100 years of X-rays and 50 years of NMR

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Discovery of X-rays by W. C. Roentgen was the result of systematic probing into a chance observation. Ever since their discovery, one hundred years ago, X-rays have contributed significantly towards human health and scientific research. These contributions will continue to grow exponentially, with the development of synchrotron sources of X-rays and solid state detectors. Nuclear magnetic resonance (NMR), discovered 50 years ago independently by Bloch and Purcell, also has evolved tremendously since its discovery. The development of Fourier transform and multidimensional NMR has enabled structures of biological macromolecules to be determined in solution, thereby providing information complementary to that obtained from X-ray techniques. The techniques of magnetic resonance imaging and X-ray transmission computed tomography are proving invaluable in the diagnosis and treatment of human disease.

THE year 1995 marks the centenary year of discovery of X-rays and also the golden jubilee year of the discovery of NMR. The discovery of X-rays[1], on 8th November, 1895 was very sensational as it allowed one to picture the interior of one's own body, as shown in Figure 1 and was the result of systematic probing into a chance observation by Wilhelm Conrad Roentgen (Figure 2). While studying gas-discharge in a cathode ray tube, the colour-blind Roentgen observed that there was fluorescence recorded on a barium platinocyanide screen kept on a neighbouring table. The fluorescence which occurred every time there was a gas discharge could not be prevented by covering the packet in an opaque cover. The agents which caused fluorescence were emanating from the gas-discharge tube and they also affected photographic film in the same way as light radiation. Roentgen termed these mysterious rays 'X-rays' and within a week's time carefully measured many of their properties and established that they were refracted the least. The properties of high penetration, low refraction and fogging of pho-

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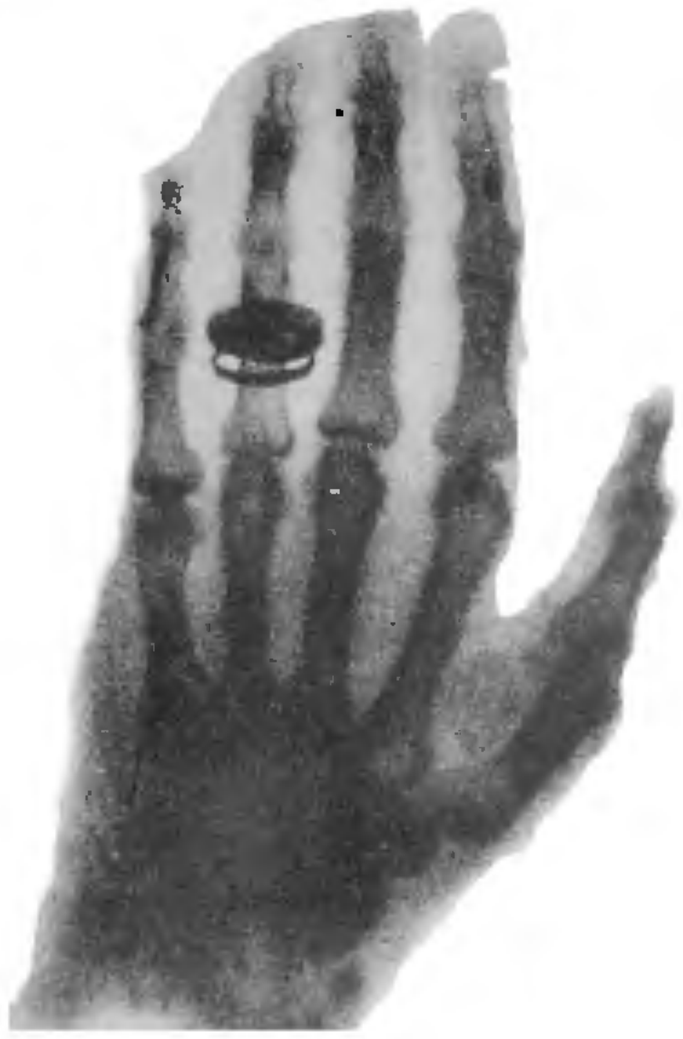

Figure 1. X-ray shadowgraph taken by Roentgen during the Wurzberg meeting where he announced his discovery of X-rays. (Source: *Radiat. Phys. Chem.*, 1995, **46**, 302.)

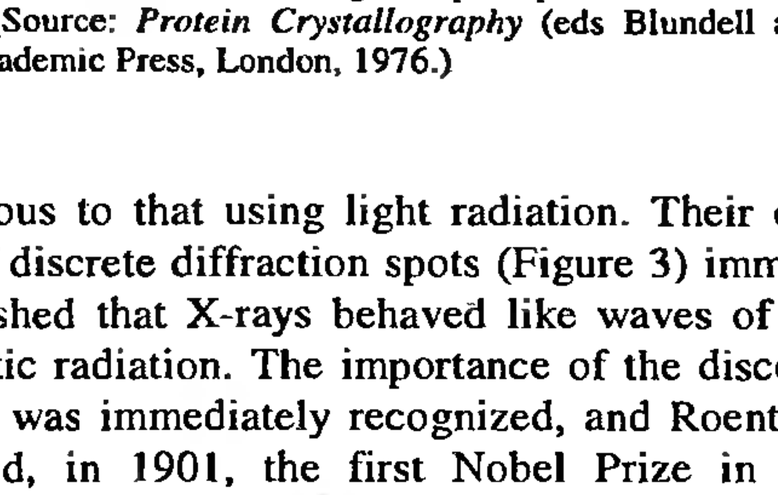

Figure 3. The first X-ray diffraction photograph recorded on samples of $CuSO_4.5H_2O$. The ring of spots proved that X-rays were waves. (Source: *Protein Crystallography* (eds Blundell and Johnson), Academic Press, London, 1976.)

Figure 2. Wilhelm Conrad Roentgen, the discoverer of X-rays was a colour-blind and shy person (Source: *Radiat. Phys. Chem.*, 1995, **46**, 299.)

tographic films suggested that these 'X-rays' were electromagnetic radiation of a low wavelength. The wave-like behaviour of X-rays, however, was confirmed when, realizing that crystalline substances were periodic arrangements of matter with very short periodicities, Max von Laue and his colleagues Friedrich and Knipping exposed crystals of $CuSO_4.5H_2O$ to these newly-discovered 'X-rays' to perform a diffraction experiment analogous to that using light radiation. Their observation of discrete diffraction spots (Figure 3) immediately established that X-rays behaved like waves of electromagnetic radiation. The importance of the discovery of X-rays was immediately recognized, and Roentgen was awarded, in 1901, the first Nobel Prize in physics, barely six years after the discovery of X-rays.

