What is NMR

- Nuclear Magnetic Resonance
NMR, what is it?

The acronym NMR stands for “Nuclear Magnetic Resonance.” Anybody who has ever had to undergo magnetic resonance imaging (MRI) in hospital will already have had first-hand experience of NMR equipment. An MRI tomograph for MRI and an NMR spectrometer are not quite the same thing, but they are based on the same physical principles.

A magnetic resonance tomograph provides the amazing opportunity to look inside a body without having to make an incision. For example, doctors can diagnose a worn meniscus without having to carry out a costly endoscopy procedure on the knee. MRI tomographs are nowadays standard medical systems that can be seen in practically any hospital. On the other hand, an NMR spectrometer is very probably still a mysterious object to most people. Nevertheless, NMR is a very powerful, versatile and well-established physical tool for the investigation of matter through the measurement of nuclear magnetic interactions, which has found, in the past half century, countless applications in many fields such as physics, chemistry, biology, biochemistry, material science, food industry, geology, pharmaceutical research and medicine.

Although NMR was first described and measured in exotic molecular beams by Isidor Rabi in 1938, the birth of NMR as a probe to study ordinary matter can be traced back to 1946. In that year, Edward Mills Purcell at Harvard University detected the first solid-state NMR signal in 1kg of paraffin wax. Almost simultaneously Felix Bloch at Stanford University carried out the first liquid-state NMR experiment in water. For these discoveries Purcell and Bloch shared the Nobel Prize in Physics in 1952. It is a remarkable fact that the discovery, development and applications of NMR have resulted in no less than 8 Nobel laureates in Physics, Chemistry and Medicine! After the United States, Switzerland is the second most prolific country in the world in NMR, boasting two Nobel laureates, Richard Ernst (1991) and Kurt Wüthrich (2002), who where both professors at ETH Zürich. Nowadays, NMR comprises a seemingly endless range of experimental methods that can give access to a wealth of information about the structures of amazingly complex molecules and their dynamics. Both NMR methods and its technologies are under continuous development in universities and industries.
What do you need NMR for?

Why do we want to know the structure of molecules? Molecules are essentially arrangements of atoms. Molecules have different structures that depend on which atoms are in the molecule and how they are bonded together. The structure of a molecule has a decisive influence on the physical and chemical properties of the respective substance and thus on its effects for instance on a living organism. So, for example, ethanol (better known as alcohol) is made of the same type and number of atoms as dimethyl ether. The former is a liquid and can be drunk, at least in moderate amount, whilst the latter is a poisonous gas. It is therefore crucial to know what the chemical structure of a compound or substance looks like. This information is also essential to create the synthetic equivalent of a substance or to create (synthesize) a completely novel compound. Countless compounds are produced synthetically nowadays in the chemical and pharmaceutical industry. They range from acetic acid, vitamins, and aspirin to nylon or plastic. The essential prerequisite for the existence of these products is a detailed knowledge of the molecular structure of the compounds. NMR is, in this respect, an extremely useful tool that provides detailed information on molecular structures.

NMR can also be used to determine the structure of very large and biologically important molecules such as proteins. This is important because proteins regulate virtually all of the body’s functions and the biochemical processes that take place in living organisms to maintain life. The protein known as insulin, for example, regulates the blood sugar levels in our body, the protein called hemoglobin carries oxygen in the blood from the lungs to the rest of the body and immunoglobulins (more commonly known as antibodies) are proteins used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses. It is important to study and understand such processes in order to produce medicines or therapies. It is necessary to know not only the structure but also the dynamics of proteins to understand how they work. NMR is the only method that can provide both structural and dynamic information on biological molecules, in their physiological environment, with atomic resolution.

Atoms, nuclei and nuclear spin

A total of 118 elements are known today, such as, for example, hydrogen, carbon, oxygen, copper, iron, etc. Every chemical element consists of atoms of its element. A piece of iron, for example, is made up of an inconceivably large number of iron atoms. The atoms in turn are not the smallest units of matter, as the Greeks had assumed in ancient times (’atomos’ is Greek for indivisible), instead they can be further divided into smaller particles. Atoms consist of a nucleus, which is positively charged and carries most of the mass of the atom, surrounded by a cloud of negatively charged electrons. Many atomic nuclei possess a physical property called spin which can be overly simplified as a rotation of the nuclei about their own axis. These spinning nuclei behave much like rotating electric charges which generate, according to fundamental physics laws, magnetic fields and hence so called magnetic moments. A magnetic moment is the physical quantity that describes the properties of a bar magnet or a compass needle, for instance. In other words, atomic nuclei can be imagined as tiny spinning tops whose main axis is a small bar magnet with north and south at opposing ends of the axis. Because of their magnetic properties, atomic nuclei with a spin (that is, nuclei that do spin) behave in all respects as compass needles that can be oriented by means of magnetic fields. Typically an ensemble of atoms does not carry any macroscopic magnetization because all these small magnets are randomly oriented and therefore point in completely different directions. Under these conditions, the magnetic fields generated by these tiny magnets mutually cancel each other. However, nuclear magnetic moments can be aligned by exposing them to a sufficiently strong magnetic field. This creates a macroscopic ensemble magnetization which is the fundamental condition that makes NMR possible.
Molecules

