Outline

1. Brief review of nuclear spin interactions, MAS, etc.
2. Basics of heteronuclear recoupling (rotary resonance)
3. REDOR & quantitative distance measurements in isolated $^{13}$C-$^{15}$N (and other low-$\gamma$) spin pairs
4. Quantitative $^{13}$C-$^{15}$N distance measurements in U-$^{13}$C,$^{15}$N peptides and proteins using frequency selective REDOR & 3D TEDOR
5. Intro to dipole tensor correlation methods for torsion angle measurements in peptides & proteins (extra slides)
Isolated Spin-1/2 System (I-S)

\[ H_{int} = H^{CS}_I + H^{CS}_S + H^J_{IS} + H^D_{IS} \]

- NMR interactions are anisotropic: can be generally expressed as coupling of two vectors by a 2\textsuperscript{nd} rank Cartesian tensor (3 x 3 matrix)
Rotate Tensors: PAS $\rightarrow$ Lab

$$\sigma_{LAB} = \mathbf{R}(\Omega) \cdot \sigma_{PAS} \cdot \mathbf{R}(\Omega)^{-1}; \quad \Omega = \left\{ \alpha, \beta, \gamma \right\}$$

- SSNMR spectra determined by interactions in lab frame
- Rotate tensors from their principal axis systems (matrices diagonal) into lab frame ($\mathbf{B}_0 \parallel z$-axis)
- In general, a rotation is accomplished using a set of 3 Euler angles $\{\alpha, \beta, \gamma\}$
Tensor Rotations: Euler Rotation Matrices

$$\sigma_{LAB} = R(\Omega) \cdot \sigma_{PAS} \cdot R(\Omega)^{-1}$$

$$\Omega = \{\alpha, \beta, \gamma\}$$

$$\sigma_{PAS} = \begin{bmatrix} \sigma_{xx} & 0 & 0 \\ 0 & \sigma_{yy} & 0 \\ 0 & 0 & \sigma_{zz} \end{bmatrix}$$

$$R(\Omega) = \begin{bmatrix} \cos \alpha \cos \beta \cos \gamma - \sin \alpha \sin \gamma & \sin \alpha \cos \beta \cos \gamma + \cos \alpha \sin \gamma & -\sin \beta \cos \gamma \\ -\cos \alpha \cos \beta \sin \gamma - \sin \alpha \cos \gamma & -\sin \alpha \cos \beta \sin \gamma + \cos \alpha \cos \gamma & \sin \beta \sin \gamma \\ \cos \alpha \sin \beta & \sin \alpha \sin \beta & \cos \beta \end{bmatrix}$$
Multiple Interactions

- In case of multiple interactions first transform all tensors into common frame (usually called molecular- or crystallite-fixed frame)

- Powder samples: rotate each crystallite into lab frame (for numerical simulations of NMR spectra trick is to accurately describe an infinite ensemble of crystallites with min. # of angles)
High Field Truncation: $H_{CS}$

\[ H^C_S = \gamma_1 B_0 \sigma^{LAB}_{zz} I_z = \gamma_1 B_0 \left( \sigma_{xx} \sin^2 \theta \cos^2 \phi + \sigma_{yy} \sin^2 \theta \sin^2 \phi + \sigma_{zz} \cos^2 \theta \right) I_z \]

\[ = \left\{ \gamma_1 B_0 \sigma_{iso} + \gamma_1 B_0 \delta \frac{1}{2} \left[ 3 \cos^2 \theta - 1 - \eta \sin^2 \theta \cos(2\phi) \right] \right\} I_z \]

\[ \sigma_{iso} = \frac{1}{3} \left( \sigma_{xx} + \sigma_{yy} + \sigma_{zz} \right) \]

\[ \delta = \sigma_{zz} - \sigma_{iso} \]

\[ \eta = \frac{\sigma_{yy} - \sigma_{xx}}{\delta} \]

- Retain only parts of $H^C_S$ that commute with $I_z$
High Field Truncation: $H_J$ and $H_D$

$$H_{IS}^J = \pi J_{IS} 2I_z S_z$$

$$H_{IS}^D = b_{IS} \frac{1}{2} \left(3 \cos^2 \theta - 1\right) 2I_z S_z$$

$$b_{IS} = -\frac{\mu_0 \gamma_I \gamma_S}{4\pi \cdot r_{IS}^3}$$

- J-anisotropy negligible in most cases
- $b_{IS}$ in rad/s; directly related to I-S distance
## Dipolar Couplings in Biomolecules

<table>
<thead>
<tr>
<th>Spin 1</th>
<th>Spin 2</th>
<th>$r_{12}$ (Å)</th>
<th>$b_{12}/2\pi$ (Hz)</th>
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<td>$^{15}\text{N}$</td>
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<tr>
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<td>$^{15}\text{N}$</td>
<td>4.0</td>
<td>~50</td>
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</table>
Dipolar Couplings and Protein Structure

• Structural studies rely on DC-based distance restraints
• Weakest couplings (~3+ Å distances) most useful

M.H. Levitt, “Spin Dynamics”
Spectra determined by magnitudes and relative orientations of CS tensors and dipolar couplings for different sites (even with efficient $^1H$ decoupling).
NMR of Rotating Samples

\[ H_{I}^{CS} = \omega_{I}(t) I_{z} \]
\[ H_{IS}^{D} = \omega_{IS}(t) 2I_{z} S_{z} \]
\[ H_{IS}^{J} = \pi J_{IS} 2I_{z} S_{z} \]
\[ \omega_{\lambda}(t) = \sum_{m=-2}^{2} \omega_{\lambda}^{(m)} \exp \{ i m \omega_{r} t \} \]
$H_D$ for Rotation at Magic Angle ($\theta_m = 54.74^\circ$)

$$H_{IS}^D = \left\{ \sum_{m=-2}^{2} \omega_{IS}^{(m)} \exp(i m \omega_r t) \right\} 2I_z S_z$$

$$\omega_{IS}^{(0)} = b_{IS} \frac{\left(3 \cos^2 \beta_{PR} - 1\right)}{2} \frac{\left(3 \cos^2 \theta_m - 1\right)}{2} = 0$$

$$\omega_{IS}^{(\pm 1)} = - \frac{b_{IS}}{2\sqrt{2}} \sin(2 \beta_{PR}) \exp\{\pm i \gamma_{PR}\}$$

$$\omega_{IS}^{(\pm 2)} = \frac{b_{IS}}{4} \sin^2 \beta_{PR} \exp\{\pm i 2 \gamma_{PR}\}$$

