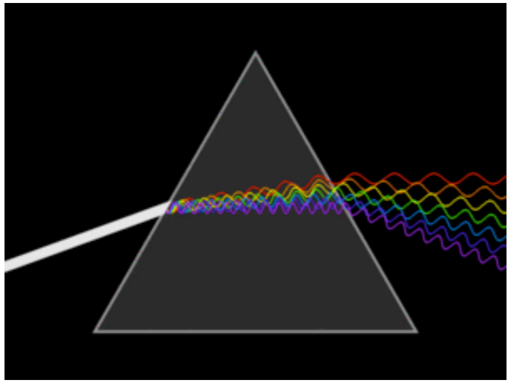
Spectroscopy

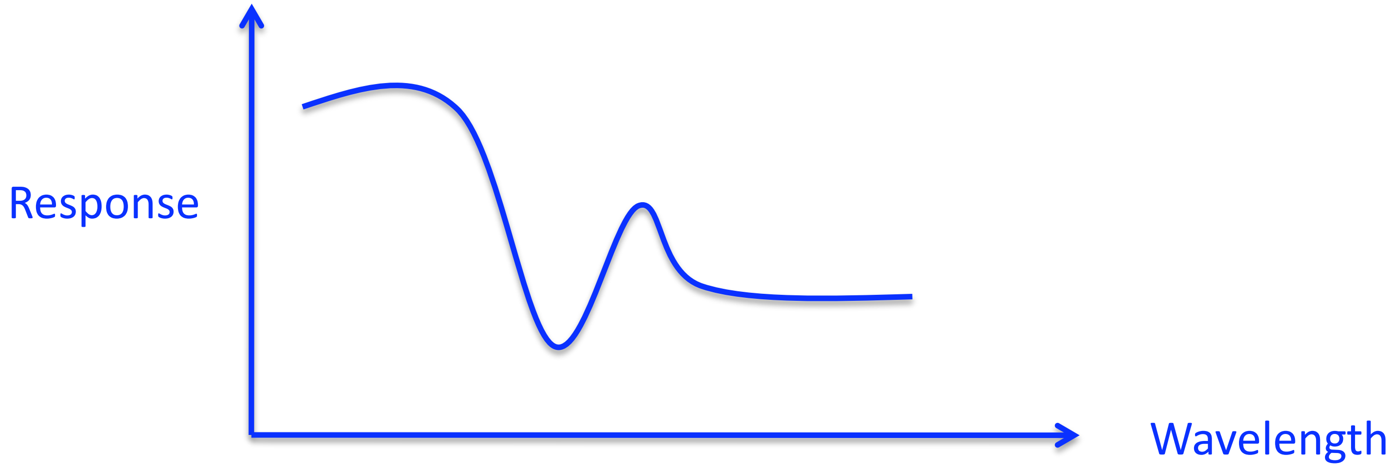


Spectroscopy

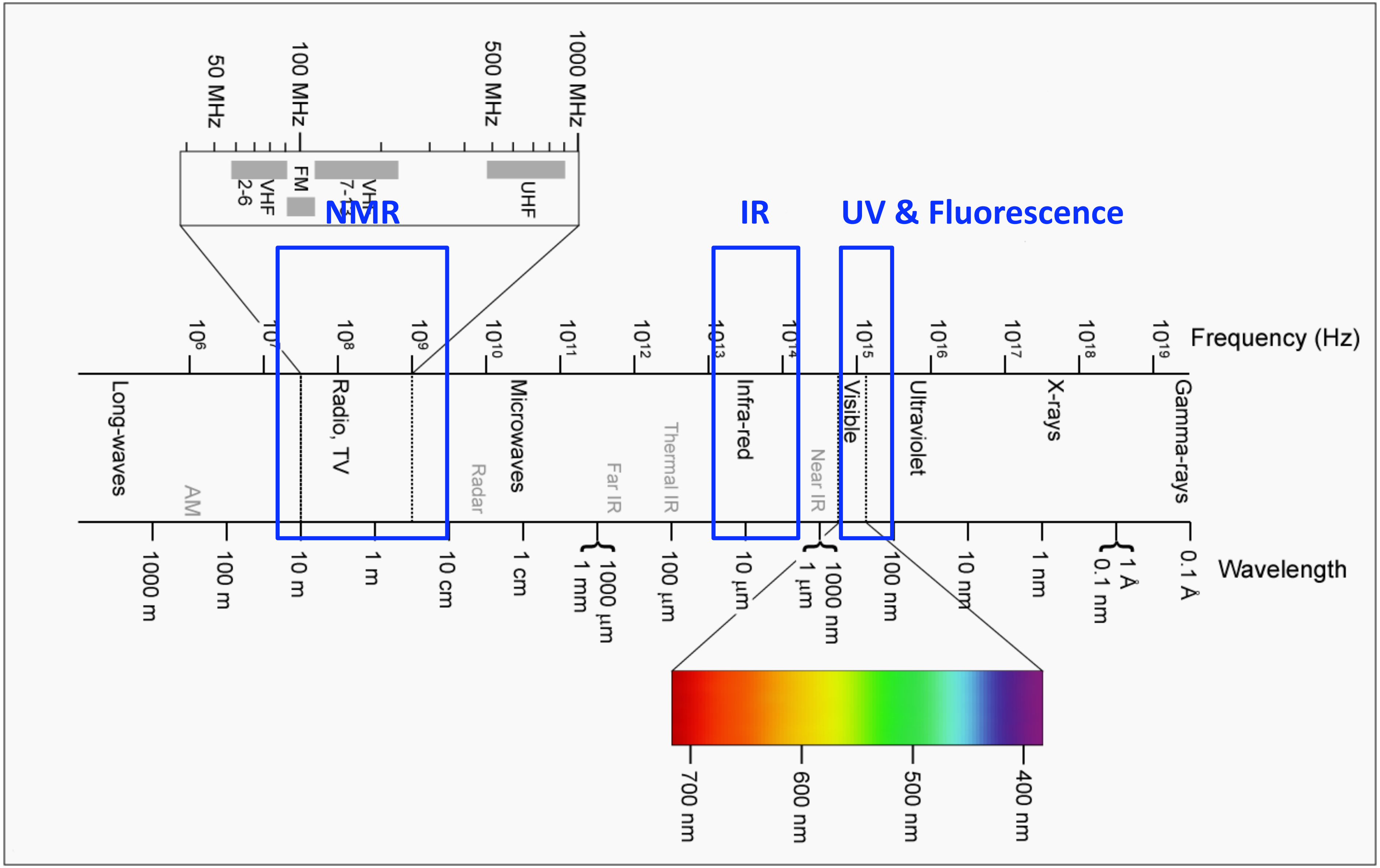
Originally the study of the interaction between radiation and matter as a function of wavelength (λ)

Later the concept was expanded to comprise any measurement of a quantity as a function of either wavelength or frequency

A plot of the response as a function of wavelength (or frequency) is referred to as a spectrum:



The electromagnetic spectrum



Infrared (IR) spectroscopy

Most compounds having **covalent bonds** absorb infrared radiation

Only bonds with **changing dipole** moments absorb IR-radiation

Absorption is **quantized** = a molecule absorbs only selected frequencies

Energy corresponds to **vibrational frequencies** of the covalent bonds in the molecule

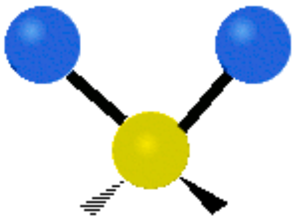
Absorption increases the **amplitude** of the vibrational motions

Each vibrational excited state has **rotational sublevels**, leading to broad peaks (bands)

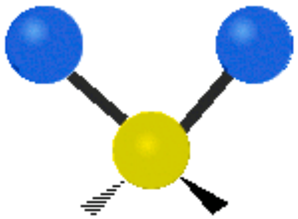
The most informative peaks in an IR-spectra arise from **fundamental absorptions**

Fundamental absorptions

STRETCHING VIBRATIONS:



Symmetric
stretching

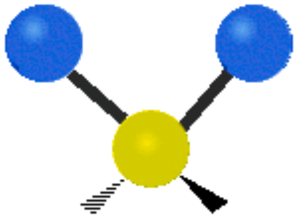


Asymmetric
stretching

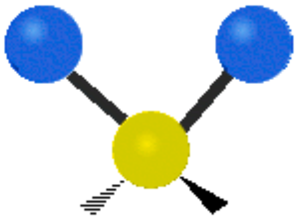
http://en.wikipedia.org/wiki/Infrared_spectroscopy

Fundamental absorptions

IN-PLANE BENDING VIBRATIONS:



Scissoring

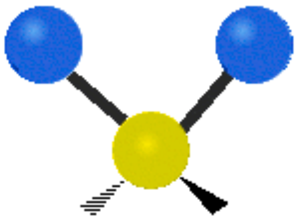


Rocking

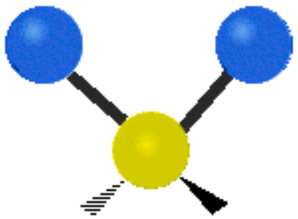
http://en.wikipedia.org/wiki/Infrared_spectroscopy

Fundamental absorptions

OUT-OF-PLANE BENDING VIBRATIONS:



