Rapid acquisition of multidimensional NMR data

'The NMR sampling problem'
Let's take stock...

Conventional, multidimensional FT NMR spectroscopy......
Drawbacks of Multidimensional FT NMR

- Measurement times scale with $\prod n_j$

1 scan / second (16 complex / dimension) =>

1D -> 1 second
2D -> ~0.5 minutes
3D -> ~0.25 hours
4D -> ~8 hours
5D -> ~12 days
6D -> ~1.1 years
The 'NMR sampling problem':

minimal measurement

'explodes' with dimensionality
3D and 4D:

**Sampling** versus **Sensitivity** Limitation

3D and 4D:

**Sampling** versus **Sensitivity** Limitation

5D +: **Sampling Limitation**

Time Domain

Frequency Domain

FT
600 MHz Cryogenic Probe at UB
First FID of 3D **HCCH-COSY**

Conventional  

Cryogenic probe
Drawbacks of Multidimensional FT NMR

- Measurement times scale with $\prod n_j$
- Low precision of chemical shift measurement in indirect dimensions
The ‘Gordian knot’:

Speeding up multidimensional NMR data acquisition, while increasing the accuracy of the measurement of NMR parameters.
RD/GFT NMR Spectroscopy
Reduced dimensionality (RD)

NMR Spectroscopy
Reduced Dimensionality in Triple-Resonance NMR Experiments

T. Szyperski, G. Wider, J. H. Bushwell, and K. Wüthrich

Institut für Molekularbiologie und Biophysik
Eidgenössische Technische Hochschule-Hönggerberg
CH-8093 Zürich, Switzerland

Received April 30, 1993

As an alternative to the conventional assignment of protein NMR\(^1\) spectra by observation of sequential NOEs\(^2\) in homonuclear 2D \([\text{H}, \text{H}]\)-NOESY or 3D and 4D heteronuclear-resolved \([\text{H}, \text{H}]\)-NOESY spectra, 3D and 4D triple-resonance experiments have been proposed for establishing intra- and interresidual connectivities via heteronuclear scalar couplings.\(^3\)\(^-\)\(^1\)\(^2\) 4D experiments of this type are conceptually particularly attractive, since only a single experiment is needed for intraresidual correlation of the four backbone spins \(\text{H}^N, \text{H}^{15}N, \text{C}^\alpha,\) and \(\text{H}^\alpha,)\) and suitable combinations of two 4D experiments can provide sequential assignments.\(^1\)\(^1\)\(^13\) However, because of the short \(T_2\) relaxation times of \(\text{C}^\alpha\) 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 \(t_{\text{m}}\) can then be chosen for the

![Figure 1. Contour plots of \((\omega_1(\text{C}^\alpha) - \omega_3(\text{H}^N))\)-strips from a 3D HA CA N HN spectrum obtained with a 2.5 mM sample of the uniformly\(^1\)\(^3\)\(^C\) and \(^1\)\(^5\)\(^N\)-labeled mixed disulfide of \(E.\) coli glutaredoxin (C14S) with glutathione\(^17\) 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 \((t_1) \times 98 (t_2) \times 512 (t_3)\) complex points were accumulated, with \(t_{\text{m}}(\text{H}^\alpha) = 16.8\) ms, \(t_{\text{m}}(\text{N}, \text{C}^\alpha) = 11.2\) ms, and \(t_{\text{m}}(\text{H}^N) = 65.5\) ms. 32 scans per increment were acquired, resulting in a total measuring time of 3.5 days. The carrier frequencies of the \(^1\)\(^5\)\(^N\) and \(^1\)\(^3\)\(^C\)\(^\alpha\) pulses were set to 105.1 and 56 ppm, respectively.](image)
Challenge: keep information upon projection
'projected' chemical shift
Challenge: keep information upon projection

"projected" chemical shift

Ω\text{carrier} \rightarrow \Omega_{S} \rightarrow \Omega_{K}
Structural genomics lead to increased interest in RD NMR

Progress

Protein NMR spectroscopy in structural genomics

Gaetano T. Montelione¹, Deyou Zheng¹, Yuanpeng J. Huang¹, Kristin C. Gunsalus¹ and Thomas Szyperski²
Reduced-dimensionality NMR spectroscopy for high-throughput protein resonance assignment


*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)
Letter to the Editor: $^1$H, $^{13}$C, and $^{15}$N resonance assignments and secondary structure of the PWI domain from SRm160 using Reduced Dimensionality NMR

