Chapter J1
Frequencies and distances

J1.1 Historical review

1924

W. Pauli proposed the theoretical basis for NMR spectroscopy. He suggested that certain atomic nuclei have properties of spin and magnetic moment and, as a consequence, exposure to a magnetic field leads to splitting of their energy levels. **W. Gerlach** and **O. Stern** observed the splitting in atomic beam experiments, providing proof for the existence of nuclear magnetic moments.

1938

I. I. Rabi and colleagues first observed NMR by applying electromagnetic radiation in atomic beam experiments. Energy was absorbed at a sharply defined frequency, causing a small but measurable deflection of the beam. **Rabi** received the Nobel prize for physics in **1944**.

1946

Research groups led by **F. Bloch** and **E. M. Purcell** reported the observation of proton NMR in liquid water and solid paraffin wax. **Bloch** and **Purcell** shared the **1953** Nobel prize for physics.

1946

F. Bloch suggested a new method of excitation using a short radio-frequency pulse and in **1949 E. L. Hahn** showed that this did indeed produce a free precession signal. **Hahn** also established that pulse sequences could be used to generate additional information in the form of a spin echo. For many years, however, these methods were of little use to chemists because of the complexity of the signal obtained. In **1956, I. J. Lowe** and **R. E. Norberg** pointed out that the time-domain signal and the frequency-domain spectrum are related by Fourier transformation. The first high-resolution multichannel Fourier transform NMR spectrum was measured by **R. R. Ernst** and **W. A. Anderson**.

1950

W. G. Proctor and **F. C. Yu** observed two unexpected ^{14}N resonance frequencies for NH_4NO_3. At about the same time, **W. C. Dickinson** noticed similar effects for ^{19}F in several compounds. In **1951 J. T. Arnold** and colleagues introduced the term *chemical shift* following the observation of several resonance peaks for ^{1}H in ethanol, with the relative intensity in each peak corresponding to the relative number of protons in each chemical environment.

1951

H. S. Gutowsky and **D. W. McCall** suggested that interactions between spins of neighbouring nuclei were responsible for multiple resonance lines. In **1951 N. F. Ramsey** and **E. M. Purcell** proposed the concept of indirect *spin–spin coupling* or *scalar coupling*. It was found that in certain cases spin coupling failed to produce the expected multiplets, leading to the development of the concept of *chemical exchange*.

1953

A. W. Overhauser explored the dynamic polarization of nuclei, in metals where the electron spin resonance had been saturated. The effect he discovered was called the 'Overhauser effect'. The potential of nuclear Overhauser enhancement (NOE) signals for providing information on the conformation of molecules in solution was first demonstrated by **F. A. L. Anet** and **A. J. R. Bourn** in **1965**. In **1970, R. A. Bell** and **J. K. Saunders** reported a direct correlation between NOE and internuclear distances and **R. E. Schrimer** with colleagues demonstrated that relative internuclear distances can be determined quantitatively from NOE measurements on a system containing three or more spins.

By the **mid-1950s** the basic physics of NMR and its potential value in chemistry had been elucidated, and commercial instruments were available. In **1956**, the observation frequency for ^{1}H NMR spectroscopy on the HR-30 Varian Spectrometer with a 0.7 T electromagnet was fixed by a crystal at 30 MHz. In order to improve sensitivity and increase chemical shift dispersion, commercial instrument development focused on increasing the magnetic field strength. The development of persistent superconducting solenoids (cryo-magnets) in the **early 1960s** constituted a major milestone for NMR applications. In the **late 1990s** 18.8 T (corresponding to a resonance frequency of 800 MHz for ^{1}H NMR) spectrometers were installed in many NMR laboratories, with the first 900 MHz (21.1 T) instruments becoming available.

1957

M. Sauders, A. Wishnia and **J. G. Kirkwood** reported the first NMR spectrum of a protein, and a small number of similar studies reports followed in the next decade. Because of technical limitations in sensitivity and spectral resolution,

however, these early NMR applications in structural biology did not bear directly on macromolecular three-dimensional structure. The fundamental theory of NMR was published in **1961** in a landmark book, *The Principles of Nulcear Magnetism*, by **A. Abragam**.