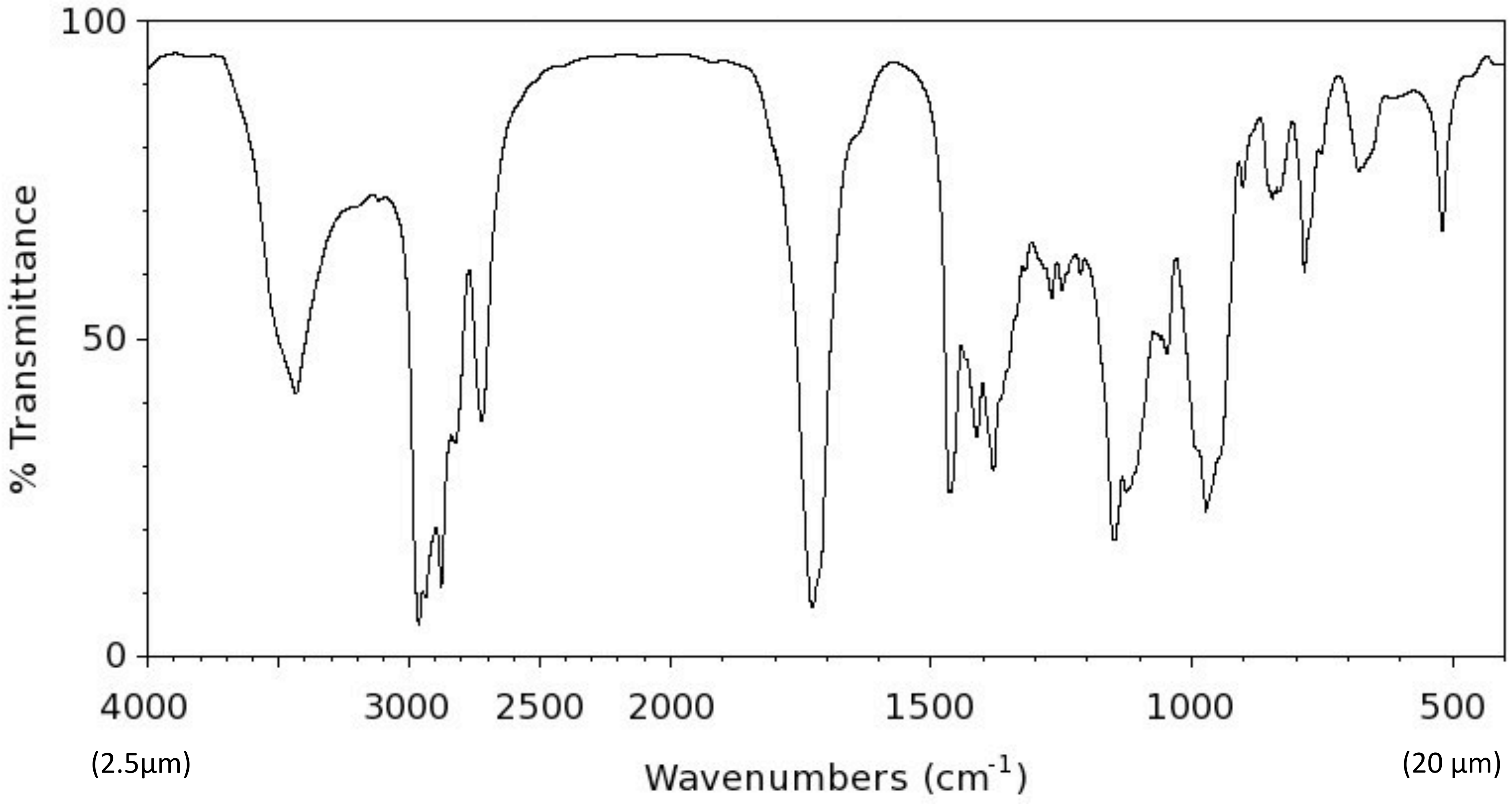
Wagging



Twisting

http://en.wikipedia.org/wiki/Infrared_spectroscopy

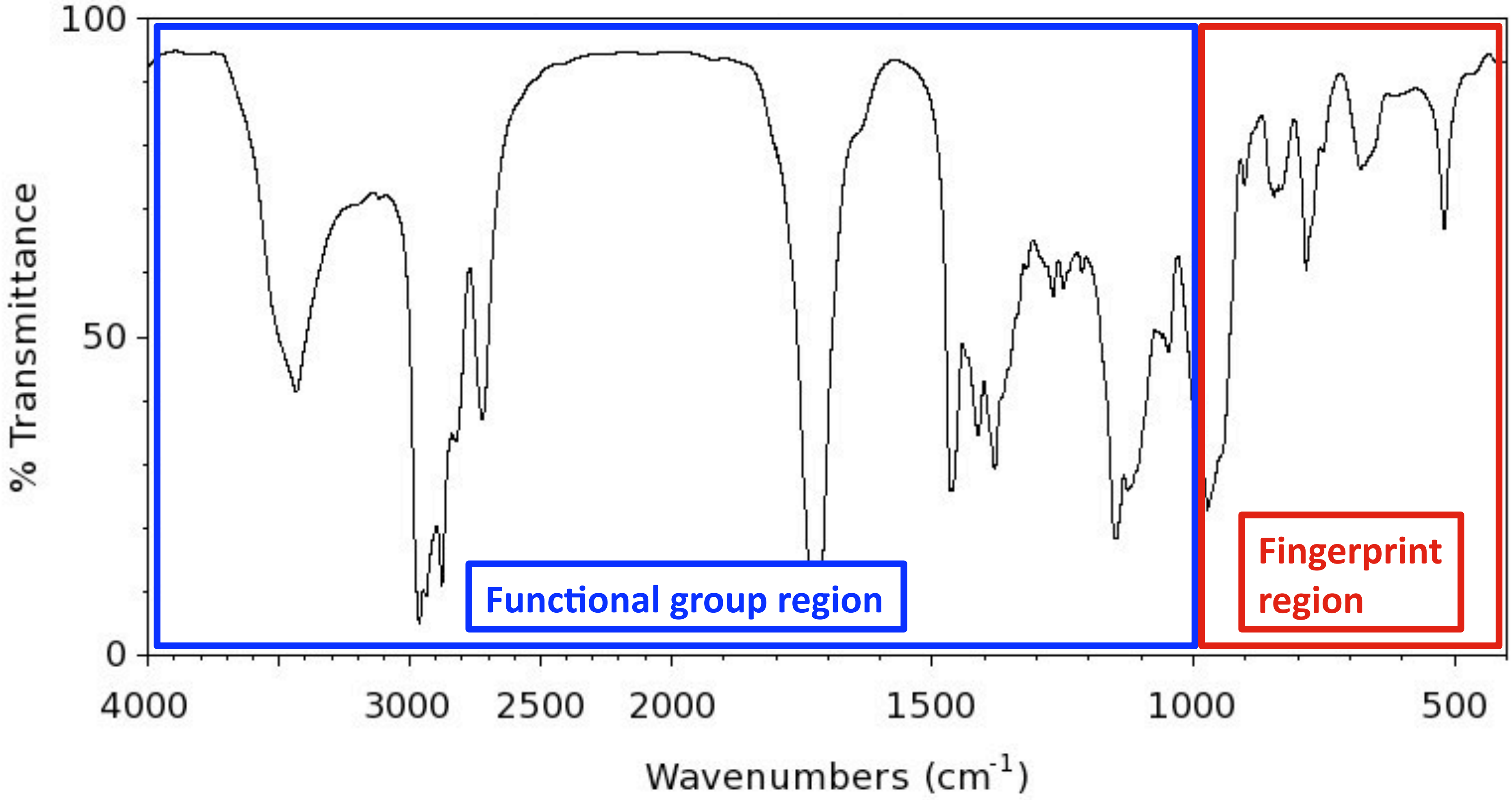
The infrared spectrum



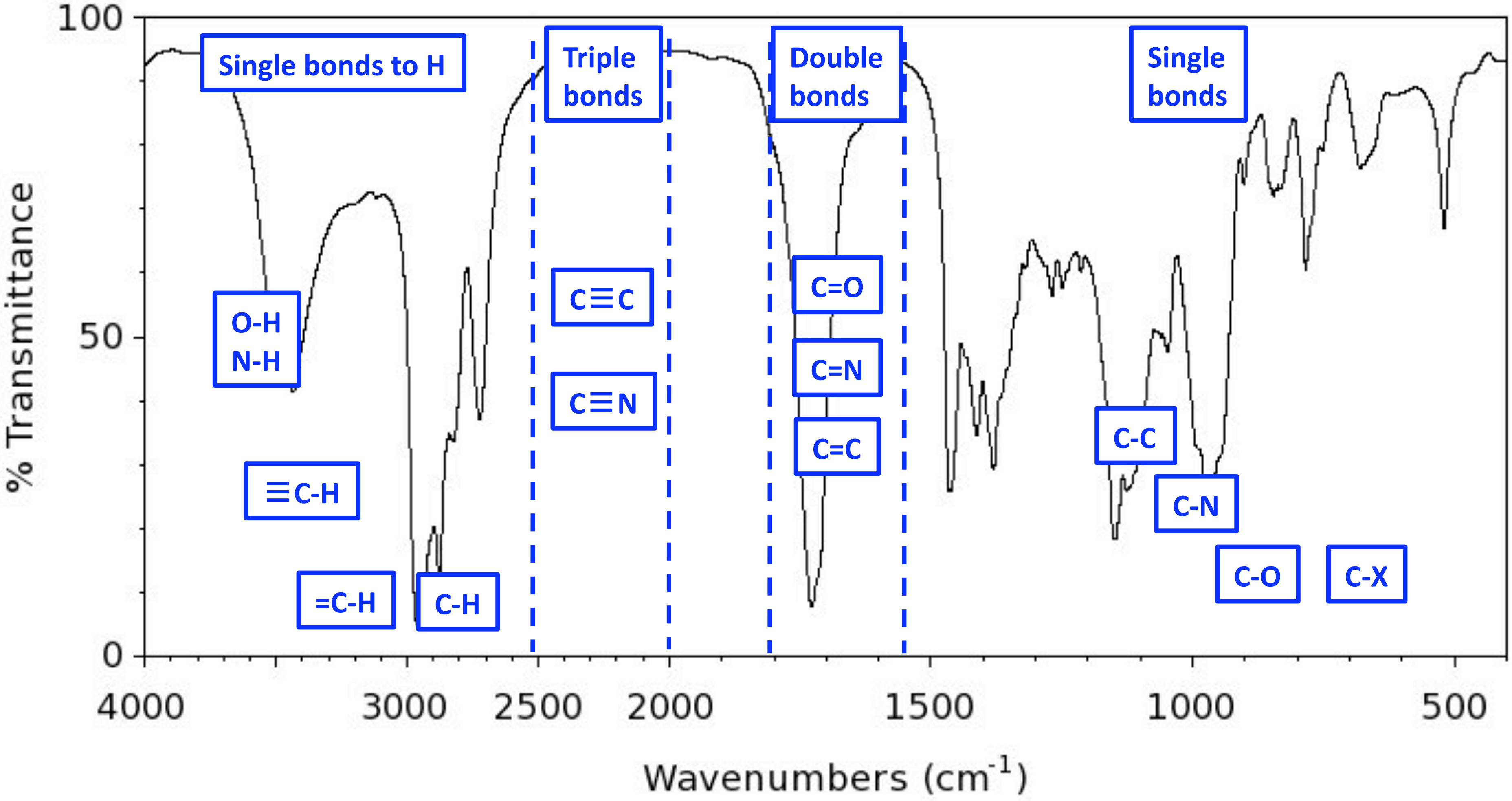
Higher frequency
Shorter wavelength
Higher energy

