Introduction to 1D and 2D NMR Spectroscopy

(3) 2D Spectroscopy

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Is 1D NMR Good Enough?

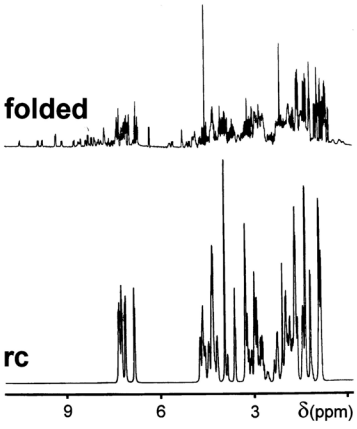

Figure 5. One-dimensional (1D) ^{1}H NMR spectra of the small protein bovine pancreatic trypsin inhibitor (BPTI, M ≈ 6000). Top: experimental spectrum of folded, active BPTI in a freshly prepared ^2H_2O-solution. Bottom: simulated spectrum for the unfolded, random coil form of the BPTI polypeptide chain.

1H NMR spectrum of BPTI (a small protein)
(Wüthrich, *J. Biomol. NMR,* **27:** 13-39, 2003)

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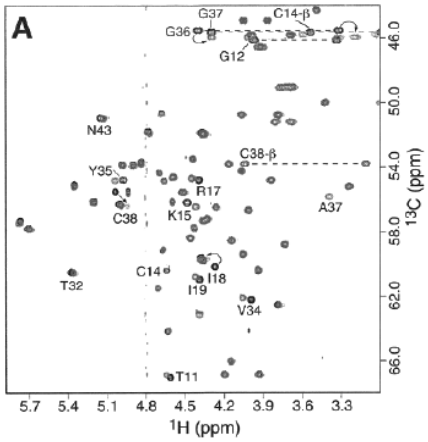
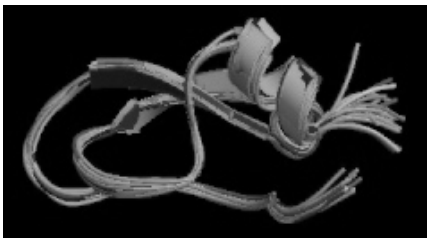
A 2D Spectrum



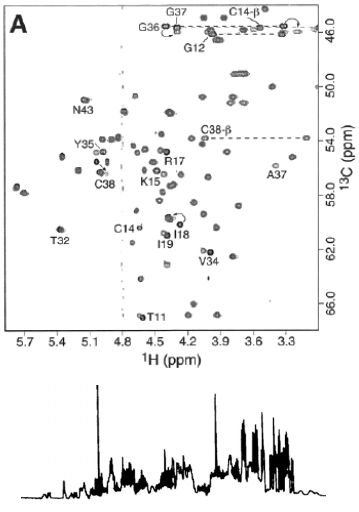
2D HSQC spectra of BPTI (black) and its G37A mutation (red)
(*Biochemistry, Vol. 41, No. 7,* 2237)



3D structure of BPTI

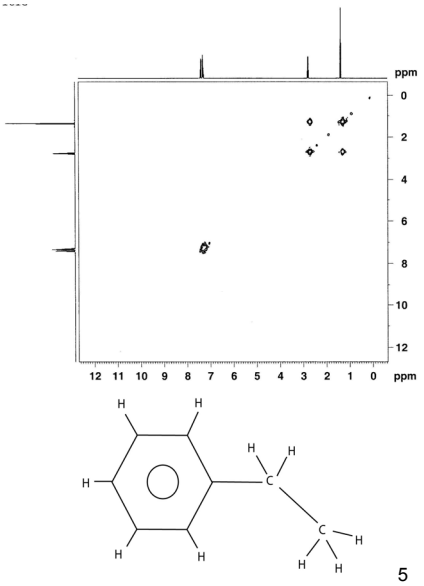
What can 2D NMR do?

