Welcome every one. This is going to be a course on principles and applications of NMR spectroscopy. I am Hanudatta Atreya from NMR Research Center at IISc Bangalore. I work primarily in the area of bimolecular NMR, looking at structures of proteins and nucleic acids. In this course, we are going to introduce you to very basic aspects of what is NMR spectroscopy, what are the principals involved, how NMR spectroscopy can be applied to different areas of chemistry and biology. I will start from the very basic applications of NMR spectroscopy. We can see that NMR spectroscopy is a very unique spectroscopic tool.

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It has applications in a wide area of science and technology. For example, it is applicable in agriculture, in medicines, where it has a very popular application called magnetic resonance imaging and that is where MRI is used. NMR spectroscopy is also used in different areas like microscopy, food technology; in fact testing of many of the food products nowadays in many companies is done using NMR spectroscopy. But the main focus, the major focus of this course, will be on what is called structural biology and structural chemistry. These are the two major applications which have come up in last thirty years and we will see how NMR spectroscopy can be used in these areas.

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Having said that NMR spectroscopy is applicable to various fields, several Nobel prizes have been given to different discoveries made in NMR. Starting from the very beginning, the 1952 Nobel Prize was given to Felix Bloch and Purcell who independently and simultaneously discovered the phenomenon of NMR spectroscopy.

Richard Ernst got Nobel Prize in 1991. Richard Ernst is a very common name in chemistry and physics. Everybody is familiar with what is called Fourier transform NMR (FT-NMR), which is used routinely in chemistry. Richard Ernst discovered and developed many of the two dimensional NMR methods which we will see during this course. Kurt Wuthrich from ETH, Switzerland, got Nobel Prize in 2002 and his main contribution was towards developing new methodologies for structure determination of biomolecules. This is when NMR took off from being a tool of chemists to a being a tool of biologists (mainly structural biologists) and this was a phenomenal growth. We will look at how NMR can be used for structure determination of small biomolecules such as peptides and proteins in this course. A major use of NMR, as I mentioned in the previous slide, is in the area of imaging. For this the Nobel Prize was awarded in 2003 to Peters Mansfield and Paul Lauterbur. They had made seminal contributions to MRI. MRI basically involves imaging of the whole body of human or animal and actually captures the anatomy of the human system. Thus, we can see the huge range of applications of NMR, right from physics to looking at chemistry and biology and looking at imaging. Why is NMR such a very unique tool? This is what we will see now in this next slide.

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The first and foremost is that NMR spectroscopy is a non-invasive and a non-destructive method. What does this mean? This means that if you take a sample of your compound, of your molecule, and record NMR data on that, you get back the sample intact. Nothing happens to the sample; it is not destroyed and therefore is non-invasive.

In fact in MRI, the same thing applies when the whole body imaging is carried out in a non-destructive and a non-invasive manner. So, NMR is unique among the other techniques in this respect.

The next point is that in NMR spectroscopy you can actually study each and every atom in a given molecule. As we know molecules are made up of atoms and there are different types of atoms in a given molecule. Almost every atom can be selectively probed or looked at in a very sensitive manner. This is the major advantage of NMR spectroscopy compared to other techniques. In other techniques, you look at the whole molecule as such; you do not actually get glimpse of each and every atom in a molecule. But that is where NMR comes in to help us in finding the structure.

The other important point about NMR spectroscopy is that you can study the sample or the compound or the molecule which you are interested in, under different conditions of pH, you can change the temperature, you can change the solvent, you can change the pressure. So, you can actually play around with any condition and keep monitoring the data or acquire the spectra under different conditions. This is a major plus point again because being a non-destructive technique, as long as the sample is valid or not degraded, you can choose any condition and probe the system.
The next important point is that NMR is a quantitative technique. You can actually quantify the amount of sample present. Since we do not destroy the sample, it is a non-destructive method, you can actually preserve the quantification and measure the amount of a particular compound present in the sample. This is very useful in many applications, for example, when we will see later what is called as ‘Metabolomics’, where we look at quantity of molecules present in a given mixture. We would like to know what are the different relative amounts present. This is where NMR spectroscopy stands out unique among other techniques.