Lower frequency
Longer wavelength
Lower energy

Regions of the infrared spectrum



Regions of the infrared spectrum



Analysing an IR-spectra

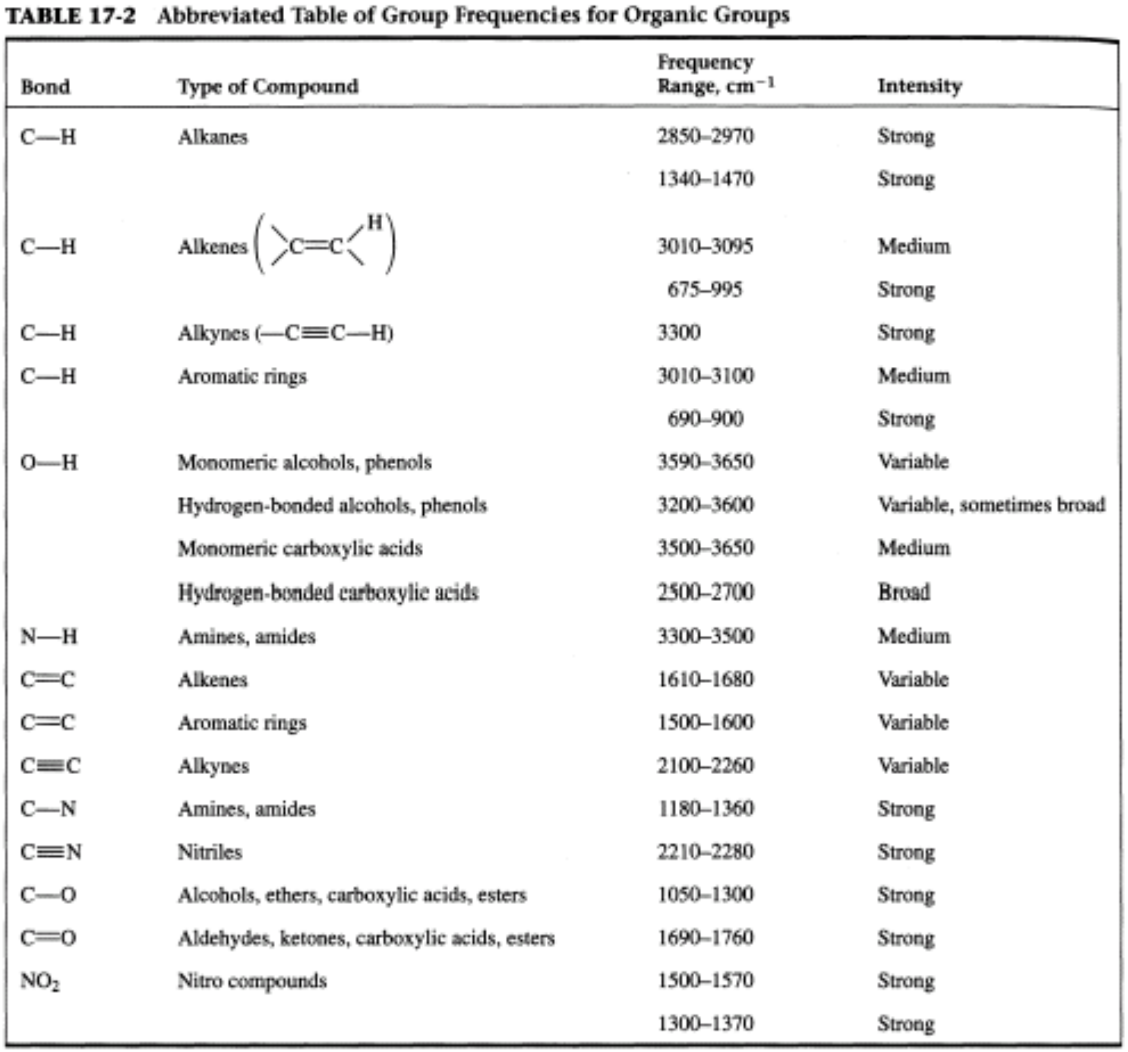


TABLE 17-2 Abbreviated Table of Group Frequencies for Organic Groups

Bond	Type of Compound	Frequency Range, cm^{-1}	Intensity
C—H	Alkanes	2850–2970	Strong
		1340–1470	Strong
C—H	Alkenes $\left(\gt C{=}C\lt^{H}\right)$	3010–3095	Medium
		675–995	Strong
C—H	Alkynes (—C≡C—H)	3300	Strong
C—H	Aromatic rings	3010–3100	Medium
		690–900	Strong
O—H	Monomeric alcohols, phenols	3590–3650	Variable
	Hydrogen-bonded alcohols, phenols	3200–3600	Variable, sometimes broad
	Monomeric carboxylic acids	3500–3650	Medium
	Hydrogen-bonded carboxylic acids	2500–2700	Broad
N—H	Amines, amides	3300–3500	Medium
C═C	Alkenes	1610–1680	Variable
C═C	Aromatic rings	1500–1600	Variable
C≡C	Alkynes	2100–2260	Variable
C—N	Amines, amides	1180–1360	Strong
C≡N	Nitriles	2210–2280	Strong
C—O	Alcohols, ethers, carboxylic acids, esters	1050–1300	Strong
C═O	Aldehydes, ketones, carboxylic acids, esters	1690–1760	Strong
NO_2	Nitro compounds	1500–1570	Strong
		1300–1370	Strong

Correlation tables/charts

Characteristic absorptions for functional groups

Frequency, intensity, shape

Analysing an IR-spectra

Do not over-interpret!
Do not try to analyse every single absorption band.

Look for C=O, O-H, N-H, C-O, C=C, C≡C, C≡N and NO_2.

If C=O is present determine if it is an acid, amide, ester, anhydride, aldehyde or ketone

If C=O is absent look for alcohol (O-H + C-O), amine (N-H) or ether (C-O)

Look for alkene and/or aromatic ring

Look for triple bonds (alkyne, nitrile)

Nitro group gives two strong absorptions at 1600-1530 and 1390-1300

What can we use IR spectroscopy for?

Structure determination/confirmation (**functional groups present**)

Characterisation of materials/thin films

Forensic science (dust, fibres, paint…)

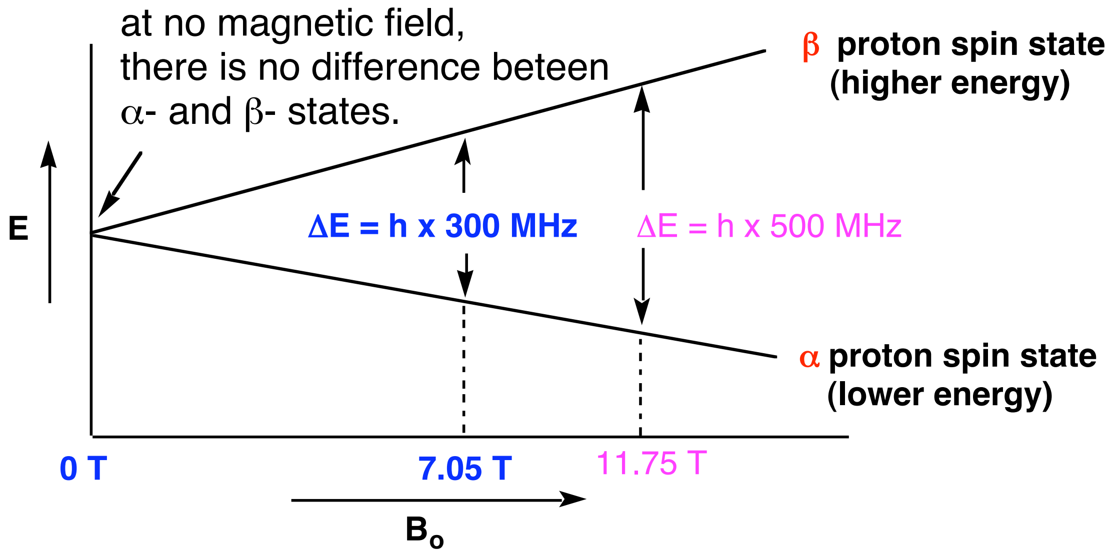
Used a lot in polymer science: process monitoring, quality control, degradation

Nuclear magnetic resonance, NMR

NMR – basic concepts

Paramagnetic nuclei (odd number of protons or neutrons, for example **^{1}H** and **^{13}C**) will behave as magnets when placed in a magnetic field. They can align with or against the magnetic field:

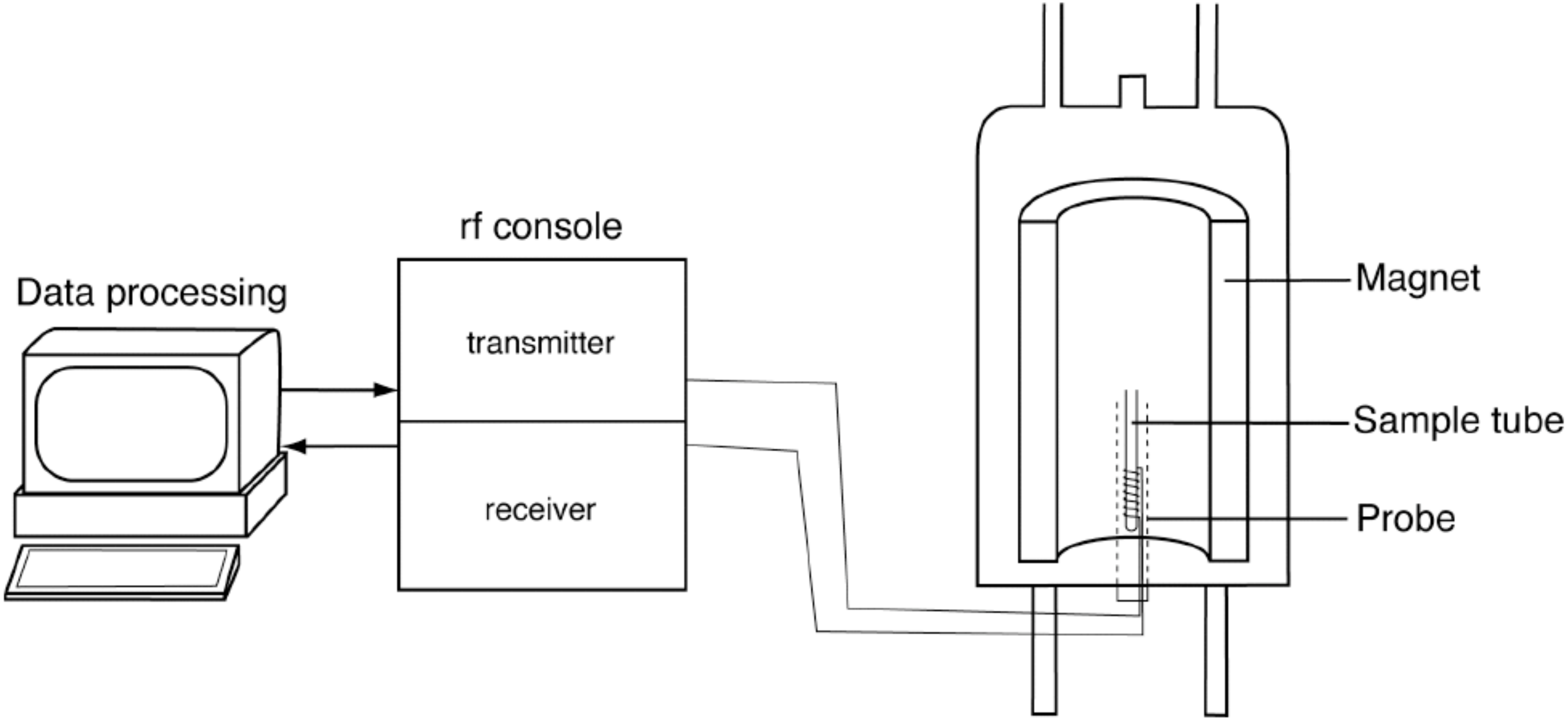
Graphical relationship between magnetic field (B_o) and frequency (ν) for ^{1}H NMR absorptions



