Homonuclear $J$-Decoupling in NMR
Resolution in SSNMR of U-$^{13}$C,$^{15}$N Proteins

• Homonuclear $J$ couplings are in many cases the dominant inhomogeneity
• Predominantly $^{1}J_{CC}$

• GB1, 9.4 T
• CTUC COSY
Homonuclear Decoupling: the Indirect Dimensions

- Selective $\pi$ pulse
- Evolution under multiple quantum coherence
- Constant-time experiments
Selective $\pi$

$\ ^{1}H$

$\ ^{13}C$

$t_{1}$

$C_{\alpha}$

$\tau_{m}$

$t_{2}$

$C'$

$C_{\alpha}$

$C_{\gamma/\delta}$

$C_{\text{aromatic}}$

$^{13}C$ Chemical Shift / ppm

Multiple-Quantum Experiments: refocused-INANDEQUATE

DQ-SQ correlation
\[ \tau = \frac{1}{4}J = 4.5 \text{ ms} \]

Refocused-INANDEQUATE

DQ-SQ correlation
\[ \tau = \frac{1}{4}J = 4.5 \text{ ms} \]

\[ I_x + S_x \xrightarrow{\frac{\tau - \pi - \tau}{2(I_y + S_y)}} 2I_yS_z + 2I_zS_y \]
\[ \xrightarrow{\pi/2(I_y + S_y)} 2I_yS_x + 2I_xS_y \]
\[ \xrightarrow{\text{DQF}} 2I_yS_x + 2I_xS_y \]
\[ \xrightarrow{t_1} \cos((\omega_I + \omega_S)t_1)(2I_yS_x + 2I_xS_y) \]
\[ \xrightarrow{\pi/2(I_y + S_y)} -\cos((\omega_I + \omega_S)t_1)(2I_yS_z + 2I_zS_y) \]
\[ \xrightarrow{\frac{\tau - \pi - \tau}{2}} \cos((\omega_I + \omega_S)t_1)(I_x + S_x) \]
\[ \xrightarrow{t_2} \text{detection} \]


Does not evolve in \( t_1 \) under active coupling

\[ \left[ 2\pi J I_z S_z, 2I_y S_z + 2I_x S_y \right] = 0 \]

Does evolve in \( t_1 \) under passive couplings

\[ \left[ 2\pi J I_z K_z, 2I_y S_z + 2I_x S_y \right] \neq 0 \]

so broadening from scalar couplings to neighboring spins in U-\( ^{13}\)C proteins
Constant-Time Experiments: CTUC COSY

Constant-time, uniform-sign cross-peak COSY

$$\tau = \frac{1}{4}J = 4.5 \text{ ms}$$

Constant-Time Experiments:

CTUC COSY

$\tau = 1/4J = 4.5 \text{ ms}$

\[ U(t_1) = e^{-i\left(\frac{\tau + \frac{\tau}{2}\right)}(\omega_I I_z + \omega_S S_z + 2\pi I_J I_z S_z)} e^{-i\frac{\tau}{2}(\omega_I I_z + \omega_S S_z + 2\pi I_J I_z S_z)} \]

\[ = e^{-i\pi(I_x + S_x)} e^{-i\pi(I_x + S_x)} e^{-i\left(\frac{\tau + \frac{\tau}{2}\right)}(\omega_I I_z + \omega_S S_z + 2\pi I_J I_z S_z)} e^{-i\tau(\omega_I I_z + \omega_S S_z + 2\pi I_J I_z S_z)} \]

\[ = e^{-i\pi(I_x + S_x)} e^{-2\pi I_z S_z(2\tau)} e^{-it_1(-\omega_I I_z - \omega_S S_z)} \]

\[ U(t_1)I_{1x}U^{-1}(t_1) = \cos(\omega_I t_1)2I_y S_z + \sin(\omega_I t_1)2I_x S_z \]
$^{13}$C Correlation in U-$^{13}$C, $^{15}$N-GB1

- CTUC COSY
- 25 kHz MAS; 150 kHz SPINAL64
- 9.4 T
• Active and passive couplings removed from CTUC COSY
Homonuclear Decoupling: the Indirect Dimensions

- Selective $\pi$ pulse
- Evolution under multiple quantum coherence
- Constant-time experiments
Homonuclear Decoupling: the Direct Dimension

- In-Phase/Anti-Phase (IPAP) and related Spin-State-Selective ($S^3$) techniques (Lyon Group and others) – intrinsic S/N increase of $\sqrt{2}/\sqrt{s}$