The year 1995 also marks the 50th anniversary of the discovery[2] of Nuclear Magnetic Resonance (NMR). The discovery was made independently by two groups, one led by Felix Bloch at Stanford and the other led by E. M. Purcell at Harvard. Bloch and his colleagues observed resonance absorption of energy by protons in water and called it nuclear induction, since the signal they detected was an induction signal due to precession of nuclear magnetic moments. On the other hand, Purcell's group observed resonance absorption of energy in solid paraffin, which they attributed to transitions between nuclear spin states rendered non-degenerate in the presence of an externally applied magnetic field. It became clear that both were essentially looking at the same phenomenon. While Bloch's interpretation was based on classical description of motion of nuclear spins in a magnetic field, Purcell invoked quantum mechanical principles, energy level diagrams, etc. Bloch and Purcell (Figure 4) shared the Nobel Prize in physics for this discovery in 1952. X-ray is a form of electromagnetic radiation which has led to development of important techniques using it and to many phenomena, while NMR is a phenomenon which also has been very valuable. Since their discoveries both have contributed towards such good scientific research that many Nobel Prizes have been won using these tools (Table 1). Techniques based on them have evolved over the years

a

b

Figure 4*a*, *b*. Discoverers of NMR, ***a***, F. Bloch and ***b***, E. M. Purcell. (Source: *Hinduja Foundation Encyclopedia of Nobel Laureates* (eds A. K. Barua *et al.*), Konark Publishers, New Delhi, 1988.)

and have often complemented each other in scientific research.

Evolution

X-ray techniques

The phenomena of X-ray absorption, X-ray scattering and/or X-ray diffraction are the basis of X-ray techniques developed for scientific research. The power and scope of each one of these techniques is enhanced manifold by progressive evolution in the technology of production and detection of X-rays. In the conventional laboratory X-ray generator, electrons accelerated across

Table 1. List of Nobel prizes won in the disciplines of X-rays or NMR

Year	Winner/s	Discipline
1901	W. C. Roentgen	X-ray, Physics
1914	M. T. F. von Laue	X-ray, Physics
1915	W. H. Bragg and W. L. Bragg	X-ray, Physics
1924	K. M. G. Siegbahn	X-ray, Physics
1927	A. H. Compton	X-ray, Physics
1952	F. Bloch and E. M. Purcell	NMR, Physics
1962	J. C. Kendrew and M. F. Perutz	X-ray, Chemistry
1962	F. H. C. Crick, M. H. F. Wilkins and J. D. Watson	X-ray, Medicine & Physiology
1964	D. C. Hodgkin	X-ray, Chemistry
1976	W. N. Lipscomb	X-ray, NMR, Chemistry
1979	A. M. Cormack and G. N. Hounsfield	X-ray, Medicine & Physiology
1985	H. A. Hauptman and J. Karle	X-ray, Chemistry
1988	R. Huber, J. Deisenhoffer and H. Michele	X-ray, Chemistry
1991	R. R. Ernst	NMR, Chemistry

a high voltage are brought to a halt at the anode. A small fraction (<0.5%) of the electron energy is converted to X-rays and the rest is dissipated as heat in the anode. Heating and vapourization of the anode sets a limit to the intensity of X-rays that can be produced by such generators. This limitation is removed in a synchrotron source where X-rays are produced when very high-energy (few GeV) electrons are momentarily deflected from their straight paths inside the storage ring. Over the last hundred years the spectral brilliance of X-rays produced has increased by about a trillion times and much of this increase has come about only in the last twenty years or so, with the development of three generations of synchrotrons. In fact, the spectral brilliance is doubling almost every two years for the past twenty years. In addition to brilliance, two other characteristics of synchrotrons make them presently the most sought-after source of X-rays for experimental research[3]: i) tunability of the maximum intensity wavelength within a short continuum and ii) very high degree of collimation. These features have enabled use of smaller and smaller sample sizes, going down to even a single molecule as in the case of X-ray microscopy. X-rays more intense than even the synchrotron radiation are produced recently by laser-driven plasmas[4] in compact table-top units. These X-rays however, have longer wavelengths and are emitted as single pulses of picosecond to nanosecond duration. The evolution in X-ray detectors has been in the form of an increased sensitivity, increased dynamic range, increased counting rate and accurate quantitative measurement of intensity. The organic and inorganic scintillators from Roentgen's times have been replaced by imaging plate systems containing memory phosphors and read-out lasers, and more recently by cooled charge

coupled detection devices combined with image intensifiers.

There have been parallel developments in the methodologies of different X-ray techniques. In the field of X-ray diffraction, the important contribution was made by the father and son team of W. H. Bragg and W. L. Bragg, when they explained that X-rays were diffracted along few specific directions simply because of interference between waves reflected from parallel lattice planes. They further predicted the directions of diffracted rays by a very simple law:

$$2d_{hkl} \sin\theta_{hkl} = \lambda,$$

where d_{hkl} is the perpendicular distance between lattice planes of Miller indices (h, k, l), $2\theta_{hkl}$ is the deviation of the diffracted beam from the incident beam, and λ is the wavelength of the incident X-rays. This explanation of the phenomenon of X-ray diffraction enabled determination of many simple crystal structures by trial and error methods, and thereby established (i) the different values of covalent, ionic and van der Waal's radii for different atoms, and (ii) the fact that two atoms in a solid or a molecule cannot approach closer than the sum of their appropriate radii. These two facts have played a major role in elucidating, theoretically, biomolecular conformation. For this contribution the Braggs were honoured with a Physics Nobel prize in the year 1915.