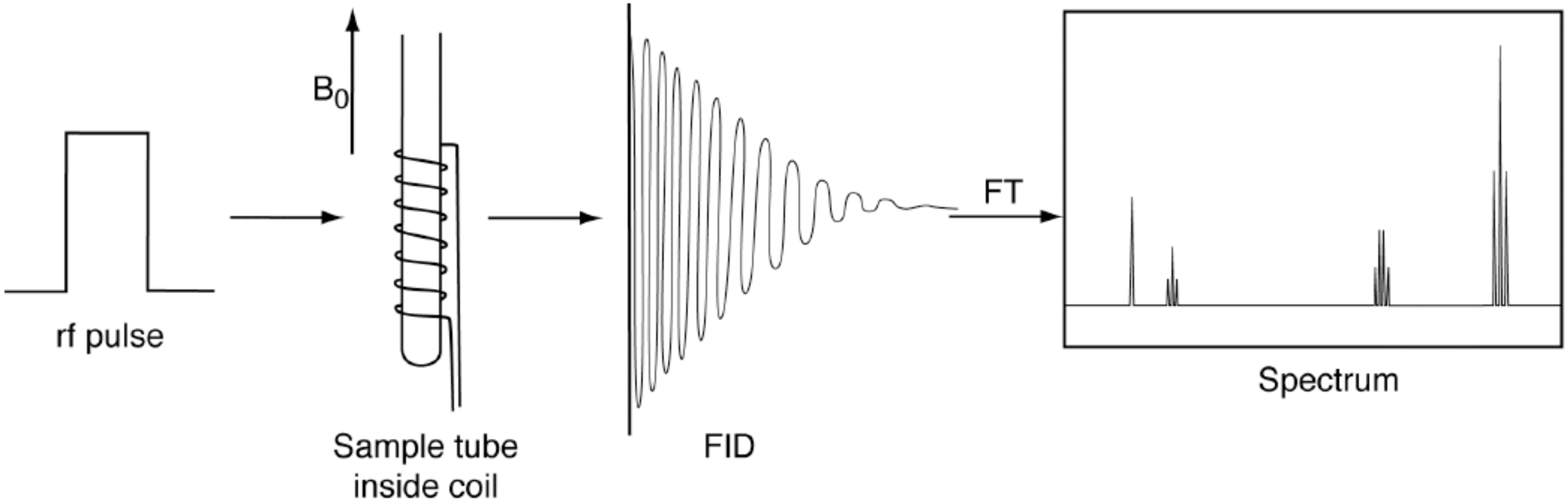
at no magnetic field, there is no difference beteen α- and β- states.

Irradiation with frequencies corresponding to the difference between the two states will excite some of the nuclei. Upon relaxation, detectable radiation is emitted.

NMR – basic concepts



NMR – basic concepts



contains all
frequencies
in the sample

NMR – basic concepts

Different nuclei in a molecule exist in different electronic environments. The nuclei are **shielded** by the electrons that surround them. The electrons generate a magnetic field opposed to the external field.

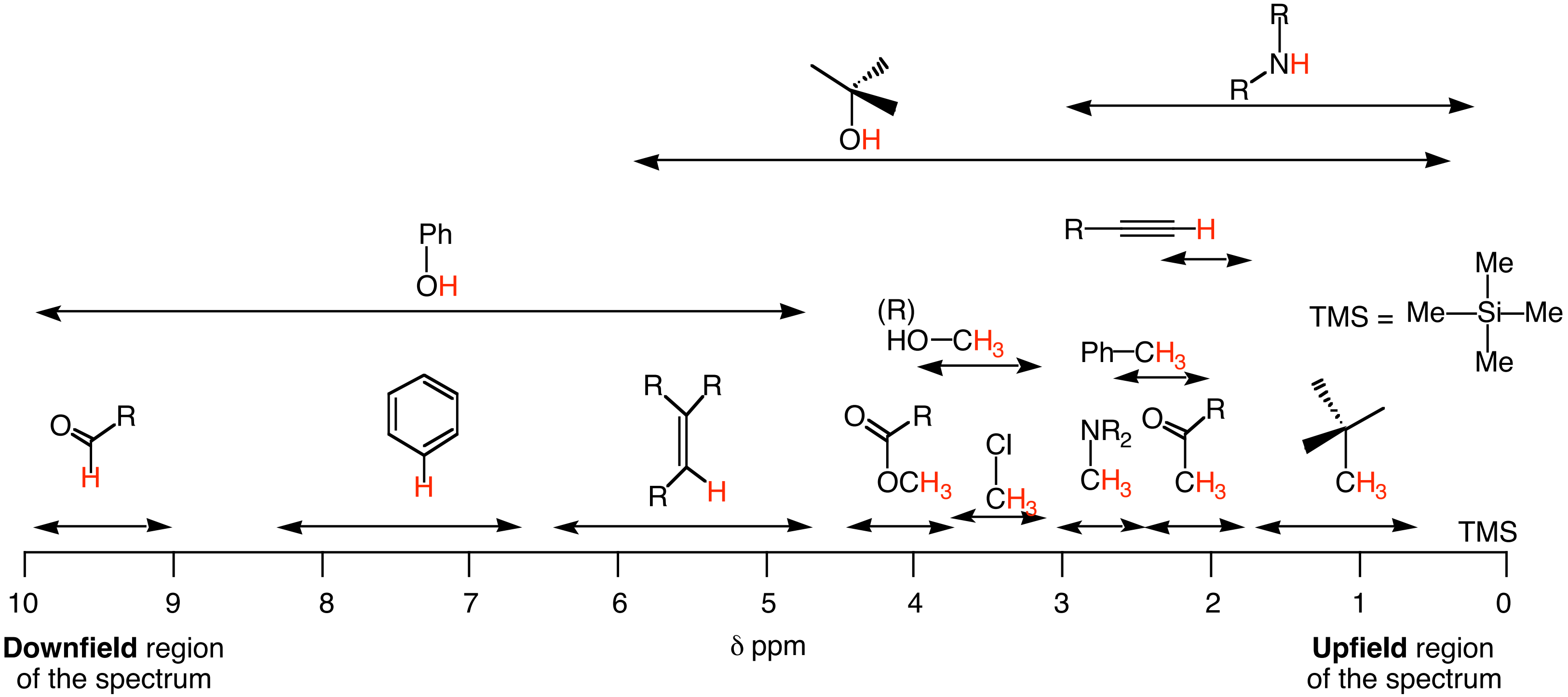
This means that nuclei in different electronic environments experience different magnetic fields and therefore absorb radiation of different frequencies. Thus, the absorption frequency of a nuclei tells us something about the environment of that proton.

The absorption frequencies in NMR are reported as ppm (parts per million) in relation to a reference (usually tetramethylsilane, TMS) and the operating frequency of the instrument. This is called the **chemical shift** (δ)

$\delta_{sample} = (\nu_{sample} - \nu_{TMS}) / \nu_{instrument}$

NMR – basic concepts

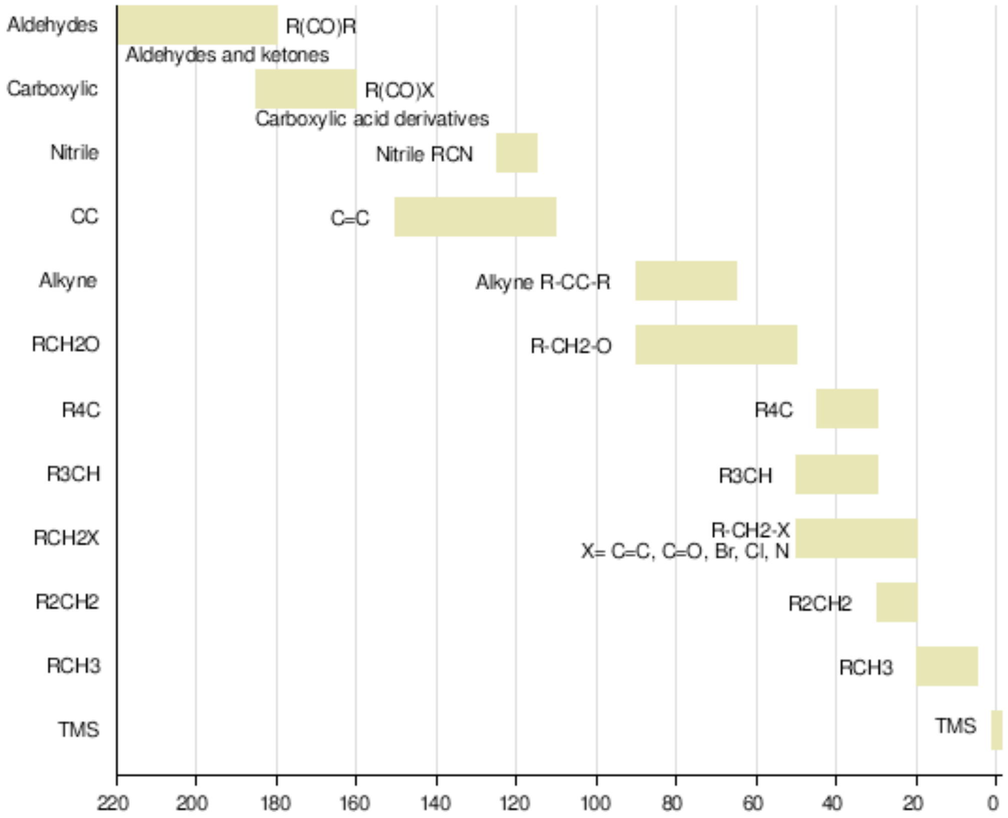
Different types of protons have characteristic chemical shifts:



In addition, the area under each peak is proportional to the number of hydrogens generating that peak. For example: $CH_3 : CH_2 : CH \Rightarrow 3 : 2 : 1$ (± 10 %)

NMR – basic concepts

^{13}C nuclei also have characteristic chemical shifts:



Note: much wider scale (220 ppm) than ^{1}H-NMR (12-14 ppm).