- MAS: time-independent dipolar (and CSA) components are zero; terms modulated at $\omega_r$ and $2\omega_r$ go to zero when averaged over the rotor cycle $\rightarrow$ high-resolution spectrum
Isolated $^{13}$C-$^1$H Dipolar Coupling Under MAS (simulation of ideal case)

- Frequency (Hz): $-30000$ to $30000$

- Static

- $v_r = 2$ kHz

- $v_r = 20$ kHz

Increasing Resolution & Sensitivity
Isolated $^{13}\text{C}-^{1}\text{H}$ Dipolar Coupling Under MAS (simulation of ideal case)

Interaction effectively “decoupled” by MAS:
Good for resolution
Bad for structural info
Dipolar Recoupling: Basic Concept

- MAS averages couplings to zero
- Multiple RF pulse sequences partially restore couplings

\[ H = \omega_D \sum_{\lambda \mu} A_{\lambda \mu} \times T_{\lambda \mu} \]

\[ \Theta = \pi/2 \]

\[ \Theta = 54.7^\circ \]

\[ H = 0 \]

Magic Angle Spinning

\[ \Theta = 0 \]

\[ \Theta = \pi/2 \]

\[ \Theta = 54.7^\circ \]
Let’s see how this works for simple case of CW RF field on one of the spins

(I) $^1H$  
\[ \text{CW}_x \]  
\[ \omega_1 = n\omega_r \]

(S) $^{13}C$

\[
H_{tot} = \omega_S^{iso} S_z + \omega_S(t) S_z + \omega_I^{iso} I_z + \omega_I(t) I_z \\
+ \pi J_{IS} 2I_z S_z + \omega_{IS}(t) 2I_z S_z + \omega_1 I_x
\]

• For average Hamiltonian analysis: RF and MAS modulations must be synchronized ($\omega_1 = n\omega_r$) to obtain cyclic propagator
AHT: Summary

\[ \rho(t) = U_{tot}(t) \rho(0) U_{tot}(t)^{-1}; \quad U_{tot}(t) = T \exp \left\{ -i \int_0^t dt' (H + H_{RF}) \right\} \]

\[ U_{tot}(t) = U_{RF}(t) U(t) = U_{RF}(t) \cdot T \exp \left\{ -i \int_0^t dt' \tilde{H} \right\}; \quad \tilde{H} = U_{RF}^{-1} H U_{RF} \]

\[ U_{tot}(t_c) = U(t_c) = T \exp \left\{ -i \int_0^{t_c} dt' \tilde{H} \right\} = \exp \left\{ -i \tilde{H} t_c \right\} \quad \text{(for } U_{RF}(t_c) = 1) \]

\[ \tilde{H} = \tilde{H}^{(0)} + \tilde{H}^{(1)} + \tilde{H}^{(2)} + \ldots \]

\[ \tilde{H}^{(0)} = \frac{1}{t_c} \int_0^{t_c} dt' \tilde{H}; \quad \tilde{H}^{(1)} = \frac{1}{2it_c} \int_0^{t_c} dt'' \int_0^{t''} dt' \left[ \tilde{H}(t''), \tilde{H}(t') \right]; \quad \ldots \]

• Main idea: calculate effective spin Hamiltonian that does not contain RF term

Haeberlen & Waugh, Phys. Rev. 1968
Interaction Frame Hamiltonian

\[ \tilde{H} = U_{RF}^{-1} H U_{RF} = \exp \{ i \omega_1 t I_x \} H \exp \{ -i \omega_1 t I_x \} \]

\[ \tilde{H} = \tilde{H}_S + \tilde{H}_I + \tilde{H}_J + \tilde{H}_D \]

\[ \tilde{H}_S = \omega_{iso} S_z + \omega_S (t) S_z \]

\[ \tilde{H}_J = \pi J_{IS} 2 S_z \left\{ I_z \cos(\omega_1 t) + I_y \sin(\omega_1 t) \right\} \]

\[ = \pi J_{IS} S_z \left\{ I_z \left( e^{i \omega_r t} + e^{-i \omega_r t} \right) - i I_y \left( e^{i \omega_r t} - e^{-i \omega_r t} \right) \right\} \]

\[ \tilde{H}_D = \omega_{IS} (t) 2 S_z \left\{ I_z \cos(\omega_1 t) + I_y \sin(\omega_1 t) \right\} \]

\[ = \omega_{IS} (t) S_z \left\{ I_z \left( e^{i \omega_r t} + e^{-i \omega_r t} \right) - i I_y \left( e^{i \omega_r t} - e^{-i \omega_r t} \right) \right\} \]
Interaction Frame Hamiltonian (Cont.)

\[
\tilde{H}_{IS}^D = \omega_{IS}(t)2S_z \left\{ I_z \cos(\omega_1 t) + I_y \sin(\omega_1 t) \right\} \\
= \omega_{IS}(t)S_z \left\{ I_z \left( e^{i\omega_r t} + e^{-i\omega_r t} \right) - iI_y \left( e^{i\omega_r t} - e^{-i\omega_r t} \right) \right\} \\
= \sum_{m=-2}^{2} \left\{ \omega_{IS}^{(m)} \left[ e^{i(m+n)\omega_r t} + e^{i(m-n)\omega_r t} \right] I_z S_z \\
- i\omega_{IS}^{(m)} \left[ e^{i(m+n)\omega_r t} - e^{i(m-n)\omega_r t} \right] I_y S_z \right\}
\]
Lowest-Order Average Hamiltonian

$$\tilde{H}^{(0)} = \tilde{H}_{S}^{(0)} + \tilde{H}_{J,IS}^{(0)} + \tilde{H}_{D,IS}^{(0)}$$

$$\tilde{H}_{S}^{(0)} = \frac{\omega_{S}^{iso} S_{z}}{\tau_{r}} \int_{0}^{\tau_{r}} dt + \sum_{m=-2}^{2} \left\{ \frac{S_{z}}{\tau_{r}} \int_{0}^{\tau_{r}} dt \omega_{S}^{(m)} e^{im\omega_{r}t} \right\} = \omega_{S}^{iso} S_{z}$$

$$\tilde{H}_{J,IS}^{(0)} = \frac{\pi J_{IS} S_{z}}{\tau_{r}} \int_{0}^{\tau_{r}} dt \left\{ I_{z} \left( e^{in\omega_{r}t} + e^{-in\omega_{r}t} \right) - iI_{y} \left( e^{in\omega_{r}t} - e^{-in\omega_{r}t} \right) \right\} = 0$$