The strategy of trial and error was too simplistic, and therefore, to determine more complicated structures[5] a different strategy had to be adopted, exploiting the fact that the crystal structure and its diffraction pattern (the set of $\mathbf{F}_{hkl}$ for all values of h, k and l) are Fourier transforms of one another;

$$\rho(x, y, z) = \sum \mathbf{F}_{hkl} \exp -2\Pi i(hx + ky + lz), \quad (1)$$

$$\mathbf{F}_{hkl} = \sum f_n (x_n, y_n, z_n) \exp 2\Pi i(hx_n + ky_n + lz_n) \quad (2)$$

$$= |\mathbf{F}_{hkl}| \exp 2\Pi i\alpha_{hkl}.$$

$\mathbf{F}_{hkl}$, is a complex quantity, and is therefore characterized by an amplitude and a phase. In a diffraction experiment, since intensities of diffracted beams are measured, only amplitudes would be obtained. In the absence of devices that can focus low wavelength X-rays, Fourier summation of equation (1) has to be explicitly computed to produce the crystal image $\rho(x, y, z)$, after obtaining the phase α_{hkl} of each reflection hkl separately. Absence of experimentally-measured phases leads to the so-called 'phase problem' in crystallography, and continuous attempts to solve this problem have been made since the discovery of X-ray diffraction (Table 2). Patterson derived a mathematical function, now known as Patterson function, which enabled one to obtain coordinates of heavy atoms (atoms with large atomic numbers) using only intensities of X-ray reflections. This Patterson function also plays a crucial role in many methods of phase determination listed in Table 2.

Some of the methods listed in Table 2 are suitable for small molecules, while others are more suitable for macromolecules. Indeed a few of the Nobel prizes listed in Table 1 are in partial or full recognition of the attempts to solve the phase problem. Important contributions in this area have also been made by Indian scientists, especially in the context of using the physical process of anomalous scattering to derive phase information. The property of tunability of X-ray wavelengths in a synchrotron source is leading to increasing use of multi-wavelength anomalous diffraction for solving macromolecular structures. Using simple devices, such as the bent crystal polychromator and the grid plate, one is able to simultaneously record diffraction patterns from a chosen number of different X-ray wavelengths, in a single exposure thereby vastly improving data compatibility. High intensity continuous X-ray spectrum at a synchrotron source has also caused reemergence of Laue method of diffraction for determining crystal structures. The accuracy of data obtained in the Laue method, however, needs to be still improved by employing better data-processing strategies. Methods are being developed also to refine phases of X-ray reflections by iteratively modifying computed electron density maps, which may or may not possess non-crystallographic symmetry. It is clear that the method development is still continuing even hundred years after the discovery of X-rays.

NMR

The principle of the NMR phenomenon is very simple and is governed by the equation (for spin = 1/2 nuclei),

$$\omega = -\gamma \mathbf{H}_0,$$

where $\mathbf{H}_0$ is the external magnetic field, ω is the frequency of resonance absorption and γ is a constant called the gyromagnetic ratio. γ is characteristic of the nucleus and a direct measure of its magnetic moment. The value of ω clearly depends on the field strengths and for the currently practical field strengths and for all the nuclei, including protons, the resonance frequencies are in the radiofrequency (RF) range.

The experimental setups in the early days of NMR consisted of an electromagnet or sometimes a permanent

Table 2. Solutions to phase problem

Method	Since year
Trial and error	1912
Direct methods	1950
Heavy atom and Fourier methods	1930
Multiple isomorphous replacement	1955
Molecular replacement method	1960
Multiwavelength anomalous diffraction method	1984

magnet, with a pole gap wide enough to contain a sample tube (5–10 mm in diameter and 3–4 cms in height). The tube was surrounded by an RF coil and the field was swept slowly to find the resonance condition. This technique was successfully used to measure magnetic moments of several nuclei and their isotopes. Further, nuclear magnetic dipoles interact with each other and the strength of the interaction depends on the distance between them and also on the orientation of the dipoles with respect to the external magnetic field. The interaction energy is given by:

$$\mathbf{E} = (\mathbf{m}_1.\mathbf{m}_2)/r^3 - 3(\mathbf{m}_1.\mathbf{r})(\mathbf{m}_2.\mathbf{r})/r^5,$$

where $\mathbf{m}_1$ and $\mathbf{m}_2$ are the magnetic moment vectors of the interacting nuclei and $\mathbf{r}$ is the internuclear vector. In a rigid lattice this interaction leads to splitting of lines and in a powered solid consisting of several small crystallites in random orientations with respect to the external magnetic field, the experimental spectrum will be a superposition of several spectra with different splittings. Measurement of the line separations and analysis of line shapes permits estimation of interproton distances in molecules. The first pioneering work in this direction was done by Pake in 1948, who estimated the H–H distance in water from the experimental spectrum of a powdered gypsum sample (Figure 5).

In the solution phase, because of rapid Brownian motion, the dipolar interaction averages to zero on the NMR time scale. Thus there will be no splitting of lines due to this interaction. However, the dipole–dipole interaction does contribute to the relaxation properties of the spin system and affects the line widths and polarization transfers from one spin to another spin when the spin system is perturbed in some way. This is the genesis of the so-called 'nuclear Overhauser effect (**NOE**)', which describes changes in intensity of one resonance line when another line in the spectrum is perturbed by RF irradiation. The magnitude of **NOE** is proportional to inverse sixth power of the distance between the two spins representing the two resonance lines. It is also proportional to the tumbling rate of the molecule. For the **NOE** to occur, the relative orientation of the nuclei should remain fixed for at least a few microseconds and the distance between the nuclei should be less than approximately 5Å. This forms the basis of macromolecular structure determination by NMR spectroscopy, discussed later in the article.

