

Today's class:

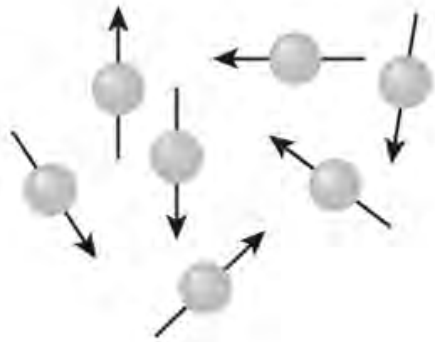
NMR Spectroscopy part 2

This lecture follows the materials from the following books

- *Physical Biochemistry by David Sheehan, 2nd Ed, Wiley, 2009*
- *Fundamentals of Biochemistry by Voet, Voet and Pratt, 5th Ed, Wiley*

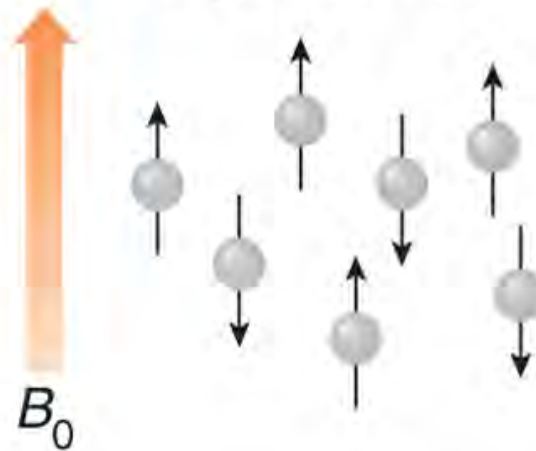
Nuclear Magnetic Resonance

With no external magnetic field...



The nuclear magnets are randomly oriented.

In a magnetic field...



The nuclear magnets are oriented **with or against** B_0 .

$$\Delta E_{\alpha\beta} \approx 0 \quad \text{if } B_0 = 0$$

$$\Delta E_{\alpha\beta} = \gamma \hbar B_0 \quad \text{if } B_0 \neq 0$$

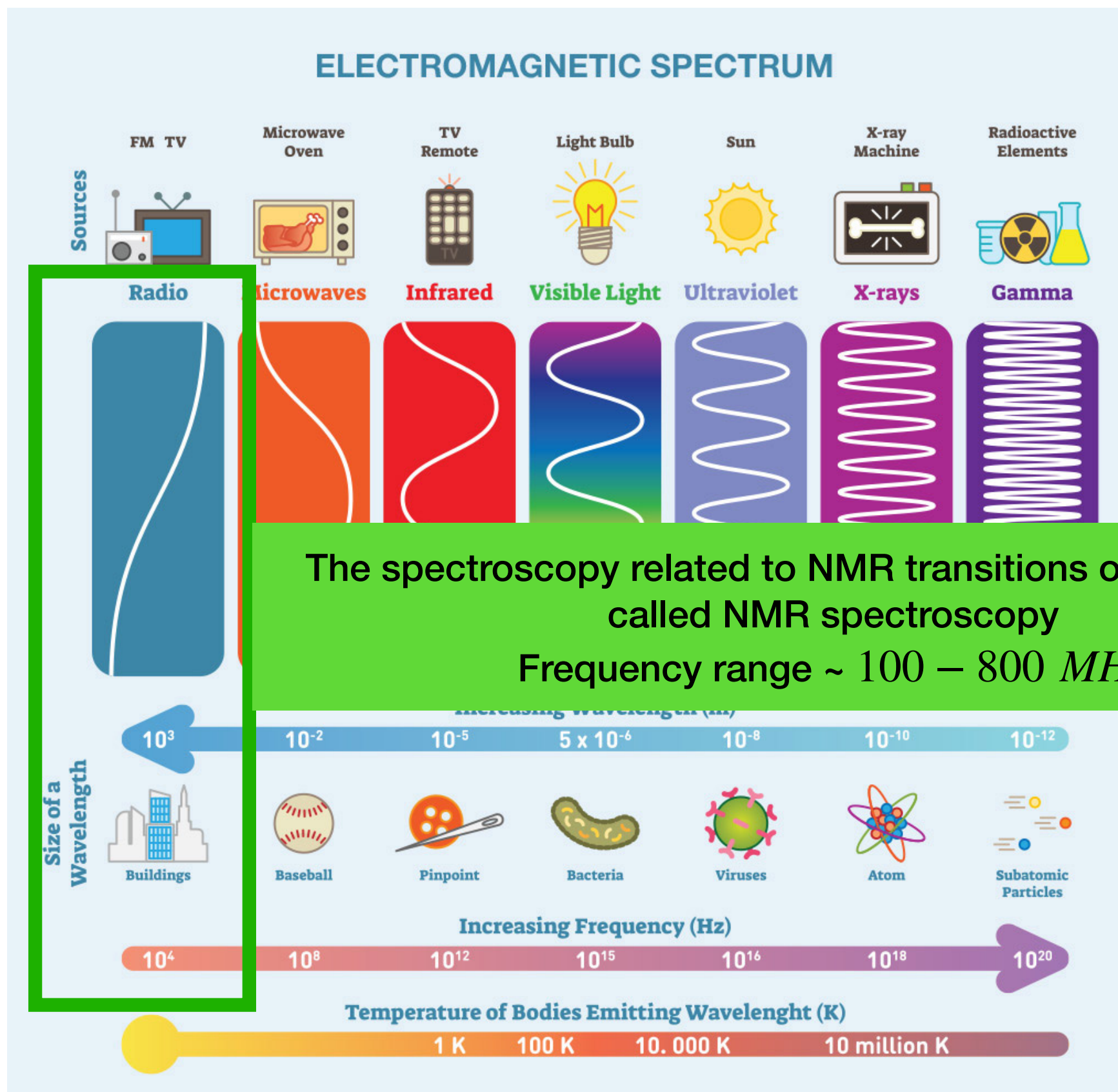
- Immediately after, population in the states are related as: $N_\beta < N_\alpha$
- If the sample is exposed to radiation of frequency ν , the energy separations come into **resonance** with the radiation when the frequency satisfies the resonance condition:

$$h\nu = \gamma \hbar B_0 \implies \nu = \frac{\gamma B_0}{2\pi}$$

- At resonance there is strong coupling between the nuclear spins and the radiation, and strong absorption occurs as the spins flip from α (low energy) to β (high energy).
- We refer to these transitions as **nuclear magnetic resonance (NMR) transitions**.

