Introduction to 1D and 2D NMR Spectroscopy

(1) Basics

Lecturer: Weiguo Hu

A328 Conte (7-1428)

weiguoh@polysci.umass.edu

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Content At a Glance – *Introduction to 1D and 2D NMR Spectroscopy*

- Experimentation
  - What's happening in the spectrometer when you type commands
  - Lock and shim
  - 1D NMR
  - 2D NMR
  - Some spectrometer commands (All Bruker commands are *italic)*
- Interpretation
  - Mechanism of chemical shift and J-coupling
  - Quantitative aspects
  - Relaxation and dynamics
- Information – NMR topics beyond the scope this class
  - NMR is rigorously based on quantum mechanics
  - Thousands of NMR techniques available for your use
  - Glimpses into some applications

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Logistics

- 7 classes in 4 weeks
- Homeworks – 2 sets
  - 1st set is Q&A of basic concepts
  - 2nd set is an unknown compound assignment
  - Please email your answers to me by deadlines
  - You will be given chances for revision if you make major mistakes
- Q and A/hands-on sessions – 10/14 and 10/28 (Wednesdays) 9-10am in LGRT Rm 29 (400MHz NMR room)
  - *<u>If you have never run a NMR experiment before, it's important that you come to the first Q&A/hands-on session</u>*
- Hands-on operation instructions: udrive.oit.umass.edu/weiguoh/nmrhandouts
  - "NMR New User Handout"
  - "2D NMR Handout"
- Exam – ½ hour on 10/29

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Concepts Review

- Atom structure
  - We are probing the nuclei to obtain information about the molecular structure
  - Nuclear spins ➔ nuclei are little magnets
- Magnetic field
  - What happens when you put two magnets close to each other?
- A pulse is a short burst of strong electromagnetic wave
  - Radio frequency
- Fourier transformation
  - Simply put, it is a mathematical process to extract frequency components out of a wave
  - Fourier transformation works for piano as well as NMR!
  - A graph showing the frequency components is called a <u>spectrum</u>
  - The longer the signal lasts, the sharper are the peaks on the spectrum

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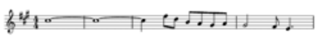
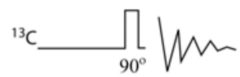
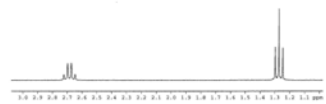
NMR – Nuclear Magnetic Resonance

Piano	**NMR**
Strings	Nuclei in sample
Tension on strings	Magnetic field
Strike the key!	Radio frequency (RF) pulse(s)
⇒ Music	⇒ Signal F (t)





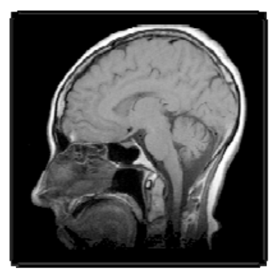
– Fourier transform ⇒ f (ω)

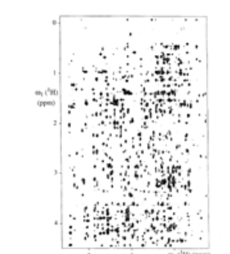


–*Also compare NMR with Fluorescence*

NMR Timeline

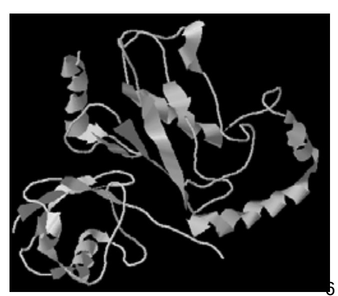
- Nobel Prizes:
  - 1952 Physics: Discovery of NMR phenomenon
  - 1991 Chemistry: 2D NMR
  - 2002 Chemistry: Protein 3D structure
  - 2003 Physiology: MRI
    - In 2003, there were ~ 10,000 MRI units worldwide, and approximately 75 million MRI scans per year performed.