Blair R. Szymczynaa,c,* Antonio Pineda-Lucenaa,c, Jeffrey L. Millsb,c, Thomas Szyperskih,c & Cheryl H. Arrowsmitha,c

aDivision of Molecular and Structural Biology, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, 610 University Ave., Toronto, ON, Canada, M5G 2M9; bDepartment of Chemistry, University at Buffalo, The State University of New York, Buffalo, New York 14260, U.S.A.; cNortheast Structural Genomics Consortium

Received 13 November 2001; Accepted 18 December 2001

Key words: pre-mRNA processing, PWI motif, reduced dimensionality NMR, resonance assignment

Total Measurement time for RD NMR spectra: 
44.5 hours
3D HCCH-COSY: 8.9 hours

© Thomas Szyperski
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**
  - 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)
  - PfR13 (12 kDa)
  - CCR19 (16 kDa)
  - HR532 (13 kDa)
  - HR2106 (11 kDa)

[Structures determined in Szyperski Lab]
Why not leaning back?
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: ~20+
- (RD) DR/TR data for 17 KDa ER75 in ~12 h
- Simultaneously: All spectral widths increase by 1.33 (~2.3-fold increased sampling demand for 3D)
RD NMR spectroscopy not fast enough
'chemical shift multiplets'
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
A pictorial approach to GFT NMR....
\( K = 2 \)  
\((N,N-2)D\)  
GFT NMR  

GFT =  
Combined \( G \)-matrix and Fourier Transformation  

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

\[ \begin{align*}  
\Omega_0 & \pm \Delta\Omega \\
\Omega_1 & \pm \Delta\Omega \\
\Omega_2 & \pm \Delta\Omega \\
\omega & \pm \Delta\omega \\
\omega_0 & \pm \Delta\omega \\
\omega_1 & \pm \Delta\omega \\
\omega_2 & \pm \Delta\omega \\
\omega^0 & \pm \frac{\Delta\omega}{\sqrt{7}} \\
\omega_1 & \pm \frac{\Delta\omega}{\sqrt{6}} \\
\omega_2 & \pm \frac{\Delta\omega}{\sqrt{4}} 
\end{align*} \]
Joint Sampling of 3D Subspace of an ND FT NMR Experiment

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

\[
\begin{align*}
\Omega_0 & \pm \Delta\Omega \\
\Omega_1 & \pm \Delta\Omega \\
\Omega_2 & \pm \Delta\Omega
\end{align*}
\]
FT NMR

3D Fourier Transformation

GFT NMR

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

\[
\begin{align*}
\hat{G} \text{ matrix} \\
\text{Transformation} \\
\text{1D Fourier} \\
\text{Transformation} \\
(\Omega_0 + \Omega_1 + \Omega_2) \pm \Delta\Omega \\
(\Omega_0 - \Omega_1 + \Omega_2) \pm \Delta\Omega \\
(\Omega_0 + \Omega_1 - \Omega_2) \pm \Delta\Omega \\
(\Omega_0 - \Omega_1 - \Omega_2) \pm \Delta\Omega \\
(\Omega_0 + \Omega_1) \pm \Delta\Omega \\
(\Omega_0 - \Omega_1) \pm \Delta\Omega \\
\Omega_0 \pm \Delta\Omega
\end{align*}
\]

Least Squares Fit

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

\[
\begin{align*}
\cos(\Omega_0 t) & \quad \cos(\Omega_1 t) \quad \cos(\Omega_2 t) \\
\cos(\Omega_0 t) & \quad \sin(\Omega_1 t) \quad \cos(\Omega_2 t) \\
\cos(\Omega_0 t) & \quad \cos(\Omega_1 t) \quad \sin(\Omega_2 t) \\
\cos(\Omega_0 t) & \quad \sin(\Omega_1 t) \quad \sin(\Omega_2 t)
\end{align*}
\]
### FT NMR

- Graph showing a 3D Fourier Transformation.
- Axes: \( t_2 \), \( t_1 \), \( t_0 \).