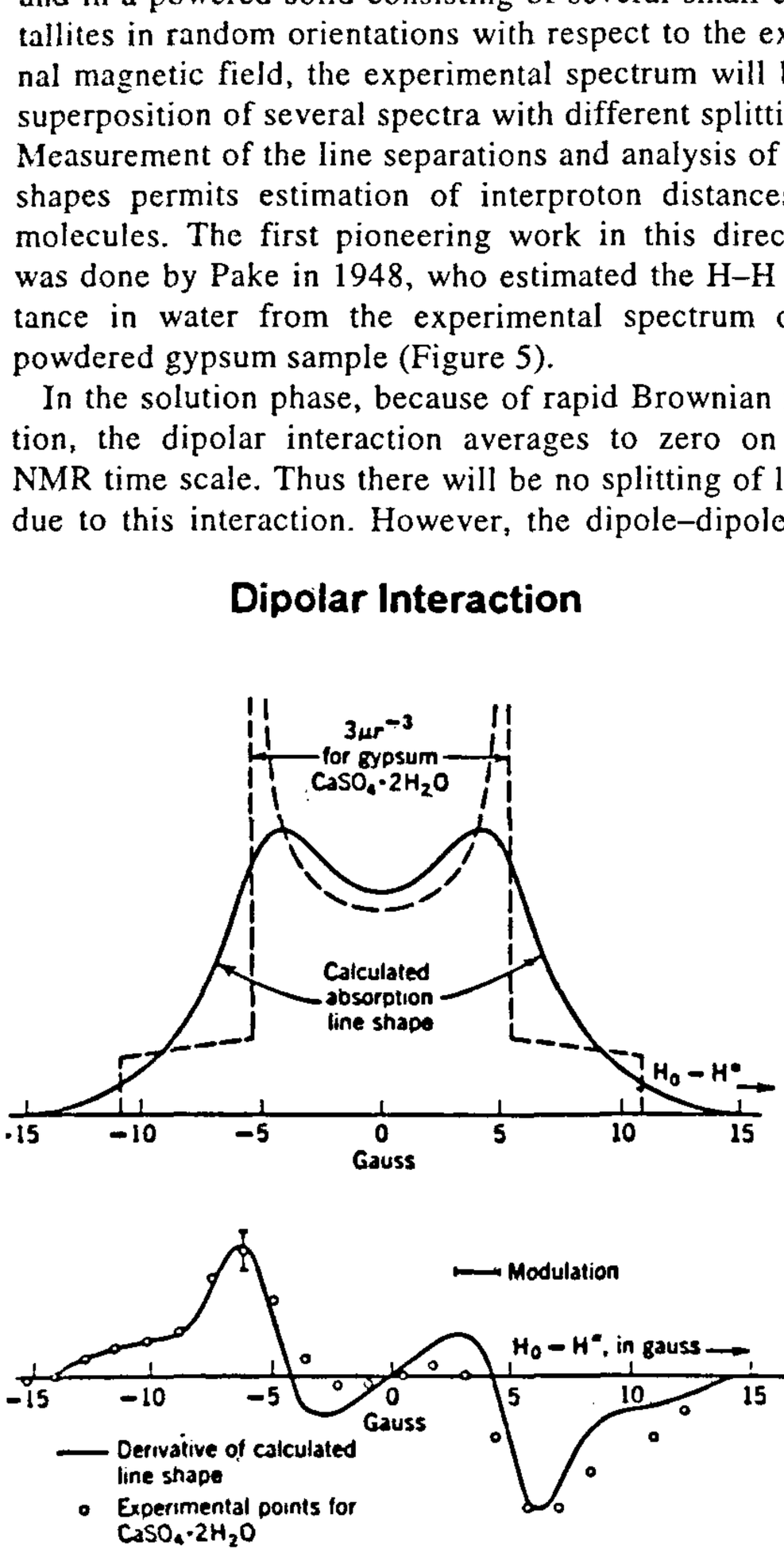

Figure 5. Interproton distances in a solid obtained through NMR by G. Pake in 1948.

Despite the great advances made by the above discoveries and consequent applications, a major hurdle was the inherently low sensitivity of the technique as compared to its optical counterparts. This limitation is because the energy quantum of a radiofrequency wave corresponding to the energy separation between the spin states is small and consequently the population difference between the levels which determines the intensity of the signal is also small. Consequently, it was necessary to have large sample quantities and with it were the associated problems of solubilities of molecules in desired solvents, field inhomogeneity in the large sample volumes, etc.

In 1965, the technique saw another major breakthrough with the discovery of Fourier Transform (FT) NMR[6] by Ernst and Anderson, then working with the VARIAN company. It was novel thinking on the methodology of the NMR experiment (Figure 6). The development of FTNMR earned Ernst a Nobel prize in the year 1991. The modern NMR spectrometer is a highly complex system with extensive automation, computer control, but it has also become relatively compact. The concepts of pulsed excitation and Fourier Transform NMR laid the foundations for most of the later developments such as broad band decoupling, two-dimensional NMR (2D NMR)[7], polarization transfer, magnetic resonance imaging, magnetic resonance spectroscopy of living systems, etc. The discovery of two-dimensional NMR in the early seventies added a new phase to molecular structure determination, by increasing the size of the molecules that could be tackled. This was possible because 2D NMR substantially reduced the overlap problems encountered in 1D spectra of large molecules by effectively spreading the signals onto a plane. Off diagonal peaks in a 2D spectrum carried information on the correlations (J or distance) between protons in different parts of the molecules. The last decade has seen further dimensions to the NMR methodology using heteronuclei such as ^{13}C and ^{15}N.