- S-spin CSA refocused by MAS, I-S J-coupling eliminated by RF field on I-spin (decoupling)
Lowest-Order Average $H_D$

$$
\tilde{H}_{D,IS}^{(0)} = \frac{1}{\tau_r} \int_0^{\tau_r} dt \sum_{m=-2}^{2} \{\omega_{IS}^{(m)} \left[ e^{i(m+n)\omega_r t} + e^{i(m-n)\omega_r t} \right] I_z S_z 
- i\omega_{IS}^{(m)} \left[ e^{i(m+n)\omega_r t} - e^{i(m-n)\omega_r t} \right] I_y S_z \}
$$

$$
\frac{1}{\tau_r} \int_0^{\tau_r} dt \omega_{IS}^{(m)} e^{i(m\pm n)\omega_r t} = \begin{cases} 
0 & \text{if } m \pm n \neq 0 \\
\omega_{IS}^{(\mp n)} & \text{if } m \pm n = 0 
\end{cases}
$$
Lowest-Order Average $H_D$

\[ n \neq 1,2 \rightarrow I-S \text{ Decoupling} \]

\[ \widetilde{H}^{(0)}_{D,IS} = 0 \]

\[ n = 1,2 \rightarrow I-S \text{ Dipolar Recoupling} \]

\[ \widetilde{H}^{(0)}_{D,IS} = \left( \omega_{IS}^{(-n)} + \omega_{IS}^{(n)} \right) I_z S_z - i \left( \omega_{IS}^{(-n)} - \omega_{IS}^{(n)} \right) I_y S_z \]

- This rotary resonance recoupling ($R^3$) effect arises from interference of MAS and I-spin RF (when $\omega_1 = \omega_r$ or $2\omega_r$)

- Additional (much weaker) resonances (for $n = 3,4,\ldots$) are also possible due to higher order average Hamiltonian terms involving the I-spin CSA
Rotary Resonance Recoupling

\[ \tilde{H}_{D,CN}^{(0)} = \left( \omega_{IS}^{-1} + \omega_{IS}^{(1)} \right) C_z N_z - i \left( \omega_{IS}^{-1} - \omega_{IS}^{(1)} \right) C_z N_y \]

\[ = \exp \left\{ -i \gamma_{PR} N_x \right\} \left( \tilde{\omega} 2C_z N_z \right) \exp \left\{ i \gamma_{PR} N_x \right\} \]

\[ \tilde{\omega} = -\frac{b_{IS}}{2\sqrt{2}} \sin(2\beta_{PR}); \quad \omega_{IS}^{(\pm)} = -\frac{b_{IS}}{2\sqrt{2}} \sin \left( 2\beta_{PR} \right) \exp \left\{ \pm i \gamma_{PR} \right\} \]

Oas, Levitt & Griffin, JCP 1988
Rotary Resonance Recoupling

\[ \rho(t) = \exp\{-i\bar{H}_{D,CN}^{(0)}t\}C_x \exp\{i\bar{H}_{D,CN}^{(0)}t\} = C_x \cos(\tilde{\omega}t) + 2C_y N_\gamma \sin(\tilde{\omega}t) \]

\[ N_\gamma = N_z \cos \gamma_{PR} - N_y \sin \gamma_{PR} \]

\[ D_{CN} = 900 \text{ Hz} \]
\[ \omega_r = 25 \text{ kHz} \]
\( R^3 \) in Real Spin Systems: Effect of CSA of Irradiated Spin

- \( R^3 \) also recouples \(^{15}\text{N} \) CSA, which doesn’t commute with dipolar term.

- Dipolar dephasing depends on CSA magnitude and orientation: problem for quantitative distance measurements.

- In experiments also have to consider effects of RF inhomogeneity.
Rotational Echo Double Resonance (REDOR)

- Apply a series of rotor-synchronized $\pi$ pulses (2 per $\tau_r$) to $^{15}\text{N}$ spins (this is usually called a dephasing or S experiment)

- Typically a reference (or $S_0$) experiment with pulses turned off is also acquired to account for $R_2$ – report $S/S_0$ ratio (or $\Delta S/S_0 = 1-S/S_0$)

Gullion & Schaefer, JMR 1989
REDOR: Summary of AHT Analysis

$$H_{IS}(t) = \omega_{IS}(t)2I_zS_z = -\frac{1}{2}b_{IS}\{\sin^2(\beta) \cos[2(\gamma + \omega_r t)] - \sqrt{2} \sin(2\beta) \cos(\gamma + \omega_r t)\}2I_zS_z$$

\[\begin{align*}
\tilde{S}_z &= \begin{cases} 
S_z & 0 < t \leq \tau \\
-S_z & \tau < t \leq \tau + \tau_r / 2 \\
S_z & \tau + \tau_r / 2 < t \leq \tau_r 
\end{cases}
\end{align*}\]

$$\tilde{H}_{IS}^{(0)} = \frac{\sqrt{2}}{\pi} b_{IS} \sin(2\beta) \sin(\gamma + \psi) \cdot 2I_zS_z; \quad \psi = \omega_r \tau \quad \text{(sequence phase)}$$

$$\tilde{H}_{IS}^{(0)} = \begin{cases} 
-\frac{\sqrt{2}}{\pi} b_{IS} \sin(2\beta) \sin(\gamma) \cdot 2I_zS_z & \text{for } \tau = \tau_r / 2 \\
\frac{\sqrt{2}}{\pi} b_{IS} \sin(2\beta) \sin(\gamma) \cdot 2I_zS_z & \text{for } \tau = 0 
\end{cases}$$

- Effective Hamiltonian changes sign as a function of position of pulses within the rotor cycle (must pay attention to this in some implementations of REDOR)
REDOR: Typical Implementation

• Rotor synchronized spin-echo on $^{13}$C channel refocuses $^{13}$C isotropic chemical shift and CSA evolution

• Recoupled $^{15}$N CSA commutes with $^{13}$C-$^{15}$N DD coupling!

