NMR in structural determination of proteins and peptides

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Abstract
Nuclear magnetic resonance (NMR) spectroscopy is one of the key techniques which gives vital information on structure determination of compounds. The NMR technique has played an instrumental role in the structural elucidation and characterization of proteins and other macromolecules. The major benefits with NMR for structure determination is in the ability to examine the biological macromolecule in solution, in its natural environment. The present article gives an insight in understanding the NMR of amino acid residues, spin systems in amino acid residues and how the NMR works for characterization of peptides. The detailed procedure of two dimensional NMR of peptides and the associated experimental schemes are explained. With the recent advances the 2D correlation spectroscopy (COSY) and 2D total correlation spectroscopy (TOCSY) NMR spectroscopy has newer ways to explain the structure determination. The article gives substantial information on the inter-nuclear distances of nuclear Overhauser effect (nOe) experiments. Important contexts related to NMR such as rotating-frame Overhauser effect spectroscopy (ROESY) and resonance assignment and structure determination in protein is explained.

Keywords: NMR, Spectroscopy, Proteins, Peptides, TOCSY

Introduction

NMR of biomolecules
To understand the function of biological macromolecules, it is important to understand their three dimensional structure. Nuclear magnetic resonance (NMR) spectroscopy is an important method for structure determination. During the last three decades several new methods have been developed which allow the determination of the structure of proteins and other macromolecules. The main advantage of using NMR for structure determination is the ability to investigate the biological macromolecule in solution, in its natural environment. This is an important complement to the other established methods for structure determination X-ray crystallography, where the molecules to be characterized are in the solid state. As an alternative to X-ray crystallography NMR has some distinct advantages.

1. There are several proteins without a crystal structure. They cannot be analyzed by XRD.

Even when crystal structure is available, it may be difficult to get satisfactory XRD patterns. As NMR studies does not require crystalline samples these problems can be avoided.

2. NMR allows us to study the behavior of peptides such as denaturation, change in conformation, and internal mobility of proteins by varying the solution conditions (e.g., pH, temperature, ionic strength, buffers and solvents).

3. NMR provides a direct and quantitative measurement of the frequencies at high activation energy motional processes which is pivotal in studying the internal dynamics of the structure of a macromolecule.

4. NMR can be employed for studying the exchange rates of labile NH protons, thus potentially elaborating a direct comparison between molecular dynamics in its different states[1].

Sequence specific resonance assignment is the initial and important step in the determination of protein structure, which relies entirely on the
knowledge of the amino acid sequence. The multidimensional NMR spectra are mapped onto the known amino acid sequence. For larger proteins, 3D and 4D heteronuclear NMR experiments are preferred. Currently 2D-NMR techniques on proteins are capable of assigning resonances of proteins with about 300 amino acid residues with high resolution. The structure of proteins containing more than 300 residues can be successfully determined by higher dimension NMR techniques like 3D and 4D-NMR methods.

In a high-resolution NMR experiment, a glass tube containing a solution (molecule of interest) is placed in a static magnetic field \( B_0 \) and is subjected to irradiation by one or several irradiation frequency (rf) fields, \( B_1, B_2, B_3 \). The nuclear spins are polarized under static magnetic field, causing macroscopic magnetization \( M_z \) in the direction of \( B_0 \). A radiofrequency with a suitably chosen duration (e.g. 90 pulse) thus rotates \( M_z \) into the \( x, y \) plane perpendicular to \( B_0 \). The transverse magnetization in the \( xy \) plane operates under the guidance of \( B_0 \) at a resonance frequency (or Larmor frequency) \( v_0 \) and brings an electric current in a detection coil, which is the primary observation in NMR experiments. Since the system tends to return to the thermodynamic equilibrium after precessing at its Larmor frequency, the magnetization once again tries to orient parallel to \( B_0 \) and the transverse magnetization decays with time. The corresponding free induction decay (FID) is recorded during a period of approximately 1s, and the NMR spectrum is obtained by Fourier transformation of these data [1].

The different parameters are as follows.

A. Chemical shift: The magnetic field in the nucleus is modified by magnetic field generated by bonding electrons, which is called the chemical environment. This slightly affects the resonance frequency of the atom in a molecule. The resonance frequency is dependent on the position of the atom within the molecule. This indirect variation, on the order of one part in a million, is called the chemical shift and provides detailed information about the structure of molecules. The chemical shift is represented in parts per million (ppm).