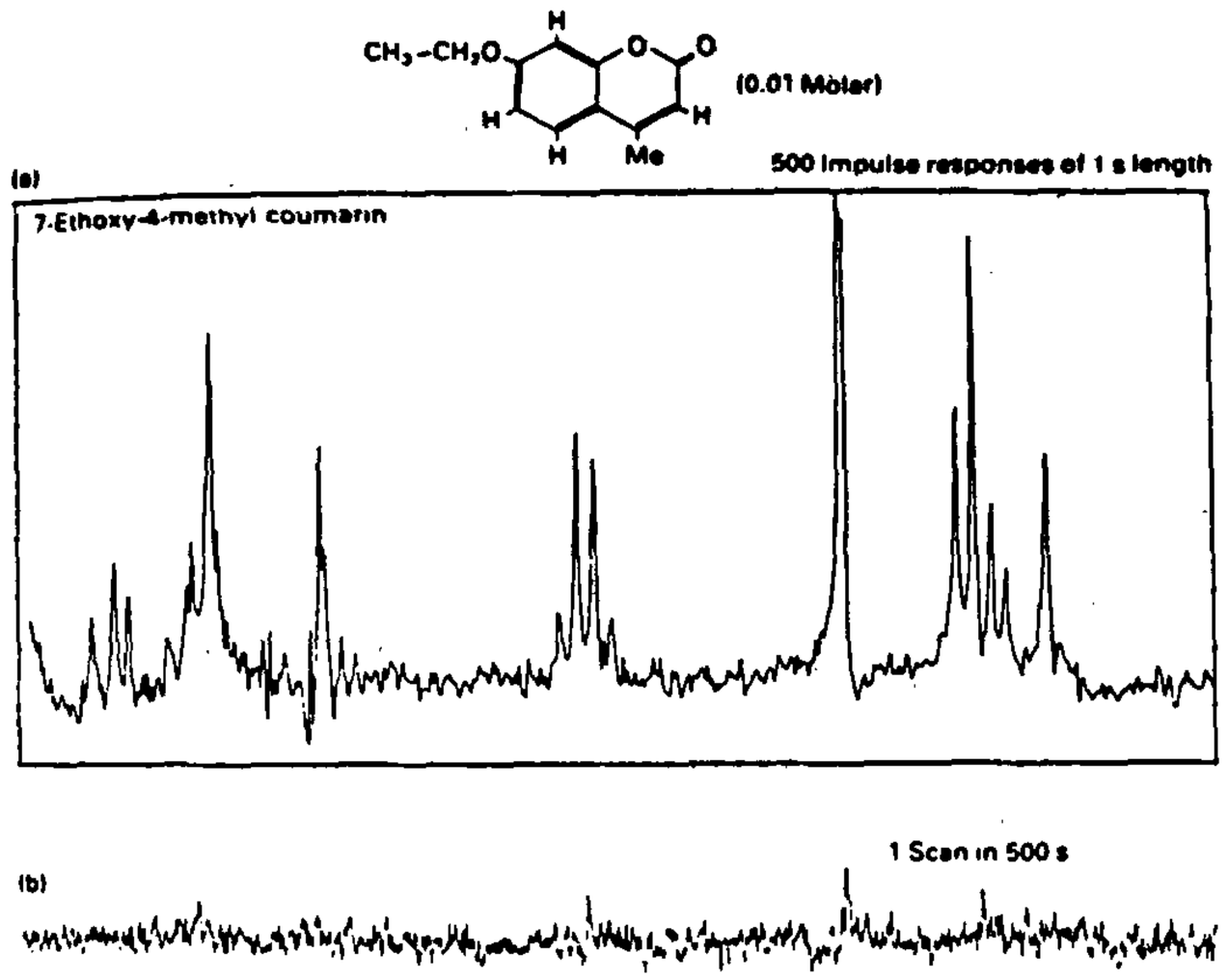

Figure 6. Comparison of continuous and FTNMR signals recorded under identical conditions. The enhancement in signal-to-noise ratio in the case of FTNMR method is dramatic. (Source: R. R. Ernst, *et al.*, *Principles of NMR in One and Two Dimensions*, Clarendon Press, Oxford, 1987.)

These developments in X-ray and NMR techniques have been facilitated by parallel growths in other areas such as computer technology, superconducting magnet technology, electronic industry, etc. X-ray and NMR techniques have found applications in a wide variety of disciplines including biology, physics, materials science, chemistry, and medicine. While X-ray diffraction could be applied to only crystalline samples, both solid and liquid samples could be used in the discipline of Nuclear Magnetic Resonance. In fact, a major advantage of NMR is that the molecule under investigation can be in solution.

Structure and interactions in materials

X-rays

X-rays have played a key role in our present understanding of the structure and interactions of matter, especially in the solid state. X-ray spectra of atomic samples recorded and analysed by Barkla, Mosley and other scientists have revealed the energy level diagram and electronic structure of the atom. Atomic level structures of inorganic, organic and biological molecules were first furnished by X-ray diffraction. It was the technique of X-ray fibre diffraction that revealed the double helical structure of DNA, in which the two helical strands recognize each other through specific hydrogen-bonding interactions between complementary bases (A:T, G:C). Such specific recognitive interactions are the foundation of biomolecular function, and also of the technology of structure-based drug design. The X-ray fibre pattern from the fibrous protein collagen was interpreted by G. N. Ramachandran, in terms of a very novel molecular model containing three interwined helices. In the case of globular proteins, Ramachandran used the twin principles established from early crystal structure analyses that every atom type has characteristic radii and that no two atoms can approach each other closer than the sum of their characteristic radii, to delineate, theoretically, the allowed backbone conformations. The results are plotted out in the form of an energy contour map for different combinations of the backbone dihedral angles ϕ and ψ, and this map is now known as the Ramachandran map. No amino acid residue in a natural protein is found to occupy disallowed regions in this map unless constrained to do so by functional necessity, even today, when more than 300 independent protein structures are known to high-resolution. The ϕ–ψ map is generally regarded as a good diagnostic of a correct protein conformation. Another method of representing protein conformation that also shows the folding is through drawing of secondary structure elements such as helices and beta strands in a schematic diagram of the polypeptide chain. Figure 7 shows the ribbon diagram of the structure of a

Figure 7. Ribbon drawing of the detailed structure of the protein gelonin. Side-chain atoms of only active site residues are drawn.