• 2$^{nd}$ group of pulses shifted by -$\tau_r/2$ relative to 1$^{st}$ group to change sign of $H_D$ and avoid refocusing the $^{13}$C-$^{15}$N dipolar coupling

• xy-type phase cycling of $^{15}$N pulses is critical (Gullion, JMR 1990)
REDOR Dipolar Evolution

\[ \rho(t) = \exp\{-i\tilde{\omega}2C_zN_zt\} C_x \exp\{i\tilde{\omega}2C_zN_zt\} \]

\[ = C_x \cos(\tilde{\omega}t) + 2C_yN_z \sin(\tilde{\omega}t) \]

\[ \tilde{\omega} = -\frac{\sqrt{2}}{\pi} b_{IS} \sin(2\beta) \sin(\gamma) \]
REDOR Dipolar Evolution

\[ \rho(t) = \exp\{-i\tilde{\omega}2C_zN_zt\}C_x \exp\{i\tilde{\omega}2C_zN_zt\} \]
\[ = C_x \cos(\tilde{\omega}t) + 2C_yN_z \sin(\tilde{\omega}t) \]
\[ \tilde{\omega} = -\frac{\sqrt{2}}{\pi} b_{IS} \sin(2\beta) \sin(\gamma) \]
• Experiment highly robust toward $^{15}$N CSA, experimental imperfections, resonance offset and finite pulse effects (xy-4/xy-8 phase cycling is critical for this)

• REDOR is used routinely to measure distances up to $\sim$5-6 Å (D $\sim$ 25 Hz) in isolated $^{13}$C-$^{15}$N spin pairs
REDOR: More Challenging Case

S112($^{13}$C')-Y114($^{15}$N) Distance Measurement in TTR(105-115) Amyloid Fibrils

$4.06 \pm 0.06 \, \text{Å}$
REDOR: $^{15}$N CSA Effects

- Simpler schemes ($xy-4$, $xy-8$) seem to perform better with respect to $^{15}$N CSA compensation than the longer $xy-16$

- Since $[\overline{H}_{D(0)}, \overline{H}_{CSA(0)}] = 0$, these effects are likely due to finite pulses and higher order terms in the average Hamiltonian expansion

Gullion, Baker & Conradi, JMR 1990

Jaroniec, Tounge, Herzfeld, Griffin JACS 2001
# REDOR at High MAS Rates

\[
\phi = \frac{2\tau_p}{\tau_r}
\]

<table>
<thead>
<tr>
<th>(\tau_p) ((\mu)s)</th>
<th>(\nu_r) (kHz)</th>
<th>(\phi)</th>
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REDOR (xy-4) at High MAS: AHT

\[ \tilde{H}_{IS}(t) = \omega_{IS}(t) \left\{ f(t)2I_zS_z + g(t)2I_zS_x + h(t)2I_zS_y \right\} \]

Jaroniec et al, JMR 2000
REDOR (xy-4) at High MAS: AHT

\[
\tilde{H}_{IS}(t) = \omega_{IS}(t) \left\{ f(t)2I_zS_z + g(t)2I_zS_x + h(t)2I_zS_y \right\}
\]

\[
\tilde{H}_{IS}^{(0)} \propto \frac{1}{\tau_r} \left\{ \int_{t_1}^{t_2} (ac'' + bc') \cos[\theta(t)] \, dt \cdot 2I_zS_z + \int_{t_2}^{t_4} (ac'' + bc') \sin[\theta(t)] \, dt \cdot 2I_zS_y - \int_{t_3}^{t_5} (ac'' + bc') \sin[\theta(t)] \, dt \cdot 2I_zS_z + \right.
\]

\[
+ \int_{t_4}^{t_6} (ac'' + bc') \cos[\theta(t)] \, dt \cdot 2I_zS_x - \int_{t_5}^{t_7} (ac'' + bc') \sin[\theta(t)] \, dt \cdot 2I_zS_y + \int_{t_6}^{t_8} (ac'' + bc') \cos[\theta(t)] \, dt \cdot 2I_zS_z + \right.
\]

\[
- \int_{t_7}^{t_9} (ac'' + bc') \sin[\theta(t)] \, dt \cdot 2I_zS_x + \int_{t_8}^{t_10} (ac'' + bc') \sin[\theta(t)] \, dt \cdot 2I_zS_y \}
\]

Jaroniec et al, JMR 2000
REDOR (xy-4) at High MAS: AHT

\[ \tilde{H}_{IS}^{(0)} = \begin{cases} 
-\sqrt{2} \frac{b_{IS}}{\pi} \cos\left(\frac{\pi}{2} \varphi \right) \sin(2\beta) \sin(\gamma) 2I_z S_z; & \text{finite pulses} \\
-\sqrt{2} \frac{b_{IS}}{\pi} \sin(2\beta) \sin(\gamma) 2I_z S_z; & \text{ideal pulses}
\end{cases} \]

\[ \kappa \equiv \frac{b_{IS}^{\text{eff}}}{b_{IS}} = \frac{\cos\left(\frac{\pi}{2} \varphi \right)}{1 - \varphi^2}; \quad \frac{\pi}{4} \leq \kappa \leq 1 \]

- For xy-4 phase cycling, finite \( \pi \) pulses result only in a simple scaling of the dipolar coupling constant by an additional factor, \( \kappa \), between \( \pi/4 \) and 1.
- For xx-4 spin dynamics are more complicated and converge to \( R^3 \) dynamics in the limit of \( \varphi = 1 \).
REDOR (xy-4) at High MAS: AHT vs. Numerical Simulations

10 $\mu$s $^{15}$N pulses
no $^{15}$N CSA
REDOR (xy-4) Experiments

- Graph 1: Plot of $S/S_0$ vs. Time (ms) with data points for $\varphi = 0.20$ (yellow) and $\varphi = 0.61$ (blue), and a simulation line.

- Graph 2: Plot of $b S_{\text{Effective}} / b S_{\text{Ideal}}$ vs. Fraction of Rotor Period ($\varphi$) with experimental data points (yellow) and a theoretical AHT line.
REDOR in Multispin Systems

\[ \bar{H}_{IS} = \omega_1 2I_z S_{1z} + \omega_2 2I_z S_{2z} \]

\[ I_x(t) = \langle \cos(\omega_1 t) \cos(\omega_2 t) \rangle \]

- Strong $^{13}$C-$^{15}$N couplings dominate REDOR dipolar dephasing; weak couplings become effectively ‘invisible’
Frequency Selective REDOR

- Use a pair of weak frequency-selective pulses to ‘isolate’ the $^{13}\text{C}^{15}\text{N}$ dipolar coupling of interest; all other couplings refocused

