Extensions to 3D
and
Improving Efficiency with Pulsed Field Gradients

BCMB/CHEM 8190
2D NMR spectra can get very crowded

2D NOESY of Staph- Nuclease 156 AA

Solution: go to 3D
3D experiments used for sequential resonance assignments: Can detect Cα in 3D by INEPT transfer from $^{13}$C to $^{15}$N then from $^{15}$N to $^1$H. Sequential connections from: HNCA plus HN(CO)CA

(HB)CBCA(CO)NH

HN(CO)CA

HNCACB
Strategy for Constructing 3D Experiments: Combine various 2D Experiments

Cavanagh et al 1996
3D TOCSY-HSQC

Cavanagh et al 1996
TOCSY-HSQC for $^{15}$N-Labeled Ubiquitin

Cavanagh et al 1996
Pulsed Field Gradients:
More Efficiency in Multidimensional Spectra

Pulsed Field Gradients – How they Work

Magnetization vectors precess at different rates depending on \( G(z) \) and \( \gamma \) for each volume element. They dephase - net \( Mx, My = 0 \). Reversal of gradient refocuses magnetization.
Effects of Gradients can be Refocussed

Application: Water Suppression

Resonance not affected by 90 refocuses

Resonance affected by 90 dephases (H$_2$O)
1D $^1$H Water-Suppressed Spectrum

$Pf$-Rubredoxin in $^1$H$_2$O
Translational Diffusion Constants for Macromolecules

- Determine aggregate size
- Determine protein-protein interactions
- Screen for bound ligands

\[
<(X_1 - X_0)^2> = nDt \text{ where } D = kT/(6\pi\eta r)
\]

- Key: if molecule moves, field is different, magnetization doesn’t refocus
Stejskal and Tanner Pulse Sequence for Diffusion Measurement

\[ \ln\left[ \frac{S}{S_0} \right] = -\gamma^2 g^2 D \delta^2 (\Delta - \delta/3) \]
Diffusion Measurement Continued

- Measurements are limited by natural $T_2$
- Improved sequence uses z storage
  (Altieri, Hinton and Byrd, 1995)

\[ \ln(S/S_0) \]

![Diagram showing a graph with two lines: one for Large molecule and one for Small molecule. The x-axis is labeled $g^2$ and the y-axis is labeled $\ln(S/S_0)$]
Coherence Selection Using Pulse Field Gradients

- $H(r) = -\sum_k \gamma_k [B_0 + B_z(r)] I_{kz}$
  (in radians s$^{-1}$ and neglecting chemical shifts)
- Effects on product operators for a $z$ gradient:
  - $I_{kz} = -\gamma_k B_z(z) I_{kz} \tau I_{kz}$
  - $I_{kx} = (I_{k+} + I_{k-})/2$
  - $I_{k+} = -\gamma_k B_z(z) I_{kz} \tau \exp[i \gamma_k B_z(z) \tau] I_{k+}$
  - $I_{k-} = -\gamma_k B_z(z) I_{kz} \tau \exp[-i \gamma_k B_z(z) \tau] I_{k-}$
- For linear gradients $B_z(z) = G_z z$
- Observables are integrals over $z$ – zero for $I_{k+}, I_{k-}$
Gradient Selected HSQC

\[ I^+(t2) \propto \text{Integral}_z \{S^+(t1) \exp[i\gamma_N 2G_1z] \exp[-i\gamma_H G_2z]\} \]

\[ \propto \text{Integral}_z \{S^+(t1) \exp[i(\gamma_N 2G_1z - \gamma_H G_2z)]\} \]

\[ I^+(t2) \text{ finite only if } \gamma_N 2G_1 = \gamma_H G_2 \]

All 1Q proton transverse magnetization eliminated
No phase cycling needed to suppress unwanted signals