B. Spin-coupling constants (J): The proton attached next to the carbon in the chain affects the proton NMR spectrum. The NMR signal splits into a multiple peak pattern under the influence of its neighboring protons. These protons are three bonds away from the proton being considered and are sometimes called “vicinal” protons. The coupling constants can be used to calculate the dihedral angle \( \phi \) using the Karplus equation.

C. The NMR line intensity is a reflection of the relative intensities of different resonance lines indicating number of nuclei manifested by these lines.

D. The nuclear Overhauser effect (nOe): The nOe is the result of direct through-space interaction between two nuclei, separated by distance not more than 5 Å. When one nucleus is irradiated with a weak radio frequency at its resonance frequency, the effect is to equalize the population of the nucleus in its two energy levels. This in turn, results in the variation of the intensity of absorbance at resonant frequency of a nearby nuclei. The effect depends on the distance between the nuclei, even if they are far apart in the bonding network. These measured distances are used to determine accurate three-dimensional structures of proteins and nucleic acids.

E. The longitudinal relaxation time or spin-lattice relaxation time (\( T_1 \)): It is a time constant, characterized by spin lattice relaxation time. In NMR, the mechanism by which the component of the magnetization vector along the direction of the static magnetic field reaches the thermodynamic equilibrium with the surroundings (lattice) is called as longitudinal relaxation.

F. The transverse relaxation time or spin-spin relaxation time (\( T_2 \)): The rate of the decay of the magnetization is quantified by this term. The nuclear spins are aligned in one direction (are said to be phase coherent) after a 90° pulse, which is lost gradually (e.g., due to field in homogeneities and/or direct interactions between the spins without energy
transfer to the lattice). \( T_1 \) and \( T_2 \) for small and large molecule is given in Figure 2.

G. The resonance linewidth determines the spectral resolution that can be attained at a given field strength \( B_0 \). Assuming a Lorentzian line shape, the linewidth is customarily presented as the half-width at half height of the line.

H. NMR time scales provides information about the dynamic processes and is largely determined by the spectrometer used, notably by the field strength \( B_0 \). Molecular rate processes with characteristic times in the range from approximately \( 10^7 \) to \( 10^{12} \) s are thus directly manifested in the relaxation parameters \( T_1 \) and \( T_2 \) (Figure 3).

NMR of amino acid residues

The residues of biopolymers were widely studied by NMR in the last three decades. The isotopes with the spin \( \frac{1}{2} \) are visible by NMR. The important parameter of these isotopes is given in Table 1.1. Study of most of the proteins are based on the observation of \( ^1H \) resonances, thus properties of this isotope are extensively discussed here. \( ^13C \) and \( ^15N \) have a low natural abundance but they provide opportunity for site specific NMR assignment using isotope labels. \( ^31P \) may be employed to study nucleic acids [1].

A. Spin systems in amino acid residues

To understand and analyze a protein NMR spectrum the reference chemical shifts of individual amino acids in random coil peptides are used (Table 3). The NMR spectrum contains the information of carbon bound hydrogens only, as the labile protons get exchanged by deuterium in D\(_2\)O solution. The spin system of amino acids is a group of spins that are connected through scalar spin-spin coupling [1]. To describe the spin systems, the notation adopted by Pople et al is followed. The characteristics of amino acids in the fingerprint region are given in Table 3.

B. NMR of Peptides in solution

Proteins contains a high number of resonances resulting in crowding in the NMR spectrum. The spectrum can be badly crowded depending on the size of the protein. For proteins up to 300 amino acids, 2D-NMR techniques can be successfully used to assign the structure, proteins with size higher than this can be assigned only by applying 3D and 4D-NMR heteronuclear techniques. The molar concentration of protein of interest should be not less than 1 mM, ideally 3-6 mM, the short relaxation time of proteins results in broadening of NMR lines compared to small molecules [2]. This can also be the result of aggregation in peptides due to viscosity, which is common in concentrated solution of proteins. Side chains in the extended polypeptide are exposed to the same solvent environment, so identical chemical shifts can be seen for multiple copies of amino acids. \(^1H \) NMR line of the amino acid side chains of denatured protein resemble highly the resonances in the principal amino acid residues. This demonstration was made by McDonalds and Philips et.al. The residues in the protein are affected by the environment such as solvent and nearest residues, which results in dispersion of the chemical shift, so different amino acid residues experience different microenvironments [1].