- This trick is possible because all relevant interactions commute

$$H = \sum_{i,j} \omega_{ij} 2I_{iz} S_{jz} + \sum_{i<j} \pi J_{ij} 2I_{iz} I_{jz}$$

$$H = \omega_{kl} 2I_{kz} S_{lz}$$
FS-REDOR Evolution

\[ H = H_0 + H_1 + H_2; \]
\[ H_0 = \sum_{i \neq k} \sum_{j \neq l} \omega_{ij} 2 I_{iz} S_{jz} + \sum_{i < j \neq k} \pi J_{ij} 2 I_{iz} I_{jz}; \]
\[ H_1 = \sum_{i \neq l} \omega_{ki} 2 I_{kz} S_{iz} + \sum_{i \neq k} \omega_{il} 2 I_{iz} S_{lz} + \sum_{i \neq k} \pi J_{ki} 2 I_{kz} I_{iz}; \]
\[ H_2 = \omega_{kl} 2 I_{kz} S_{iz}; \]

\[ [H_0, H_1] = [H_0, H_2] = [H_1, H_2] = 0 \]
\[ [H_0, I_{kx}] = [H_0, S_{lx}] = 0 \]

for each term in \( H_1 \) \( [ , I_{kx}] \neq 0 \) or \( [ , S_{lx}] \neq 0 \)
\[ [H_2, I_{kx}] \neq 0 \) and \( [H_2, S_{lx}] \neq 0 \)

\[ U(t) = e^{-iH(t/2)} \underbrace{e^{-i\pi I_{kx}} e^{-i\pi S_{lx}}} e^{-iH(t/2)} \underbrace{e^{i\pi S_{lx}} e^{i\pi I_{kx}}} e^{-i\pi I_{kx}} e^{-i\pi S_{lx}} \]
\[ = e^{-iH(t/2)} e^{-iH_0(t/2)} e^{-iH_2(t/2)} e^{iH_1(t/2)} e^{-i\pi I_{kx}} e^{-i\pi S_{lx}} \]
\[ = e^{-iH_0 t} e^{-iH_2 t} e^{-i\pi I_{kx}} e^{-i\pi S_{lx}} \]

\[ \rho(0) = I_{kx}; \]
\[ [\rho(0), e^{-i\pi S_{lx}}] = [\rho(0), e^{-i\pi I_{kx}}] = [\rho(0), e^{-iH_0 t}] = 0 \]
\[ \rho(t) = e^{-iH_2 t} I_{kx} e^{iH_2 t} = I_{kx} \cos(\omega_{kl} t) + 2 I_{ky} S_{lz} \sin(\omega_{kl} t) \]

Dipolar evolution under only \( b_{kl} \)
$^{13}$C Selective Pulses: U-$^{13}$C Thr

![Graph showing $^{13}$C selective pulses with different Gauss times](image)

- **Reference**: $\tau_{\text{Gauss}} = 0.6$ ms
- $\tau_{\text{Gauss}} = 3.0$ ms
- $\tau_{\text{Gauss}} = 3.0$ ms
- $\tau_{\text{Gauss}} = 0.6$ ms

$^{13}$C Chemical Shift (ppm)

180 160 140 120 100 80 60 40 20
FS-REDOR: U-$^{13}$C, $^{15}$N Asn
FS-REDOR: U-$^{13}$C,$^{15}$N Asn
FS-REDOR: U-$^{13}$C, $^{15}$N Asn

(a) Structural diagram of Asn with distances marked.

(b) 13C chemical shift spectrum with peaks at 160, 120, 80, 40 ppm.

(c) 15N chemical shift spectrum with peaks at 160, 120, 80, 40 ppm.

(d) 1 ms duration data showing peak at N$^{\delta 2}$.

(e) 6 ms duration data showing peak at N$^{\delta 2}$.

(f) 10 ms duration data showing peak at N$^{\delta 2}$.

Frequency of $^{15}$N Selective π Pulse (kHz)
FS-REDOR: U-$^{13}$C, $^{15}$N Asn
FS-REDOR: U-\textsuperscript{13}C,\textsuperscript{15}N Asn
FS-REDOR: U-\textsuperscript{13}C,\textsuperscript{15}N-f-MLF
FS-REDOR: U-\textsuperscript{13}C,\textsuperscript{15}N-f-MLF

Leu C\textsuperscript{\beta}-Leu N

Leu C\textsuperscript{\beta}-Phe N

Met C\textsuperscript{\beta}-Phe N

X-ray: 2.50 Å
NMR: 2.46 ± 0.02 Å

X-ray: 3.12 Å
NMR: 3.24 ± 0.12 Å

X-ray: 4.06 Å
NMR: 4.12 ± 0.15 Å
• 16 $^{13}$C-$^{15}$N distances measured in MLF tripeptide

• Selectivity of $^{15}$N pulse + need of prior knowledge of which distances to probe is a major limitation for U-$^{13}$C, $^{15}$N proteins
Simultaneous $^{13}\text{C}-^{15}\text{N}$ Distance Measurements in U-$^{13}\text{C},^{15}\text{N}$ Molecules

General Pseudo-3D HETCOR
(Heteronuclear Correlation) Scheme

- I-S coherence transfer as function of $t_{\text{mix}}$ via $D_{\text{IS}}$
- Identify coupled I and S spins by chemical shift labeling in $t_{1}, t_{2}$
Transferred Echo Double Resonance (TEDOR)

3D TEDOR Pulse Sequence

\[ S_x \xrightarrow{\text{REDOR}} 2I_zS_y\sin(\omega_{IS}t_{\text{mix}}/2) \xrightarrow{(\pi/2)I_x+(\pi/2)S_x} (\pi/2)I_x+ (\pi/2)S_x \]

\[ -2I_yS_z\sin(\omega_{IS}t_{\text{mix}}/2) \xrightarrow{\text{REDOR}} I_x\sin^2(\omega_{IS}t_{\text{mix}}/2) \]

- Similar idea to INEPT experiment in solution NMR
- Cross-peak intensities formally depend on all $^{13}\text{C}-^{15}\text{N}$ couplings
- Experiment not directly applicable to U-$^{13}\text{C}$-labeled samples (π pulse train on $^{13}\text{C}$ channel recouples $D_{CC}$ and causes loss of magnetization)
3D TEDOR: U-\textsuperscript{13}C,\textsuperscript{15}N Molecules

‘Out-and-back’ 3D TEDOR Pulse Sequence

\[ I_x \xrightarrow{\text{REDOR}} 2I_yS_z \sin(\omega_{IS}t_{mix}/2) \frac{(\pi/2)S_x+(\pi/2)I_x}{I_x} \]

\[ -2I_zS_y \sin(\omega_{IS}t_{mix}/2) \frac{(\pi/2)S_x-(\pi/2)I_x}{I_x} \]

\[ -2I_yS_z \sin(\omega_{IS}t_{mix}/2) \xrightarrow{\text{REDOR}} I_x \sin^2(\omega_{IS}t_{mix}/2) \]

Michal & Jelinski, JACS 1997
3D TEDOR: U-$^{13}$C, $^{15}$N Molecules

$N$-acetyl-$Val$-$Leu$

- Cross-peak intensities roughly proportional to $^{13}$C-$^{15}$N dipolar couplings
3D TEDOR: U-$^{13}$C,$^{15}$N N-ac-Val-Leu

- Spectral artifacts (spurious cross-peaks, phase twisted lineshapes) appear at longer mixing times as result of $^{13}$C-$^{13}$C J-evolution
Improved Scheme: 3D Z-Filtered TEDOR

3D ZF TEDOR Pulse Sequence

- Unwanted anti-phase and multiple-quantum coherences responsible for artifacts eliminated using two z-filter periods

Jaroniec, Filip & Griffin, JACS 2002
Results in N-ac-VL

3D TEDOR

$t_{mix} = 10.8 \text{ ms}$
• 3D ZF TEDOR generates purely absorptive 2D spectra
• Cross-peak intensities give qualitative distance information
ZF-TEDOR Cross-Peak Trajectories

Intensities depend on all spin-spin couplings to particular $^{13}$C.

