# Nuclear Magnetic Resonance

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NMR is a spectroscopic technique that exploits the magnetic properties of nuclei. It detects the change in nuclear spin energy in the presence of an external magnetic field as a result of absorption of electromagnetic radiation in the radio-frequency region. Its prominence as a biophysical technique lies in its ability to reveal the atomic structure of macromolecules in solution, provided that highly concentrated solutions (1 mM, or 15 mg ml-1 for a 15-kD protein) can be obtained. NMR depends on the fact that certain atomic nuclei are intrinsically magnetic; only a limited number of isotopes relevant to biochemistry display this unique property, known as *spin* ($^{1}H$, $^{13}C$, $^{14}N$, $^{31}P$ etc.). Protons, electrons and neutrons possess a property called spin. Spin is expressed in multiples of 1/2 and can be + or –. Some atomic nuclei also have spin. If a particular nucleus is composed of p protons and n neutrons, its total mass is $p + n$, its total charge is +p and its total spin will be a vector combination of p + n spins each of magnitude 1/2. If the number of both the protons and neutrons in a nucleus is even, then there is no overall spin. All nuclei with an even mass number (total number of protons and neutrons in the nucleus) and an even atomic number (number of protons in the nucleus) have thus a nuclear spin of zero. Any atomic nucleus that possesses either odd mass number, odd atomic number or both will possess a spin value.

**Table 1. The atomic number, number of neutrons and spin quantum number of some elements**

| Element | $^{1}H$ | $^{12}C$ | $^{13}C$ | $^{14}N$ | $^{15}N$ | $^{16}O$ | $^{19}F$ | $^{31}P$ |
|---|---|---|---|---|---|---|---|---|
| Atomic number | 1 | 6 | 6 | 7 | 7 | 8 | 9 | 15 |
| Number of neutrons | 0 | 6 | 7 | 7 | 8 | 8 | 10 | 16 |
| Spin quantum number | ½ | 0 | ½ | 1 | ½ | 0 | ½ | ½ |

The spinning of a proton generates a magnetic moment. This moment can take either of two orientations or spin states (called α and β), upon application of an external magnetic field. The energy difference between these states is proportional to the strength of the imposed magnetic field. The α state has slightly lower energy and hence is slightly more populated because it is aligned with the field. A spinning proton in the α state can be raised to an excited state (β state) by applying a pulse of electromagnetic radiation of radio wave frequency (RF, pulse), provided the frequency corresponds to the energy difference between the α and the β states. Radiowaves flick the nucleus from the lower energy state to the higher state; the spin orientation will change from α to β; i.e., resonance will be obtained. The nucleus, now eager to return to the lower, more stable energy state; in doing so energy will be dissipated again and this is what is detected by the spectrometer. A resonance spectrum for a molecule can be obtained by varying the magnetic field at a constant frequency of electromagnetic radiation or by keeping the magnetic field constant and varying electromagnetic radiation.