Selection rule for NMR: $\Delta m_l = \pm 1$

NMR transitions absorb radiowaves!

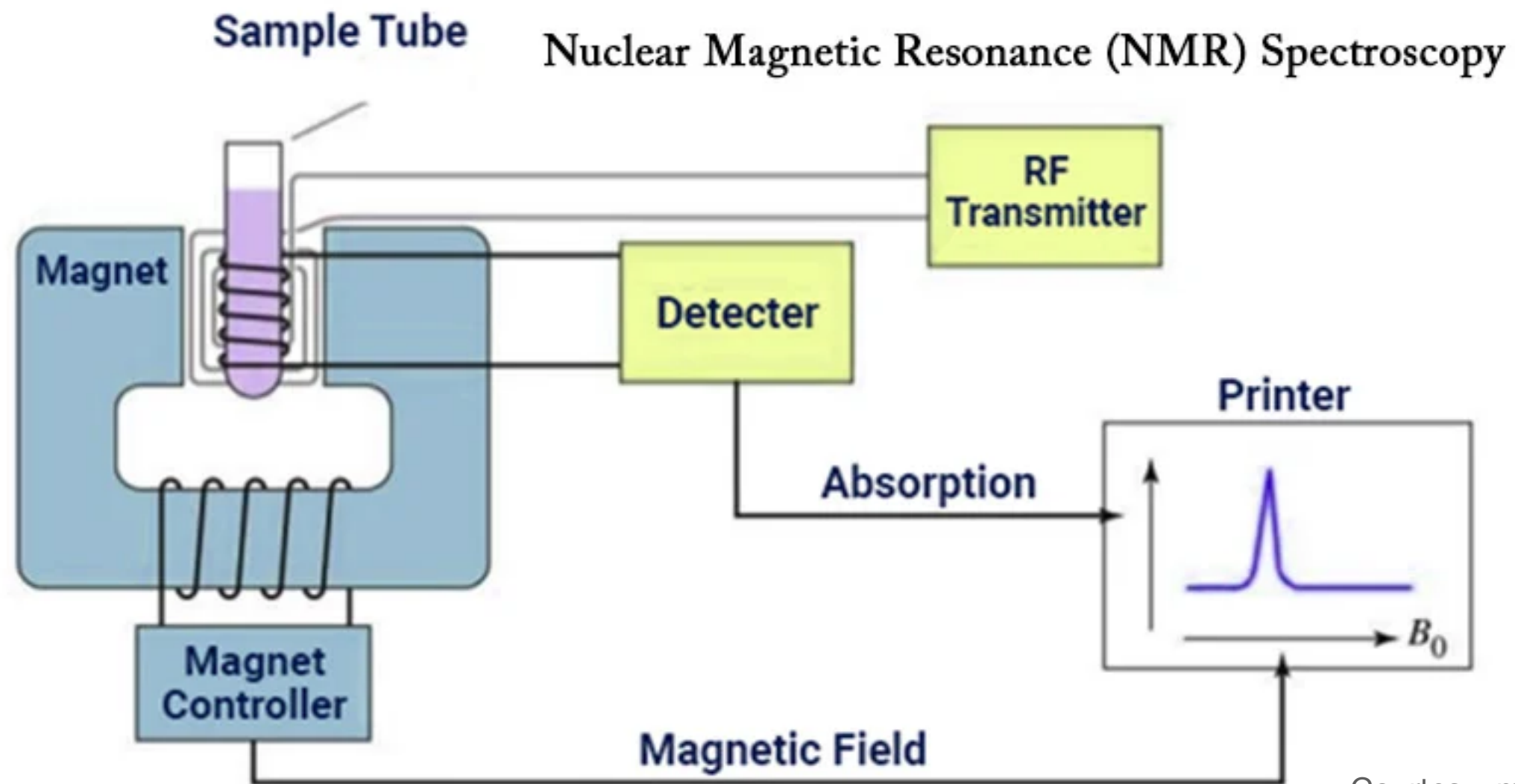


NMR transition frequencies for important nuclei

Table 3.6. Magnetic properties of some nuclei important in biochemistry

Nucleus	I	Natural abundance (%)	γ rad·s ⁻¹ T ⁻¹	NMR ν at $T =$ 2.3488 (MHz)
¹ H	1/2	99.98	26.752	100
² H	1/2	0.015	4.107	15.35
¹² C	0	98.9	—	—
¹³ C	1/2	1.10	6.7283	25.144
¹⁴ N	1	99.63	1.9338	7.224
¹⁶ O	0	99.76	—	—
³² S	0	95.02	—	—
³¹ P	1/2	100	10.8394	40.481
³⁵ Cl	3/2	75.77	2.642	9.798
¹⁵ N	1/2	0.37	-2.7126	10.133