- Separate overlapping peaks
- Tell how atoms are bonded
- Tell through-space proximity information between atoms



COSY (Correlation Spectroscopy)

- Detects connectivity between protons that have J coupling
- Both dimensions are ^1H
- Vertical is always 1st dim.
- 3 diagonal peaks
  - CH_3, CH_2, Aromatic
  - provide no new information
- Cross peaks at (CH_3, CH_2) and (CH_2, CH_3) indicate J-coupling (^3J)
  - Cross peak intensity is determined by population and coupling strength
- *Why are there no cross peaks at (Ar, CH_2) and (Ar, CH_3)?*



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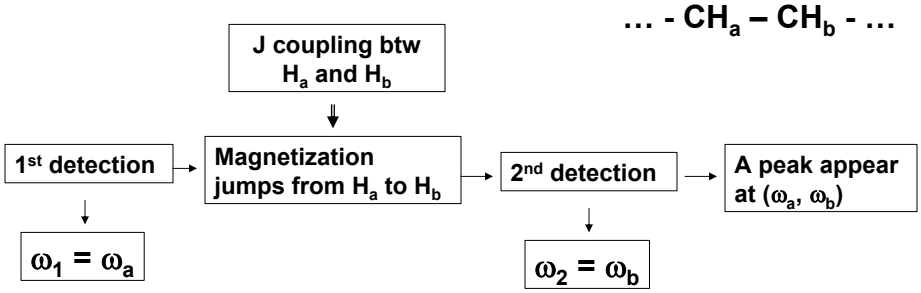
COSY (Correlation Spectroscopy)

- Principle of COSY: transfer of magnetization ("polarization transfer") between J-coupled protons
  - Part of magnetization jumps between sites, resulting in cross peaks
  - All 2D NMR experiments work via some kind of polarization transfer
  - *How about the rest of magnetization that stays in the same nuclei?*

… - CH_a – CH_b - …



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HMQC (Heteronuclear Multi-Quantum Correlation)

- Detects correlation between directly bonded ^{1}H and ^{13}C
  - ^{13}C in 1st dimension ("indirect dimension")
  - ^{1}H in 2nd dimension ("direct dimension")
  - Correlation is through $^{1}J_{13CH}$ coupling
- A 2D that could take less time than a 1D ^{13}C!
  - ^{1}H has higher γ than ^{13}C
    - γ (^{1}H) / γ (^{13}C) ~ 4
    - Signal intensity $\propto \gamma^3$
- Unprotonated carbons don't show up on spectrum
- Protons connecting to ^{12}C don't show up on spectrum

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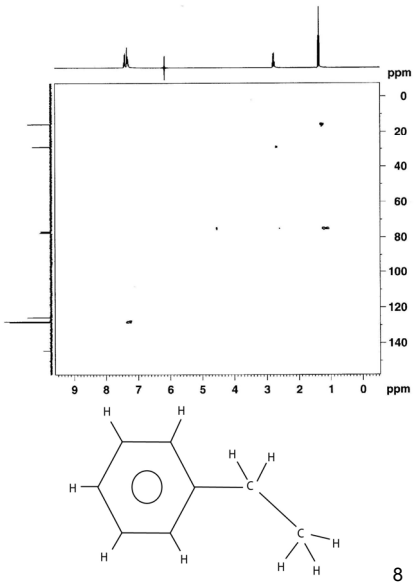
HMQC of Ethylbenzene

- A 2D spectrum taken in 10 minutes
- No diagonal peaks
  - Diagonal peaks could only exist when both dimensions are the same type of nuclei
- Shows direct C-H connectivity
- Non-protonated carbons don't have a peak on the spectrum
- Beware of artifacts
  - Along the "ridges" of big peaks
  - In the middle lines of spectrum window



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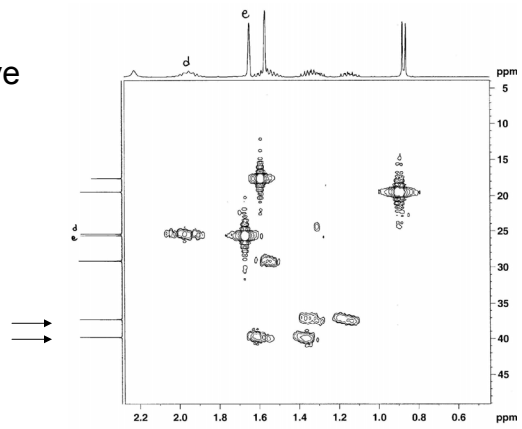
One Carbon Correlated With Two Protons

- Peaks at 37 and 40 ppm: each has two directly attached protons that have different chemical shift