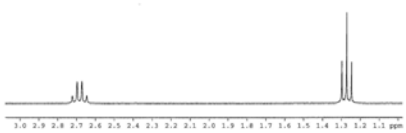
Wüthrich, *J. Biomol. NMR,* **27:** 13-39, 2003

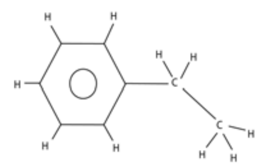
2FUH



How is NMR useful for chemists and physicists?

- Signal frequency of nucleus is a sensitive probe of local chemical structure
  - Chemical shift
    - "Chemical shift": shift of signal frequency due to chemical structure
  - J-coupling between nuclear spins ⇒ connectivity information
- Peaks are very sharp
  - e.g. ^{13}C: peaks are typically 0.005 ppm wide in a 200-ppm window
- Can be quantitative
- Gives information about molecular motion (dynamics)
  - Sensitive to motional rate of $10^0 – 10^9\ s^{-1}$
- Non-invasive test – due to penetrating property of magnetic field
  - Important for MRI





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Applications – A Glimpse

- Solution NMR
  - Chemical structure
  - Dynamics
  - Polymer tacticity
  - Protein 3D structure
  - Host-ligand interaction
  - Imaging
  - Diffusion
- Solid-state NMR
  - Chemical structure
  - Dynamics
  - Miscibility
  - Catalysis mechanism
  - Petroleum exploration
  - Crystallinity
  - Crystal structure

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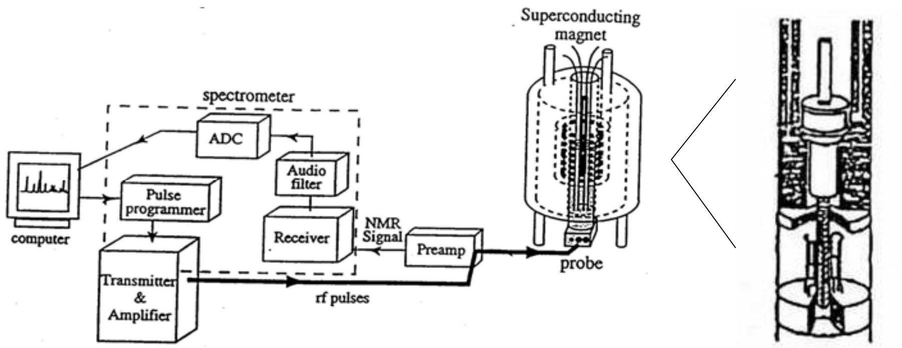
A NMR Spectrometer

- Magnet
  - Superconducting coil
  - Helium dewar
  - Nitrogen dewar
- Console
  - Pulse generation
  - Signal amplification and A/D conversion
- Probe
  - RF coil: “shines” pulses on sample; receives signal



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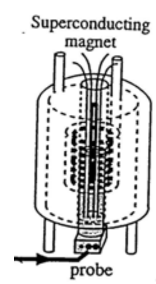
Important Safety Issues

- Very strong magnetic field!
  - ca. 100,000 times stronger than earth magnetic field
  - Never get iron object close to magnet
    - Tools, gas cylinders, safety shoes
  - Keep credit cards, cell phone, watches, etc. away
  - People with pacemakers should stay away
- Never insert sample without ejecting air



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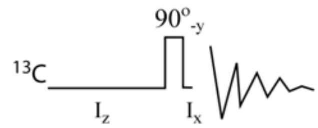
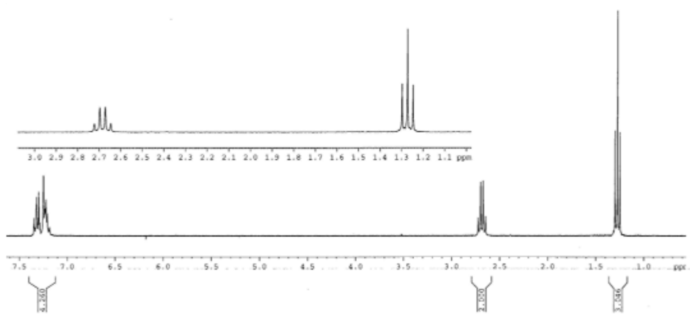
Getting a NMR spectrum

zg:

