Omega(\text{${}^1\text{H}$}) t_1] \cos[\Omega(\text{${}^{13}\text{C}$}) t_2] \cos[\Omega(\text{${}^{15}\text{N}$}) t_2].$$  \hspace{1cm} (4)

A 90°-pulse on $\text{${}^{13}\text{C}$}$ then creates single-quantum coherence on $\text{${}^{15}\text{N}$}$, which is antiphase with magnetization is antiphase with respect to $\text{${}^1\text{H}$}^N$ at the end of $\tau_2$, and the reverse INEPT leads to

$$\sigma(e) = I_y^N \cos[\Omega(\text{${}^1\text{H}$}) t_1] \cos[\Omega(\text{${}^{13}\text{C}$}) t_2] \cos[\Omega(\text{${}^{15}\text{N}$}) t_2].$$  \hspace{1cm} (5)

Using the addition theorems for trigonometric functions, $\sigma(e)$ can be re-written as

$$\sigma(e) = I_y^N \cos[\Omega(\text{${}^1\text{H}$}) t_1] \{\cos[\Omega(\text{${}^{15}\text{N}$}) + \Omega(\text{${}^{13}\text{C}$})] t_2\} + \cos[\Omega(\text{${}^{15}\text{N}$}) - \Omega(\text{${}^{13}\text{C}$})] t_2\}$$  \hspace{1cm} (6)

Phase-sensitive detection of both the double-quantum and zero-quantum components contained in the two-spin coherence requires the generation of the corresponding imaginary term

$$\sigma^i(e) = I_y^N \cos[\Omega(\text{${}^1\text{H}$}) t_1] \{\sin[\Omega(\text{${}^{15}\text{N}$}) + \Omega(\text{${}^{13}\text{C}$})] t_2\} + \sin[\Omega(\text{${}^{15}\text{N}$}) - \Omega(\text{${}^{13}\text{C}$})] t_2\}. $$  \hspace{1cm} (7)

This is achieved by applying the States-TPPI method (Marion et al., 1989) to all pulses on $\text{${}^{15}\text{N}$}$ applied before $t_2$ ($\phi_4$, $\phi_8$ and $\phi_{10}$ in Fig. 2). This phase-incrementation yields

$$\sigma(e) = I_y^N \cos[\Omega(\text{${}^1\text{H}$}) t_1] \cos[\Omega(\text{${}^{13}\text{C}$}) t_2] \sin[\Omega(\text{${}^{15}\text{N}$}) t_2], $$  \hspace{1cm} (8)

which is, according to the addition theorems for trigonometric functions, equal to Eq. 7. Thus the complex acquisition of the two-spin coherence leads to the observation of $\Omega(\text{${}^{15}\text{N}$}) \pm \Omega(\text{${}^{13}\text{C}$})$ along $\omega_2$. 

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magnetization. During $t_2$ the two-spin coherence evolves with both chemical shifts, $\Omega^{(15N)}$ and $\Omega^{(13C)}$, while $^1H^a$ and $^1C=O$ are decoupled by 180°-pulses, yielding

$$\sigma(d) = A_y N_y \cos[\Omega^{(1H^a)}t_1] \cos[\Omega^{(13C)}t_2] \cos[\Omega^{(15N)}t_2]. \quad (4)$$

A 90°-pulse on $^{13}C^a$ then creates single-quantum coherence on $^{15}N$, which is antiphase with magnetization is antiphase with respect to $^{1H^N}$ at the end of $\tau_2$, and the reverse INEPT leads to

$$\sigma(e) = I^N_y \cos[\Omega^{(1H^a)}t_1] \cos[\Omega^{(13C)}t_2] \cos[\Omega^{(15N)}t_2]. \quad (5)$$

Using the addition theorems for trigonometric functions, $\sigma(e)$ can be re-written as

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Phase-sensitive detection of both the double-quantum and zero-quantum components contained in the two-spin coherence requires the generation of the corresponding imaginary term (7)

$$\bar{\sigma}(e) = I^N_y \cos[\Omega^{(1H^a)}t_1] \{\sin\{[\Omega^{(15N)} + \Omega^{(13C)}]t_2\} + \sin\{[\Omega^{(15N)} - \Omega^{(13C)}]t_2\}\}. \quad (7)$$

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$$\sigma(e) = I^N_y \cos[\Omega^{(1H^a)}t_1] \cos[\Omega^{(13C)}t_2] \sin[\Omega^{(15N)}t_2], \quad (8)$$

which is, according to the addition theorems for trigonometric functions, equal to Eq. 7. Thus the complex acquisition of the two-spin coherence leads to the observation of $\Omega^{(15N)} \pm \Omega^{(13C)}$ along $\omega_2$. 

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(4)

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magnetization. During $t_2$ the two-spin coherence evolves with both chemical shifts, $\Omega^{(15N)}$ and $\Omega^{(13C\alpha)}$, while $^1H$ and $^{13}C=O$ are decoupled by $180^\circ$-pulses, yielding

$$\sigma(d) = A_N x_N \cos[\Omega^{(1H\alpha)}t_1] \cos[\Omega^{(13C\alpha)}t_2] \cos[\Omega^{(15N\alpha)}t_2].$$  \hspace{1cm} (4)$$

A $90^\circ$-pulse on $^{13}C\alpha$ then creates single-quantum coherence on $^{15}N$, which is antiphase with magnetization is antiphase with respect to $^1H^N$ at the end of $t_2$, and the reverse INEPT leads to

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Phase-sensitive detection of both the double-quantum and zero-quantum components contained in the two-spin coherence requires the generation of the corresponding imaginary term (7)

$$\sigma^i(e) = I_N^N \cos[\Omega^{(1H\alpha)}t_1] \{ \sin\{[\Omega^{(15N\alpha)} + \Omega^{(13C\alpha)}]t_2 \} + \sin\{[\Omega^{(15N\alpha)} - \Omega^{(13C\alpha)}]t_2 \} \}.$$  \hspace{1cm} (7)$$