### 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>
<td>0°</td>
<td>90°</td>
</tr>
<tr>
<td>90°</td>
<td>90°</td>
</tr>
<tr>
<td>0°</td>
<td>-</td>
</tr>
<tr>
<td>90°</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- Diagram illustrating the transformation of \( \hat{G} \) matrix.
- 1D Fourier Transformation:
  - \( (\Omega_0 + \Omega_1 + \Omega_2) \pm \Delta \Omega \)
  - \( (\Omega_0 - \Omega_1 + \Omega_2) \pm \Delta \Omega \)
  - \( (\Omega_0 + \Omega_1 - \Omega_2) \pm \Delta \Omega \)
  - \( (\Omega_0 - \Omega_1 - \Omega_2) \pm \Delta \Omega \)

- Least Squares Fit:
  - \( \Omega_0 \pm \frac{\Delta \Omega}{\sqrt{7}} \)
  - \( \Omega_1 \pm \frac{\Delta \Omega}{\sqrt{6}} \)
  - \( \Omega_2 \pm \frac{\Delta \Omega}{\sqrt{4}} \)

- Formulas:
  - \( \cos(\Omega_0 \, t) \)  \( \cos(\Omega_1 \, t) \)  \( \cos(\Omega_2 \, t) \)
  - \( \cos(\Omega_0 \, t) \)  \( \sin(\Omega_1 \, t) \)  \( \cos(\Omega_2 \, t) \)
  - \( \cos(\Omega_0 \, t) \)  \( \cos(\Omega_1 \, t) \)  \( \sin(\Omega_2 \, t) \)
  - \( \cos(\Omega_0 \, t) \)  \( \sin(\Omega_1 \, t) \)  \( \sin(\Omega_2 \, t) \)
FT NMR

GFT NMR

\( \phi_1 \quad \phi_2 \)

- 
- 
- 

\( \hat{G} \) matrix
Transformation

(\( \Omega_0 + \Omega_1 + \Omega_2 \) ± \( \Delta \Omega \))
(\( \Omega_0 - \Omega_1 + \Omega_2 \) ± \( \Delta \Omega \))
(\( \Omega_0 + \Omega_1 - \Omega_2 \) ± \( \Delta \Omega \))
(\( \Omega_0 - \Omega_1 - \Omega_2 \) ± \( \Delta \Omega \))
(\( \Omega_0 + \Omega_1 \) ± \( \Delta \Omega \))
(\( \Omega_0 - \Omega_1 \) ± \( \Delta \Omega \))
\( \Omega_0 \pm \frac{\Delta \Omega}{\sqrt{2}} \)
\( \Omega_1 \pm \frac{\Delta \Omega}{\sqrt{6}} \)
\( \Omega_2 \pm \frac{\Delta \Omega}{\sqrt{4}} \)

\( \cos(\Omega_0 \ t) \quad \cos(\Omega_1 \ t) \cos(\Omega_2 \ t) \)
\( \cos(\Omega_0 \ t) \quad \sin(\Omega_1 \ t) \cos(\Omega_2 \ t) \)
\( \cos(\Omega_0 \ t) \quad \cos(\Omega_1 \ t) \sin(\Omega_2 \ t) \)
\( \cos(\Omega_0 \ t) \quad \sin(\Omega_1 \ t) \sin(\Omega_2 \ t) \)

Least Squares Fit
### 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>
<td>0°</td>
<td>90°</td>
</tr>
<tr>
<td>90°</td>
<td>90°</td>
</tr>
</tbody>
</table>

- $t$

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

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

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

- Least Squares Fit

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

---

3D Fourier Transformation
FT NMR

GFT NMR

\[ \phi_1 \quad \phi_2 \]
\[ 0^\circ \quad 0^\circ \]
\[ 90^\circ \quad 0^\circ \]
\[ 0^\circ \quad 90^\circ \]
\[ 90^\circ \quad 90^\circ \]
\[ 0^\circ \quad - \]
\[ 90^\circ \quad - \]
\[ - \quad - \]

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

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

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

\[ \Omega_0 \pm \Delta \Omega \]

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

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

Least Squares Fit
\[ \cos(\Omega_0 t) \]

**FT NMR**

\[ t_2 \]

\[ t_1 \]

\[ t_0 \]

\[ \omega_2 \]

\[ \omega_1 \]

\[ \omega_0 \]

\[ \Omega_0 \pm \Delta \Omega \]

\[ \Omega_1 \pm \Delta \Omega \]

\[ \Omega_2 \pm \Delta \Omega \]