Atoms naturally tend to bond together to form molecules. Molecules are therefore aggregates of atoms which stick together to form a very specific structure. The molecular structure is a unique feature of any compound, responsible for its physical and chemical properties. Molecules exist in all shapes and sizes. Small molecules consist of only a few atoms, such as water molecules. A water molecule consists of two hydrogen atoms, both of which are connected with an oxygen atom. There are about $4 \times 10^{21}$ (= 4,000,000,000,000,000,000) water molecules in a raindrop. So if a water molecule were as big as a football, a raindrop would be about as big as the Earth. Other molecules may consist of many more atoms, up to hundreds of thousands, but they are still so incredibly small that they cannot be seen even with the most powerful conventional microscope. Therefore, other methods are required to resolve the structure of molecules. NMR is one of the best suited methods for this purpose. When a molecule is exposed to an external magnetic field, each atom will feel a slightly modified field due to the magnetic shielding effects created by neighboring electric charges (nuclei and electrons). The magnetic field experienced by each atom is therefore a local property of the molecule that depends on its geometry. NMR essentially measures the response of an atom to the local magnetic field, which bears information on the molecular structure. Modern NMR spectrometers are sensitive to local magnetic field variations as small as 1 part in 1 billion. With NMR even the structures of very large and complex molecules, such as proteins, can be determined with breathtaking accuracy. This knowledge is of great importance for drug research. Professor Kurt Wüthrich from the ETH Zürich was awarded the Nobel Prize for Chemistry in 2002 for his contributions in this field.

NMR instrumentation

The fundamental component of any NMR equipment is a magnet to generate the field in which the nuclear magnetic moments of a sample are aligned and a magnetization is created. The laws of physics put serious limits to the magnetization that can be achieved since only a very small fraction of the nuclear spins in the sample can be effectively aligned to the field. Since the magnetization is approximately proportional to the intensity of the magnetic field, it is very important that the applied field is as strong as possible. Nowadays, superconducting magnets are used in all NMR spectrometers and fields up to 24 T can be achieved (almost 1 million times stronger than the Earth magnetic field). These magnets are immersed in a bath of liquid helium in a cryostat and operate at temperatures around 2-4 K, about -270° C. Radio wave pulses are generated by a system of coils built into the probe, the physical interface between the sample and the magnet. The probe contains the sample, either liquid or solid, at the center of the radio wave coils and the electronics for the first stage of the signal detection. The electronics of modern probes can be cooled down to 10-20 K in order to reduce the electronic noise and be more sensitive to small signals that would otherwise be invisible. These probes are known as cryoprobes. This requires an external cooling unit. The NMR set-up also requires a pre-amplifier and a console that contains the sophisticated electronics for the generation of radio wave pulses and for the detection of the NMR signal. Finally, a software to control both data acquisition and processing is needed.
How does NMR work?

Many atomic nuclei possess an intrinsic property called spin. It is common practice to think of nuclear spins as rotations of the nuclei about their own axis. Although this picture is an oversimplification, it is widely accepted as an intuitive way to grasp the basic principles of NMR. Therefore, as shown here, a rotating electrically charged nucleus generates a magnetic field and hence carries a magnetic moment, represented graphically by a green and red arrow. In simpler words, atomic nuclei can be seen as tiny bar magnets that rotate around a magnetic axis as a spinning top, and - just like compass needles - they can be manipulated by means of magnetic fields.