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$$\sigma(e) = I_N^N \cos[\Omega^{(1H\alpha)}t_1] \cos[\Omega^{(13C\alpha)}t_2] \sin[\Omega^{(15N\alpha)}t_2],$$  \hspace{1cm} (8)$$

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Two-Spin Coherence Spectroscopy  
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\[ K_x S_y e^{i\Omega t} \cos(\Omega_s t) \]

RD NMR Spectroscopy  
(JACS 115, 1993)

\[ K_x e^{i\Omega t} \ldots S_y \cos(\Omega_s t) \]
Improved 3D Triple-Resonance NMR Techniques
Applied to a 31 kDa Protein

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Received September 6, 1991

Recently proposed 3D triple-resonance techniques (1–5) make it possible to obtain sequential assignment of the backbone $^1$H, $^{15}$N, and $^{13}$C resonances in proteins that can be isotopically labeled with $^{13}$C and $^{15}$N. This approach has been used successfully for a number of systems, including calmodulin (16.7 kDa) (1), calmodulin complexed with a 26-residue peptide (≈20 kDa) (6), and the phospho-carrier protein III$^{Glc}$ (7) (18 kDa). All of these triple-resonance experiments rely on transfer of magnetization via heteronuclear one-bond $J$ couplings and the sensitivity of the experiments depends strongly on the ratio of the size of the $J$ coupling and the linewidths of the nuclei involved in the magnetization transfer process. Our original experiments were designed to minimize the number of RF pulses required for a particular pulse sequence, and thus to minimize the effect of pulse imperfections. Subsequent experience with these experiments has indicated that the effect of pulse imperfections is not as severe as originally expected, provided that pulses are properly calibrated. Hence, in order to optimize the experiments it is more important to minimize the relaxation of transverse magnetization by keeping the dephasing and rephasing delays as short as possible, especially for larger proteins. Here we demonstrate the applicability of three improved triple-resonance experiments to the study of interferon-γ, a dimer with 134 residues per monomer and a total molecular weight of 31.4 kDa.
Fig. S1

Supplementary material, p. 2
Reduced Dimensionality in Triple-Resonance NMR Experiments

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Received April 30, 1993

As an alternative to the conventional assignment of protein NMR spectra by observation of sequential NOEs in homonuclear 2D $[^1H,^1H]$-NOESY or 3D and 4D heteronuclear-resolved $[^1H,^1H]$-NOESY spectra, 3D and 4D triple-resonance experiments have been proposed for establishing intra- and interresidual connectivities via heteronuclear scalar couplings. 4D experiments of this type are conceptually particularly attractive, since only a single experiment is needed for intraresonial correlation of the four backbone spins $^1H\text{N}$, $^{15}N$, $^{13}C\text{α}$, and $^1H\text{α}$, and suitable combinations of two 4D experiments can provide sequential assignments. However, because of the short $T_2$ relaxation times of $^{13}C\text{α}$ in bigger molecules, the use of 4D triple resonance experiments in practice limited to proteins with molecular weights below approximately 15 000, where such spectra are only sparsely populated with cross peaks and hence dispersion in four dimensions is not really needed. Therefore, the development of variant triple-resonance experiments that provide the same connectivity information in spectra with reduced dimensionality is attractive, since larger values of $t_{2\text{max}}$ can then be chosen for the

Figure 1. Contour plots of $(\omega_1(^{13}C\alpha) - \omega_3(^{1H}\text{N}))$-strips from a 3D HA CA N HN spectrum obtained with a 2.5 mM sample of the uniformly $^{13}C$- and $^{15}N$-labeled mixed disulfide of E. coli glutaredoxin (C14S) with glutathione in 90% H$_2$O/10% D$_2$O, 100 mM potassium phosphate, pH 6.5, at $T = 20$ °C. A Bruker AMX 600 spectrometer equipped with four channels was used. 24 ($t_1$) * 98 ($t_2$) * 512 ($t_3$) complex points were accumulated, with $t_{1\text{max}}(^{1H}\text{N}) = 16.8$ ms, $t_{2\text{max}}(^{15}N,^{13}C\alpha) = 11.2$ ms, and $t_{3\text{max}}(^{1H}\text{N}) = 65.5$ ms. 32 scans per increment were acquired, resulting in a total measuring time of 3.5 days. The carrier frequencies of the $^{15}N$ and $^{13}C\text{α}$ pulses were set to 105.1 and 56 ppm, respectively.
Reduced Dimensionality in Triple-Resonance NMR Experiments

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![Contour plots of \((\omega_1(13Cα) - \omega_3(1H^N))-strips from a 3D HA CA N HN spectrum obtained with a 2.5 mM sample of the uniformly \(^13\)C- and \(^15\)N-labeled mixed disulfide of \(E.\ coli\) glutaredoxin-(C14S) with glutathione\(^7\) in 90% \(H_2O/10% D_2O\), 100 mM potassium phosphate, pH 6.5, at \(T = 20 °C\). A Bruker AMX 600 spectrometer equipped with four channels was used. 24 \((t_1) * 98 (t_2) * 512 (t_3)\) complex points were accumulated, with \(t_{\text{acq}}(1H^α) = 16.8\) ms, \(t_{\text{acq}}(15N,13Cα) = 11.2\) ms, and \(t_{\text{acq}}(1H^N) = 65.5\) ms. 32 scans per increment were acquired, resulting in a total measuring time of 3.5 days. The carrier frequencies of the \(^15\)N and \(^13\)C\(^α\) pulses were set to 105.1 and 56 ppm, respectively.](image)
Reduced Dimensionality in Triple-Resonance NMR Experiments