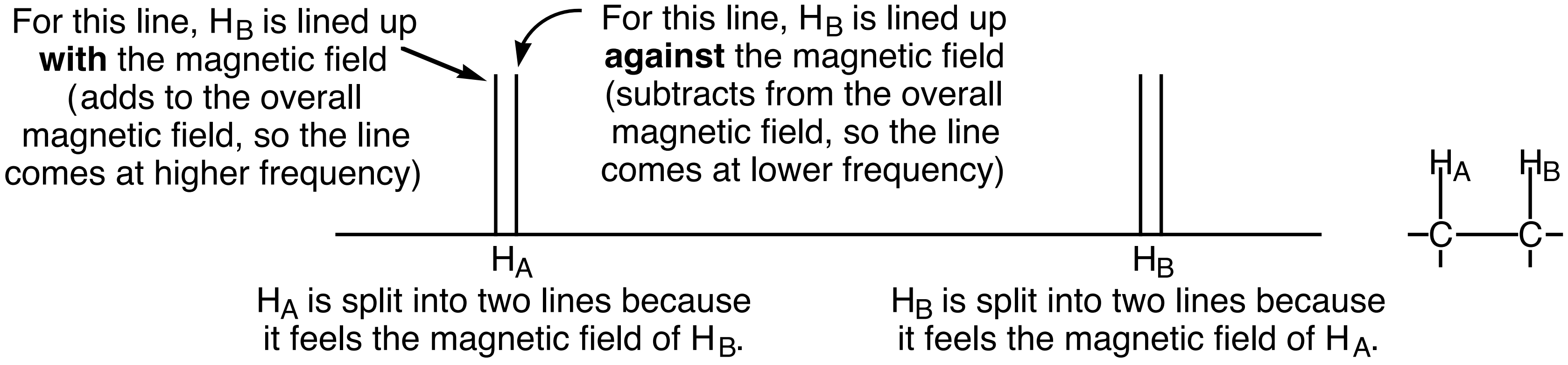
Overlap much less likely meaning every unique carbon will produce a separate peak.

However, ^{13}C-spectra are generally **not integrated**

NMR – basic concepts

^{1}H-NMR spectra provides additional information about other protons in the vicinity (within 3 bonds). This is called ^{1}H-^{1}H **coupling** (also spin-spin **splitting** or J-coupling).

Neighbouring protons either add or subtract from the magnetic field depending on their orientation. Therefore, a proton may experience several different fields:



For this line, H_B is lined up **with** the magnetic field (adds to the overall magnetic field, so the line comes at higher frequency)

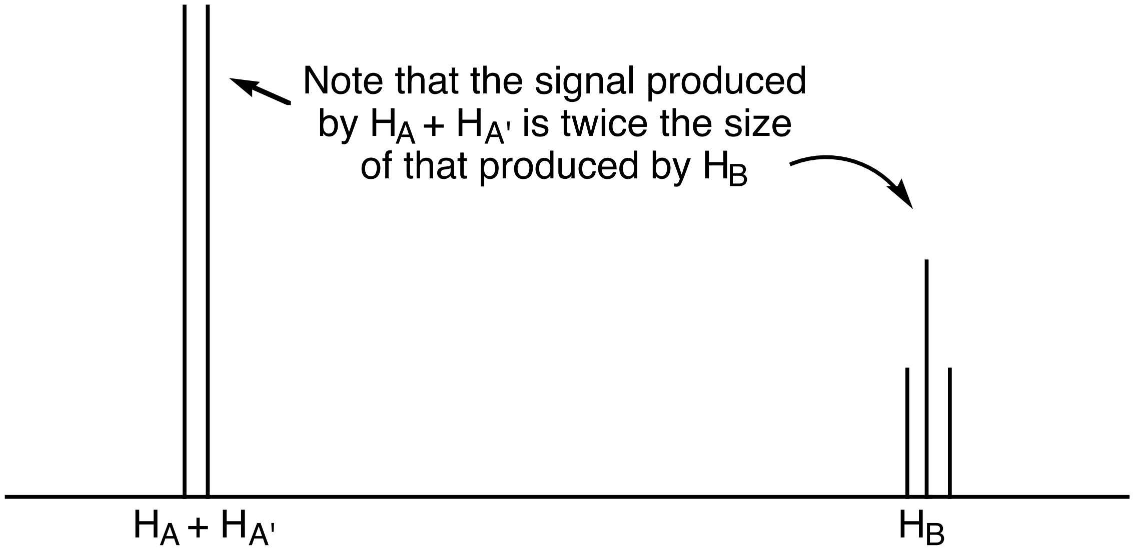
For this line, H_B is lined up **against** the magnetic field (subtracts from the overall magnetic field, so the line comes at lower frequency)

H_A is split into two lines because it feels the magnetic field of H_B.

H_B is split into two lines because it feels the magnetic field of H_A.

More ^1H - ^1H Coupling

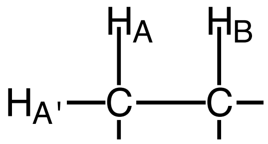
What happens when there is more than one proton splitting a neighboring proton? We get more lines. Consider the molecule below where we have two protons on one carbon and one proton on another.



Note that the signal produced by $H_A + H_{A'}$ is twice the size of that produced by H_B

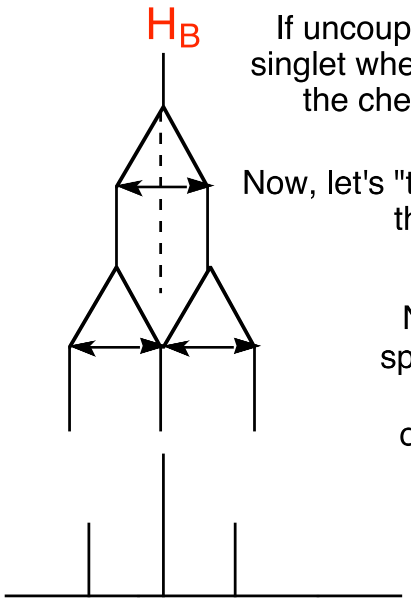
H_A and $H_{A'}$ appear at the same chemical shift because they are in identical environments
They are also split into two lines (called a doublet) because they feel the magnetic field of H_B.

H_B is split into three lines because it feels the magnetic field of H_A and $H_{A'}$



Why are There Three Lines for H_B?

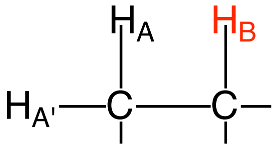
H_B feels the splitting of both H_A and $H_{A'}$. So, let's imagine starting with H_B as a single line, then let's "turn on" the coupling from H_A and $H_{A'}$ one at a time:



If uncoupled, H_B would appear as a singlet where the dashed line indicates the chemical shift of the singlet.

Now, let's "turn on" H_B - H_A coupling. This splits the single line into two lines

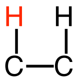
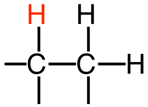
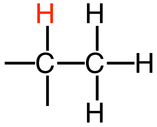
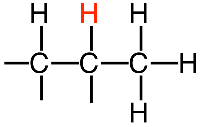
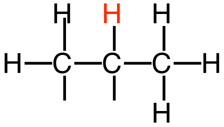
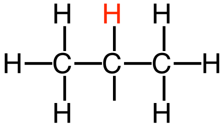
Now, let's "turn on" H_B - $H_{A'}$ coupling. This splits each of the two new lines into two lines, but notice how the two lines in the middle overlap. Overall, we then have three lines.



Because the two lines in the middle overlap, that line is twice as big as the lines on the outside. More neighboring protons leads to more lines as shown on the next slide.

Splitting Patterns with Multiple Neighboring Protons

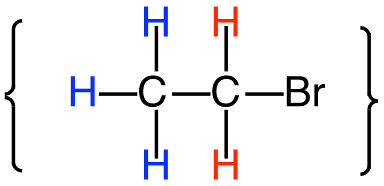
If a proton has n neighboring protons *that are equivalent,* that proton will be split into **n+1** lines. The lines will not be of equal intensity, rather their intensity will be given by Pascal's triangle:

no. of neighbors	relative intensities	pattern	example
0	1	singlet (s)	
1	1 1	doublet (d)	
2	1 2 1	triplet (t)	
3	1 3 3 1	quartet (q)	
4	1 4 6 4 1	pentet	
5	1 5 10 10 5 1	sextet	
6	1 6 15 20 15 6 1	septet	

