Simplifying proton NMR spectra by spatially-selective pure-shift experiments

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The quality of NMR spectra depends on:

**Sensitivity**

- proportional to $B_0^{3/2}$ (700/300 MHz ~ x3.6)
- cryogenic probes (~ x4)
- low volume NMR tubes (e.g. Shigemi ~ x2)
- in total ~ x30 more sensitivity in the past decades

**Resolution**

- linear with $B_0$ (700/300 MHz ~ x2.3)
Resolution problem in 1D $^1$H spectra

Proton-decoupled $^{13}$C NMR

$^1$H homonuclear broadband decoupled

Simulated "pure-shift" spectrum
Proton-decoupled $^1$H NMR
Resolving highly overlapped signals by pure-shift NMR

At 500 MHz, two signals are separated by 4 Hz, and the multiplet width is ~23 Hz for each. These two multiples would be separated in a coupled $^1$H above 3 GHz.
Pure shift-NMR:

- Collapsing multiplets to singlets

⇒ Homonuclear broadband decoupling:
  - Constant time experiment (Bax, Freeman, 1981)
  - J-resolved spectroscopy (Aue, Karhan, Ernst, 1976)
  - Time-reversal experiments (Sørensen, Griesinger, Ernst 1985)
  - Diagonal peaks of anti z-COSY spectra (Pell, Edden, Keeler, 2007)
  - BIRD decoupling (Garbow Weitekamp, Pines, 1982)
  - Slice selective decoupling (Zangger, Sterk, 1997)

- Trading sensitivity for resolution
- Often additional processing necessary
Spatially-selective excitation

- only 1 frequency excited in each slice, but the whole spectrum is excited in the NMR tube
  homonuclear system becomes pseudo heteronuclear

- sensitivity is reduced compared to regular spectra
Slice-selective decoupling

Each signal is detected in a single slice

in this slice it is selectively decoupled from all other signals
Slice selective decoupling

Evolution of scalar coupling is much slower than chemical shift evolution
not significant up to ~10-20 ms

Several data chunks need to be recorded sequentially (pseudo decoupling dimension)

Special data processing necessary

Constructing the decoupled FID from several FID data chunks

Pseudo-2D data acquisition

Slice-selectively decoupled spectrum of sucrose
**Instant homonuclear decoupling**

- Decoupling during acquisition
  - No additional processing; up to 50 times faster

Example: Azithromycin
Pure-shift for reaction mixtures

2,4,6-triisopropylbenzensulfonyl chloride (T)
1-propanol (P)
2-propanol (I)
2-pentanone (N)
diethylsulfone (S)
ethanol (E)
Parameters for real-time slice-selective pure-shift

should be ~15-25 ms; suggested: 25 ms
Parameters for real-time slice-selective pure-shift

- Use longer pulses when coupled signals are close in the spectrum; suggested: 20 ms

- 2 % corresponds to ~ 4300 Hz; use lower values for smaller spectral width to increase sensitivity; suggested: 2 %
Decoupling sidebands can be suppressed by variation of $aq/n$ between scans.
Suppression of data chunking artifacts

Real-time slice-selectively decoupled spectrum...

...with artifact suppression
Real-time pure-shift spectra of strychnine

Real-time pure-shift

artifact suppressed

regular 1D $^1$H
Extension to 2D experiments

Instant decoupled TOCSY

Decoupling of the direct or indirect dimension possible
Example: Azithromycin (TOCSY)

Overlay of regular and decoupled TOCSY

Regular TOCSY

Instant-decoupled

Overlay of regular and decoupled TOCSY
Example: CM15 peptide
2D TOCSY of CM15 peptide
Broadband proton-decoupling for intrinsically-disordered proteins

**CT-instant decoupled HSQC**

Decoupling both dimensions of HSQC spectra at once
Double-decoupled HSQC of alpha-synuclein (140 aa)

Pure-shift DOSY spectra

2D DOSY with slice-selective decoupling

...with real-time decoupling
Real-time J down-scaling

Acquisition of a completely coupled 1D spectrum, but uniform down-scaling of scalar coupling comparable to off-resonance $^1$H decoupling of $^{13}$C spectra

Scalar coupling is scaled down by $\lambda = (d - \tau) / d$
Continuous scaling of $J$ in real time

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