**GFT NMR**

\[ \phi_1 \phi_2 \]

\[ 0^\circ 0^\circ \]

\[ 90^\circ 0^\circ \]

\[ 0^\circ 90^\circ \]

\[ 90^\circ 90^\circ \]

\[ 0^\circ - \]

\[ 90^\circ - \]

\[ - - \]

- **3D Fourier Transformation**
- **1D Fourier Transformation**
- **\( \hat{G} \) matrix Transformation**
- **Least Squares Fit**

\[ \Omega_0 \pm \frac{\Delta \Omega}{\sqrt{7}} \]

\[ \Omega_1 \pm \frac{\Delta \Omega}{\sqrt{6}} \]

\[ \Omega_2 \pm \frac{\Delta \Omega}{\sqrt{4}} \]
FT NMR

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>
<td>0°</td>
<td>90°</td>
</tr>
<tr>
<td>90°</td>
<td>90°</td>
</tr>
<tr>
<td>0°</td>
<td>-</td>
</tr>
<tr>
<td>90°</td>
<td>-</td>
</tr>
</tbody>
</table>

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

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

\[ \Omega_{GFT} \]

\[ \Omega_{0} \]

\[ \Omega_{1} \]

\[ \Omega_{2} \]

\[ \Omega_{\text{direct}} \]
'Exhaustive' sampling of linear combinations of chemical shifts:

Basic spectra

1\textsuperscript{st} order central peaks

2\textsuperscript{nd} order central peaks
FT NMR

GFT NMR

\[ \hat{\mathbf{G}} \text{ matrix} \]

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

\[ \Omega_0 \pm \frac{\Delta \Omega}{\sqrt{7}} \]
\[ \Omega_1 \pm \frac{\Delta \Omega}{\sqrt{6}} \]
\[ \Omega_2 \pm \frac{\Delta \Omega}{\sqrt{4}} \]
Reduction of minimal measurement time

\[ \prod_{i=1}^{K} n_i \ast \frac{2^K}{\sum_{i=1}^{K} n_i} - 1 \]

Graph showing the reduction of minimal measurement time with ND, (N,N-1)D, (N,N-2)D, and (N,N-3)D.
An example.....
(5,2)D HACACONHN

H$^\beta$ – C$^\beta$

C$^\alpha$ – C – N – C$^\alpha$

H$^\alpha$

O

H

H$^\alpha$
8 Basic Spectra

4 First Order
Central Peak Spectra

2 Second Order
Central Peak Spectra

1 Third Order
Central Peak Spectra
complex G-matrix

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

real G-matrix for \( K = 3 \)

\[
\begin{bmatrix}
T1r & 1 & 0 & 0 & -1 & 0 & -1 & -1 & 0 & 0 & -1 & -1 & 0 & -1 & 0 & 0 & 1 \\
T1i & 0 & 1 & 1 & 0 & 1 & 0 & 0 & -1 & 1 & 0 & 0 & -1 & 0 & -1 & -1 & 0 \\
T2r & 1 & 0 & 0 & 1 & 0 & -1 & 1 & 0 & 0 & -1 & 1 & 0 & -1 & 0 & 0 & -1 \\
T2i & 0 & 1 & -1 & 0 & 1 & 0 & 0 & 1 & 1 & 0 & 0 & 1 & 0 & -1 & 1 & 0 \\
T3r & 1 & 0 & 0 & -1 & 0 & 1 & 1 & 0 & 0 & -1 & -1 & 0 & 1 & 0 & 0 & -1 \\
T3i & 0 & 1 & 1 & 0 & -1 & 0 & 0 & 1 & 1 & 0 & 0 & -1 & 0 & 1 & 1 & 0 \\
T4r & 1 & 0 & 0 & 1 & 0 & 1 & -1 & 0 & 0 & -1 & 1 & 0 & 1 & 0 & 0 & 1 \\
T4i & 0 & 1 & -1 & 0 & 1 & 0 & -1 & 0 & 0 & -1 & 1 & 0 & 0 & 1 & 0 & -1 \\
T5r & 1 & 0 & 0 & -1 & 0 & -1 & -1 & 0 & 0 & 1 & 1 & 0 & 1 & 0 & 0 & -1 \\
T5i & 0 & 1 & 1 & 0 & 1 & 0 & 0 & -1 & -1 & 0 & 0 & 1 & 0 & 1 & 1 & 0 \\
T6r & 1 & 0 & 0 & 1 & 0 & -1 & 1 & 0 & 0 & -1 & 0 & 1 & 0 & 1 & 0 & 0 \\
T6i & 0 & 1 & -1 & 0 & 1 & 0 & 0 & 1 & -1 & 0 & 0 & -1 & 0 & 1 & -1 & 0 \\
T7r & 1 & 0 & 0 & -1 & 0 & 1 & 1 & 0 & 0 & 1 & 1 & 0 & -1 & 0 & 0 & 1 \\
T7i & 0 & 1 & 1 & 0 & -1 & 0 & 0 & 1 & -1 & 0 & 0 & 1 & 0 & -1 & 1 & 0 \\
T8r & 1 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & 1 & -1 & 0 & -1 & 0 & -1 & 0 & 0 \\
T8i & 0 & 1 & -1 & 0 & 1 & 0 & -1 & 0 & 0 & -1 & -1 & 0 & 0 & -1 & 0 & 1 \\
\end{bmatrix}
\]