… - CH_2 – CHR - …



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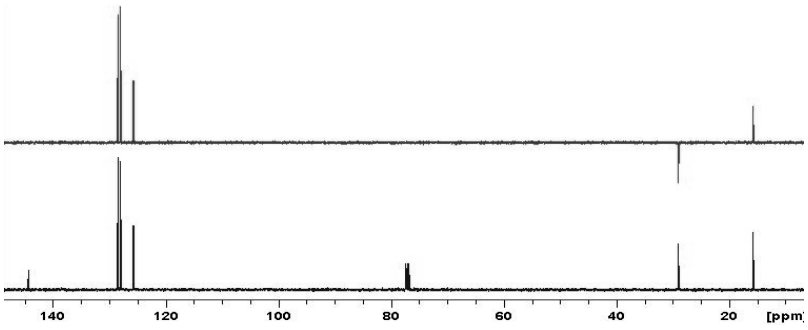
DEPT (Distortionless Enhancement by Polarization Transfer)

- Discern methyl, methylene, methine, and quaternary carbons
  - A 1D technique, with several variations. Most popular is DEPT-135
  - Mechanism: C-H J-coupling for odd- and even-numbered protons are different
- DEPT-135
  - CH and CH_3 peaks are positive; CH_2 peaks are negative
    - Due to different ^{13}C-^{1}H J-coupling
  - Carbons with no directly bonded protons do not have signals



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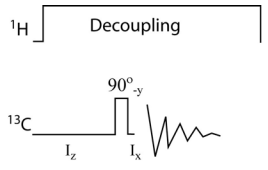
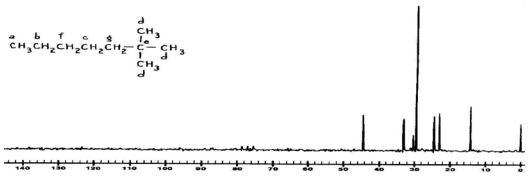
Decoupling

- In ^{13}C experiment, ^{13}C-^{1}H J-coupling is usually removed by a "decoupling" pulse sequence
  - Pulsing at ^{1}H during detection of ^{13}C signal
    - i.e. Set pulsing frequency to ^{1}H and detector frequency to ^{13}C
  - Decoupling resolves overcrowding due to large ^{1}J splitting
    - The large ^{1}J couplings usually don't provide useful information
  - Decoupling reduces ^{13}C multiplet to singlets, which improves S/N
- Exception: HMBC





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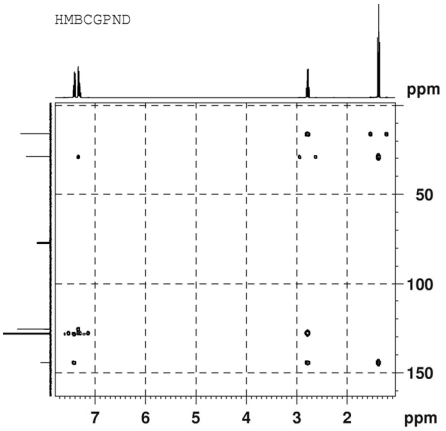
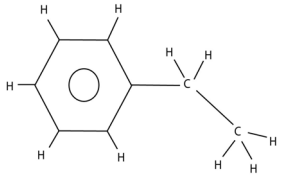
HMBC (Heteronuclear Multiple Bond Correlation)

- The pulse sequence is designed to suppress ^{1}J peaks and show $^{2}J_{C\text{-}H}$ and $^{3}J_{C\text{-}H}$ peaks
  - Complete suppression of ^{1}J peaks is difficult
  - So experiments are run without ^{1}H decoupling, so that ^{1}J peaks are easy to recognize (wide doublets)
- Similar to HMQC, protons bonded to ^{12}C don't have signals
- Quaternary carbons do have correlation peaks (and they are important!)
- Some ^{3}J peaks can be as strong as ^{2}J's





Why are there no peaks at (7.3,16) and (1.4,127)?

Absence of cross peaks can be important information too.

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2D NMR: Implementation

- 2D NMR signal is composed of a series of 1D signals ("slices"):
  - $f(t_1, t_2) = \{ f(t_0, t_2),$
    $f(t_0+\Delta t, t_2),$
    $f(t_0+2\Delta t, t_2),$
    …
    $f(t_0+N\Delta t, t_2) \}$
  - Actual detection is only conducted during t_2
  - *Works exactly like a CRT TV!*
- 2D Fourier transformation:
  - $f(t_1, t_2) \rightarrow F(\omega_1, \omega_2)$
  - *How would you improve the resolution of a TV?*
  - *How would you improve the resolution of a 2D spectrum?*