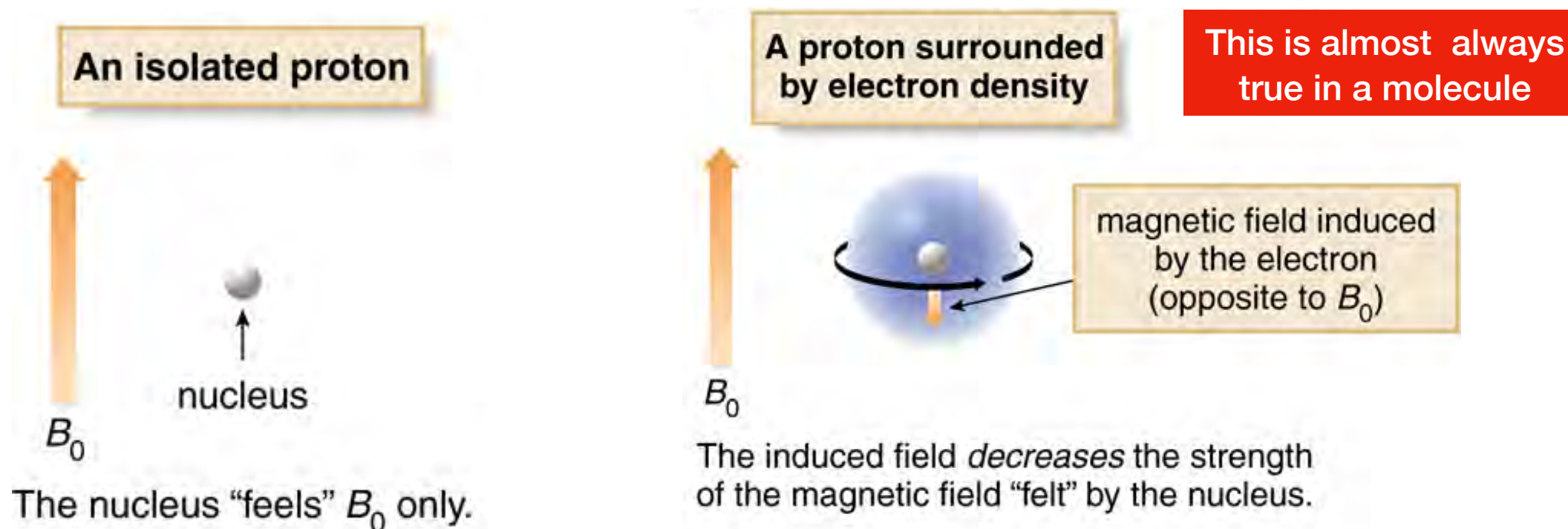
NMR spectroscopy set up



Courtesy: microbenotes.com

- The RF transmitter generates a pulse of radio waves
- The magnetic controller sweeps a range of B_0 to supply the energy required for NMR transitions
- When the excited nuclei relaxes back the emitted energy is recorded
- This frequency of transition is used to generate the NMR spectrum

Shielding by electrons and chemical shift



Courtesy: chemistry.msu.edu

The induced field is written as:

$$\delta B = -\sigma B_0$$

Thus the effective field experienced by the nucleus:

$$B_{eff} = B_0 + \delta B = B_0(1 - \sigma)$$

The modified resonance condition becomes:

$$\nu = \frac{\gamma}{2\pi} B_0(1 - \sigma)$$

Due to this the resonating frequency is always lower than what it should be - called Shielding Effect

Shielding by electrons and chemical shift ...*contd*

The chemical shift of a nucleus is the difference between its resonance frequency and that of a reference standard.



The reference standard for protons is the proton resonance in tetramethylsilane, $\text{Si}(\text{CH}_3)_4$, commonly referred to as TMS

Reason for choosing TMS as standard: TMS shows a strong peak from 12 protons at almost the lowest frequency compared to any known protons