C. Two Dimensional NMR of Peptides

Basic information such as the chemical shift and spin-spin coupling of the individual resonances in the spectrum can be easily obtained from a normal 1D-NMR experiment. Additional information on through-bond, through space, dipolar connectivities between individual spins, which is the basis for resonance assignment and conformational studies in proteins can be acquired using homonuclear as well as heteronuclear multidimension NMR experiments, based on the size of protein as described earlier.

Using 2D-NMR techniques, the limitation of 1D-NMR, which is an overcrowding of the peaks, can be nullified. In 2D-NMR, as in 1D-NMR, the FID is recorded during the detection period \( t_2 \) after the observation pulse. Additional non-selective pulses are applied before the observation pulses. By repeating the experiment with incrementation of \( t_1 \), an additional time dimension can be
generated. For each value of \(t_1\), an FID recorded against \(t_2\) is stored so that a data matrix \(s(t_1, t_2)\) is acquired. A 2D FT of \(s(t_1, t_2)\) is then Fourier transformed into the desired 2D frequency spectrum \(S(\omega_1, \omega_2)\) [1].

**Experimental schemes for 2D-NMR Spectroscopy**

A common scheme for 2D-NMR comprises four consecutive time periods preparation, evolution, mixing and detection (Figure 4).

1. Preparation period: Generating magnetization in the \(x-y\) plane by exciting nucleus \(A\).
2. Evolution period: Measuring the chemical shift indirectly for nucleus \(A\).

**2D Correlation Spectroscopy (COSY)**

It is homonuclear spectroscopy that is used to identify spins that are coupled to each other. The pulse train (Figure 5) for 2D COZY is just \(90^\circ - t_1 - 90^\circ - FID\). It relates 1 proton (Ha) with additional (Hb) by a sole J-coupling, which can be 2 bonds (geminal), 3 bonds (neighboring), or in rare cases 4 or 5 bonds (longer range). Consider the interaction of two J-coupled protons, Ha and Hb, in which the preliminary pulse revolves the Ha magnetization from the \(z\)-axis to the \(x-y\)-plane. The Ha magnetization takes place in the rotating frame with a speed that depends on its displacement of the chemical shift \(\Omega a\) during the evolution period \(t_1\). Simultaneously, Ha magnetization due to J-coupling evolution occurs, which is out of phase with its J-coupling with Hb. The Hb magnetization takes place at its characteristic rate \((\Omega b)\) in the rotating frame and induces a voltage in the probe coil during the detection period (FID). The FT in F2 and then in F1 leads to a 2D data matrix with a cross peak at \(F_1 = \Omega a, \quad F_2 = \Omega b\) [3].

**2D TOCSY (TOTAL CORRELATION SPECTROSCOPY)**

It is useful to identify the larger interconnected spin systems. A repetitive series of pulses are inserted during the mixing period which causes isotropic mixing, longer isotropic mixing times results in polarization that extends through increasing number of bonds. The pulse sequence is similar to the COSY, but instead of the second \(90^\circ\)-pulse a spin lock sequence is used. A more refined sequence is the MLEV-17 sequence, which is assembled from combination of \(90^\circ-x-180^\circ-y-90^\circ-x\) pulses (R) and the inverted sequence \(90^\circ-x-180^\circ-y-90^\circ-x\) \((r)\) in a cycle of RRrr rRRr rrRR RrrR. Spectrum. When the 2D-TOCSY is compared to a COSY spectrum, the sequence is finished with a \(60^\circ\)-pulse to invert possible phase errors and repeated to ensure a coherence transfer over the whole spin system [3].

**D. Nuclear Overhauser Enhancement in peptides**

The data on inter-nuclear distances are mainly obtained by nuclear Overhauser effect (nOe) experiments. This can be correlated directly with molecular conformations. These can be more directly correlated with the molecular conformation or with the structure of multi-molecular complexes. In 2D NOESY experiments (Figure 8) the nOe are expressed by cross peaks in \((\omega_1, \omega_2)\).

The equation given below represents the relation between nOe intensity to the distance \(r\)

\[\text{nOe} \propto 1/(r^{6}).f(\tau_c)\]

Where \(\tau_c\) is a function of the correlation time.