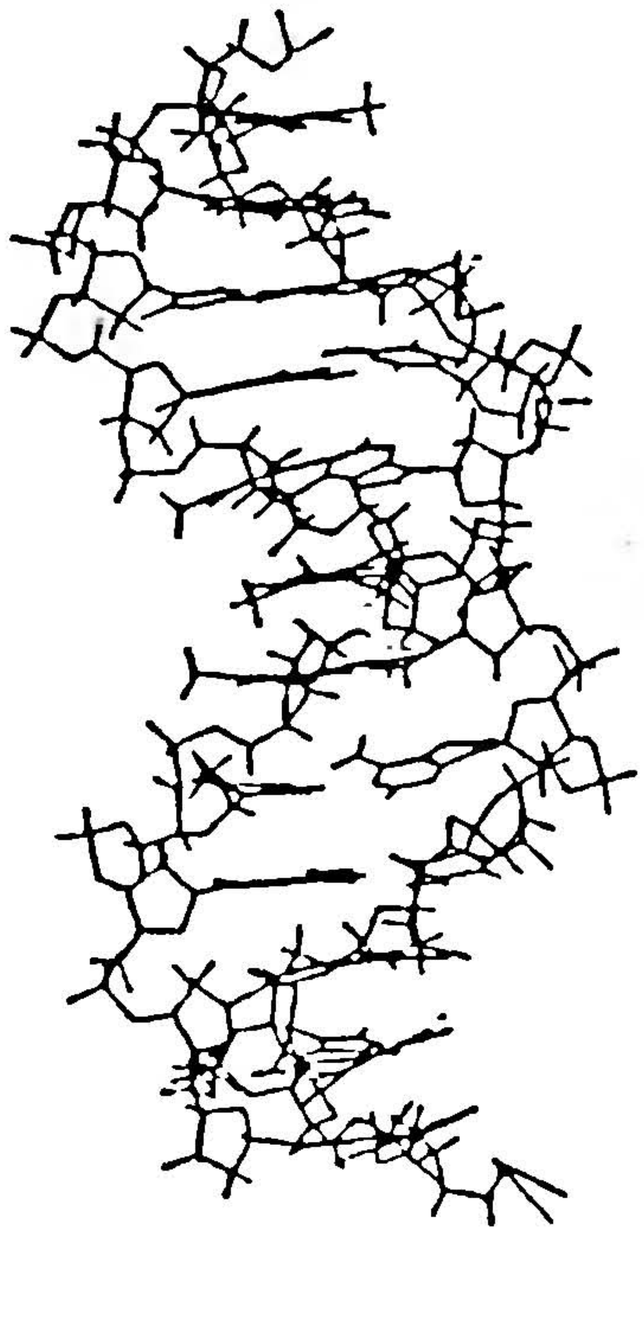

Figure 8. Structure of the DNA fragment specifically recognized by the oncogenic protein c-Myb, as obtained by multidimensional NMR.

medicinally-important protein called gelonin, solved to 1.8Å resolution at BARC recently, by the method of single crystal X-ray diffraction[9]. In addition to the molecular conformation, X-ray diffraction studies on single crystals provide very accurate geometrical information on intra- and intermolecular interactions. The technique of X-ray absorption fine structure (XAFS) spectroscopy has been used to probe local environments and interactions around a selected metal atom. By choosing appropriate energy from the continuous synchrotron spectrum, element-specific environments/ interactions, even in non-crystalline samples such as catalysts, ceramics and other materials have been studied.

NMR

Three-dimensional structures of macromolecules can also be obtained now by NMR[10], thanks to the development of multi-dimensional NMR. The general strategy for structure determination of a biological macromolecule consists of basically two steps: (i) sequence-specific resonance assignments and (ii) quantification of the interactions between the assigned nuclei. In the latter, there are two types of interactions namely, J-coupling which provides torsional angle information, and NOE which provides internuclear distance information. These inputs are then used to construct a unique structure for the molecules by different algorithms. Figure 8 shows the structure of c-myb DNA fragment determined at the Tata Institute of Fundamental Research[11]. Currently, as many as 100–150 DNA and protein structures are determined every year to atomic resolution, by NMR methods.

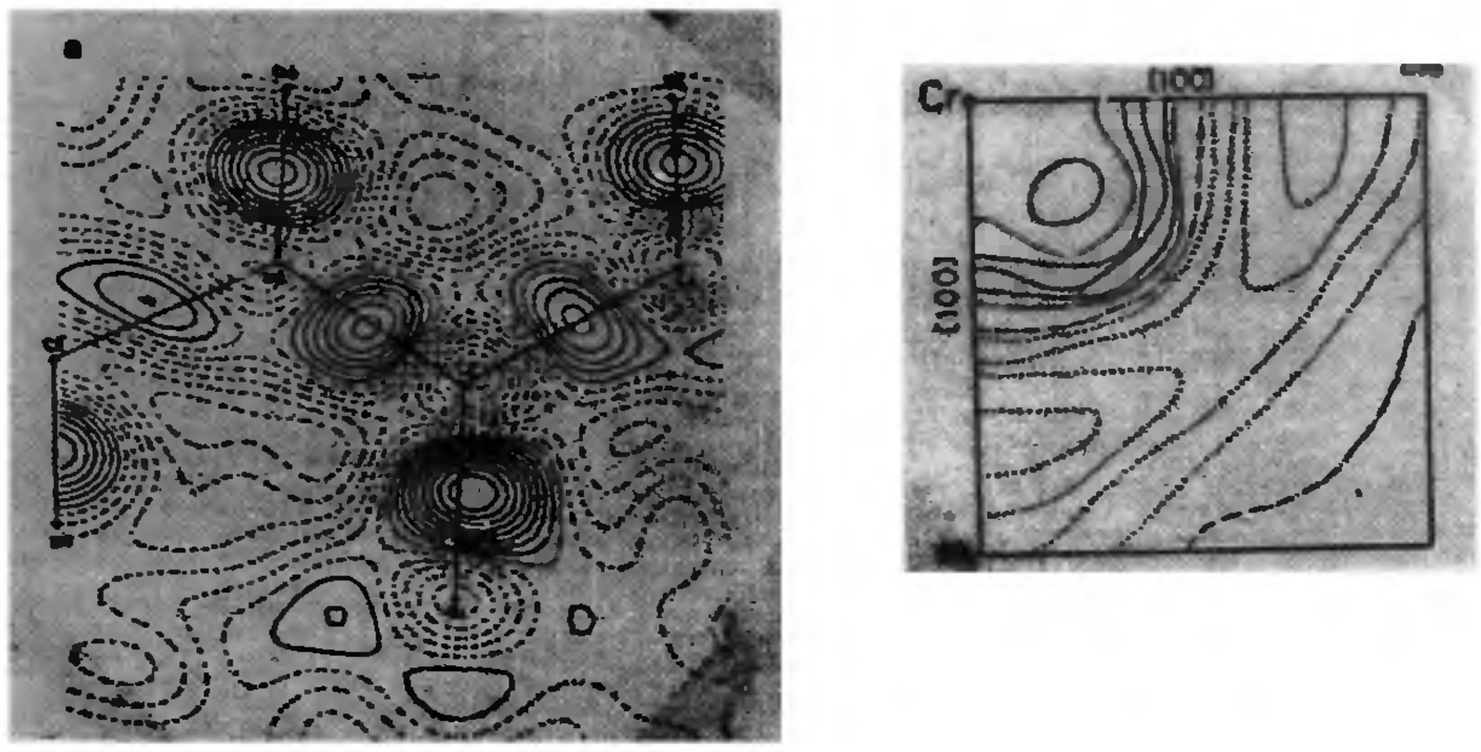

Figure 9. Charge density in a peptide bond and near a chromium nucleus revealed by X–N maps. (Reprinted with permission from J. F. Griffith and P. Coppens, *J. Am. Chem. Soc.*, 1975, **97**, 3496. Copyright 1975 American Chemical Society; and S. Ohba *et al.*, *Acta Crystallogr.*, 1982, A38, 103–108.)