© Thomas Szyperski
4D Information
+52.8 min

© Thomas Szyperski

GRC 6/22/04
How about the increased precision of the shift measurements?
GFT NMR: Increased precision

- **Overdetermination**
  \[ \sigma_{\text{GFT}}(\Omega_j) = \left[ \frac{1}{\sqrt{n}} \right] \sigma_{\text{edited}} (..\Omega_j \pm \Omega_k \pm ..) \]

- **Constant-time chemical shift evolution**
  \[ \sigma_{\text{edited}} = \sigma_{\text{FT}}(\Omega_j) \]
  \[ \rightarrow \sigma_{\text{GFT}}(\Omega_j) = \left[ \frac{1}{\sqrt{n}} \right] \sigma_{\text{FT}}(\Omega_j) \]
<table>
<thead>
<tr>
<th><strong>GFT</strong></th>
<th><strong>FT</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(5,2)D HACACONHN</strong></td>
<td><strong>5D HACACONHN</strong></td>
</tr>
<tr>
<td>- 15*53(t₁)*512(t₂)</td>
<td>- 10(t₁)*11(t₂)*13(t₃)*13(t₄)*512(t₅)</td>
</tr>
<tr>
<td>- 15*512(ω₁)*512(ω₂) [16 Mbyte]</td>
<td>- 32(ω₁)*32(ω₂)*32(ω₃)*32(ω₄)*512(ω₅) 2.1 Gbyte</td>
</tr>
<tr>
<td>- Minimal measurement time: 33 min</td>
<td>- 96(ω₁)*96(ω₂)*256(ω₃)*128(ω₄)*512(ω₅) [618 Gbyte]</td>
</tr>
<tr>
<td>- Precision of chemical shift measurement: ~2–3 fold increased</td>
<td>- Minimal measurement time: 5.8 days</td>
</tr>
</tbody>
</table>

© Thomas Szyperski  
GRC 6/22/04
Features of GFT NMR

- Generally applicable acquisition scheme
- 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
• Greatly accelerated processing speed
• Robustness of data analysis
• Combine with other approaches to reduce the 'sampling demand':
  - Non-linear sampling (Three-way decomposition) and MEM, Filter diagonalization,
  - Hadamard NMR, Single-scan ND acquisition,
• ‘Orthogonal’ to TROSY: **GFT-TROSY**
New high-throughput NMR

Proteins are the stuff of life and discovering their structures can be a significant step towards the development of new drugs. However, determining protein structures is a difficult task that requires a great deal of expertize, sophisticated instruments -- and a lot of time.

Now, a new method of analyzing data acquired by nuclear magnetic resonance (NMR) instrumentation promises to cut down -- dramatically -- the amount of time needed to identify protein structures. As a result, the new, high-throughput method devised by scientists at the University at Buffalo (http://www.buffalo.edu) will make drug development considerably faster, the researchers claim [1].

Determining protein structures

So far, two methods have been used primarily to determine protein structures: NMR spectroscopy and X-ray crystallography. The vast majority of protein structures have been solved using X-ray crystallography, a method that enables scientists to explore the details of protein structures but requires crystallization of the protein being studied.

NMR does not entail crystalization but is usually slower than crystallography and is limited to solving the structures of small and medium-sized molecules. NMR uses powerful magnets to determine the chemical shifts of the atoms that make up the protein molecules. The technique involves conducting a series of multi-dimensional experiments, which measure the resonance frequencies of -- and the distances between -- the nuclei of the atoms.