In an NMR spectrometer a sample of the substance to be examined is placed in a strong static magnetic field which forces the nuclear magnetic moments to align themselves parallel to the applied field (the latter is represented by yellow arrows). The sample acquires a macroscopic magnetization and it is said to be polarized or magnetized. Although in modern spectrometers magnetic fields can be as large as 24 Tesla (almost 1 million times stronger than the Earth magnetic field!), the total fraction of aligned spins is on the order of 1 out of 10,000 only! The experiment has to rely on those few nuclei to retrieve information on the molecules under investigation. On the other hand, NMR uses probes, the nuclear magnetic moments, which are already built into the object of interest. Nuclear magnetic moments are very weak, and they barely interfere with the molecular degrees of freedom. This is of utmost importance, because molecules are not altered by NMR spectroscopy.

In a typical NMR experiment the sample is irradiated with a short electromagnetic pulse characterized by a broad spectrum of frequencies in the range of radio waves. This radio wave pulse has the effect of forcing the nuclear magnetic moments (that is, the bar magnets) out of their equilibrium position (parallel to the magnetic field) and dragging them into a plane perpendicular to the magnetic field. Different nuclei in a molecule will be tilted away from their equilibrium by radio waves of slightly different, but well defined, frequencies called resonance frequencies. The specific resonance frequencies required to tilt the nuclear magnetic moments provide information on the type of atoms in that molecule and their positions. It is a remarkable physics fact that a broad range of different resonance frequencies can all be carried by a single radio wave pulse, which can be as short as a microsecond (1/1,000,000 of a second); this is the fundamental idea of the so called Fourier Transform NMR method (FT-NMR), in which all nuclei in a sample are stimulated simultaneously by one electromagnetic pulse.
After the radio wave pulse, the deflected nuclear magnetic moments (that is, the bar magnets) start a rotational motion (called a precession) around the static magnetic field (represented by the yellow arrows). The frequency of this precession ($\omega$ in the figure) depends mainly on the strength of the magnetic field and on the type of atom, and to a very small but detectable degree on its position in the molecule. Therefore, the characteristic precession frequencies of the nuclei in a molecule contain valuable information from which one can determine the structure of the molecule itself. How can we measure these frequencies?

Firstly, in an NMR spectrometer, the sample is placed in a small coil of wire. Just like in a bicycle dynamo, the rotating (precessing) magnetic moments induce an alternating voltage in the coil, the so-called NMR signal. This signal is often the overlay of many oscillating electric signals. The frequency of each oscillating signal corresponds to the precession frequency of the magnetic moment that generates it. The amplitude of the observed voltage decays because the magnetic moments spontaneously and progressively return to their equilibrium state parallel to the vertical axis. The NMR signal is extremely weak, as a consequence of the tiny initial sample magnetization. If the input radio frequency power used to stimulate the nuclei were as large as the Mount Everest, the output signal measured in an NMR experiment would be 10,000 times smaller than the diameter of a human hair. This fact requires the use of highly sophisticated electronics.

Finally, a mathematical method known as Fourier transform (FT) allows one to present the NMR signal in the clear form of an NMR spectrum, in which the characteristic frequencies of the precessing nuclear magnetic moments appear as distinct peaks, called resonance lines.

For example, the NMR spectrum of ethanol shows two distinct families of peaks. Their positions, that is, their frequencies, are determined by the atomic structure of the molecule. From the analysis of such a spectrum one can trace the molecular structure of ethanol.
The difference between CW- and FT-NMR can be best explained by using the example of a tuning fork. The resonance frequency of a tuning fork can be determined with a synthesizer: the frequency is varied until the tuning fork starts to make a sound. But there is a far simpler method: after the fork is given a quick tap, it will spontaneously emit its own resonance frequency (Alexander Wokaun: “Multidimensional nuclear magnetic resonance spectroscopy,” NZZ dated 27.11.1991).

The FT-NMR method is practically the only method used in NMR since the 1960’s when it superseded the old method of continuous wave (CW) NMR, where radio waves of different frequencies were sent into the sample individually. The FT method is both much faster and more sensitive than the previous CW-NMR method and opens up an incredibly wide range of experimental possibilities. Professor Richard Ernst from the ETH Zürich was awarded the Nobel Prize in Chemistry in 1991 for, among other things, the discovery of this new NMR method in the 1960’s. Since then hundreds of NMR experiments have been developed based on the principle described above. Two dimensional (2D) NMR is one of those powerful developments.

Two-dimensional NMR spectrum of approximately 200 micrograms of a steroid hormone. The hormone is a complex molecule with many resonance lines that strongly overlap. This type of spectra is one of many extensions of the NMR measurement principles presented in this brochure to overcome this problem. It allows resolving the structure of larger molecules with a few thousands of atoms. Typical acquisition times: a few minutes to a few hours, depending on the instrumentation and quality requirement.