More About Coupling

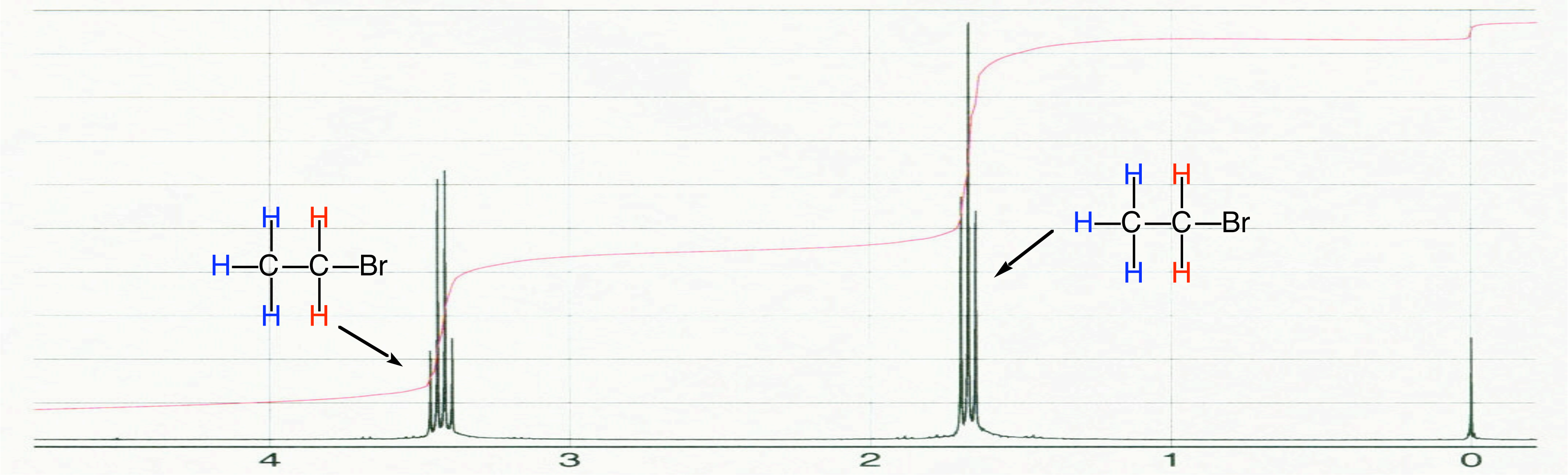
When two protons appear at the same chemical shift, they do not split each other. So, in EtBr, we have a CH_3 next to a CH_2, and each proton of the CH_3 group is *only* coupled to the protons of the CH_2 group, not the other CH_3 protons because all the CH_3 protons come at the same chemical shift (**magnetically equivalent**).

The blue protons all come at the same chemical shift and do not split each other



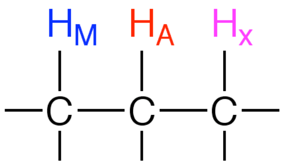
$\mathrm{H{-}CH_2{-}CH_2{-}Br}$

The red protons both come at the same chemical shift and do not split each other

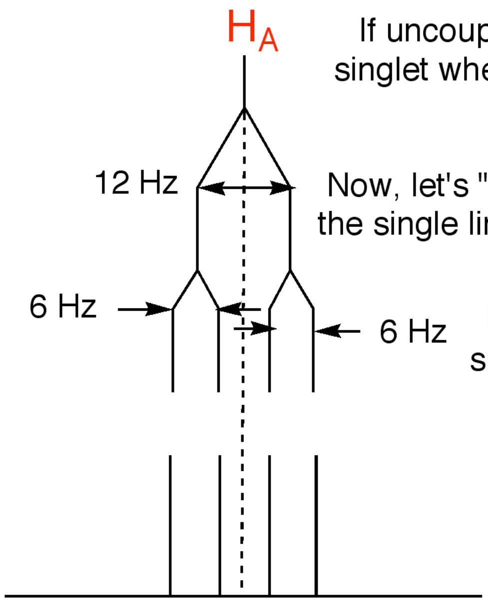


Not all Couplings are Equal

When protons couple to each other, they do so with a certain intensity. This is called the "coupling constant." Coupling constants can vary from 0 Hz (which means that the protons are not coupled, even though they are neighbors) to 16 Hz. Typically, they are around 7 Hz, but many molecules contain coupling constants that vary significantly from that. So, what happens when a molecule contains a proton which is coupled to two different protons with different coupling constants? We get a different pattern as described in the diagram below.



Coupling constant H_A- H_X = 12
Coupling constant H_A- H_M = 6



If uncoupled, H_A would appear as a singlet where the dashed line indicates

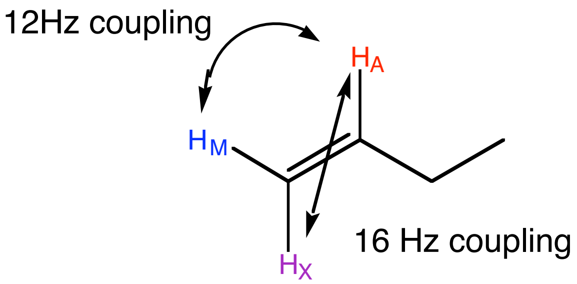
Now, let's "turn on" H_A - H_X coupling. This splits the single line into two lines that are 12 Hz appart

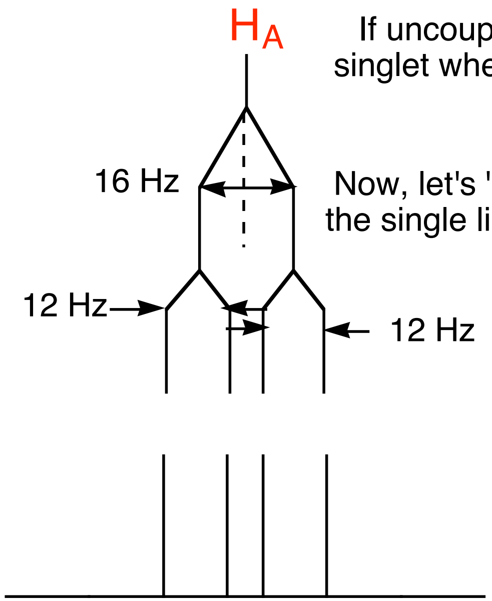
Now, let's "turn on" H_A - H_M coupling. This splits each of the two new lines into two lines that are 6 Hz appart for a total of four lines

So, if the protons are not equivalent, they can have different coupling constants and the resulting pattern will not be a triplet, but a "doublet of doublets." Sometimes, nonequivalent protons can be on the same carbon as described on the next slide.

Coupling Constants in Alkenes

Coupling constants in alkenes can also differ depending on whether the protons are cis or trans to each other. Note that in a terminal alkene (i.e., an alkene at the end of a carbon chain), the cis and trans protons are NOT equivalent. One is on the same side as the substituent, the other is on the opposite side. The coupling of *trans* protons to each other is typically very large, around 16 Hz, while the coupling of *cis* protons, while still large, is a little smaller, around 12 Hz. This leads to the pattern shown below, and an example of a molecule with this splitting pattern is shown on the next slide.