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As an alternative to the conventional assignment of protein NMR\(^1\) spectra by observation of sequential NOEs\(^2\) in homonuclear 2D \(^{1}{H},{^{1}}{H}\)-NOESY or 3D and 4D heteronuclear-resolved \(^{1}{H},{^{1}}{H}\)-NOESY spectra, 3D and 4D triple-resonance experiments have been proposed for establishing intra- and interresidual connectivities via heteronuclear scalar couplings.\(^3\)–\(^{12}\) 4D experiments of this type are conceptually particularly attractive, since only a single experiment is needed for intraresidual correlation of the four backbone spins \(^{1}H\), \(^{15}N\), \(^{13}C\), and \(^{13}H\), and suitable combinations of two 4D experiments can provide sequential assignments.\(^{11}\)\(^{,}\)\(^{13}\) However, because of the short \(T_2\) relaxation times of \(^{13}C\) in bigger molecules, the use of 4D triple resonance experiments\(^6\)\(^,\)\(^9\)\(^,\)\(^11\) is in practice limited to proteins with molecular weights below approximately 15 000,\(^6\) where such spectra are only sparsely populated with cross peaks and hence dispersion in four dimensions is not really needed. Therefore, the development of variant triple-resonance experiments that provide the same connectivity information in spectra with reduced dimensionality is attractive, since larger values of \(t_2\) can then be chosen for the axis \(\omega_2\) in the 3D HA CA N HN spectrum. Since the \(^{15}N\) chemical shift is extracted from the difference between \(\Omega(\bar{^{13}}C)\) – \(\Omega(\bar{^{15}}N)\) and \(\Omega(\bar{^{13}}C\bar{^{13}}C) + \Omega(\bar{^{15}}N)\), the \(^{15}N\) carrier must be at the edge of the \(^{15}N\) spectral range to obtain unambiguous \(^{15}N\) assignments. As the sweep width for \(^{15}N\) (<2000 Hz at 14.1 T) is significantly smaller than that for \(^{13}C\) (∼4500 Hz at 14.1 T), the thus required sweep width along \(\omega_2\) is only about one-third larger than in a corresponding 4D experiment. (This increase in sweep width could be circumvented if the in-phase splitting due to \(\Omega(\bar{^{15}}N)\) were scaled down using a smaller increment for \(^{15}N\) than for \(^{13}C\), which would, however, also reduce \(t_{\text{max}}(\bar{^{15}}N)\).)
Methodological extensions of RD NMR Spectroscopy

- Scaling of projected chemical shift evolution
- Time proportional phase incrementation (TPPI) to shift carrier to the edge of spectral range
- Phase-sensitive RD NMR
- Symmetrization of RD NMR spectra
- 'Double' RD NMR
- Central peak detection & combined shift scaling and TPPI
- RD NMR combined with simultaneous data acquisition
- RD NMR for high throughput
Methodological extensions of RD NMR Spectroscopy

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$K_x e^{i\Omega_K t} \ldots \cdot S_y \cos(\Delta \Omega_S t)$
\[ K_x \ e^{i\Omega K t} \quad \ldots \quad S_y \cos(\Delta \Omega_s t) \]

\[ K_x \ e^{i\Omega K t} \quad \ldots \quad S_y \cos(\kappa \Delta \Omega_s t) \]
$K_x e^{i\Omega K t}$ ...... $S_y \cos(\Delta \Omega_S t)$

$K_x e^{i\Omega K t}$ ...... $S_y \cos[(\Delta \Omega_S + \Delta \Omega') t]$
Methodological extensions of RD NMR Spectroscopy

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Time proportional phase incrementation (TPPI) to shift carrier to the edge of spectral range

High-resolution 3D HNCOCA experiment applied to a 28 kDa paramagnetic protein

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Accepted 23 January 1995

Keywords: Cytochrome c'; Resonance assignment; HNCOCA; Multiple quantum coherence
Methodological extensions of RD NMR Spectroscopy

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- Symmetrization of RD NMR spectra
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- Central peak detection & combined shift scaling and TPPI
- RD NMR combined with simultaneous data acquisition
- RD NMR for high throughput
$K_x e^{i\Omega_K t} \cdots S_y \cos(\Delta\Omega_S t)$

$K_x e^{i\Omega_K t} \cdots S_y \sin(\Delta\Omega_S t)$
'Edited phase-sensitive' RD NMR

Determinement of an Initial Set of NOE-Derived Distance Constraints for the Structure Determination of $^{15}\text{N}/^{13}\text{C}$-Labeled Proteins

**Bernhard Brutscher, Nathalie Morelle, Florence Cordier, and Dominique Marion**

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Received August 11, 1995
FIG. 2. Slices of sum (A) and difference (B) spectra of the 3D $^{13}$C/$^{15}$N-filtered NOESY experiment of *Rhodobacter capsulatus* ferrocytochrome c$_2$. The slices are taken at the frequency $H^C = 4.44$ ppm. The NOE correlation peaks could be automatically assigned to (1) 106°/2N, (2) 42°/42N, (3) 106°/107N, (4) 42°/43N, (5) 23°/24N, (6) 109°/110N, (7) 109°/109N, (8) 106°/106N, (9) 109°/111N, (10) 23°/23N, (11) 106°/109N. Unassigned peaks have their intensity maximum in neighboring slices.
Methodological extensions of RD NMR Spectroscopy

- Scaling of projected chemical shift evolution
- Time proportional phase incrementation (TPPI) to shift carrier to the edge of spectral range
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- RD NMR for high throughput
Fig. 2. $^{13}$C strip of residue V6, drawn at the noise level (A). The intensity of the two correlation peaks is close to the noise level. Nevertheless, the symmetry of the two peaks with respect to a known center point allows one to distinguish between signal and noise. This is shown in part (B), where spectrum (A) has been added to its mirror image (reflected on the CO frequency) resulting in an increased S/N ratio for the two correlation peaks. All other peaks, which do not display the symmetry with regard to the same CO frequency (e.g., the signal at 172 ppm), are more or less cancelled, together with the experimental artefacts.
Methodological extensions of RD NMR Spectroscopy

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- ‘Double’ RD NMR
- Central peak detection & combined shift scaling and TPPI
- RD NMR combined with simultaneous data acquisition
- RD NMR for high throughput
A new triple-resonance experiment for the sequential assignment of backbone resonances in proteins