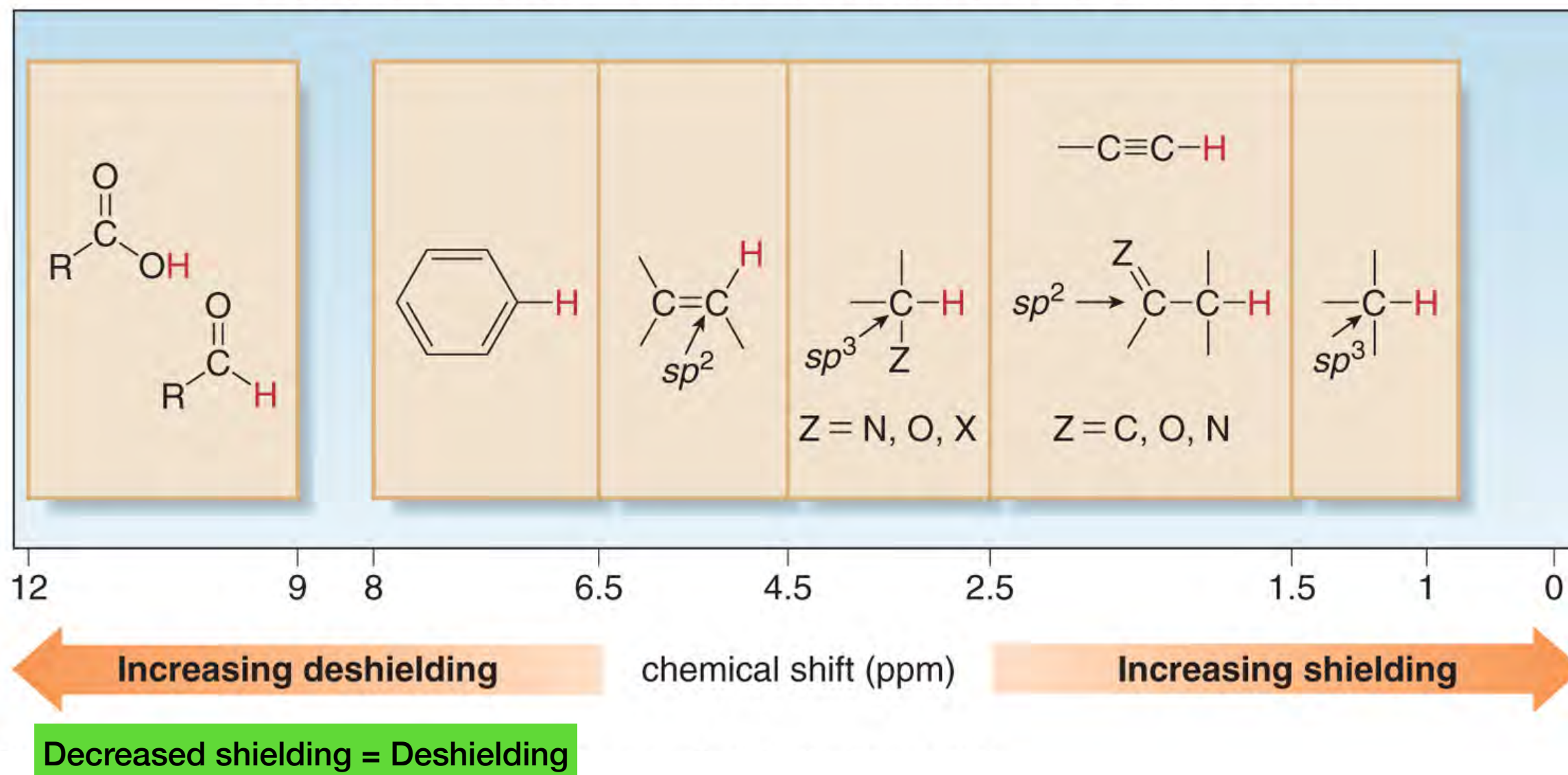
The chemical shift w.r.t TMS is then

$$\delta = \frac{\nu - \nu_0}{\nu_0} \times 10^6$$

expressed in ppm scale

ν_0 is the resonance frequency of the standard

Regions in the ^1H NMR spectrum

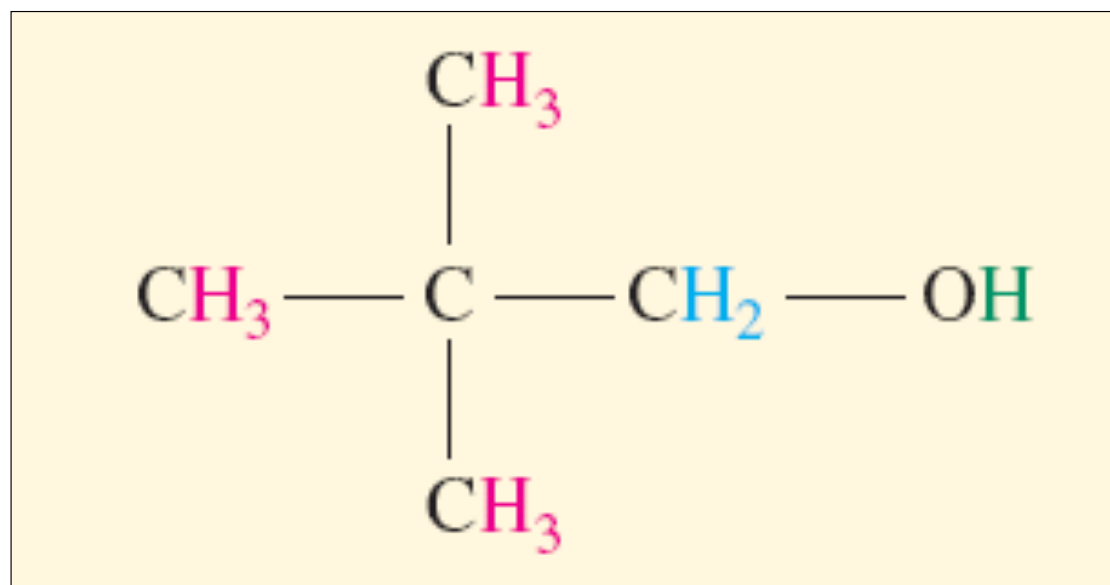


Structural information from features of a ^1H NMR spectrum

- **Position of signals:** indicates what types of protons the molecule contains
- **Number of signals:** indicates the number of different types of protons
- **Intensity of signals:** indicates the relative amounts of each kind of protons
- **Splitting of signals:** gives further information of the neighboring environment of various protons

Position of signals reports chemical equivalence

Chemically equivalent hydrogens in a molecule all have identical electronic environments and therefore show NMR peaks at the same position.



In the NMR spectrum of 2,2-dimethyl-1-propanol, there are three different peaks due to absorptions by:

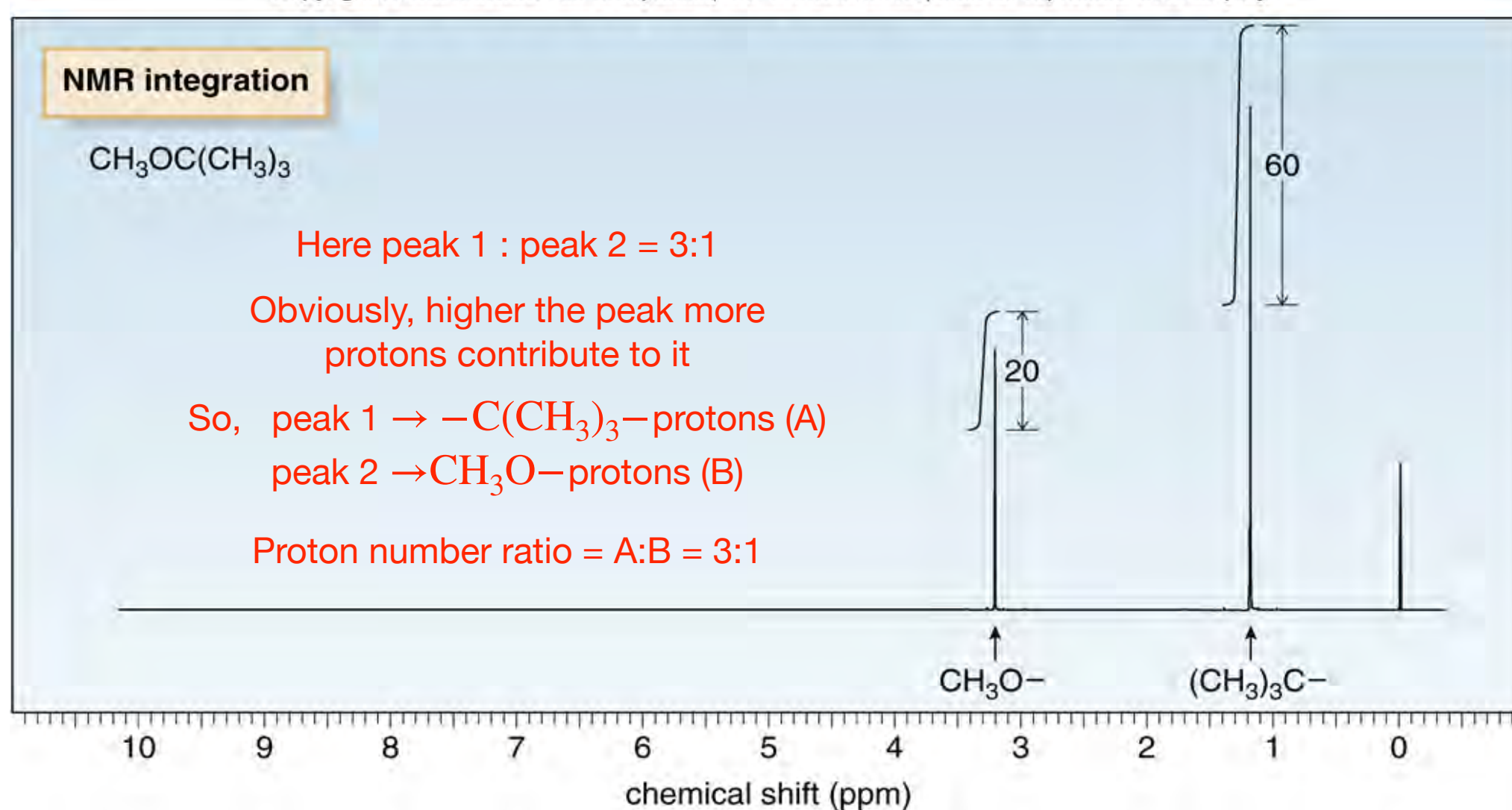
- Nine equivalent methyl hydrogens on the butyl group (most shielded);