- Band-Selective Homonuclear Decoupling (Reif) – intrinsic S/N increase of 1.2 /$\sqrt{s}$

- Long-Observation-Window Band-Selective Homonuclear Decoupling (LOW BASHD; Mueller) – intrinsic S/N increase of 1.7-1.8/$\sqrt{s}$
Homonuclear Decoupling: IPAP and Related $S^3$ Techniques

- In-phase and anti-phase spectra are recorded (or orthogonal mixed-phase)
- Sum and difference combinations give two independent, single multiplet lines
- Shift and combine
- May require additional delays
- In its most general form, the final increase in intrinsic S/N of $\sqrt{2}/\sqrt{s}$
In-Phase/Anti-Phase

- Experimentally often see full theoretical enhancement of $\sqrt{2}$ per $\sqrt{s}$
Homonuclear Decoupling: the Direct Dimension

• Band-Selective Homonuclear Decoupling (Reif Group)

• Fast, stroboscopic detection interleaved with Dante excitation

• S/N suffers from low duty cycle

  ~ Increase of 1.2 per √s

Reif et al., *JMR* **2005**, 172, 56
Long-Observation-Window Band-Selective Homonuclear Decoupling (LOW BASHD)

- Long (8 ms) observation windows punctuated by short (200 µs) refocusing pulses on partner spin
- Avoids deleterious effects of low duty cycle
LOW BASHD

- U-^{13}C,^{15}N-Gly
- 9.4 T
Decoupling Sidebands

- Partial evolution under $J$-coupling
- Modulated by the decoupling cycle time
- Balance sideband intensity vs. accumulation of off-resonance pulse effects
Decoupling Sidebands

- $\pi$ pulses:
  - 200 $\mu$s Gaussian
  - cosine modulated at 13 kHz (130 ppm)
  - minimizes 1st order, off-resonance pulse effects
Mind the Gap

Convolution of signal with PSF

sampling sidebands
Mind the Gap
Mind the Gap

Time-domain digital filter

\[ \ast \]
Cut-and-Stitch
Cut-and-Stitch
Crevasse Method

- Digitize entire signal including holes
- Fill them back in with post processing

Convolution of signal with PSF

13C Chemical Shift / ppm
LOW-BASHD Window Function

Deconvolution in the frequency domain by the application of a suitable time-domain window function

Filter only depends on acquisition parameters (dwell and sampling schedule), not the sample or signal
LOW-BASHD Window Function
LOW-BASHD Window Function
LOW BASHD

- Collapses multiplet in a single scan
- Uses only 5-8 pulses
- Can replace standard detection in any sequence that detects on carbon
LOW BASHD: NCO

- GB1, 9.4 T
- NCO with Specific CP
LOW BASHD: NCO

- GB1, 9.4 T
- NCO with Specific CP
LOW-BASHD: \textit{J-CACO}

LOW BASHD \quad S/N \times 1.7 \quad \text{Standard}

- GB1, 9.4 T
- CTUC COSY
LOW-BASHD: $J$-CACO

*LOW BASHD*

- GB1, 9.4 T
- CTUC COSY
LOW-BASHD: NCA

LOW BASHD

S/N × 1 - 1.7

Standard

• GB1, 9.4 T
• NCO with Specific CP
LOW-BASHD: NCA

LOW BASHD

- GB1, 9.4 T
- NCO with Specific CP
LOW-BASHD: \textit{J-CBCACO}

\(\alpha\)-subunit of tryptophan synthase

\(27\ \text{kDa}\)

\textit{LOW BASHD} \hspace{1cm} \textit{S/N \times 1.7 \sqrt{\text{Hz}}} \hspace{1cm} \textit{Standard}

- \(\alpha\)-TS, 9.4 T
- Constant time \textit{J-MAS CBCACO}; only 26 ms direct dimension
LOW-BASHD: $J$-CBCACO

- $\alpha$-TS, 9.4 T
- Constant time $J$-MAS CBCACO; only 26 ms direct dimension
LOW-BASHD: NCO

\[ \alpha \]-subunit of tryptophan synthase

27 kDa

\[ S/N \times 1.55 \sqrt{Hz} \]

- \( \alpha \)-TS, 9.4 T
- NCO; only 26 ms direct dimension
Long-Observation-Window
Band-Selective
Homonuclear Decoupling

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Bruker BioSpin: Jochem Struppe

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