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Keywords: Triple-resonance NMR spectroscopy; Sequential assignment; Reduced dimensionality; Desulfovibrio vulgaris flavodoxin
Fig. 5. Expansion of an F1,F2-slice of the HCACOCANH spectrum obtained with the pulse sequence from Fig. 1C. The intraresidual correlation for Cys$^{90}$ (residue i) of D. vulgaris flavodoxin and the sequential Ala$^{89}$/Cys$^{90}$ correlation are shown. The midpoints of the rectangles appear at the $^{15}$N chemical shift of Cys$^{90}$ and the $^{13}$C$^{\alpha}$ chemical shifts of Cys$^{90}$ and Ala$^{89}$, which can be read directly from the scales at the F1 and F2 axes. $^{1}$H$^{\alpha}$ and $^{13}$CO chemical shifts are obtained from the splittings along the two indirectly detected dimensions. The splittings (in Hz) are scaled down using the factors $x = 0.625$ and $y = 0.2$, such that 1 ppm splitting in F1 corresponds to $\Delta \Omega(H^{\alpha}) = 0.2$ ppm and 1 ppm splitting in F2 corresponds to $\Delta \Omega(CO) = 1$ ppm.
Novel 2D Triple-Resonance NMR Experiments for Sequential Resonance Assignments of Proteins

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Received January 17, 2002; revised March 19, 2002; published online June 20, 2002

FIG. 6. Expansion of the boxed region in Fig. 5A comprising the cross peaks of K28/E27 and T49/A48 in 2D HN(CO)CAHA. The centers for the cross-peak
Methodological extensions of RD NMR Spectroscopy

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- RD NMR combined with simultaneous data acquisition
- RD NMR for high throughput
A Novel Reduced-Dimensionality Triple-Resonance Experiment for Efficient Polypeptide Backbone Assignment, 3D CO HN N CA

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Received May 26, 1995
Useful Information from Axial Peak Magnetization in Projected NMR Experiments

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Received March 27, 1996

Axial peaks arise from magnetization which is not frequency-labeled in at least one indirect dimension of a multidimensional NMR experiment. Because they correspond to incomplete coherence transfers, axial peaks represent a considerable magnetization reservoir. Here we show that the previously introduced projection technique allows the derivation of useful information from axial coherences. In these experiments the evolution of chemical shifts in the projected dimension gives information on relaxation of the axial magnetization which depends on the interaction between the nuclei involved.

Figure 1. Magnetization transfer pathways of reduced-dimensionality triple-resonance experiments. (a) “Out-and-back” 3D HS<KL> experiment for bifurcated one-bond scalar coupling topology. (b) Corresponding 3D HSKL experiment for linear topology. (c) “Out-and-stay” 3D HCSH experiment. H and C denote proton and carbon spins, S a heterospin (e.g., $^{13}$C or $^{15}$N), and K and L either protons or heterospins. The two nuclei observed in a common dimension are in a shaded box, and among these the nucleus detected in quadrature is marked with an asterisk. Uni- and bidirectional magnetization transfers are represented by arrows and double arrows, respectively.

Figure 2. Cross sections along $\delta_1(C)$ taken from two subspectra of a 3D H$^1$/C$^{13}$/H$^1$(CO)HN experiment recorded on a Bruker AMX-600 spectrometer with acquisition of central peaks. The protein studied is...
Methodological extensions of RD NMR Spectroscopy

- Scaling of projected chemical shift evolution
- Time proportional phase incrementation (TPPI) to shift carrier to the edge of spectral range
- Phase-sensitive RD NMR
- Symmetrization of RD NMR spectra
- 'Double' RD NMR
- Central peak detection & combined shift scaling and TPPI
- RD NMR combined with simultaneous data acquisition
- RD NMR for high throughput
Alternative Projection Techniques and Their Combination with RD NMR

Simultaneous phase sensitive detection
(Xia et al., J. Biomol. NMR 24, 2002)
Challenge: keep information upon projection
TM1112
10.5 kDa
\( t_c = 8.5 \text{ ns} \)
2 * 16.5 hours
at 500 MHz
Combination of 3D HNN[CAHA] / HNN(CO)[CAHA] with 3D RD HNNCACO / HNNCOCA

- $\omega_3 (^1\text{HN-G46}) = 8.63$ (ppm)
- $\omega_2 (^{15}\text{N-G46}) = 109.5$ (ppm)
- $\omega_1 (^{13}\text{C} \alpha\text{-G46}) = 45.25$ (ppm)
- $\omega_1 (^{13}\text{C} \alpha\text{-E45}) = 55.37$ (ppm)
Methodological extensions of RD NMR Spectroscopy

- Scaling of projected chemical shift evolution
- Time proportional phase incrementation (TPPI) to shift carrier to the edge of spectral range
- Phase-sensitive RD NMR
- Symmetrization of RD NMR spectra
- ‘Double’ RD NMR
- Central peak detection & combined shift scaling and TPPI
- RD NMR combined with simultaneous data acquisition
- RD NMR for high throughput
Reduced-dimensionality NMR spectroscopy for high-throughput protein resonance assignment

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*Departments of Chemistry and Structural Biology, State University of New York, Buffalo, NY 14260; and §Center for Advanced Biotechnology and Medicine, Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ 08854

Communicated by Herbert Hauptman, Hauptman–Woodward Medical Research Institute, Buffalo, NY, April 12, 2002 (received for review November 15, 2001)
RDpack Parameter Set Options

- $\text{H}_2\text{O}$ Presaturation
- Use of Spin-lock Purge Pulses
- $^{13}\text{C}$-Steady state magnetization for central peak acquisition
- TROSY
- $^2\text{H}$-Decoupling
Backbone

HNNCAHA
HACA(CO)NHN
HNNCACB
HαβCαβ(CO)NHN
Backbone Assignment: HACA(co)NHN(add I, sub II) & HNNCAHA(III), (A114-L121)
Backbone

HNNCAHA
HACA(CO)NHN
HNCACB
HαβCαβ(CO)NHN
Backbone Assignment: $\text{H}\alpha\beta\text{C}\alpha\beta$ (co)NHN(add I) & HNCACB(II), (A114-L121)
**Backbone**