1477-3437/03 © 2003 Elsevier Science Ltd. All rights reserved. Ph: 1477-3437/030346-7

Structural Biology

Propelled by Recent Advances, NMR Moves Into the Fast Lane

A speedy new NMR technique could finally help structural genomics groups achieve their goal of devising factory-style approaches to mapping protein structures at high speeds.

Key advances were reported this year across a broad span of chemistry subdisciplines, ranging from carbohydrate chemistry to surface science.
Major developments 1997-2003

1. TROSY and large systems
2. Residual dipolar couplings
3. Rapid data collection
4. Functional protein dynamics

References


The 2003 Scientific American 50 List of Winners

RESEARCH

RESEARCH LEADER OF THE YEAR
Roderick MacKinnon
Professor of molecular neurobiology and biophysics, Rockefeller University; investigator, Howard Hughes Medical Institute
Elucidated the structure and function of ion channels, particularly the potassium ion channel.

AEROSPACE
Larry Cornman and Robert Sharman
Project scientists, research applications program, National Center for Atmospheric Research, Boulder, Colo.
Discovered an algorithm that allows aircraft radar to better detect turbulence.

AGRICULTURE
Joanne Chory
Professor of plant molecular and cellular biology, Salk Institute for Biological Studies, San Diego; investigator, Howard Hughes Medical Institute
Pinpointed a gene that may allow shaded plants to grow more productively.

AUTOMOTIVE
Khalil Amine
Group leader, Battery Technology Development, Argonne National Laboratory, Argonne, Ill.
Made superior lithium-based batteries for hybrid vehicles and medical devices.

CHEMICALS AND MATERIALS
Thomas Szyperski
Associate professor of chemistry and biochemistry, State University of New York, Buffalo
Adapted nuclear magnetic resonance techniques to map a protein’s atomic structure in hours, not days.

COMMUNICATIONS
David E. Culler
Professor of computer science, University of California, Berkeley, former director of the Berkeley laboratory of Intel Research
Field-tested networks of sensors for military and environmental applications.

COMPUTING
Armando Fox
Assistant professor of computer science, Stanford University
Showed how software could protect networks from disastrous crashes in individual servers.

DEFENSE
Frank X. Hursey
Size

Measurement time

Sampling limited

Sensitivity limited

© Thomas Szyperski

GRC 6/22/04
G-matrix Fourier transform NMR spectroscopy for complete protein resonance assignment

Hanudatta S. Atreya and Thomas Szyperski*

Departments of Chemistry and Structural Biology, State University of New York, Northeast Structural Genomics Consortium, Buffalo, NY 14260

Communicated by Herbert Hauptman, Hauptman-Woodward Medical Research Institute, Buffalo, NY, May 18, 2004 (received for review December 14, 2003)

A G-matrix Fourier transform (GFT) NMR spectroscopy-based strategy for resonance assignment of proteins is described. Each of the GFT NMR experiments presented here rapidly affords four-, five-, or six-dimensional spectral information in combination with precise measurements of chemical shifts. The resulting high information content enables one to obtain nearly complete assignments by using only four NMR experiments. For the backbone amide proton detected “out-and-back” experiments, data collection was further accelerated up to ~2.5-fold by use of longitudinal 1H relaxation optimization. The GFT NMR experiments were acquired for three proteins with molecular masses ranging from 8.6 to 17 kDa, demonstrating that the proposed strategy is of key interest for automated resonance assignment in structural genomics.

Nearly complete resonance assignments are generally considered a necessity for NMR-based protein structure determination (e.g., refs. 11 and 12). Here we describe a strategy for complete protein resonance assignment based on GFT NMR experiments affording accurate 4D, 5D, and 6D spectral information. Applications are presented for proteins with molecular masses ranging from 8.6 to 17 kDa.

Materials and Methods

NMR Spectrometer and Protein Samples. All measurements were performed at 25°C on Varian INOVA 600 and 750 MHz spectrometers, equipped with conventional 1H/13C/15N triple-resonance probes, by using ~1 mM solutions in 95% H2O/5%
3D HCC-CH COSY

$t_1,GF (^{1}H(1), ^{13}C(1); ^{13}C(2))$

$t_2(^{13}C(2))$

$t_3(^{1}H(2))$
Higher Precision for Shift Measurements
Gain is precision of shift measurements in (5,3)D HCC-CH versus 3D (H)CCH and 3D H(C)CH.