Charge density distribution

A combination of X-ray and neutron diffraction has been used to probe atomic asphericity and redistribution of valence electronic charge (Figure 9) on covalent bond formation[12]. In this method, X-ray structure factors and neutron atom parameters are used to calculate difference density maps, the so-called X–N maps. These maps would reveal departure from sphericity of electronic charge around atoms, since atoms are assumed to be spherical while calculating the X-ray structure factors. X–N maps have provided direct evidence for: (i) redistribution of valence electrons into covalent bonds through orbital overlap, (ii) existence and spatial orientation of electron lone-pairs and (iii) different modes of orbital hybridization. Accurate measurements of the charge density provide a critical input to the refinement of parameters in quantum chemical calculations of atomic wave functions. The atomic partial charges are important for accurate calculation of interaction energies, in the emerging area of structure-based drug design. X–N maps have revealed in the case of metals presence of *d*-electron density very close to the atomic nucleus (Figure 9).

The bond densities and presence of electron density near a nucleus also affect the NMR spectra. In paramagnetic systems such as radicals, and metals with free conduction electrons, there is a net electron spin polarization and direct first order effects of electron–nuclear coupling show up in the NMR spectrum. In free radicals, they appear as hyperfine structures in the spectra and in metals the effects appear in the form of the so-called Knight shift, named after the discoverer, Walter Knight. The interaction manifests as an extra field ΔH which aids the externally applied field H_0 and the nuclear resonance of the metal gets shifted to a higher frequency as compared to that of the same nucleus in an insulator. For example, the resonance frequency of ^{63}Cu in metallic copper is at a frequency which is 0.23% higher than in diamagnetic CuCl. The fractional shift measured in field units is given by the relation,

$$\Delta H/H_0 = 8\Pi/3\ \langle|\mu_k(o)|^2\rangle\chi_e,$$

where the central term on the right represents the *s*-electron density at the nucleus and the last term is the total electronic spin susceptibility. Thus the measurement of the fractional shift or the Knight shift permits estimation of the *s*-electron density at the site of the nucleus in metals. In 1950, Arnold, Dharmatti and Packard observed that the three groups of protons in ethyl alcohol (CH_3CH_2OH) had different resonance frequencies (Figure 10), suggesting different chemical (electronic) environments for the three groups of protons. While X-ray based studies provide a direct map of the electron densities in different parts of a molecule or a solid, in NMR, this information is obtained in an indirect manner, through measurement of chemical shifts and coupling constants. In diamagnetic systems such as isolated molecules with no unpaired electrons or insulators, the chemical shift of a nucleus reflects the electron density distribution within a few Angstroms around the nucleus. The electron densities depend on the nature of the bonds, whether single, double, etc. and also on the nature of the groups, whether electron withdrawing, electron donating etc. A proton in the vicinity of an electron-withdrawing group has lower electron density around it and is consequently less shielded. Similarly, if a covalent bond between two atoms has a larger *s* character, there is a greater electron density around the nu-

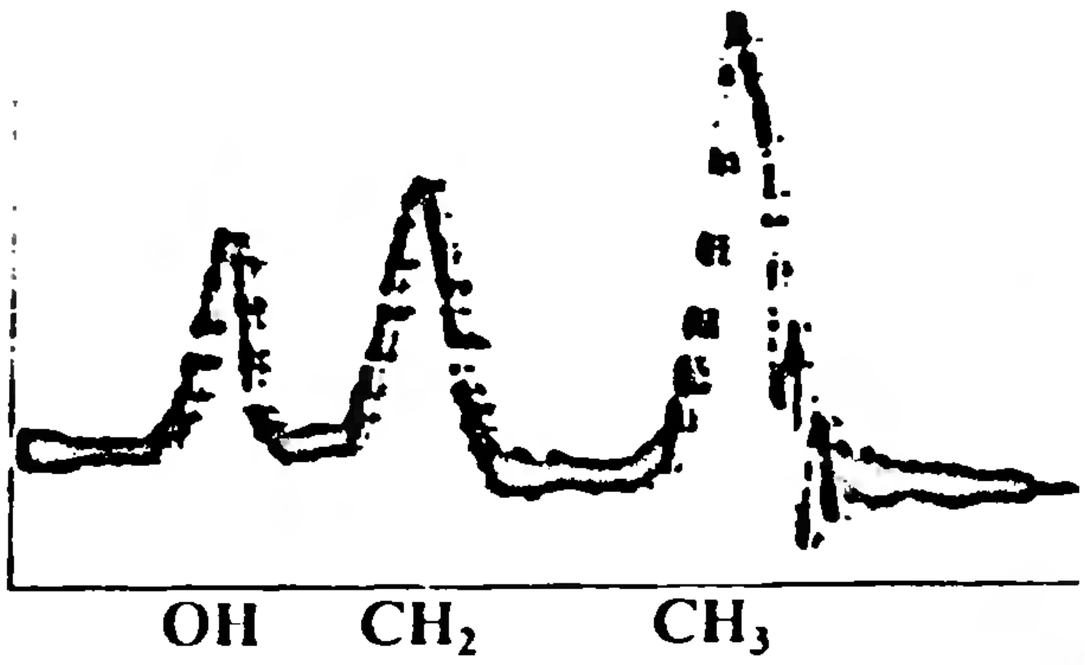

Figure 10. The first NMR spectrum revealing different electronic charge environments around CH_3, CH_2 and OH protons in ethyl alcohol. (Source: J. T. Arnold, S. S. Dharmatti and M. E. Packard, *J. Chem. Phys.*, 1950, **19**, 507.)

cleus of the atom, involved in the bond. Consequently the nucleus is shielded to a larger extent. Likewise, the spin–spin coupling seen in a diamagnetic system is a consequence of the electron–nuclear coupling to second order. Thus two nuclei separated by a double bond or a triple bond have greater *s*-electron densities and consequently have larger coupling constants than in the case of a single bond.