HNNCAHA
HACA(CO)NHN
HNCACB

HαβCαβ(CO)NHN

**Side-Chain**

HCCH-COSY (TOCSY)
HBCB(CGCD)HD
1H-TOCSY-HCH-COSY
Backbone to Side-Chain, PRO 4, HabCab(CO)NHN(add I, sub II) to HCCCH-COSY (add III, sub IV)
Survey: RD NMR Data for NESGC

- **Proteins from Toronto**
  - MT1362 (8 kDa)
  - SH3-peptide complex (12.5 kDa)
  - SRm160 domain (13 kDa)
  - MT1598 (15 kDa)
  - VT1 (17 kDa)
  - TT212 (14 kDa)

- **Proteins from Rutgers**
  - Z-domain (8 kDa)
  - WR4 (14 kDa)
  - WR33 (21 kDa)
  - MT467 (13 kDa)
  - SR17 (18 kDa)
  - ER14 (12 kDa)
  - ER75 (17 kDa)
  - QR6 (14 kDa)
  - GR2 (7 kDa)
  - MR19 (15 kDa)
  - SR64 (17 kDa)
  - HR41 (22 kDa)
R: Rutgers (Montelione)
T: Toronto (Arrowsmith)
From conventional 600 MHz to 800 MHz spectrometer w/cryoprobe

- Conventional 600:  
  S/N 1,200:1
- 800 w/cryoprobe:  
  S/N 7,700:1
- Reduction in measurement time: ~25+
- (RD) DR/TR data for ER75 in ~10 h
- Simultaneously: All spectral widths increase by 1.33 (~2.3-fold increased sampling demand for 3D)
...RD NMR spectroscopy not fast enough
GFT NMR Spectroscopy

Acquiring multidimensional NMR spectral information with high speed and precision
Challenges:
- keep information of conventional experiment
- avoid spectral crowding
**GFT NMR**

- **Speed**: Phase-sensitive joint sampling of $K+1$ dimensions and ‘recursive central peak detection’

- **Alternative data processing**: Editing of resulting ‘chemical shift multiplets’ ($G$-matrix) and Fourier Transformation

- **Precision**: Least squares fit to obtain shifts from edited multiplets
K=2

\[(N, N-2)D\]

GFT = Combined $G$-matrix and Fourier Transformation
**Joint Sampling of 3D Subspace of an NMR Experiment**

**FT NMR**

- $t_0$ to $t_1$ to $t_2$
- 3D Fourier Transformation

**GFT NMR**

<table>
<thead>
<tr>
<th>$\phi_1$</th>
<th>$\phi_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$0^\circ$</td>
<td>$0^\circ$</td>
</tr>
<tr>
<td>$90^\circ$</td>
<td>$0^\circ$</td>
</tr>
<tr>
<td>$0^\circ$</td>
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<tr>
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<td>$90^\circ$</td>
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<tr>
<td>$0^\circ$</td>
<td>$-$</td>
</tr>
<tr>
<td>$90^\circ$</td>
<td>$-$</td>
</tr>
<tr>
<td>$-$</td>
<td>$-$</td>
</tr>
</tbody>
</table>

- $\hat{G}$ matrix Transformation
- 1D Fourier Transformation

\[
\begin{align*}
\Omega_0 \pm \Delta \Omega & \quad \Omega_1 \pm \Delta \Omega & \quad \Omega_2 \pm \Delta \Omega \\
(\Omega_0 + \Omega_1 + \Omega_2) \pm \Delta \Omega & \quad (\Omega_0 - \Omega_1 + \Omega_2) \pm \Delta \Omega & \quad (\Omega_0 + \Omega_1 - \Omega_2) \pm \Delta \Omega \\
(\Omega_0 - \Omega_1 + \Omega_2) \pm \Delta \Omega & \quad (\Omega_0 + \Omega_1 - \Omega_2) \pm \Delta \Omega \\
(\Omega_0 + \Omega_1) \pm \Delta \Omega & \quad (\Omega_0 - \Omega_1) \pm \Delta \Omega \\
\Omega_0 \pm \Delta \Omega & \quad \\
\end{align*}
\]

- Least Squares Fit

\[
\begin{align*}
\Omega_0 \pm \frac{\Delta \Omega}{\sqrt{7}} & \quad \Omega_1 \pm \frac{\Delta \Omega}{\sqrt{6}} & \quad \Omega_2 \pm \frac{\Delta \Omega}{\sqrt{4}} \\
\end{align*}
\]
FT NMR

3D Fourier Transformation

GFT NMR

\[
\begin{array}{cc}
\phi_1 & 0^\circ \\
\phi_2 & 90^\circ \\
0^\circ & 0^\circ \\
90^\circ & 0^\circ \\
0^\circ & 90^\circ \\
90^\circ & 90^\circ \\
0^\circ & - \\
90^\circ & - \\
- & - \\
\end{array}
\]

\[
\begin{align*}
\varepsilon &= \cos(\Omega_0 \, t) \\
\varepsilon' &= \cos(\Omega_1 \, t) \cos(\Omega_2 \, t) \\
\varepsilon'' &= \cos(\Omega_0 \, t) \sin(\Omega_1 \, t) \cos(\Omega_2 \, t) \\
\varepsilon''' &= \cos(\Omega_0 \, t) \cos(\Omega_1 \, t) \sin(\Omega_2 \, t) \\
\varepsilon'''' &= \cos(\Omega_0 \, t) \sin(\Omega_1 \, t) \sin(\Omega_2 \, t)
\end{align*}
\]

\[
\begin{align*}
\varepsilon &= \frac{\Delta \Omega}{\sqrt{7}} \\
\varepsilon' &= \frac{\Delta \Omega}{\sqrt{6}} \\
\varepsilon'' &= \frac{\Delta \Omega}{\sqrt{4}}
\end{align*}
\]
**FT NMR**

- $t_0$, $t_1$, $t_2$
- $\Omega_0 \pm \Delta\Omega$, $\Omega_1 \pm \Delta\Omega$, $\Omega_2 \pm \Delta\Omega$

**GFT NMR**

$$
\begin{array}{cc}
\phi_1 & \phi_2 \\
0^\circ & 0^\circ \\
90^\circ & 0^\circ \\
0^\circ & 90^\circ \\
90^\circ & 90^\circ \\
0^\circ & - \\
90^\circ & - \\
- & - \\
\end{array}
$$