Use approximate models based on Bessel expansions of REDOR signals to describe cross-peak trajectories (Mueller, *JMR* 1995).

\[
V_{ij} = V_i(0) \prod_{l \neq i}^{m_i} \cos^2 \left( \pi J_{il} \tau \right) \times \left( \sin^2 \left( \omega_{ij} \tau \right) \prod_{k \neq j}^{N_j} \cos^2 \left( \omega_{ik} \tau \right) \right)
\]
3D ZF-TEDOR: N-ac-VL

Val C$^{11}$ Cross-peaks

Cross-peak intensity vs. Mixing Time (ms)

- Val(N): X-ray: 2.95 Å, NMR: 3.1 Å
- Leu(N): X-ray: 4.69 Å, NMR: 4.7 Å

Val C$^{12}$ Cross-peaks

Cross-peak intensity vs. Mixing Time (ms)

- Val(N): X-ray: 3.81 Å, NMR: 4.0 Å
- Leu(N): X-ray: 3.38 Å, NMR: 3.5 Å
3D ZF-TEDOR in Small Peptides: Summary

- Typical uncertainties in measured distances are ~ ±10% due to the use of approximate analytical simulation model
Application to TTR(105-115) Amyloid Fibrils

Slice from 3D ZF TEDOR Expt.

Cross-Peak Trajectories (T106)

Ensemble of 20 NMR Structures

- ~70 $^{13}$C-$^{15}$N distances measured by 3D ZF TEDOR in several U-$^{13}$C,$^{15}$N labeled fibril samples (30+ between 3-6 Å)

Jaroniec et al, PNAS 2004
**$^{13}$C-$^{13}$C J-Evolution Effects in ZF-TEDOR**

Simulated $^{13}$C Cross-Peak Buildup

4 Å C-N Distance ($D_{CN} = 50$ Hz)

- $^{13}$C-$^{15}$N cross-peak intensities reduced 2- to 5-fold
- Effective dipolar evolution times limited to ~10-14 ms
• $^{13}$C-$^{13}$C J-couplings refocused using band-selective $^{13}$C pulses (no z-filters required)

• Most useful for strongly J-coupled sites with unique chemical shifts (e.g., $^{13}$CO) – less so for C$\alpha$/C$\beta$ and other aliphatic C’s due to overlapping chemical shift ranges (e.g., C$\beta$ range is ~20-70 ppm)
ZF-TEDOR vs. BASE-TEDOR

Gly $^{13}\text{C}_\alpha-^{15}\text{N}$

- REDOR 3D
- Sim: $D=892$ Hz, $J=60$ Hz
- REDOR 3D (J-decoupled)
- Sim: $D=892$ Hz

Gly $^{13}\text{C}'-^{15}\text{N}$

- REDOR 3D
- Sim: $D=189$ Hz, $J=60$ Hz
- REDOR 3D (J-decoupled)
- Sim: $D=189$ Hz

Thr $^{13}\text{C}_\gamma-^{15}\text{N}$

- REDOR 3D
- Sim: $D=58$ Hz, $J=22$ Hz
- REDOR 3D (J-decoupled)
- Sim: $D=58$ Hz
Cross-Peak Trajectories in TEDOR Experiments

**3D ZF TEDOR**

\[
V_{ij} = V_i(0) \prod_{l \neq i}^{m_i} \cos^2 \left( \pi J_{il} \tau \right) \times \\
\left\langle \sin^2 \left( \omega_{ij} \tau \right) \prod_{k \neq j}^{N_i} \cos^2 \left( \omega_{ik} \tau \right) \right\rangle
\]

**3D BASE TEDOR**

\[
V_{ij} = V_i(0) \left\langle \sin^2 \left( \omega_{ij} \tau \right) \prod_{k \neq j}^{N_i} \cos^2 \left( \omega_{ik} \tau \right) \right\rangle
\]
3D BASE TEDOR: N-ac-VL

Val C' Cross-peaks

X-ray: 2.39 Å
NMR: 2.4 Å
Val(N)

Leu(N)

Ac C' Cross-peaks

X-ray: 1.33 Å
NMR: 1.3 Å
Val(N)

X-ray: 1.33 Å
NMR: 1.4 Å
Val(N)

X-ray: 4.28 Å
NMR: 4.1 Å
Leu(N)
3D SCT-TEDOR Pulse Scheme

- Dipolar $^{13}$C-$^{15}$N SSNMR version of HMQC
- Constant time spin-echo ($2T + 4\delta = \sim 1/J_{CC}$) used to refocus $^{13}$C-$^{13}$C J-coupling, with encoding of $^{15}$N shift ($t_1$) and $^{13}$C-$^{15}$N DD coupling ($\tau_{CN}$)
- Experiment can offer improved resolution and sensitivity
- Need isolated $^{13}$C-$^{13}$C pairs ($C'$-$C\alpha$, $C^{\text{methyl}}$-$C^{\text{aliphatic}}$)

Helmus et al, JCP 2008
3D SCT-TEDOR: Methyl $^{13}$C in GB1

500 MHz, ~2.7 h

$\delta^{(15)N}$ (F₁, ppm) vs $\delta^{(13)C}$ (F₂, ppm)

- Effects of $^{13}$C-$^{13}$C J-couplings and relaxation removed from trajectories – longer dipolar evolution times accessible
• ~2-3 fold gains in sensitivity predicted for CT-TEDOR for
~4-5 Å $^{13}$C-$^{15}$N distances at typical $^{13}$C methyl $R_2$ rates
3D SCT-TEDOR: GB1

A) I6N-I6δ1
B) T11N-T11γ
C) K13N-L12δ
D) A20N-A20β
E) V21N-A20β
F) D40N-V39γ
G) T44N-T44γ
H) T49N-A48β
I) T49N-T49γ

Cross-Peak Intensity (a.u.)