- One hydrogen on the OH;

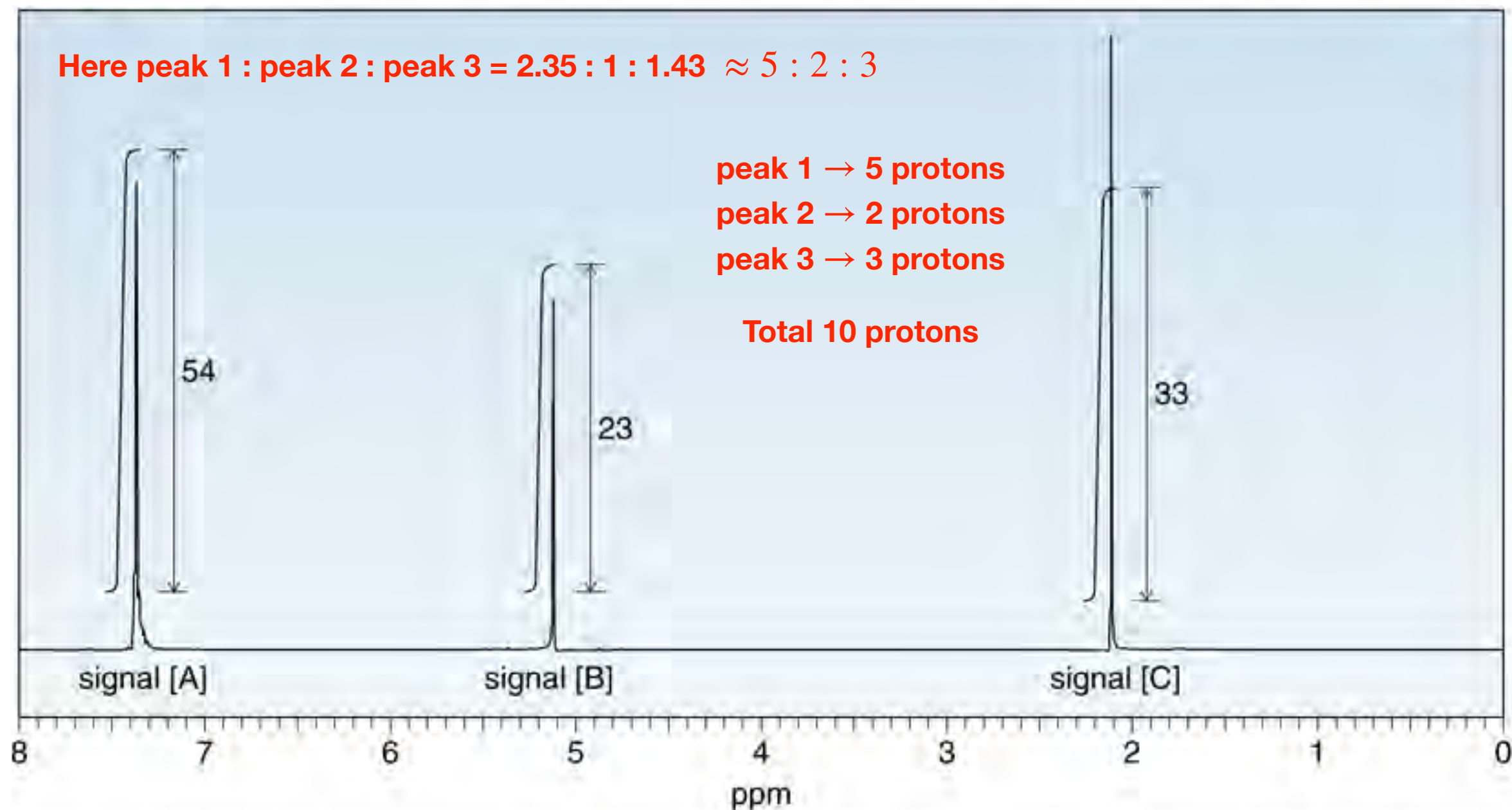
- Two equivalent methylene hydrogens.

Signal height and width reports relative number of protons of different types

- Modern NMR spectrometers automatically calculate and plot the value of integral of each peak in arbitrary units.
- The ratio of integrals to one another gives the ratio of absorbing protons
- Note that this gives a ratio, and not the absolute number, of absorbing protons.