- $\hat{G}$ matrix Transformation
- 1D Fourier Transformation
- Least Squares Fit

$$
\begin{align*}
\cos(\Omega_0 \, t) & \quad \cos(\Omega_1 \, t) \cos(\Omega_2 \, t) \\
\cos(\Omega_0 \, t) & \quad \sin(\Omega_1 \, t) \cos(\Omega_2 \, t) \\
\cos(\Omega_0 \, t) & \quad \cos(\Omega_1 \, t) \sin(\Omega_2 \, t) \\
\cos(\Omega_0 \, t) & \quad \sin(\Omega_1 \, t) \sin(\Omega_2 \, t) \\
\end{align*}
$$

$$
\Omega_0 \pm \frac{\Delta\Omega}{\sqrt{7}} \quad \Omega_1 \pm \frac{\Delta\Omega}{\sqrt{6}} \quad \Omega_2 \pm \frac{\Delta\Omega}{\sqrt{4}}
$$
**FT NMR**

- $t_2$ axis
- $t_1$ axis
- $t_0$ axis
- $\omega_2$ axis
- $\omega_1$ axis

- $\Omega_0 \pm \Delta \Omega$
- $\Omega_1 \pm \Delta \Omega$
- $\Omega_2 \pm \Delta \Omega$

**GFT NMR**

<table>
<thead>
<tr>
<th>$\phi_1$</th>
<th>$\phi_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0°</td>
<td>0°</td>
</tr>
<tr>
<td>90°</td>
<td>0°</td>
</tr>
<tr>
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<td>90°</td>
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<tr>
<td>90°</td>
<td>90°</td>
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<tr>
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</tr>
<tr>
<td>90°</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- $\hat{G}$ matrix
- Transformation
- 1D Fourier Transformation

- $(\Omega_0 + \Omega_1 + \Omega_2) \pm \Delta \Omega$
- $(\Omega_0 - \Omega_1 + \Omega_2) \pm \Delta \Omega$
- $(\Omega_0 + \Omega_1 - \Omega_2) \pm \Delta \Omega$
- $(\Omega_0 - \Omega_1 - \Omega_2) \pm \Delta \Omega$
- $(\Omega_0 + \Omega_1) \pm \Delta \Omega$
- $(\Omega_0 - \Omega_1) \pm \Delta \Omega$
- $\Omega_0 \pm \Delta \Omega$

- Least Squares Fit

- $\cos(\Omega_0 \, t)$
- $\cos(\Omega_1 \, t)$
- $\cos(\Omega_2 \, t)$

- $\cos(\Omega_0 \, t)$
- $\sin(\Omega_1 \, t)$
- $\cos(\Omega_2 \, t)$

- $\cos(\Omega_0 \, t)$
- $\cos(\Omega_1 \, t)$
- $\sin(\Omega_2 \, t)$

- $\cos(\Omega_0 \, t)$
- $\sin(\Omega_1 \, t)$
- $\sin(\Omega_2 \, t)$

- $\frac{\Delta \Omega}{\sqrt{7}}$
- $\frac{\Delta \Omega}{\sqrt{6}}$
- $\frac{\Delta \Omega}{\sqrt{4}}$
FT NMR

GFT NMR

\[
\begin{align*}
\Phi_1 &= 0^\circ \\
\Phi_2 &= 0^\circ \\
\Phi_1 &= 90^\circ \\
\Phi_2 &= 0^\circ \\
\Phi_1 &= 0^\circ \\
\Phi_2 &= 90^\circ \\
\Phi_1 &= 90^\circ \\
\Phi_2 &= 90^\circ \\
\Phi_1 &= 0^\circ \\
\Phi_2 &= - \\
\Phi_1 &= 90^\circ \\
\Phi_2 &= - \\
\Phi_1 &= - \\
\Phi_2 &= - \\
\end{align*}
\]

\[
\begin{align*}
\cos(\Omega_0 t) \cos(\Omega_1 t) \\
\cos(\Omega_0 t) \sin(\Omega_1 t)
\end{align*}
\]
FT NMR

GFT NMR

\[
\Phi_1 \quad \Phi_2
\]

\[
\begin{array}{c|c}
0^\circ & 0^\circ \\
90^\circ & 0^\circ \\
0^\circ & 90^\circ \\
90^\circ & 90^\circ \\
0^\circ & - \\
90^\circ & - \\
- & - \\
\end{array}
\]

\[\cos(\Omega_0 t) \cos(\Omega_1 t)\]

\[\cos(\Omega_0 t) \sin(\Omega_1 t)\]

\[
\Omega_0 \pm \Delta \Omega \\
\Omega_1 \pm \Delta \Omega \\
\Omega_2 \pm \Delta \Omega
\]

\[
\Omega_0 \pm \frac{\Delta \Omega}{\sqrt{7}} \\
\Omega_1 \pm \frac{\Delta \Omega}{\sqrt{6}} \\
\Omega_2 \pm \frac{\Delta \Omega}{\sqrt{4}}
\]

\[
\hat{G} \text{ matrix} \\
\text{Transformation} \\
\downarrow \\
\text{1D Fourier Transformation}
\]

\[
(\Omega_0 + \Omega_1 + \Omega_2) \pm \Delta \Omega \\
(\Omega_0 - \Omega_1 + \Omega_2) \pm \Delta \Omega \\
(\Omega_0 + \Omega_1 - \Omega_2) \pm \Delta \Omega \\
(\Omega_0 - \Omega_1 - \Omega_2) \pm \Delta \Omega \\
(\Omega_0 + \Omega_1) \pm \Delta \Omega \\
(\Omega_0 - \Omega_1) \pm \Delta \Omega \\
\Omega_0 \pm \Delta \Omega
\]

\[
\text{Least Squares Fit}
\]
\[ \cos(\Omega_0 t) \]