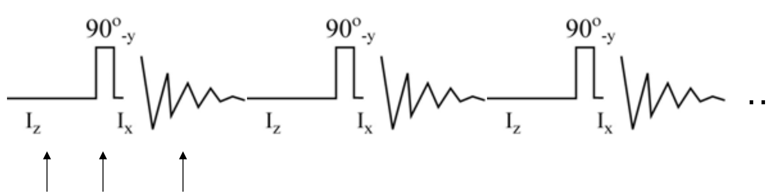


Signal f (t): *aka* FID – *Free Induction Decay*

↓ ***efp***



A Complete Multi-Scan Experiment



Recycle Delay (*d1*)

pulsing

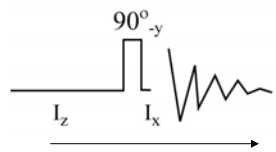
Signal acquisition (*aq*)

S/N ∝ sqrt (ns)

Recycle delay (*d1*)	1-100 s
Pulse(s)	~ 10 μs
Detection (*aq*)	0.5-5 s

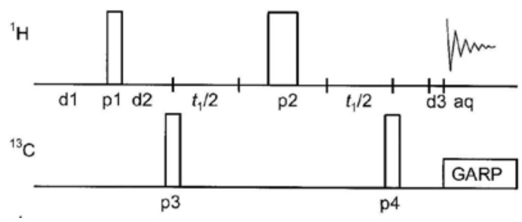
Pulse Sequence: Examples

Single pulse ^1H:



HMQC:

(a 2D ^1H-^{13}C expt)



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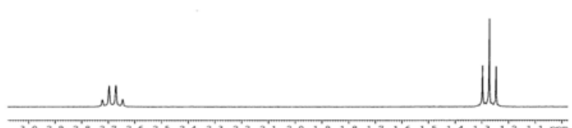
What's on a NMR Spectrum

- Position of peaks: resonance frequency
- Integration (area) of peaks

- **Frequency** often expressed in "ppm" for convenience
  - For DPX300, spectrometer base frequency ~ 300.1323970 MHz
  - ^1H in $CHCl_3$ frequency is ~ 300.1318534 MHz
  - The frequency difference between peaks on ^1H spectrum is ~ 10-1000 Hz
    => *Define chemical shift in ppm =* $(\omega - \omega_0)/\omega_0 * 10^6$
  - Reference: TMS (tetramethylsilane) = 0 ppm
  - For 300MHz instrument, 1 ppm on ^1H = 300 Hz
- **Integration** reflects population of structure
  - However, obtaining quantitative NMR spectra needs some NMR knowledge



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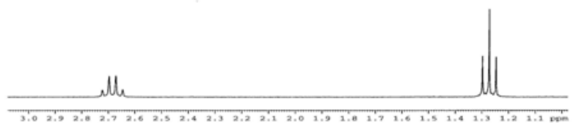
An Important Equation

$$\omega = \gamma B$$ *(Larmor Equation)*

- γ (gyromagnetic ratio) is a property of the nucleus
  - All nuclei of the same isotope have the same γ, regardless of its chemical environment
    - *Then why are there peaks of different frequency on a ^{1}H spectrum?*
  - γ (^{13}C) ~ ¼ of γ (^{1}H)
    - On a 400MHz spectrometer, frequency of ^{13}C ~ 100MHz
      - A “600 NMR” means that its ^{1}H frequency is 600MHz
      - *What would be the ^{13}C frequency on a 600MHz instrument?*
- B is magnetic field strength