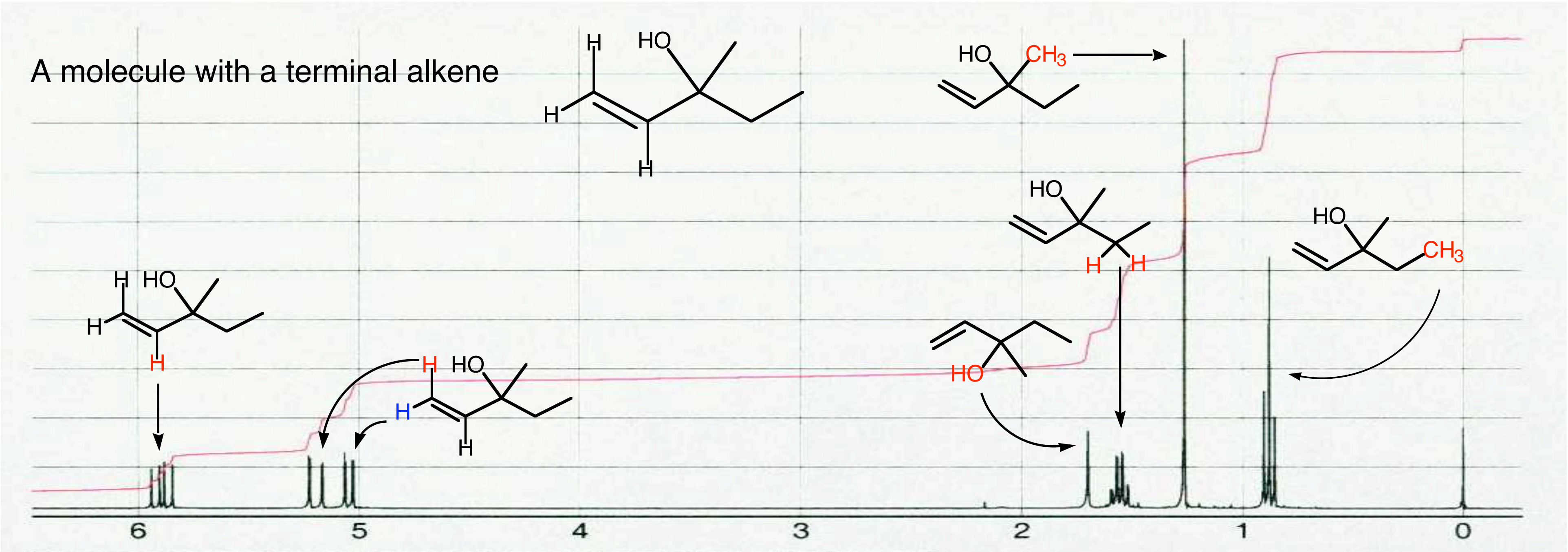


If uncoupled, H_A would appear as a singlet where the dashed line indicates

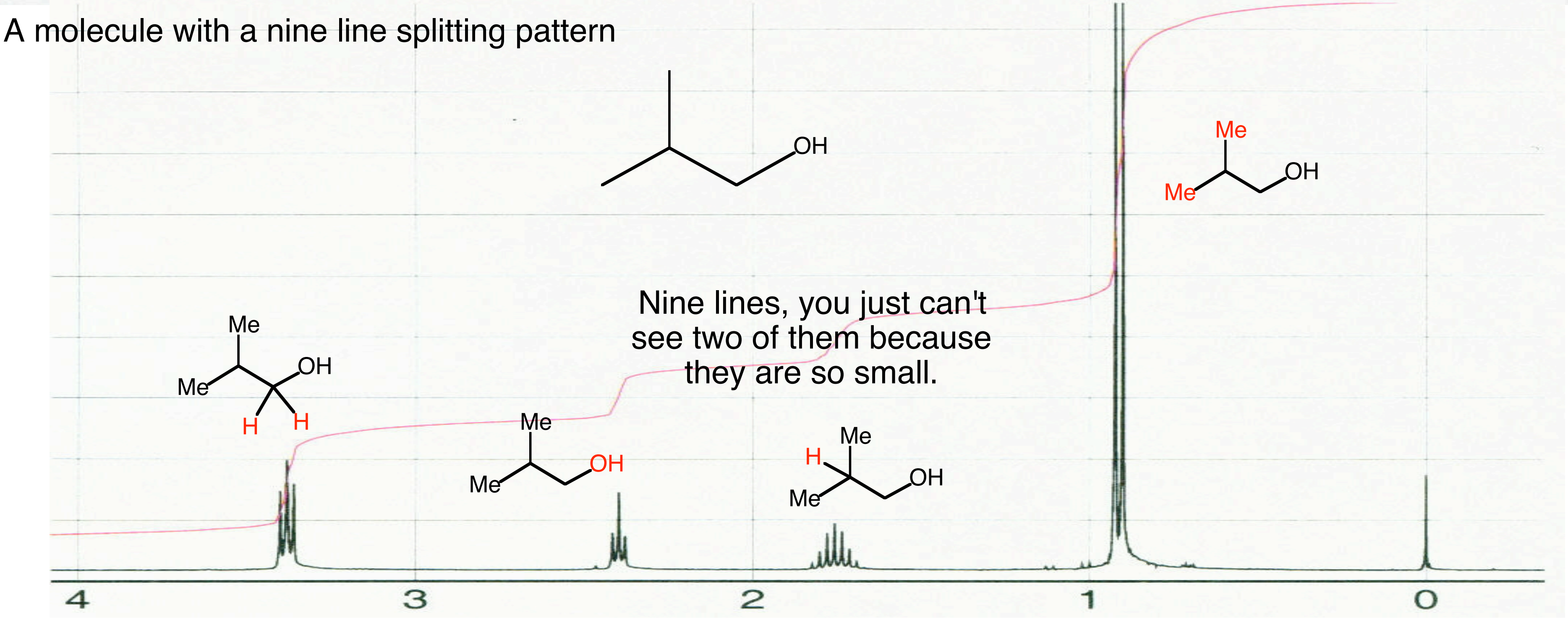
Now, let's "turn on" H_A - H_X coupling. This splits the single line into two lines that are 16 Hz appart

Now, let's "turn on" H_A - H_M coupling. This splits each of the two new lines into two lines that are 12 Hz appart for a total of four lines

A molecule with a terminal alkene



A molecule with a nine line splitting pattern


Nine lines, you just can't see two of them because they are so small.

NMR – summary

^{1}H-NMR:

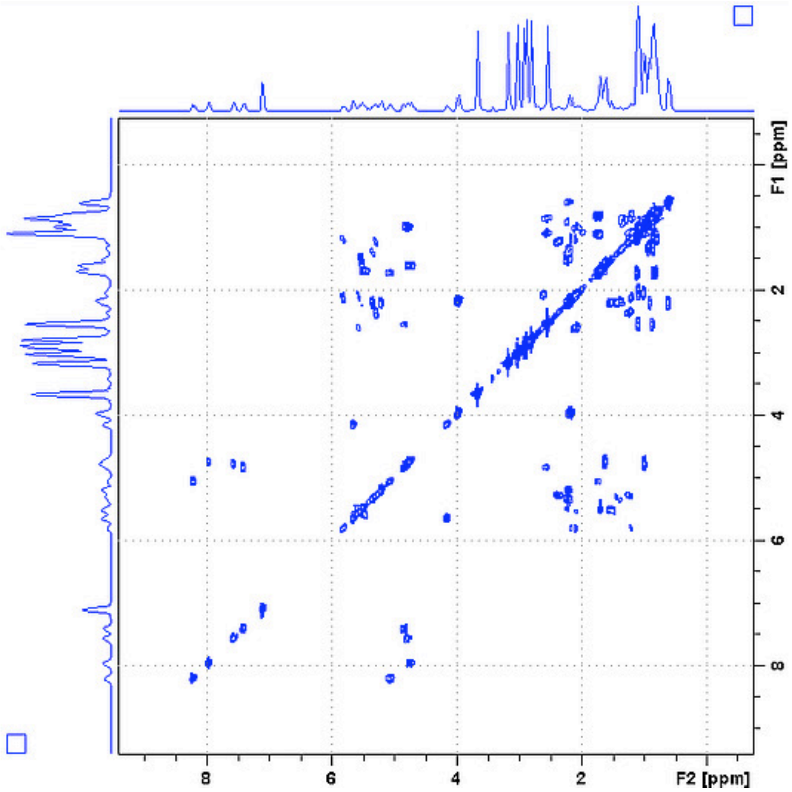
- **Number of signals** – number of unique groups of hydrogen atoms
- **Intensity of signals** (area) – number of hydrogens giving each signal
- **Position of signals** – type of hydrogens
- **Splitting of signals** – number of adjacent hydrogens (n+1 rule)
- **Spacing of splitting** – coupling constant

^{13}C-NMR

- **Number of signals** – number of unique carbon atoms
- **Position of signals** – types of carbons

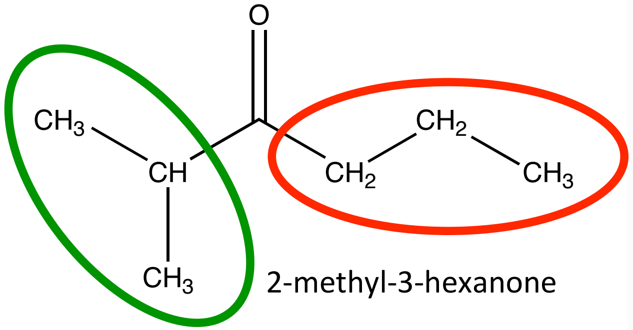
Two dimensional NMR

By clever combinations of multiple RF pulses and time delays it is possible to generate two dimensional NMR-spectra. This is very useful for determination of complicated structures (peptides, proteins, polysaccarides…)

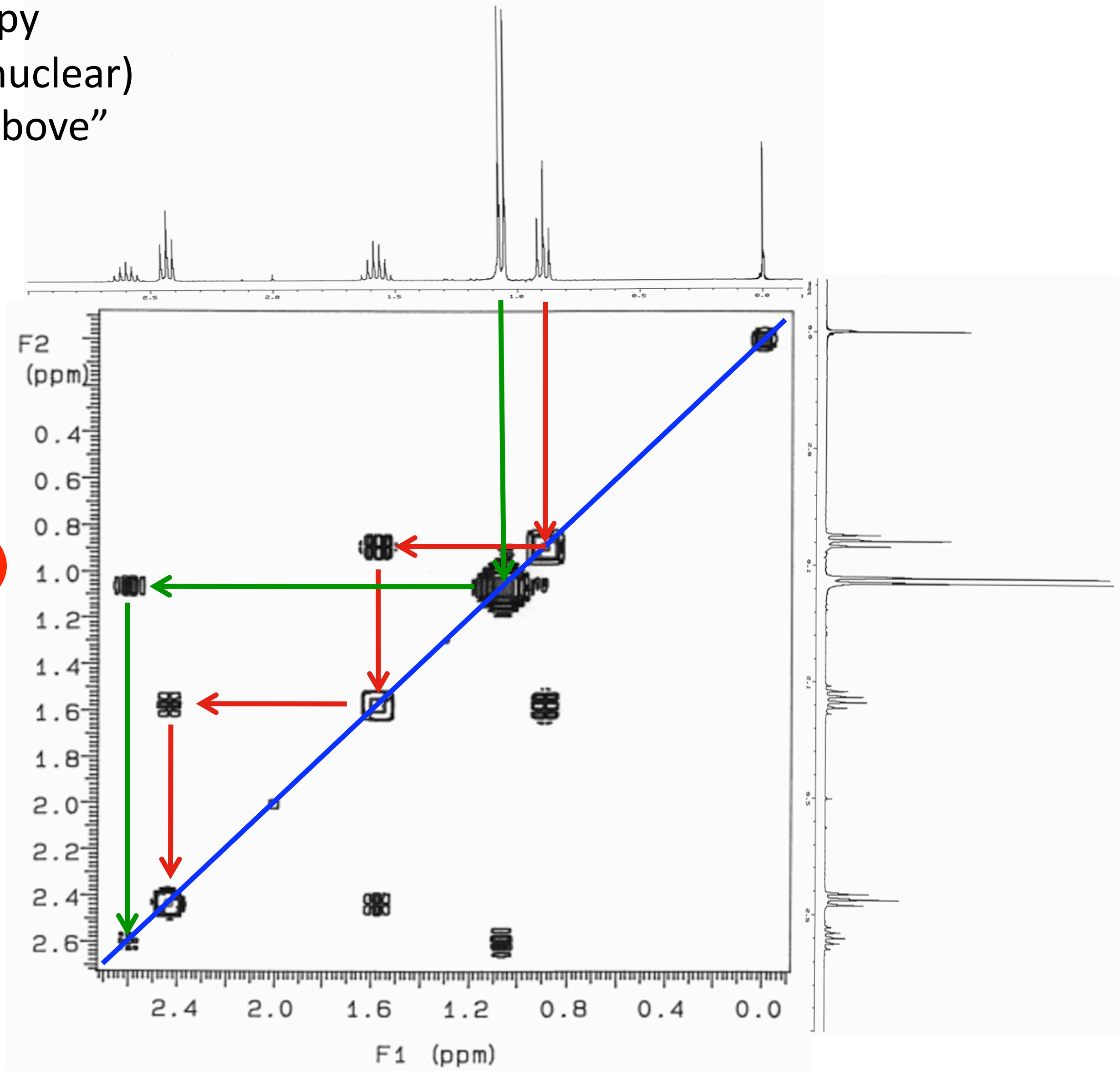


Two dimensional NMR

COSY – correlation spectroscopy
^{1}H in both dimensions (homonuclear)
Diagonal = 1D-spectra ”from above”
Cross-peaks = J-coupling