**FT NMR**

- 3D Fourier Transformation

**GFT NMR**

\[
\begin{array}{c|c|c}
\phi_1 & \phi_2 \\
0^\circ & 0^\circ \\
90^\circ & 0^\circ \\
0^\circ & 90^\circ \\
90^\circ & 90^\circ \\
0^\circ & - \\
90^\circ & - \\
- & - \\
\end{array}
\]

- 1D Fourier Transformation
- Least Squares Fit

\[
\begin{array}{c|c|c|c}
\Omega_0 & \Omega_1 & \Omega_2 \\
\pm \frac{\Delta \Omega}{\sqrt{7}} & \pm \frac{\Delta \Omega}{\sqrt{6}} & \pm \frac{\Delta \Omega}{\sqrt{4}} \\
\end{array}
\]

- 3D Graph with labels: $t_0, t_1, t_2, \Omega_0, \Omega_1, \Omega_2$
FT NMR

3D Fourier Transformation

GFT NMR

<table>
<thead>
<tr>
<th>$\omega$</th>
<th>$\phi_1$</th>
<th>$\phi_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_2$</td>
<td>0°</td>
<td>0°</td>
</tr>
<tr>
<td>$t_1$</td>
<td>90°</td>
<td>0°</td>
</tr>
<tr>
<td>$t_0$</td>
<td>0°</td>
<td>90°</td>
</tr>
<tr>
<td>$\omega$</td>
<td>90°</td>
<td>90°</td>
</tr>
<tr>
<td>$\omega$</td>
<td>0°</td>
<td>-</td>
</tr>
<tr>
<td>$\omega$</td>
<td>90°</td>
<td>-</td>
</tr>
<tr>
<td>$\omega$</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$\hat{G}$ matrix
Transformation

1D Fourier Transformation

$\Omega_0 \pm \Delta\Omega$  $\Omega_1 \pm \Delta\Omega$  $\Omega_2 \pm \Delta\Omega$

Least Squares Fit

$\Omega_0 \pm \frac{\Delta\Omega}{\sqrt{7}}$  $\Omega_1 \pm \frac{\Delta\Omega}{\sqrt{6}}$  $\Omega_2 \pm \frac{\Delta\Omega}{\sqrt{4}}$
'Exhaustive' Sampling of linear combinations of chemical shifts:

Basic spectra
1st order central peaks
2nd order central peaks
FT NMR

GFT NMR

\[ \Phi_1 \quad \Phi_2 \]
\[ 0^\circ \quad 0^\circ \]
\[ 90^\circ \quad 0^\circ \]
\[ 0^\circ \quad 90^\circ \]
\[ 90^\circ \quad 90^\circ \]
\[ 0^\circ \quad - \]
\[ 90^\circ \quad - \]
\[ - \quad - \]

\[ \hat{G} \text{ matrix Transformation} \]
\[ 1D \text{ Fourier Transformation} \]

\[ (\Omega_0 + \Omega_1 + \Omega_2) \pm \Delta \Omega \]
\[ (\Omega_0 - \Omega_1 + \Omega_2) \pm \Delta \Omega \]
\[ (\Omega_0 + \Omega_1 - \Omega_2) \pm \Delta \Omega \]
\[ (\Omega_0 - \Omega_1 - \Omega_2) \pm \Delta \Omega \]
\[ (\Omega_0 + \Omega_1) \pm \Delta \Omega \]
\[ (\Omega_0 - \Omega_1) \pm \Delta \Omega \]
\[ \Omega_0 \pm \Delta \Omega \]

\[ \Omega_0 \pm \frac{\Delta \Omega}{\sqrt{7}} \quad \Omega_1 \pm \frac{\Delta \Omega}{\sqrt{6}} \quad \Omega_2 \pm \frac{\Delta \Omega}{\sqrt{4}} \]

3D Fourier Transformation

Least Squares Fit
Phase Sensitive RD (K=1): Quadrature Detection of joint evolution of $\Omega_0$ and $\Omega_1$

\[
\begin{align*}
\cos(\Omega_0 t_i) \cos(\Omega_1 t_i) & = \cos[(\Omega_0 + \Omega_1) t_i] + \cos[(\Omega_0 - \Omega_1) t_i] \quad (1) \\
\sin(\Omega_0 t_i) \cos(\Omega_1 t_i) & = \sin[(\Omega_0 + \Omega_1) t_i] + \sin[(\Omega_0 - \Omega_1) t_i] \quad (2) \\
\cos(\Omega_0 t_i) \sin(\Omega_1 t_i) & = \sin[(\Omega_0 + \Omega_1) t_i] - \sin[(\Omega_0 - \Omega_1) t_i] \quad (3) \\
\sin(\Omega_0 t_i) \sin(\Omega_1 t_i) & = -\cos[(\Omega_0 + \Omega_1) t_i] + \cos[(\Omega_0 - \Omega_1) t_i] \quad (4)
\end{align*}
\]

‘Cosine RD’:
Quadrature for cosine modulated RD of $\Omega_1$

\[
\begin{align*}
\cos[(\Omega_0 + \Omega_1) t_i] + \cos[(\Omega_0 - \Omega_1) t_i] \rightarrow (1): S1_r \\
\sin[(\Omega_0 + \Omega_1) t_i] + \sin[(\Omega_0 - \Omega_1) t_i] \rightarrow (2): S1_i
\end{align*}
\]

‘Sine RD’:
Quadrature for sine modulated RD of $\Omega_1$

\[
\begin{align*}
\cos[(\Omega_0 + \Omega_1) t_i] - \cos[(\Omega_0 - \Omega_1) t_i] \rightarrow (4): -S2_i \\
\sin[(\Omega_0 + \Omega_1) t_i] - \sin[(\Omega_0 - \Omega_1) t_i] \rightarrow (3): S2_r
\end{align*}
\]