**2D Rotating-Frame Overhauser Effect Spectroscopy (ROESY)**

NOESY experiments perform with both very low and high molecular weight molecules. NOESY fails with molecules with molecular weights of around 1000 - 2000 g / mol at typical field strengths, since the nOe for these molecules are very close to zero. Both NOESY and ROESY
give very similar results for high molecular weight molecules [3].

In ROESY experiment, homonuclear nOe effects are measured under spin-lock conditions. In such circumstances the nOe is close to zero, however the rotating-frame nOe (or ROE) is always positive and shows subsequent increase with increase in the values of τc. In ROESY the spin-lock period is the mixing time. The exchange occurs between spin-locked magnetization components of different nuclei during this time spin.

Distance Measurement

During mixing, the initial rate of increase in nOe intensity is directly proportional to $1/r_{ab}^6$, where $r_{ab}$ is the distance between Ha and Hb. In a NOESY spectrum. There are multiple limitations in measuring distances using cross-peak intensities. First, calibration of cross peak intensity is required with a pair of protons (or even better, several pairs) with a precisely known distance within the molecule as these cross peak intensities (volumes) are given in arbitrary units. Second, isolated spin pair hypothesis which means an assumption that there are only two nuclei, Ha and Hb, linked by a nOe interaction. In reality, there are other neighboring protons within 5 Å. This in turn results to a process called spin diffusion, in which a perturbation of the populations ($z$-magnetization) of one nucleus affects the populations of neighboring nuclei, which in turn disrupts the populations of their neighbors in an expanding process.

E. Resonance assignment and structure determination in protein

NOE-Observable $^1$H-$^1$H Distances in Proteins

Short $^1$H-$^1$H distances obtained by nOe measurements provides a basis for conformational studies of proteins. (Figure 11)

\[
\begin{align*}
\delta_{aN}(i,j) &= d(\alpha H_i ,\alpha H_j) \\
\delta_{NN}(i,j) &= d(NH_i,NH_j) = d_{NN}(j,i) \\
\delta_{NN}(i,j) &= \min\{d(\beta H_i,NH_j)\}c \\
\delta_{aa}(i,j) &= d(\alpha H_i,\alpha H_j) = d_{aa}(j,i) \\
\delta_{ab}(i,j) &= \min\{d(\alpha H_i,\beta H_j)\}
\end{align*}
\]

Sequential distances are between backbone protons or between a backbone proton and a β proton in residues that are adjacent neighbors in the amino acid sequence. i+2, i+3, i+4 and are collectively called as medium range distances. nOes connecting residues separated by more than 5 residues are long-range backbone distances.

Sequential $^1$H- $^1$H Distances

The sequential distances $\delta_{aN}$, $\delta_{NN}$ and $\delta_{a\beta}$ depend on one or two intervening torsion angles about single bonds. For L-amino acids, $\delta_{aN}$ varies between approximately 2.20 and 3.55 Å for $\phi$ values between –180 and 180°. For β pleated sheets, $\delta_{aN}$ is close to the minimum value of 2.2 Å, for a regular α helix it is approximately 3.5 Å. Depending on the intervening backbone torsion angles $\phi$ and $\psi$, $\delta_{NN}$ varies between the van der Waals limit of 2.0 Å and approximately 4.7 Å. In the sterically allowed region A of the ($\phi$ and $\psi$) plane (Figure 12), $\delta_{NN}$ is shorter than 3.0 Å, with a value of 2.8 Å for the regular α helix. In region B, $\delta_{NN}$ varies between 2.0 and 4.8 Å, with values of 4.3 and 4.2 Å for the antiparallel and parallel β structures, respectively. In region C, $\delta_{NN}$ covers the range 2.5-3.5 Å (Figure 11) [1].

Identification of the Amino Acid $^1$H Spin Systems

Identification of individual amino acids generally starts with a COSY or a TOCSY spectrum in deuterated solution, in which all the labile protons are replaced by deuterium. Normally, for a protein with small size such TOCSY spectra. All 20 amino acids have unique geometric patterns which do not match with each other.
Sequential Assignments via $^1$H-$^1$H Overhauser Effects

The information required to obtain the sequential connectivities is obtained from analysis of the regions containing the NH-$\alpha$H, NH- NH, and NH-$\beta$H cross peaks in NOESY spectra recorded in H$_2$O. Both NOESY (and ROESY) and TOCSY, and suitable combination and a visual display of information of through-space and through-bond connectivities is crucial. All three sequential connectivities comprise at least one potentially exchangeable amide proton. A complete set of sequential assignments can therefore only be obtained from NOESY or ROESY spectra recorded in H$_2$O under conditions of pH and temperature[1].