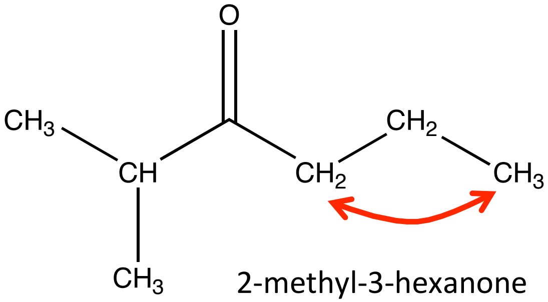


2-methyl-3-hexanone

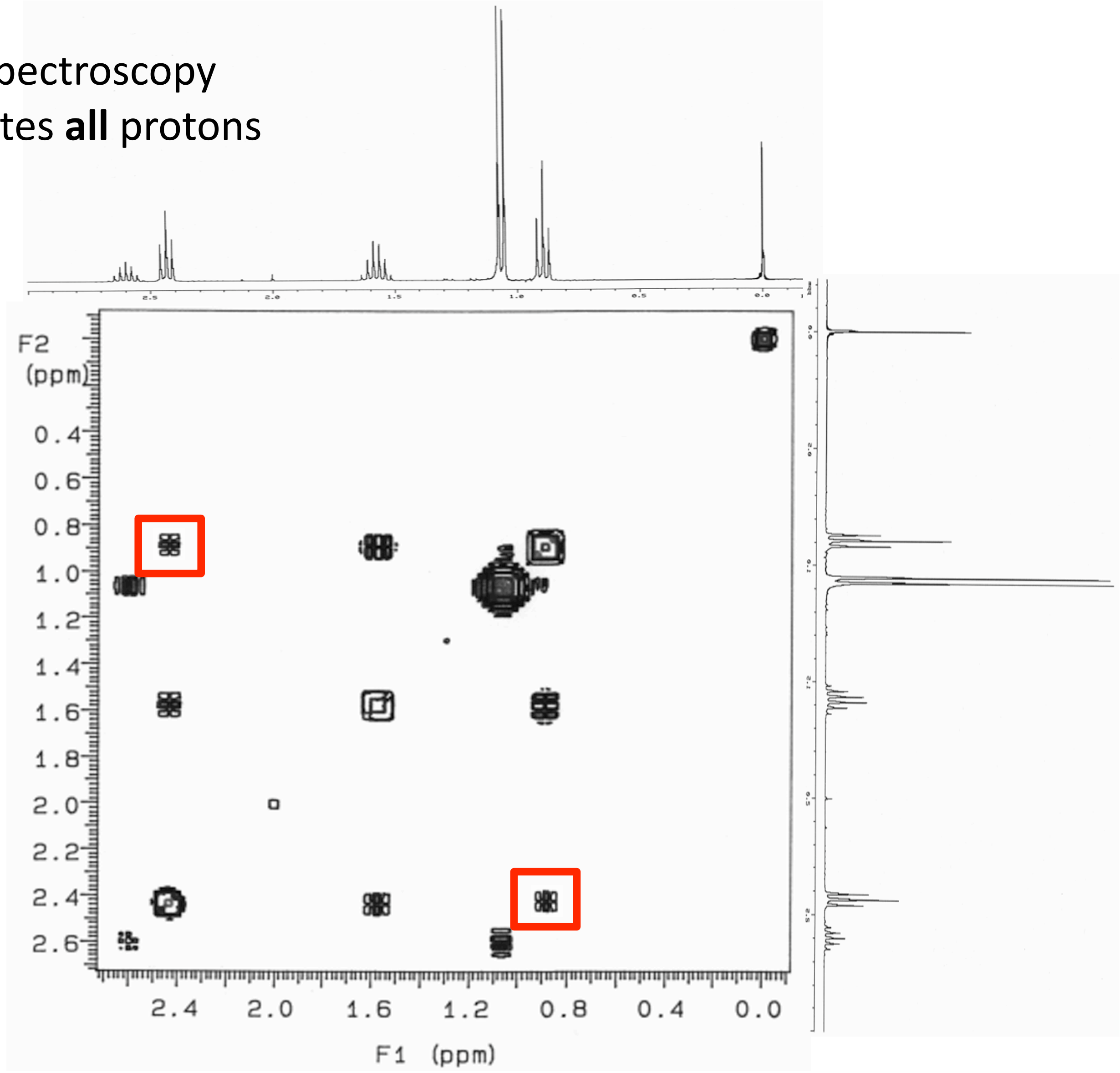


Two dimensional NMR

TOCSY – total correlation spectroscopy
Similar to COSY but correlates **all** protons
in a **spin system**.

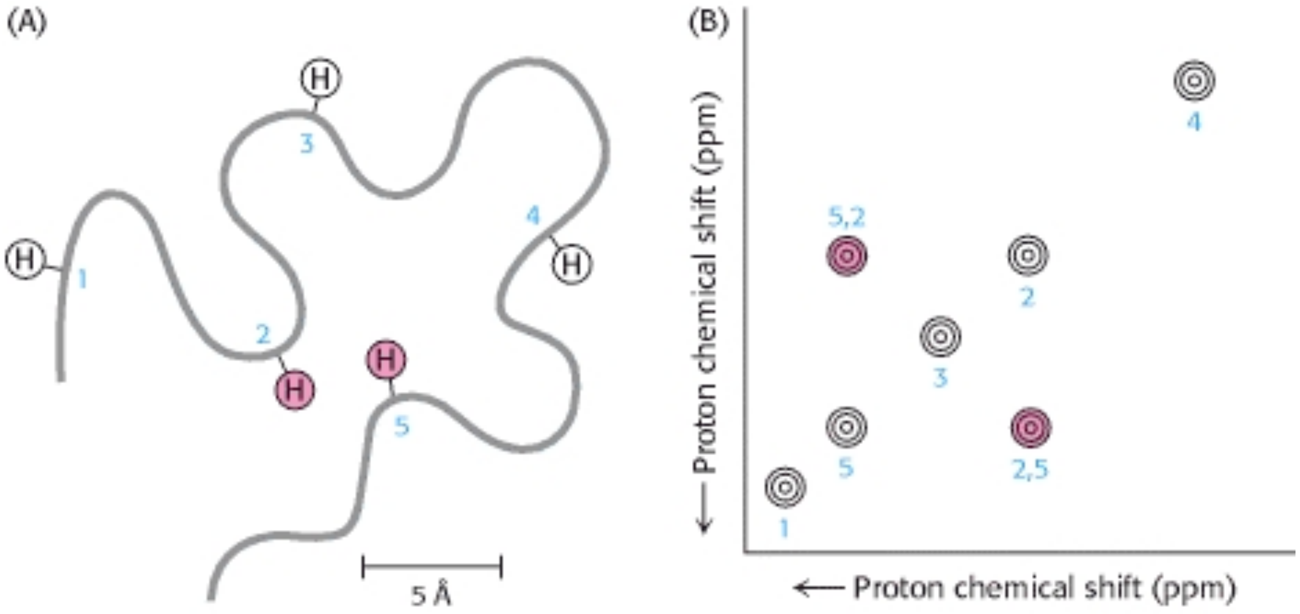


2-methyl-3-hexanone



Two dimensional NMR

NOESY – Nuclear Overhauser spectroscopy
Correlates protons that are within 5Å

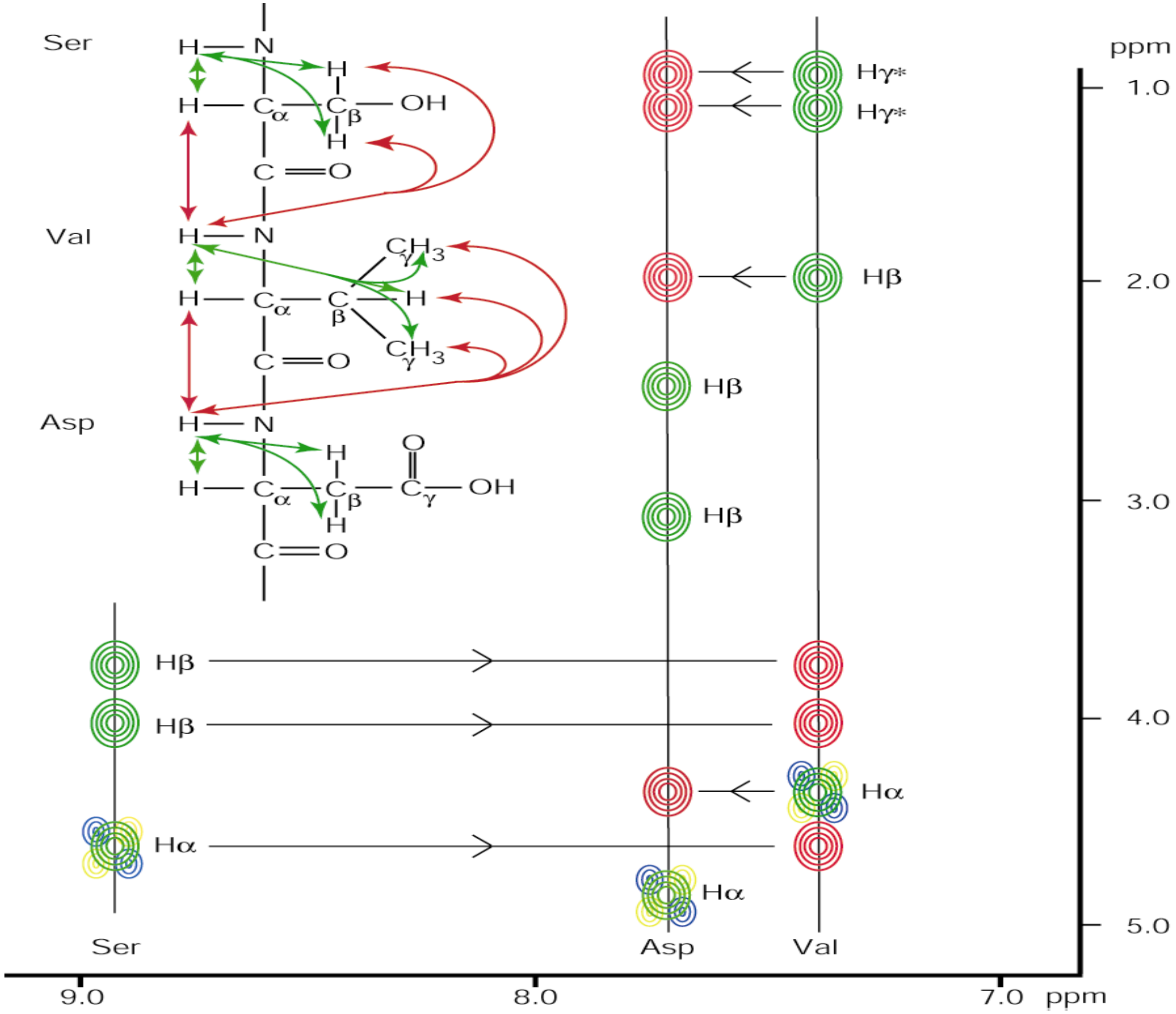


Structure determination of proteins

COSY
Cross-peaks between protons connected by no more than 3 bonds.

TOCSY
Cross-peaks between protons in a pathway of no more than 3 bonds between each pair in the pathway.

NOESY
Through Space Interactions:
Cross-peaks between protons closer than 5A in the 3D structure.



What can we use NMR for?

Structure determination

Host-guest interaction studies

- -Where does an interaction take place?
- -How strong is it?
- -What is the stoichiometry?