Editing:

\[
\begin{align*}
\cos[(\Omega_0 + \Omega_1) t_i] \rightarrow (1) - (4): T1_r \\
\sin[(\Omega_0 + \Omega_1) t_i] \rightarrow (2) + (3): T1_i \\
\cos[(\Omega_0 - \Omega_1) t_i] \rightarrow (1) + (4): T2_r \\
\sin[(\Omega_0 - \Omega_1) t_i] \rightarrow (2) - (3): T2_i
\end{align*}
\]
For $K = 1$: two dimensions being jointly sampled

\[
\begin{bmatrix}
T1_r & T1_i & T2_r & T2_i
\end{bmatrix}
= \begin{bmatrix}
1 & 0 & 0 & -1 \\
0 & 1 & 1 & 0 \\
1 & 0 & 0 & 1 \\
0 & 1 & -1 & 0
\end{bmatrix} \cdot \begin{bmatrix}
S1_r & S1_i & S2_r & S2_i
\end{bmatrix}
\implies T(K) = G(K)S(K)
\]

For arbitrary $K$: $K + 1$ dimensions being jointly sampled

\[
\begin{bmatrix}
e^{iK} & e^{i1} & e^{i0}
\end{bmatrix}
\otimes
\begin{bmatrix}
e^{iK} & e^{i1} & e^{i0}
\end{bmatrix}
= \begin{bmatrix}
1 & i & 0 \\
1 & i & 0 \\
1 & i & 0
\end{bmatrix}
\otimes
\begin{bmatrix}
1 & i & 0 \\
1 & i & 0 \\
1 & i & 0
\end{bmatrix}
\cdot
\begin{bmatrix}
c_K & c_1 & c_0
\end{bmatrix}
\otimes
\begin{bmatrix}
s_K & s_1 & s_0
\end{bmatrix}
\]

with

\[
c_j = \cos(\Omega_j \cdot t), \quad s_j = \sin(\Omega_j \cdot t) \quad \text{and} \quad e^{i\Omega_j t} = e^{ij}
\]

\[
e^{ij} = c_j + i \cdot s_j = \begin{bmatrix} 1 \\ i \\ s_j \end{bmatrix} \cdot \begin{bmatrix} c_j \\ 0 \\ s_j \end{bmatrix}
\]
complex $G$-matrix

$$\hat{G}_c(K) = \left[\begin{array}{c}
1 \\
1
\end{array}\right] \otimes \ldots \otimes \left[\begin{array}{c}
i \\
-1
\end{array}\right]$$

real $G$-matrix for $K=3$

$$
\begin{array}{cccccccccccc}
T1r & 1 & 0 & 0 & -1 & 0 & -1 & -1 & 0 & 0 & -1 & 0 & 1 \\
T1i & 0 & 1 & 1 & 0 & 1 & 0 & 0 & -1 & 0 & 0 & -1 & 0 \\
T2r & 1 & 0 & 0 & 1 & 0 & -1 & 1 & 0 & 0 & -1 & 0 & -1 \\
T2i & 0 & 1 & -1 & 0 & 1 & 0 & 0 & 1 & 1 & 0 & 0 & 1 \\
T3r & 1 & 0 & 0 & -1 & 0 & 1 & 1 & 0 & 0 & -1 & -1 & 0 \\
T3i & 0 & 1 & 1 & 0 & -1 & 0 & 0 & 1 & 1 & 0 & 0 & -1 \\
T4r & 1 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & 1 & 0 & 0 & 1 \\
T4i & 0 & 0 & 1 & -1 & 0 & 0 & -1 & -1 & 0 & 1 & 0 & 1 \\
T5r & 1 & 0 & 0 & 1 & 0 & -1 & 1 & 0 & 0 & -1 & -1 & 0 \\
T5i & 0 & 1 & 1 & 0 & 1 & 0 & 0 & -1 & 0 & -1 & 0 & 1 \\
T6r & 1 & 0 & 0 & 1 & 0 & -1 & 0 & 1 & 0 & 0 & -1 & -1 \\
T6i & 0 & 0 & 1 & 1 & 0 & 0 & -1 & 0 & -1 & -1 & 0 & 1 \\
T7r & 1 & 0 & 0 & -1 & 0 & 1 & 1 & 0 & 0 & -1 & -1 & 0 \\
T7i & 0 & 0 & 1 & 0 & -1 & 0 & 0 & -1 & -1 & 1 & 0 & 1 \\
T8r & 1 & 0 & 0 & 1 & 0 & 1 & 0 & -1 & 0 & -1 & 0 & -1 \\
T8i & 0 & 0 & 1 & -1 & 0 & 0 & -1 & 1 & 1 & 0 & -1 & 0 \\
\end{array}
$$
Application: (5,2)D HACACONHN
2D Information: 13.8 min

© Thomas Szyperski
4D Information: +52.8 min

© Thomas Szyperski
5D Information: +108 min
© Thomas Szyperski
GFT
(5,2)D HACACONHN
- 15*53(t₁)*512(t₂)
- 15*512(w₁)*512(w₂) [16 Mbyte]
- Minimal measurement time: 33 min
- Precision of chemical shift measurement: 3-4 fold increased

FT
5D HACACONHN
- 10(t₁)*11(t₂)*13(t₃)*13(t₄)*512(t₅)
- 32(w₁)*32(w₂)*32(w₃)*32(w₄)*512(w₅) 2.1 Gbyte
- 96(w₁)*96(w₂)*256(w₃)*128(w₄)*512(w₅) [618 Gbyte]
- Minimal measurement time: 5.8 days
Gain in Measurement time

\[ \sum_{i=1}^{K} \prod_{j=1}^{n_i} \left( \frac{2^k - 1}{\sum n_i} \right) \]

- ND
- (N,N-1)D
- (N,N-2)D
- (N,N-3)D
Features of GFT NMR

- Generally applicable acquisition scheme
- Accurate adaptation of measurement times to sensitivity requirement without sacrificing digital resolution or high dimensional correlation
- Realize 5+ D
- High Precision of Shift Measurements
  - Systems with high shift degeneracy (RNA, Lipids)
Features of GFT NMR cont..

- No additional hardware required
- Data size reduction
- Accelerated processing speed
- Robustness of data analysis
- Combine with other approaches to reduce the ‘sampling demand’:
  - Non-linear sampling and MEM, Filter diagonalization, Hadamard NMR, Single-scan 2D/3D acquisition
- ‘Orthogonal’ to TROSY: GFT-TROSY