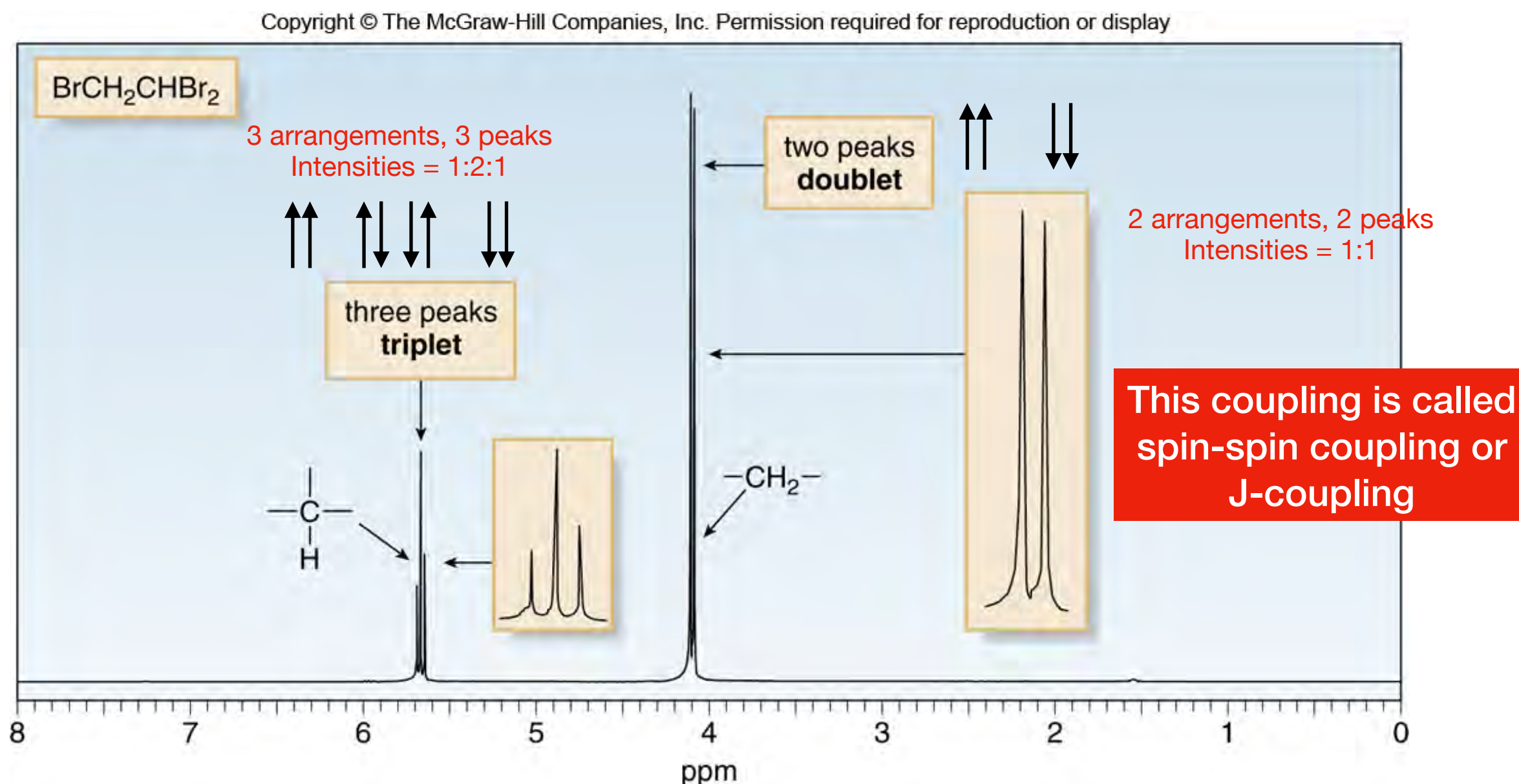
Test problem: A compound $\text{C}_9\text{H}_{10}\text{O}_2$ gives rise to the following NMR spectrum. How many protons give rise to each signal?



Splitting of signals NMR peaks due to spin coupling

Often signals for different protons are split into more than one peak.

This splitting happens due to coupling spins of neighboring protons



Features of spin-spin coupling

- When non-equivalent H atoms are not separated by at least one carbon or oxygen atom “spin-spin coupling” occurs.
- Instead of single peaks, more complex patterns called multiplets (doublets, triplets or quartets) are observed.
- The number and kind of hydrogen atoms directly adjacent to the absorbing nuclei can be deduced from the multiplicity of the peak.

The number of peaks to be expected from any nucleus:

$$N_{peaks} = 2 \times n \times I + 1$$

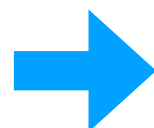
where n = number of equivalent nuclei in the neighboring spin-coupled chemical group
 I = total spin of the equivalent nuclei in the neighboring spin-coupled chemical group

Example:



$$N_{peaks}(Y) = 2 \times n \times I_X + 1$$

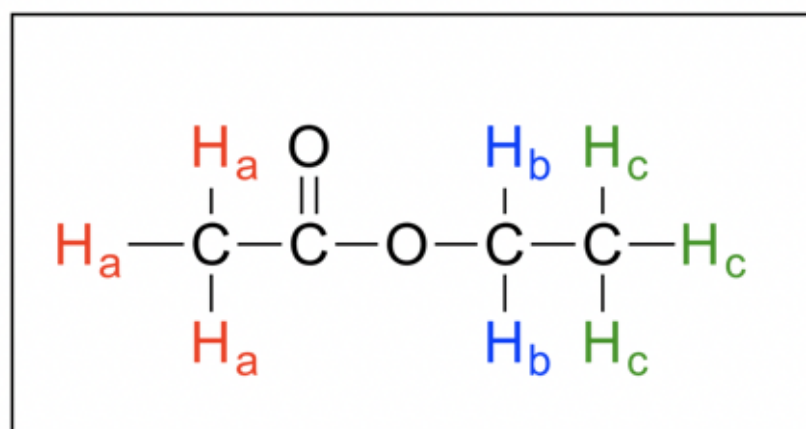
For protons, $I = 1/2$



$$\begin{aligned} \text{number of split peaks is} \\ = 2 \times n \times \frac{1}{2} + 1 = n + 1 \end{aligned}$$

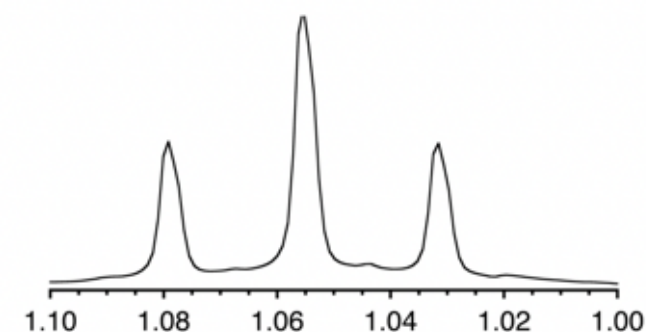
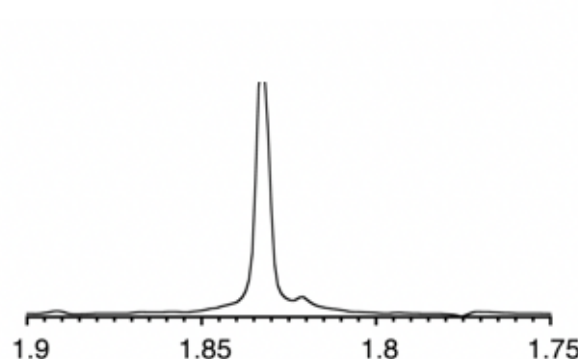
- Equivalent nuclei do not exhibit mutual spin-spin splitting.
- Splitting is observed only between nuclei with different chemical shifts.