1966

Ernst proposed a Fourier transform method, which provided a major leap forward with respect to the amount of information accessible by NMR. The inherent advantages of greater sensitivity, high resolution, and the absence of line-shape distortions contributed to make Fourier spectroscopy the preferred experimental technique in the field.

1971

J. Jeener first suggested the idea of two-dimensional Fourier transform NMR (FT-NMR), based on the Fourier transformation of signals in two independent time domains to yield a plot with respect to two orthogonal frequency axes. In **1975, R. R. Ernst** with colleagues reported the first two-dimensional NMR (2D-NMR) ^{13}C spectrum of hexane. This was followed in **1976** by a seminal publication presenting a comprehensive theoretical treatment of 2D-NMR correlation spectroscopy (COSY). **Ernst** received the **1991** Nobel Prize for chemistry for his many contributions to NMR.[†]

1972

P. C. Lauterbur demonstrated the feasibility of macroscopic imaging by NMR. In the same year, **R. Damadian** used the method for investigations of the human body, in particular for cancer detection, paving the way for the non-invasive imaging of entire biological organisms. In **2003 Lauterbur** and **P. Mansfield** shared the Nobel Prize for medicine or physiology for contributions to magnetic resonance imaging (MRI).

1983

T. A. Cross and **S. J. Opella** showed that high-resolution structural constraints could be obtained from solid-state NMR experiments, and the potential of the approach was rapidly established.

1985

K. J. Wüthrich and coworkers reported the complete three-dimensional structure of a protein, BPTI, in solution based on NOE distance constraints only. There has since been spectacular progress in the development and application

[†] J. Jeener originated the idea of two-dimensional NMR spectroscopy in 1971. Unfortunately, his first two-dimensional spectra were never published; the only reference to the work is in a set of lecture notes for a summer school.

of NMR methodology to protein structure determination in solution. Because of the growing number of peaks and larger peak widths with increasing molecular mass, the method was initially limited to macromolecules of a few tens of kilodaltons. Isotopic labelling extended the molecular mass range to 40–50 kDa. In **1998**, **Wüthrich** with coworkers discovered that in very high magnetic fields narrow resonance peaks can result from interference between dipole–dipole coupling and chemical shift anisotropy, and proposed the technique called transverse relaxation optimised spectroscopy (TROSY). TROSY experiments should make possible the determination of three-dimensional structures of proteins close to 100 kDa in molecular mass. **Wüthrich** was the **2002** Nobel laureate in chemistry

Late 1980s

H. Oschkinat, D. Marion, A. Bax and **G. W. Vuister** introduced a third frequency dimension in NMR spectra (3D-NMR), and in the **early 1990s L. E. Kay** and coworkers and **G. M. Clore** and coworkers expanded the technique to 4D-NMR. By using isotopic enrichment 3D- and 4D-NMR have become powerful experimental approaches, widely applicable in structural biology.

2000 to present

NMR has become one of the most powerful spectroscopic techniques in physics, chemistry and biology. Powerful experimental methods have been devised for observing different NMR phenomena in detail. NMR in structural biology maintains all the typical signs of a young, emerging field of research, with fundamental contributions continuing to be made by many scientists, including **A. Bax, S. Grzesiek, A. M. Groenborn, D. Marion**, and others.

The field of NMR proved to be remarkable through the number of revolutionary innovations that have occurred since the first experimental observation of the phenomenon more than 55 years ago. The introduction of the second frequency dimension constituted a critical step for biological applications. A large variety of experimental schemes were developed to extend NMR applications to the characterisation of complex molecules, such as small synthetic polymers, peptides and sugars. Small proteins and oligonucleotides became accessible to study following the introduction of new procedures. The addition of a third frequency dimension (3D- NMR) was the next important development with advances in genetic engineering enabling the overproduction of proteins and their labelling in microorganisms with NMR- stable isotopes. Isotope enrichment, in fact, increased NMR resolution sufficiently that it became possible to add a fourth frequency dimension to the spectra.

NMR now occupies a very special place in the armoury of physical techniques available to biologists. At the end of 2004 the PDB contained more than 4200 NMR-derived structures out of a total of about 24 500. NMR also provides information on protein and nucleic acid dynamics in a time domain spanning from picoseconds to days. Its unique versatility for the study of molecular structure