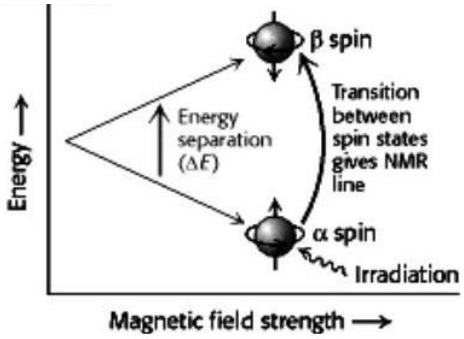


**Figure 1. The two allowed spin states for a $^{1}H$ nuclei**

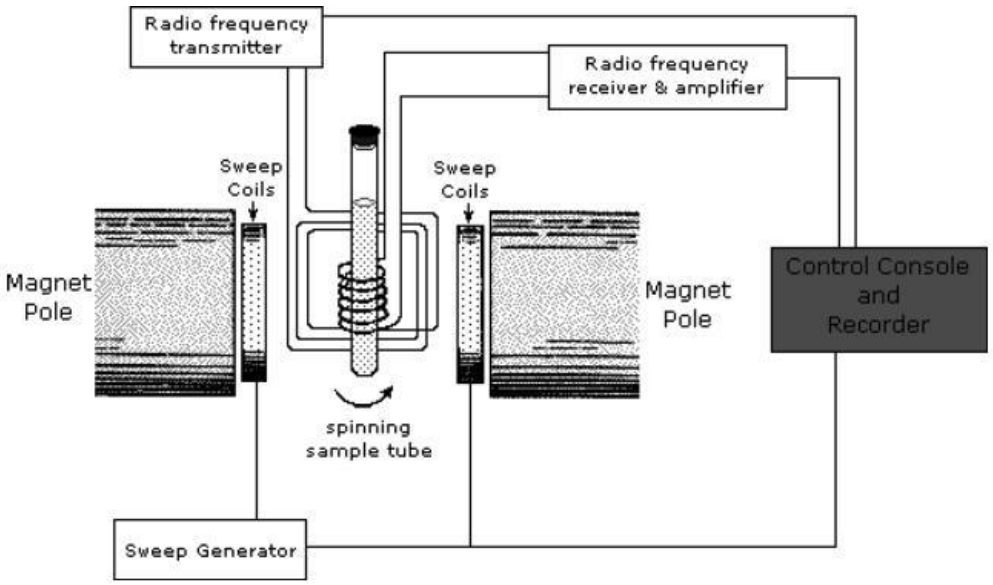


**Figure 2. Working model for NMR Spectroscopy**

Now in an applied magnetic field, not all atoms like for e, g. hydrogens and carbons in an ethanol or propanol molecule resonate at exactly the same frequency. This variability exists due to the fact that the hydrogens and carbons in a molecule are surrounded by electrons and exist in minutely different electronic environments from one another. The flow of electrons around a magnetic nucleus generates a small local magnetic field that opposes the applied field (diamagnetic anisotropy). Each nucleus is surrounded by electrons, and in a magnetic field these will set up a tiny electric current. This current will set, up its own magnetic field, which will oppose the magnetic field that we apply. The electrons are said to shield the nucleus from the external magnetic field. If the electron distribution varies from say, $^{13}C$ atom to $^{13}C$ atom in an ethanol molecule, so does the local magnetic field, and so does the resonating frequency of the $^{13}C$ nuclei. Thus, the changes in the distribution of electrons around a nucleus effect:

I. The local magnetic field that the nucleus experiences.

II. The frequency at which the nucleus resonates.

III. The chemistry of the molecule at that atom.

This variation in frequency is known as the *chemical shift* and it is denoted by δ. The chemical shift of a nucleus depends on many factors, but the surrounding electron density is often the dominant one. A high electron density causes a large shielding effect. Let us consider ethanol; the carbon attached to the -OH group will have relatively fewer electrons around it compared to the other carbon (oxygen atom is more electronegative and draws electrons towards it, away from the carbon atom). The external magnetic field that this carbon nucleus feels will, therefore, be slightly greater than the felt by the other carbon with more electrons. Since this carbon is less shielded (deshielded) from the applied external magnetic field, it feels a stronger magnetic field and there will be a greater energy difference between the two energy states of the nucleus. The greater the energy difference, the higher is the resonance frequency. So, for ethanol, it is expected the carbon with the OH group attached to resonate at a higher frequency than the other carbon; the same is revealed by the $^{13}C$ NMR spectrum.

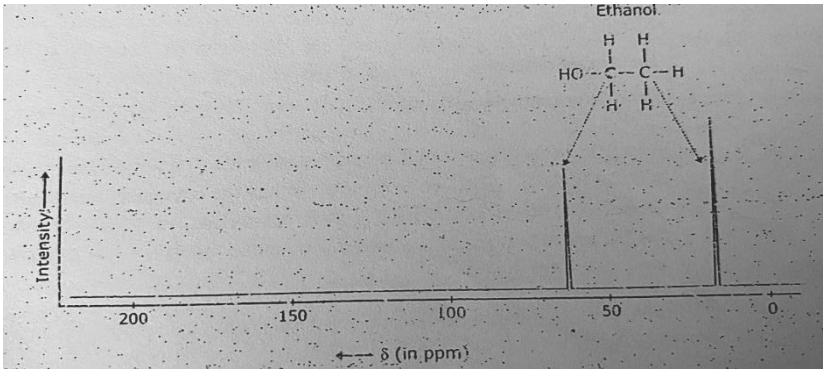


**Figure 3. A typical NMR spectrum for ethanol**

These different frequencies, termed chemical shifts, are expressed in fractional unit $\delta$ (parts per million, or ppm) relative to the shifts of a reference compound, such as a water-soluble tetramethylsilane (TMS), that is added with the sample. TMS is simply a silane ($SiH_4$) derivative with each of the hydrogen atoms replaced by methyl groups to give $Si(CH_3)_4$. Because of molecular symmetry, all 12 protons of TMS absorb at the same frequency and all 4 carbons absorb at the same frequency. The frequency of absorption for a nucleus of interest is measured relative to the frequency of absorption of a TMS standard. For instance, the chemical shift of the $^1H$ nuclei in the $^1H$ NMR Spectrum or $^{13}C$ nuclei in the $^{13}C$ NMR spectrum of TMS appears at = 0 ppm. Typically, it increases from 0 on the right-hand side of the spectrum to 10 ppm on the left-hand side of a $^1H$ NMR spectrum or from 0 on the right-hand side to 200 ppm on the left-hand side of a $^{13}C$ NMR spectrum. The reason frequencies of absorption are recorded on the δ-scale relative to those of a standard molecule since it makes the position of absorption independent of the spectrometer used to record the spectrum independent of the strength of the magnetic field of the spectrometer.