Example: determine the spin splitting pattern in the following NMR spectrum



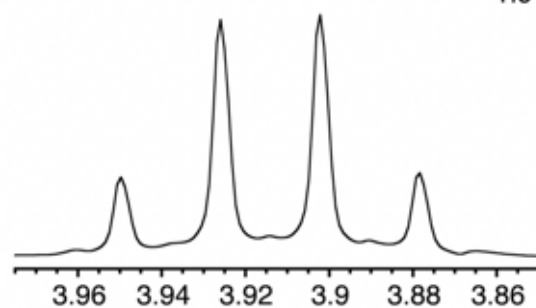
H_a is a single peak because
on protons in the adjacent
carbon to couple to

H_a



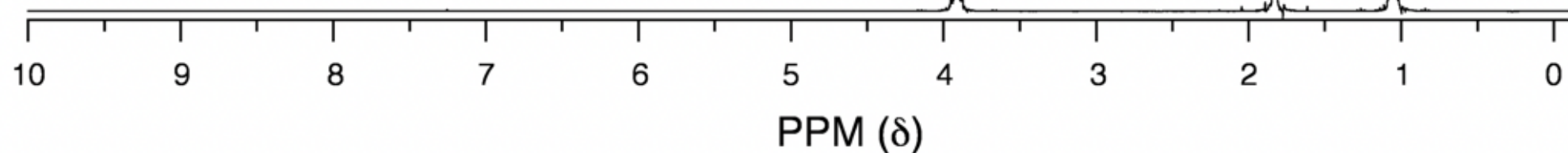
H_c

H_c is a triplet because they
are coupled to two H_b ,
hence $2+1=3$ peaks



H_b

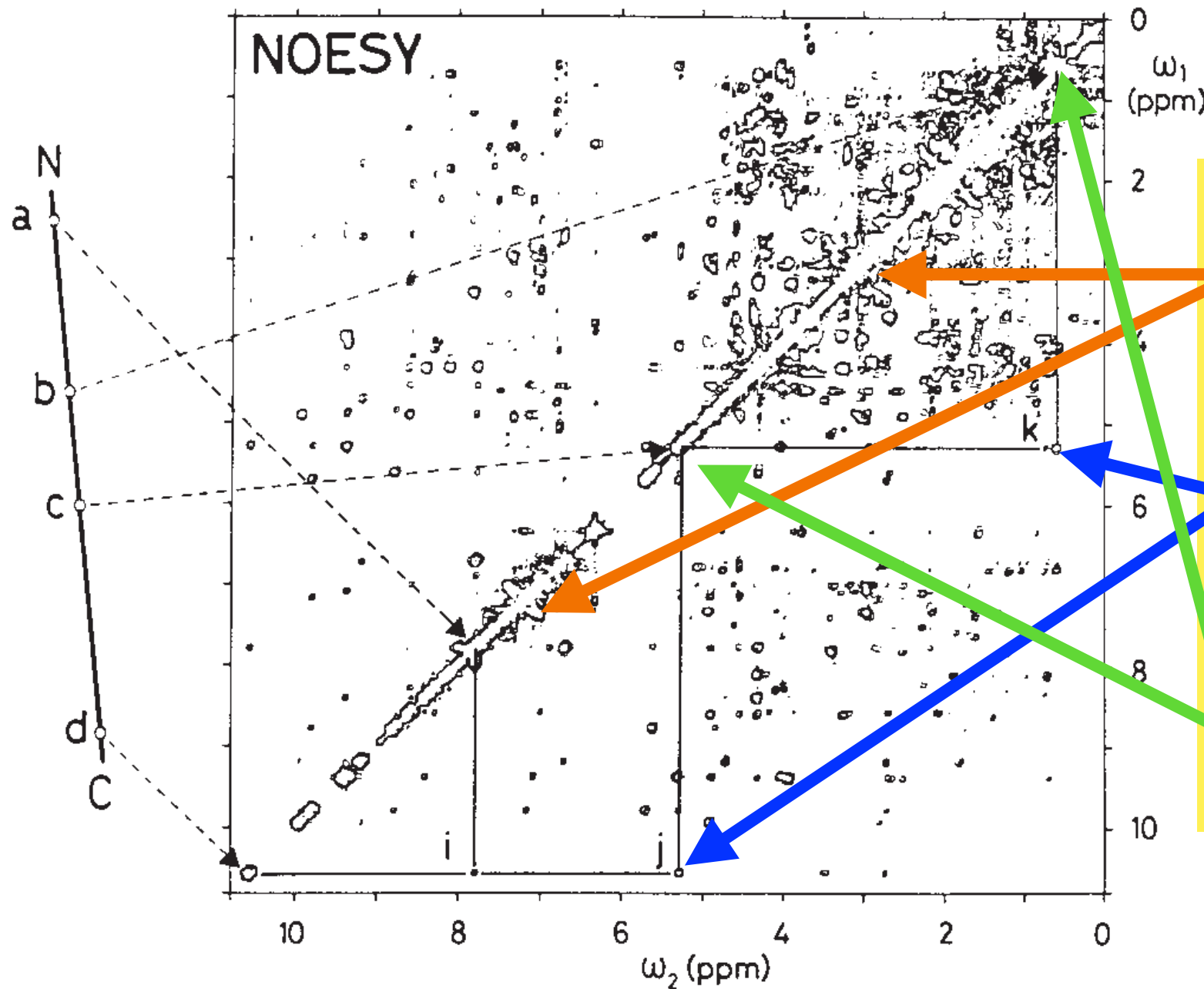
H_b is a quartet because
they are coupled to three
 H_c , hence $3+1=4$ peaks