Mixing Time, \( \tau_{CN} \) (ms)
• Most NMR distances are within ~10% of X-ray structure
• Useful information about protein side-chain rotamers
T11 Side-Chain Conformation

<table>
<thead>
<tr>
<th>Rotamer</th>
<th>$\chi_1$ (°)</th>
<th>N-C$_\gamma$ distance (Å)</th>
<th>% occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+60</td>
<td>2.94</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td>-60</td>
<td>3.81</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>180</td>
<td>2.94</td>
<td>9</td>
</tr>
<tr>
<td>X-ray</td>
<td>-82</td>
<td>3.77</td>
<td>-</td>
</tr>
<tr>
<td>NMR</td>
<td>-</td>
<td>3.1</td>
<td>-</td>
</tr>
</tbody>
</table>

4D SCT-TEDOR Pulse Scheme

- Additional “relaxation-free” CT $^{13}$C methyl chemical shift labeling period easily implemented for improved resolution
4D SCT-TEDOR: N-Acetyl-Valine

- N-Cγ distances detected via both N-Cγ and N-Cβ correlations
- Distances within ~0.1 Å of NAV X-ray structure
- 4D recorded in ~37 h
4D SCT-TEDOR: GB1

$F_2^{(13}C) = 15.9 \text{ ppm}$

$\tau_{CN} = 15.84 \text{ ms}$
J-Decoupling by $^{13}$C ‘Spin Dilution’

GB1 spectra from Rienstra group, UIUC
ZF-TEDOR: $1,3^{-13}C,^{15}N$ GB1

Nieuwkoop et al, JCP 2009
ZF-TEDOR: 1,3-$^{13}\text{C}$, $^{15}\text{N}$ GB1

~750 $^{13}\text{C}$-$^{15}\text{N}$ distances + dihedral restraints (TALOS)

Nieuwkoop et al, JCP 2009
Proton assisted heteronuclear recoupling: PAIN-CP

- Superficially similar to CP but RF fields satisfy different matching conditions
- Polarization transferred from $^{15}\text{N}$ to $^{13}\text{C}$ via a 2$^{nd}$ order mechanism using a cross term involving a $^{1}\text{H}$ assisting spin ($H_{\text{eff}} \sim N^{+}C-H_{z}$)
- Works best at higher MAS rates (~20+ kHz)

Lewandowski, De Paepe and Griffin JACS 129, 728-29 (2007)
De Paepe et al. JCP 134, 095101 (2011)
• Very flexible recoupling – by appropriately combining RF strength, offset and mixing times broadband or selective recoupling can be achieved

*De Paepe et al. JCP 134, 095101 (2011)*
Comparison of TEDOR and PAIN-CP transfer: 1,3-^{13}C,^{15}N GB1
PAIN-CP: Applications to Larger Proteins

- >5Å $^{15}$N-$^{13}$C contacts between secondary structure elements

De Paepe, Lewandowski, Bockmann, Griffin & co-workers
PAIN-CP: Applications to Larger Proteins

\[ \frac{\omega_r}{2\pi} = 20 \text{ kHz}, \quad \frac{\omega_{1H}}{2\pi} = 900 \text{ MHz} \]

5 ms PAR

\[ \text{15}^N \text{ chemical shift (ppm)} \]
\[ \text{13}^C \text{ chemical shift (ppm)} \]

15 ms PAIN-CP

MMP-12 protein (17.6 kDa)

Bertini, Griffin & co-workers JACS 132, 1032 (2010)
Chemical Shifts and Secondary Structure

• Characteristic deviations from ‘random coil’ shifts for α-helix and β-sheet secondary structures form the basis for TALOS and other CS-based structure prediction programs

Spera & Bax, JACS 113 (1991) 5491
TALOS CS-Based Torsion Angle Prediction: TTR(105-115) Amyloid Fibrils

Cornilescu, Delagion & Bax JBNMR 1999
Dipolar Evolution in Multispin Systems

\[ \bar{H}_{IS} = \omega_1 I_z S_{1z} + \omega_2 I_z S_{2z} ; \quad I_x(t) = \langle \cos(\omega_1 t) \cos(\omega_2 t) \rangle \]

- Evolution under several orientation-dependent DD couplings is detrimental to schemes where main goal is to determine weak DD couplings (e.g., REDOR)

- Put this to good use for determining projection angles between DD tensors by “matching” dipolar phases (i.e., make \( \omega_1 t \sim \omega_2 t \) above) – such expts. yield dihedral restraints that can be combined with CS-based data
R-symmetry based sequences (Levitt) have been shown to be highly effective for N-H/C-H recoupling with H-H decoupling at high MAS rates (e.g., Polenova 2011).

In highly deuterated proteins other (e.g., REDOR-type) sequences can also be used.
T-MREV Recoupling in NH and NH$_2$ Groups


FT of dipolar dephasing trajectory in time domain

Hohwy et al. JACS 2000
HC-CH Experiment (Levitt et al, CPL 1996)

\[ \Theta = \text{projection angle} \]
**HC-CH Experiment (Levitt et al, CPL 1996)**

Double-quantum coherence (correlated $C_1$-$C_2$ spin state)

\[ \rho \sim C_1^+ C_2^+ + C_1^- C_2^- \]

\[ \rho \sim C_1^+ + C_2^+ \]

\( \Theta = \) projection angle
**HC-CH Experiment (Levitt et al, CPL 1996)**

\( \Theta = \text{projection angle} \)

Dipolar phases depend on the relative orientations of the two dipolar coupling tensors (most pronounced changes in signal as function of projection angle for \( \Theta = 0 \) or \( 180^\circ \pm \sim 30^\circ \) due to diff. term)

Double-quantum coherence (correlated \( C_1 \)-\( C_2 \) spin state)

\[
\rho \sim C_1^+C_2^+ + C_1^-C_2^-
\]

\[
\rho \sim C_1^+ + C_2^+
\]

\[
S(t_1) \approx \left\langle \cos(\Phi_1) \cos(\Phi_2) \right\rangle = \frac{1}{2} \left\langle \cos(\Phi_1 - \Phi_2) + \cos(\Phi_1 + \Phi_2) \right\rangle
\]

\[
\overline{H}_{MREV} \approx \kappa(\omega_1(t_1)2C_{1z}H_{1z} + \omega_2(t_1)2C_{2z}H_{2z})
\]

\[
\Phi_\lambda(t_1) = \kappa \int_0^{t_1} dt \omega_\lambda(t); \quad \omega_\lambda(t) = \sum_{m=-2}^{2} \omega^{(m)}_\lambda \exp\{im\omega_r t\}
\]
**HC-CH Experiment (Levitt et al, CPL 1996)**

\[ \Theta \approx 60^\circ \quad \Theta \approx 0^\circ/180^\circ \]