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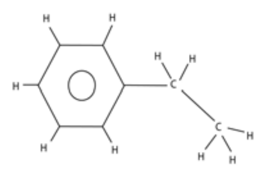
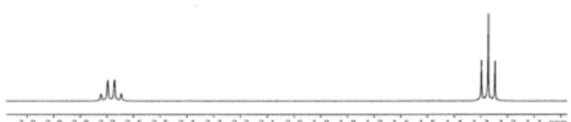
Magnets in Your Sample

- A given nucleus senses magnetic field from many sources:
  - From the “big magnet” and shimming coils (B_0)
  - From sample tube
  - From electrons in the neighborhood – “shielding effect”
    - This is the physical mechanism of chemical shift
  - From neighboring nuclei – “J-coupling”



$$\omega = \gamma B$$



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How To Obtain A High-Resolution Spectrum - Locking

- High resolution of peaks requires high homogeneity of magnetic field
  - $\omega = \gamma B_0$
  - Peak width of 0.3 Hz requires B be homogeneous and constant to the order of $\underline{10^{-9}}$
- In reality, magnetic field is not constant
  - The big magnet is always slowly losing field
  - Typically, loss rate is 1-10 Hz/hour
- We need a mechanism to keep the field constant

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How To Obtain A High-Resolution Spectrum - Locking

- Locking: track ^2H signal to compensate for the drift
  - *This is one reason why we need deuterated solvent to do NMR!*
    - *<u>What's another reason?</u>*
  - To lock, spectrometer looks for ^2H signal using a pulse along with a sweeping magnetic field (*lockdisp*)
  - Once find the ^2H signal, spectrometer locks the signal by adjusting the field
    - Similar to the anti-shaking mechanism in camcorders
  - Level of lock signal = ^2H signal peak height
  - The stronger the ^2H signal, the easier to lock
  - "*lock* [solvent name]" also calibrates chemical shift

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How To Obtain A High-Resolution Spectrum - Shimming

- Another set of small coils - "shim coils" - compensates inhomogeneity of superconducting magnet
  - Each coil generates a unique spatial magnetic field gradient
  - Lower orders are more important than higher orders
    - z > z2 > z3 > z4
- Shimming uses ^{2}H lock signal for optimization
  - *lockdisp*: lock level is ^{2}H signal height
  - Taller peak means sharper peak (why?)
- Manual shimming: visual iteration of curve fitting
- Gradient shimming (*topshim*): directly detect coefficients
- *If the solvent peak is sharp but all other peaks are broad, is this indicative of a shimming problem?*

$$B(x, y, z) = a_0 + a_1 z + a_2 z^2 + a_3 z^3 + ... + b_1 x + c_1 y + ...$$

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Active Nuclei

- Spin number of nuclei:
  - ^{1}H: ½
  - ^{2}H: 1
  - ^{13}C: ½
  - ^{12}C and ^{16}O: 0 $\Rightarrow$ No NMR signal!
  - ^{17}O: 5/2
- Spin > ½: Quadrupolar effect
- Natural abundance
  - ^{13}C = 1.1%; ^{29}Si = ~5%; ^{17}O = 0.04%; ^{1}H, ^{19}F and ^{31}P ~ 100%
- Isotopically enriched (labeled) materials are often used for in-depth ^{13}C, ^{15}N, ^{2}H studies

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Resonance Frequency

- γ is a property of nuclei type
- At magnetic field of 7.0 tesla:
  - ^{1}H: 300 MHz
    - 1 ppm = 300 Hz
  - ^{13}C: ~ 75 MHz
    - 1 ppm = 75 Hz
  - ^{29}Si: ~ 60 MHz
  - …
  - *Why don't we see peaks from deuterium or ^{13}C on proton spectra?*

$$\omega = \gamma B_0$$

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Summary of Important Concepts

- Basic components of NMR experiment
  - Pulse sequence
  - Recycle delay
  - Detection
- Basic components of NMR spectrometer
- An important equation: $\omega = \gamma B_0$
- Locking: feedback mechanism to locate the drifting magnetic field
- Shimming: fitting of a 3-dimensional function
- Active nuclei and (1) spin number; (2) natural abundance; (3) resonance frequency

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