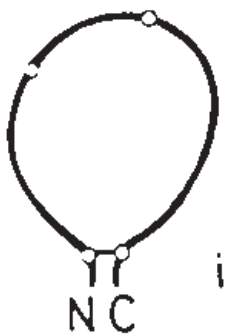
Protein Structures Can Be Determined by NMR

- A protein's conventional (one-dimensional) proton (^1H) NMR spectrum is crowded with overlapping peaks, since even a small protein has hundreds of protons.
- This problem is addressed by two-dimensional (2D) NMR spectroscopy, which yields additional peaks arising from the interactions of protons that are $< 5 \text{ \AA}$ apart.
- Correlation spectroscopy (COSY) provides interatomic distances between protons that are covalently connected through one or two other atoms, such as the H atoms attached to the N and C_α of the same amino acid (corresponding to the ϕ torsion angle).
- Nuclear Overhauser spectroscopy (NOESY) provides interatomic distances for protons that are close in space, although they may be far apart in the protein sequence.

NOESY spectrum of a protein

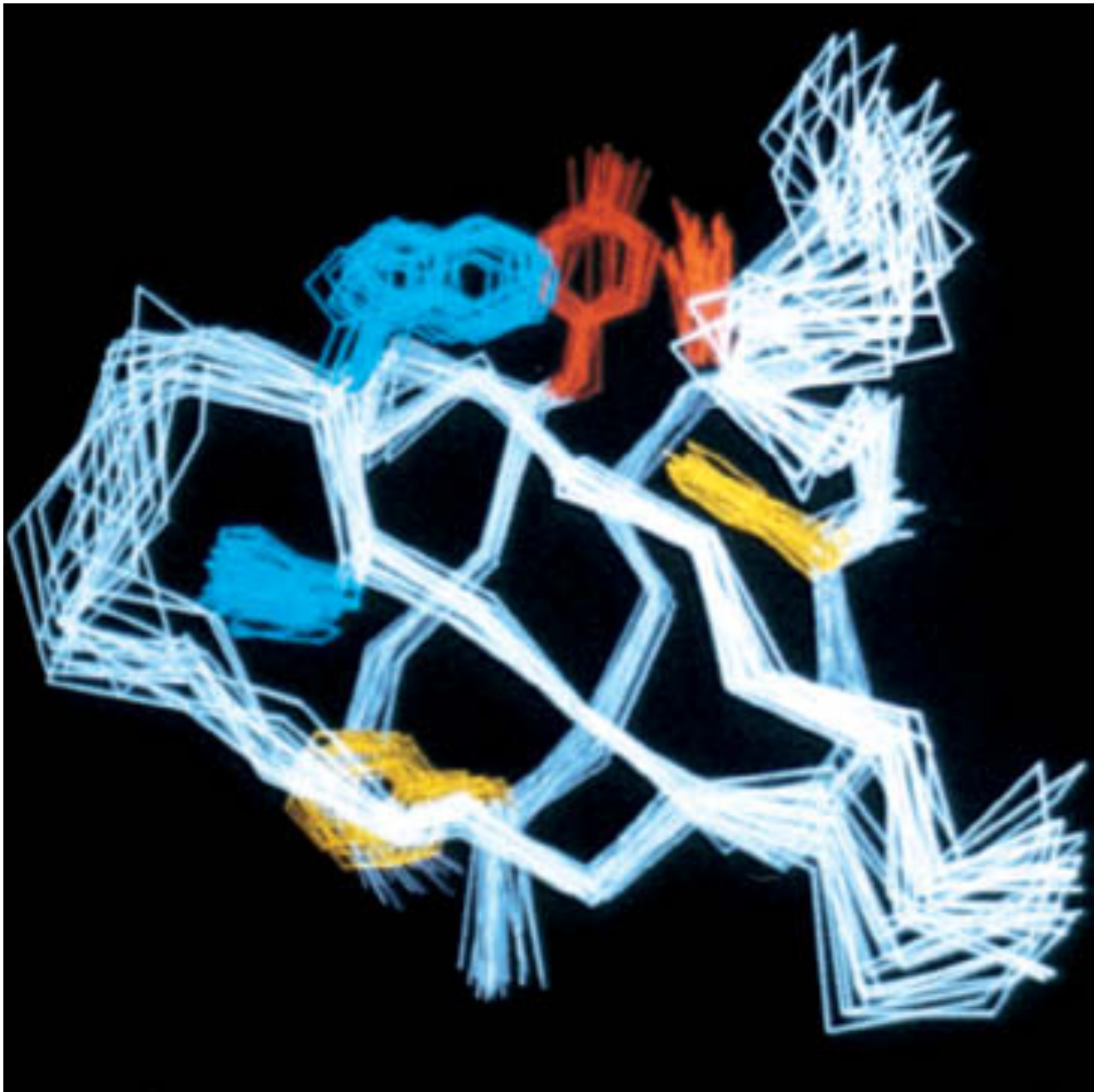


- The diagonal represents the conventional 1D NMR spectrum presented as a contour plot.
- The cross (off-diagonal) peaks each arise from the interaction of two protons that are $< 5 \text{ \AA}$ apart in space.
- Their 1D NMR peaks are located where horizontal and vertical lines intersect the diagonal.



NMR structure of a protein represents an ensemble of structures

Courtesy Stuart Schrieber, Harvard University



- Inter-proton distance measurements are imprecise
- So they cannot imply a single structure rather a set of closely related structures
- NMR structure of a protein is presented as an ensemble of structures that best represent the data
- The ‘tightness’ of a bundle of structures shows how accurate the measurement of structure is.
- Typically, good NMR structures are comparable to X-ray crystal structures of resolution $\sim 2\text{-}2.5 \text{ \AA}$.
- NMR structures of proteins $> 30 \text{ kD}$ are determined by multidimensional NMR techniques replacing the common C and N atoms by ^{13}C and ^{15}N .