F. Secondary structure of peptide by NMR

After successful sequence specific resonance assignment, further measurements, such as nOe's, spin-spin coupling constants and amide proton exchange rates are required to be measured to specified locations in the sequence. Specific patterns of NMR parameters in the polypeptide chain is then representative of particular secondary structures. The data thus obtained may be used as a preliminary point for resolving the tertiary structure [1].

Secondary structure prediction by spin-spin couplings $^3$J$_{\text{HN-H\alpha}}$

the conformation of the peptide can be identified by finding the $^3$J$_{\text{HN-H\alpha}}$ coupling constant which is a function of the dihedral angle $\phi$ [1]. The Karplus equation is used to calculate the dihedral angles $\phi$ from the $^3$J$_{\text{HN-H\alpha}}$ values [4, 5]. The size of the spin-spin coupling constant $^3$J and the intervening torsion angle $\psi$ are important factors for studies of molecular conformations (Table 5). Mostly the dihedral angles $\phi$, for all amino acid residues (in proteins) are in the range of -30 to -180° except glycine [1].

Helical structure shows a uniqueness of above 80%, attained with segments of three to five subsequent residues with $^3$J$_{\text{HN-H\alpha}} < 6.0$ Hz. In $\beta$ sheet structures uniqueness is lower, primarily because globular proteins comprise extended peptide segments also outside of $\beta$ sheets. $^3$J$_{\text{HN-H\alpha}}$ present supporting evidence for secondary structures identified by short $^1$H-$^1$H distances.

Hydrogen Bonds and Amide-Proton Exchange

The common polypeptides have characteristic formation of H-bonds between backbone amide proton and backbone carbonyl oxygen. The CO$_i$-…NH$_{i+4}$ and CO$_i$-…NH$_{i+3}$ bonds are formed in the $\alpha$ helix and the $3_{10}$ helix, respectively. However dense network of hydrogen bonds are formed in $\beta$ sheets between neighboring peptide strands. All amide protons are involved in hydrogen bonds in the regular secondary structures, with few exceptions like; the first four residues in a helix; the first three residues in a $3_{10}$ helix; every second residue in the peripheral strands of $\beta$ sheets [6-7].

Temperature coefficients of amide proton resonances

The chemical shifts are temperature-dependent therefore; Temperature coefficients of amide protons are prominent in the presence of an intramolecular hydrogen bond [8]. These Temperature coefficient values of the amide proton gives prediction of hydrogen bonding. Intramolecular hydrogen bonds usually gives Small values of the temperature coefficients (<3.0 ppb / K) in amide protons [9-11].

G. Three dimensional structure by NMR

The data obtained from the NMR measurements are the prime parameters to arrive at the 3D structure. A three-dimensional molecular model is constructed with the use of NMR constraints to determine the relative spatial locations of the secondary structure elements. A second method uses a mathematical technique called embedding, which generates spatial polypeptide structures that are an approximate fit to the ensemble of all conformational constraints gathered by the NMR data. This approximate fit is then further improved by numerical optimization. Both procedures can be supplemented with structure
refinement routines, for example, using classical techniques of energy minimization or molecular dynamics simulation. A third method uses restrained molecular dynamics calculations. Independent of the choice of technique for the structural analysis, the outcome of a structure determination will always depend primarily on the correct NMR assignments and the quality of the additional NMR data [1].

Molecular dynamics simulations usually estimate the instantaneous forces present in a molecular mechanical system by the repeated numerical calculation. The MM system comprise of a series of particles that move in response to their interactions according to Newtonian mechanics.

Molecular dynamics simulate a number of experimental conditions. Initially, the protein simulations [12] considers the molecules as isolated entities. However, later simulations involved explicit water and neighboring protein molecules in a crystal environment. After a certain period, a number of factors determines the length of a simulation viz the cost of evaluating interactions, the number of interactions, the length of that time step, and the number of degrees of freedom that must be propagated [13]. Various values for physical conditions such as pressure and temperature can easily be taken into account in the simulations [14].

Long-range electrostatic interactions play a dominant role in the stability of the protein structure and molecular associations. A theoretically rigorous approach for evaluation of electrostatic interactions in infinite periodic systems is the Ewald summation method.