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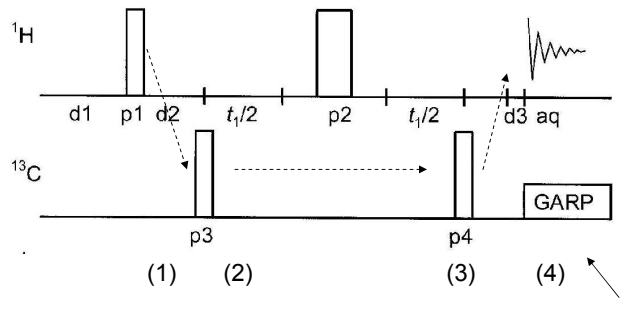
Pulse Sequence of HMQC

$t_1 = t_0,\ t_0+\Delta t,\ t_0+2\Delta t,\ t_0+3\Delta t, \ldots\ t_0+N\Delta t$



(1) Magnetization transfers from ^1H to ^{13}C
(2) The signal gets a prefactor with ^{13}C chemical shift info
(3) Magnetization transfers from ^{13}C back to ^1H
(4) ^1H signal (with a prefactor containing ^{13}C info) is detected

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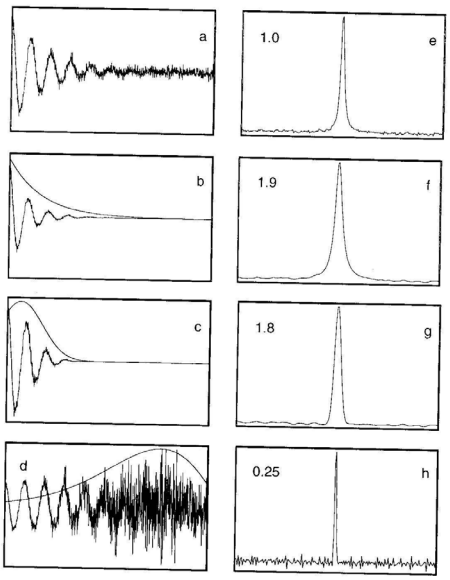
Window Function (Apodization)

- Window functions: weighting of time domain signal
  - FT: faster decay gives broader line
- Balancing between S/N and resolution
  - Beginning-favored weighting enhances S/N
  - End-favored weighting enhances resolution
- Proper apodization is useful for both 1D and 2D



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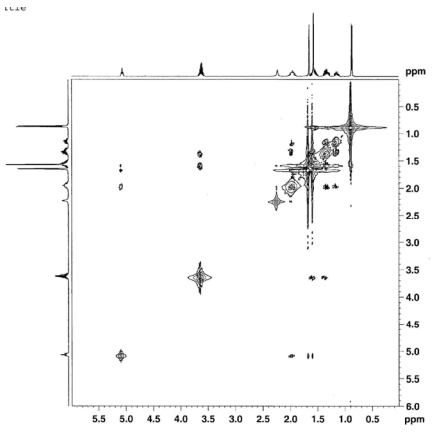
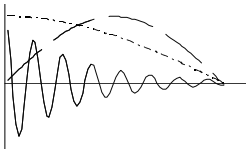
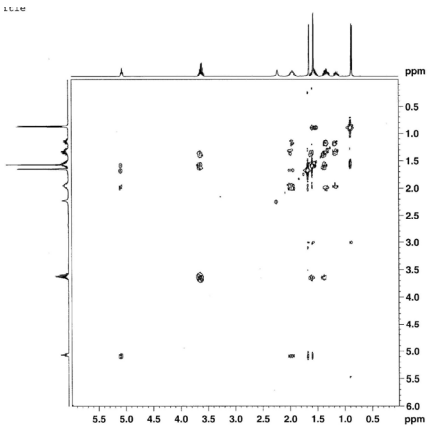


- Cosine window function
  - emphasizes beginning of signal
  - *edp*: SINE with SSB=2





- Sine window function
  - emphasizes middle of signal
  - *edp*: SINE with SSB=1

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Summary

- COSY: cross peaks due to J-coupled ^{1}H pairs
  - Diagonal peaks are trivial
  - Cross peaks are mostly due to ^{2}J or ^{3}J, sometimes due to ^{4}J
- HMQC: cross peaks due to directly bonded ^{1}H-^{13}C pairs
  - Carbon with equivalent directly-bonded protons shows one cross peak
  - Carbon with two non-equivalent directly-bonded protons shows two cross peaks
- DEPT: distinguish carbon types by number of protons bonded
- 2D spectrum is composed of a series of 1D spectra
  - 1^{st} detection is done indirectly
  - Cross peak is due to polarization transfer between the two detections
- NOESY, HMBC
- Experimental issues
  - How to enhance resolution by window functions

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