- Large differences in dipolar dephasing trajectories for *cis* and *trans* topologies (in both time and frequency domain)

- Dipolar spectrum generated from time domain data for one \( \tau_r \), by multiplying signal by \( \exp(Rt) \), repeating many times, applying an overall apodization function followed by FT
Backbone & Side-chain Torsion Angle Measurements in Peptides and Proteins

- Create various correlated spin states involving two nuclei (e.g., $\text{CO}_i - \text{C}\alpha_i$, $\text{N}_i - \text{C}\alpha_i$, $\text{N}_{i+1} - \text{C}\alpha_i$, $\text{N}_i - \text{N}_{i+1}$, etc.) and evolve for some period of time under selected dipolar couplings (e.g., N-H$\alpha$, N-CO, etc.)

- Evolution trajectories highly sensitive to relative tensor orientations for projection angles around 0°/180° (more info: Hong & Wi, in *NMR Spect. of Biol. Solids* 2005; Ladizhansky, *Encywl. NMR*, 2009)

Observable signal

$$S(t) \approx \langle f_{\text{mix}} \cos(\Phi_1) \cos(\Phi_2) \rangle$$

$$\Phi_\lambda \equiv \Phi_\lambda(b_\lambda, \Omega_\lambda, t)$$
Measurement of $\phi$ in Peptides: HN-CH

Multiple-quantum coherence (correlated N-C spin state; combination of ZQC & DQC)

$$\rho \sim C_y N_x$$

$$S(t_1) \approx \langle \cos(\Phi_{CH}) \cos(\Phi_{NH}) \rangle$$

$$\Phi_\lambda(t_1) = \kappa \int_0^{t_1} dt \omega_\lambda(t)$$

Hong, Gross & Griffin, JPC B 1997
Hong et al. JMR 1997

N-ac-Val
Measurement of $\phi$ in Peptides: HN-CH

$\Theta = 90^\circ$

- Experiment most sensitive around $\phi = -120^\circ$ ($\Theta \sim 165^\circ$); resolution of ca. $\pm 10^\circ$
- Useful structural restraints despite degeneracies (use proj. angles directly in structure refinement)
- Implementations using ‘passive’ evolution under DD couplings limited to slow MAS rates (<5-6 kHz)

$\Phi_{CH} \sim 2\Phi_{NH}$ (CH coupling dominates; interference less pronounced)

Hong et al. *JPC B* 1997
Hong et al. *JMR* 1997
Torsion Angles: 3D HN-CH

Hohwy et al. JACS 2000
Rienstra et al. JACS 2002

$S(t_2) \approx \langle \cos(\omega_{CH} t_2) \cos(r \omega_{NH} t_2) \rangle$

- Expts. that employ active recoupling sequences to reintroduce DD couplings are better suited for applications to high MAS rates & U-$^{13}$C,$^{15}$N samples, and typically offer higher angular resolution.
3D HN-CH: PrP23-144 Amyloid Fibrils

- Record series of 2D N-Cα spectra
- Observe cross-peak volumes as function of $t_2$ (DD evolution time)

$t_2 = 0$ (reference spectrum)
3D HN-CH: PrP23-144 Amyloid Fibrils
Measurement of $\psi$ in Peptides: NC-CN

\[ \psi \approx \langle \cos(\Phi_{N\alpha}) \cos(\Phi_{NCO}) \rangle; \quad \Phi_\lambda(t_1) = \tilde{\omega}_\lambda t_1 \]

Costa et al., CPL 1997
Levitt et al., JACS 1997
Trajectories & Projection Angle vs. $\psi$

- Dephasing of $^{13}$C-$^{13}$C DQ coherence is very sensitive to the relative orientation of $^{13}$C-$^{15}$N dipolar tensors for $|\psi| \approx 150-180^\circ$
Trajectories & Dipolar Spectra vs. $\psi$

'zero-frequency' feature from $\cos(\Phi_1-\Phi_2)$ term
Application to TTR(105-115) Fibrils

Reference DQ-SQ correlation spectrum for U-$^{13}$C,$^{15}$N YTIA labeled sample

- $^{13}$C-$^{13}$C DQ coherences evolve under the sum of CO and $C\alpha$ resonance offsets during $t_1$
TTR(105-115): Dephasing Trajectories

(a) $\psi_{Y105} = \pm 164^\circ \pm 4^\circ$

(b) $-140^\circ < \psi_{T106} < 140^\circ$

(c) RMSD

(d) RMSD
Measurement of $\psi$ in $\beta$-sheet peptides

- $\beta$-sheet protein backbone conformation ($\psi \sim 120^\circ$) falls outside of the sensitive region of NCCN experiment – correlate other set of couplings
Measurement of $\psi$ in $\beta$-sheet peptides: 3D HN(CO)CH experiment

- Same idea as original 3D HNCH with exception that N and C$\alpha$ belong to adjacent residues and magnetization transfer relayed via CO spin

Jaroniec et al. *PNAS* 2004
TTR(105-115) Fibrils: 3D HN(CO)CH trajectories

- $-180^\circ < \psi_{Y105} < 90^\circ$
  $150^\circ < \psi_{Y105} < 180^\circ$

- $90^\circ < \psi_{T106} < 150^\circ$

- Complementary to NCCN data for same residues
Measurement of $\psi$ in $\alpha$-helical peptides: 3D HCCN experiment

- N-CO and C$\alpha$–H$\alpha$ tensors are nearly co-linear for $\psi \sim -60^\circ$

Ladizhansky et al., JMR 2002
HN-NH Tensor Correlation: $\phi$ and $\psi$

$S(t_1) \approx \langle \cos(\Phi_{NH_i})\cos(\Phi_{NH_{i+1}}) \rangle$

Reif et al., JMR 2000
HN-NH Tensor Correlation: GB1

Projection angle depends on intervening $\phi$ and $\psi$ angles (see Reif et al. JMR 2000)

Franks et al., JACS 2006
HN-NH Tensor Correlation: GB1

Franks et al., JACS 2006
Fold of GB1 Refined with Projection Angle Restraints

Franks et al., PNAS 2008

H-H, C-C, N-N distances only (~8,000 restraints!!!); RMSD bb ~ 1 A

+ TALOS & ~110 angle restraints (HN-HN, HN-HC, HA-HB); RMSD bb ~ 0.3 A