The software packages that can be selected today are very different. Only a few research groups limit their simulations to a single software package. The commonly used software for structure generation by NMR are AMBER and GROMACS, as they easily allow one to apply the restraints in a molecular dynamic simulation. Both distance and dihedral restraints can be applied. AMBER also has features to generate the structure directly using the obtained peak volumes.

<table>
<thead>
<tr>
<th>Isotope</th>
<th>$\gamma \times 10^{-7}$</th>
<th>$\nu$ at 11.4 T (MHz)</th>
<th>Natural abundance</th>
<th>Sensitivity Rel$^a$</th>
<th>Abs$^b$</th>
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<td>13C</td>
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<td>$1.6 \times 1.8 \times 10^4$</td>
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<td>202.4</td>
<td>100</td>
<td>$6.6 \times 10^{-2}$</td>
<td>$6.6 \times 10^{-2}$</td>
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</table>

Table 1. Nuclear properties of selected isotopes important in NMR. $a$ is relative sensitivity at constant field for equal no of nuclei and $b$ is product of relative sensitivity and natural abundance.
Table 2. Chemical shift of individual amino acids.

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<th>Residue</th>
<th>NH</th>
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<th>2.13</th>
<th>1.90</th>
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<th>1.48, 1.19</th>
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Table 3. Characteristics of amino acids in fingerprint region.

<table>
<thead>
<tr>
<th>Amino acid residues</th>
<th>Characteristics in the fingerprint region of TOCSY spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly, Thr, Val, Ile, Leu, Ser, Asp, Asn, Cys, Trp, Phe, Tyr, His, Met, Gln, Glu, Lys, Arg, Pro</td>
<td>Possible doublet arising from non-degenerate Hα1, Hα2; Upfield single cross peak from HN-Hβ for Alal or HN-Hγ2 for Thr; H N-Hα-Hβ-Hγ-Hδ coupling system is observable with long mixing time. Val two γ-methyls observed around 0.9 ppm and one Hβ around 2.13 ppm; No Hγ proton; Hβ downfield shift of ~2.5 ppm; resonances arising from aromatic rings will normally need NOESY spectrum for assistance; Hβ upfield shift of ~2.5 ppm.; HN-Hγ where Hγ is upfield of Hβ; HN-Hγ where Hγ is downfield of Hβ; Lys, Arg; Pro; No HN correlation in TOCSY fingerprint</td>
</tr>
</tbody>
</table>
Table 4. The torsion angle $\varphi$ from the spin-spin coupling for secondary structure of peptides.

<table>
<thead>
<tr>
<th>secondary structure</th>
<th>$\varphi$ (in degrees)</th>
<th>$^3J_{\text{NH} \alpha H}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$ helix</td>
<td>-57</td>
<td>3.9 Hz</td>
</tr>
<tr>
<td>$3_{10}$ helix</td>
<td>-60</td>
<td>4.2 Hz</td>
</tr>
<tr>
<td>antiparallel $\beta$-sheet</td>
<td>-139</td>
<td>8.9 Hz</td>
</tr>
<tr>
<td>parallel $\beta$-sheet</td>
<td>-119</td>
<td>9.7 Hz</td>
</tr>
</tbody>
</table>

Figure 1. (A) NMR sample under magnetic field effect. (B, C) impact of magnetization leading to Decay of phase coherence.

Figure 2. Effect of molecular size on relaxation times $T_1$ and $T_2$. 
Figure 3. Protein dynamics—time scale and NMR experiments

Figure 4. Common pulse sequence for 2D-NMR.

Figure 5. Pulse sequence of COSY

Figure 6. A typical spectrum presenting COSY (blue colored) and TOCSY (orange colored) peaks.

Figure 7. 2D-TOCSY pulse sequence.

Figure 8. 2D NOESY pulse sequence.
Conclusion
The article reviews the basic principle involved in NMR spectroscopy to give relevant data on prime parameters to arrive at the 3D structure for bio macromolecules. The three-dimensional molecular model are used to determine the relative spatial locations of the secondary structure elements of these compounds.

Conflict of Interest
The authors declare no conflict of interest.

Disclaimer
The views, thoughts and opinions expressed in this review belong solely to the authors, and not necessarily to the author’s employer, organization, committee or other group or individual.
References