Generally, a -CH3 proton typically exhibits a chemical shift (δ) of 1 ppm, compared with a chemical shift of 7 ppm for an aromatic proton. The chemical shifts of most protons in protein molecules fall between 0 and 9 ppm.

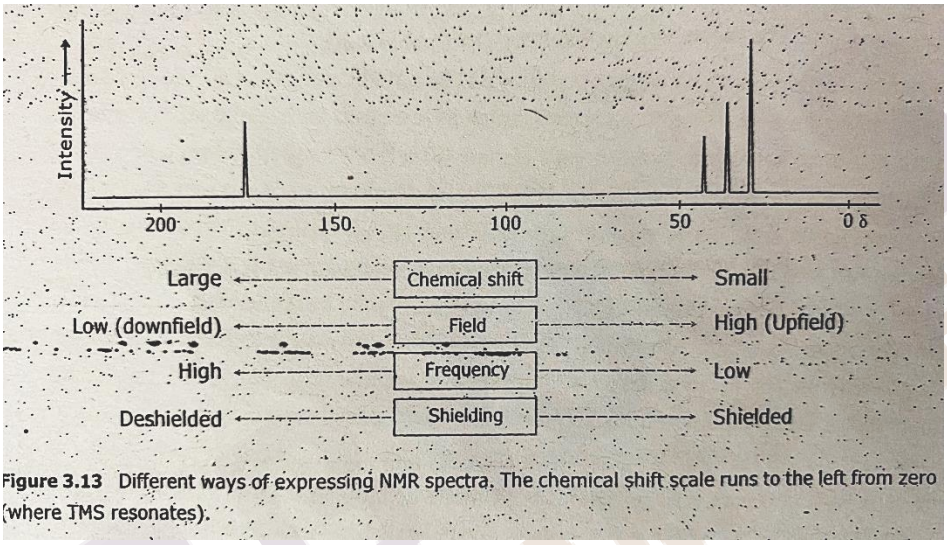


Figure 3.13 Different ways of expressing NMR spectra. The chemical shift scale runs to the left from zero (where TMS resonates).

**Figure 4. Expression of parameters associated with NMR Spectra**

Apart from this standard *One Dimensional NMR* with which most protons in a variety of protein samples can be resolved. Furthermore, sufficiently greater information can also be extracted by examining how the spins on different protons affect their neighbours. This is made possible by inducing a transient magnetization in a sample through the application of a radio-frequency pulse, which will alter the spin on one nucleus and hence, enable examination of the effect on the spin of a neighbouring nucleus. It reveals a **two- dimensional spectrum** obtained by Nuclear Over-Hauser Enhancement Spectroscopy (NOESY), which graphically displays pairs of protons that are in close proximity, even if they are not close together in the primary structure. The basis for 2-D NMR is the Nuclear Over Hauser effect (NOE), an interaction between nuclei that is proportional to the inverse sixth power of the distance between them. Magnetization is transferred from an excited nucleus to an unexcited one if they are less than about 5 Å apart. To sum up, the NOE effect thus provides a means of detecting the location of atoms relative to one another in the three-dimensional structure of the protein.

**Applications**

- Study of molecular structure.
    - Interactions between proteins and lipid bilayers in membranes. The structure of certain membrane proteins has been related to their predicted biological function, examples of such proteins include gramicidin A, bacteriorhodopsin and rhodopsin, phage coat proteins and alamethicin.
    - Structural studies of nucleic acids, DNA and RNA. Investigations of interactions between various drugs and DNA and between binding proteins and DNA.
    - Peptide and protein structural studies, e.g.lac repressor, antiviral proteins, etc.

- Deduce changes to a particular chemical group under different conditions, such as the conformational change of a protein from a disordered structure to an α helix in response to a change in pH.
    - Protein folding studies e.g. ribonuclease A, cytochrome c, bamase, α-lactalbumin, lysozyme, ubiquitin and Bovine pancreatic trypsin inhibitor (BPTI).
    - NMR advantage- useful in studying molecular behaviour in solution. Produces more useful information than the constrained structures available from X-ray crystallographic studies.
- Investigate certain types of kinetic changes
    - Study of enzyme kinetics both in vivo and in vitro. The groups of enzymes studied include chymotrypsin, trypsin, papain, pepsin, thermolysin; adenylate, creatinine and pyruvate kinases; alkaline phosphatase, ATPase and ribonuclease. Other examples are glycogen phosphorylase, dihydrofolate reductase and triosephosphate isomerase.
- Drug metabolism studies
    - Molecular modelling (in combination with X-ray diffraction data) to elucidate drug action.
- Isotope $^{31}P$ has been used extensively in studies of phosphate metabolism. The relative and changing concentrations of AMP, ADP and ATP can be measured and hence their metabolism is studied in living cells and tissues.
- Magnetic Resonance Imaging (MRI) used for the diagnosis and evaluation of diseases is based on NMR spectroscopy principles.

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