Imaging

Imaging of matter, in general, is of enormous interest in experimental research, and both X-rays and NMR have been used for this purpose. In magnetic resonance imaging (MRI)[13], the water distributions in different parts of the object under investigation (a human body for example) are monitored by applying different frequency labels to water in different parts of the body. This is achieved by applying linear *Z*-field gradients along the three Cartesian axes, i.e. the *z*-magnetic field at different parts of the sample is arranged to be different and consequently the corresponding water resonance frequencies will also be different. The intensities of the different signals then report the spatial distribution of water. Thus, bones can be readily distinguished from the soft tissues. Even among the soft tissues, good contrast is obtained by making use of the facts that water in different parts has slightly different characteristics such as line widths, relaxation times, extent of ordering, viscosity, flow, etc. Techniques have been developed to obtain images of any section of the body very quickly in a matter of few minutes. Thus, MRI has become an invaluable clinical tool and has already found a place in most of the modern hospitals. An offshoot of the MRI technique is the so-called NMR microscopy where emphasis is on higher-resolution. Specially-designed RF coils are used and data is collected from a smaller section of the sample. MRI has also been performed using compounds other than water and this is referred to as chemical shift imaging. This way, the distribution of certain metabolites during some functions can be monitored. This has been particularly useful for monitoring brain function and has led to the development of a discipline known as functional imaging.

Figure 11. X-ray transmission-computed tomographs recorded and processed by G. S. Ramakrishna and colleagues in the Isotope Division of BARC, using locally-developed hardware and software.

The properties of high penetration and atomic number-dependent absorption make X-rays very useful in high-resolution imaging of matter. X-ray imaging is playing a major role in medical diagnostics and treatment. In the early days, X-ray imaging was in the form of contact X-ray shadowgraphs, which were used to identify fractured bones. The ability to produce collimated X-ray beams of very narrow cross section has made it possible to produce high-resolution images through the scanning techniques. Sets of transmitted X-ray intensity patterns recorded digitally by scanning an object in different sections and directions are appropriately recombined, using a computer, to produce a detailed three-dimensional view of the object, to a spatial resolution of about 1 micron. This technique of X-ray imaging, known as computed tomography (CT)[14,15], is being widely used in planning and administration of radiation therapy to cancer patients. The location of the tumour within the body is precisely determined by CT, to ensure that during radiation therapy only tumour cells are exposed to the killer radiation.

X-rays are used in heavy industries for purposes of non-destructive testing of materials. This was first crudely demonstrated by Roentgen himself by taking an X-ray picture of a defective double barrel gun. The present-day fully automated technique of computed tomography has increased severalfold the accuracy and sensitivity of this method of testing. Figure 11 is an X-ray tomograph obtained at the Isotope Division of BARC, using a locally-developed hardware and software system.

Other methods of imaging which are undergoing rapid development are those of microscopy and holography using X-rays. Microscopy is the method of choice when the sample is non-periodic in nature. It is found that the wavelength region of 10–50 Å is most useful for microscopic work. The resolution attainable in these studies is in between those achievable by electron microscopy and light microscopy. A serious limitation of this technique is the damage caused to the sample by prolonged exposure to radiation. In X-ray holography, both amplitude and phase of the scattered waves are recorded into a hologram by allowing the scattered waves to interfere with a coherent reference beam. Very recently the reference beam is generated from within the sample by selected atom X-ray fluorescence (14.1 keV strontium line), and the hologram so recorded is processed to reveal atomic-resolution structure of strontium atoms in the crystal[16]. This approach could be of considerable use in locating heavy atoms in macromolecular crystals in the future.

Future directions

A glance at the list of Nobel laureates shows that many prizes have been awarded for either developing a technique or for determining biomolecular structures using X-rays. This trend will continue and progress in the important field of biophysics is likely to speed up with the advent of synchrotron rings as sources of X-rays. Many synchrotron rings are being built all over the world. In India, the Department of Atomic Energy is building two synchrotrons at Indore. The low-energy ring (INDUS1, energy 450 MeV) will have a critical wavelength of about 60 Å, while the high-energy ring (INDUS2, energy 2 GeV) will generate high-energy X-rays of wavelength suitable for protein crystallography. The high intensity on a synchrotron source permits use of even smaller crystals for data collection. The reduced exposure times result in reduced radiation damage, thereby leading to more accurate, more complete and higher resolution intensity data sets. The pulsed and at the same time very intense X-rays make possible study of enzyme reactions and dynamics of proteins by the method of time-resolved X-ray diffraction. Combined with development of faster computers and recombinant DNA technology, the future trends in the use of X-rays will be:

1) to determine, by diffraction or other X-ray techniques, three-dimensional structures of (a) more complex and bigger biological systems such as multienzyme complexes, enveloped viruses, ribosomes etc., (b) molecules while in function using time-resolved Laue diffraction technique, (c) medicinally and industrially important proteins, even if they are naturally not very abundant,
2) for accurate measurements of charge density in molecules, and of electronic correlations in atoms,
3) medical imaging of broken bones, tumours and blocked arteries,
4) direct measurement of handedness using circularly polarized X-rays, and
5) for structure-based design of drugs, artificial enzymes and other molecules.

Similarly, in the field of materials science, X-rays will be used:

1) to study phase transitions at surfaces and interfaces in solids, as a function of pressure, temperature, etc.
2) to study dislocations, strain patterns and lattice constant inhomogeneities, for purposes of design of electronic devices, and
3) to study atomic-level and *in-situ* structure–function relationships in catalysts, alloys, ceramics, etc., for purposes of design of advanced materials.

Moreover, as with X-rays, NMR research would also become more and more interdisciplinary, with the thrust being on determination of detailed structures of biological molecules. Presently, due to overlaps in the spectra, the largest molecule that can be studied by NMR is one with a molecular weight of about 25 kD. Efforts are be-

ing made to push this limit as high as possible, by developing higher-frequency spectrometers and also by developing filtering techniques to extract proper signals from overlapped spectra. Magnetic resonance imaging and *in-vivo* spectroscopy are the other important developing areas where substantial effort will be put in to derive functional information.

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