We can study all different states of matter such as solids, liquids and gases and this is an important point because many of the samples such as polymers, different materials are present in solid form, you cannot make them in liquid form. So, you can use solid state NMR spectroscopy to study that. Liquids, solution state NMR, is what 90% or most of the people do when they use NMR spectroscopy. So solution state NMR is the standard approach and of course in some very rare cases you can also look at gases. But our focus in this course will be mainly on liquids, where we will focus on the solution state and for a few examples, if required, we will also look at solid state NMR spectroscopy.

NMR can be used for studying dynamics and this is very useful because in many different methods such as X-ray crystallography, what happens is you look at the sample in a solid form or you study it in the crystal form and what you get is only the structural information. In NMR what you are doing is you are looking at the sample in solution form. In a solution form, sample is in its native state. Therefore, it has all the properties what you would expect to have in its native state and dynamics is one such important property of a molecule.

Most of the molecules in solutions are very dynamic. They are never static as we see in pictures. Therefore, understanding dynamics is a very important part of understanding the function of the molecule and this is where NMR spectroscopy stands out and helps us to look at dynamics at various times scales. You can go from as short as picoseconds to as long as a few seconds.

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You can see there is a wide range of time scales of dynamic processes that can be studied by NMR. We will look at that in this course.

Before we go on to the details of the course I would like to suggest some books which you can refer to during this course and some of them are available online and you can have a look at them. There is a very nice book called ‘NMR spectroscopy explained’ by Neil Jacobsen. James Keeler’s book, which is ‘Understanding NMR spectroscopy’ is the most popular book today among students and is very nice and can be looked at. If you are interested in biological systems, which we will focus a little bit in this course, the book by Professor Chary and Govil is an important book. While all these books are going to be referred to in some way or the other, we will not cover all the details. So, these books can be used as reference materials or for a deeper insight if you would like to have during the course.

Let us then begin with this course. Before we start with NMR spectroscopy, as the word implies, it is a spectroscopic technique. It is similar in some aspects to other spectroscopic techniques. So, we need to first understand, what is spectroscopy? In general, what does spectroscopy mean? What do we actually do in spectroscopy?

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Let us go through the basic concepts in spectroscopy first before we move on to NMR. Spectroscopy involves the study of interaction of radiation with matter. When we say radiation, we basically are referring to what is called as electromagnetic radiation and this is also called as ‘light’, like for example, sunlight. Sunlight consists of electromagnetic radiation of different wavelengths. As you can see here, there is this picture of an electromagnetic wave which is a schematic drawing and shows its two components.

One is called the electric field, shown in blue color, and other in the red color the magnetic field. These two fields are oriented perpendicular to each other and what we can see here is what characterizes the wave: the wavelength, the number of waves which are present per meter or per second. So, when we talk about number of wave per second, we refer to it as frequency.
Frequency is how many waves are traveling per second and wavelength is the length of the wave. So, these are basically the two properties which we will be very much using throughout our course, because this forms the basic aspect of spectroscopy. So, essentially what happens in spectroscopy is you take this kind of electromagnetic field or radiation and you shine it on a sample.

Let us say that you are seeing, in the center, a sample. We can call it as matter or you can call it as your molecule or compound. So, when you shine light on this molecule, the molecule starts responding to the incoming light. This incoming light we write it as $h\nu$. This is a photon. So, one photon of energy is $h\nu$. Where ‘h’ is what is called as Planck’s constant and $\nu$ is the frequency of the wave.

Whenever there is an electromagnetic wave, its energy is given by $h\nu$. So, this energy $h\nu$ is now shown on the sample and the sample then responds to this wave. It can do several things: number one, it can absorb that wave, it can absorb that energy into the system or it can scatter that energy, it just reflects the energy or it just does not do anything and the wave simply passes through. When it simply passes through it means the matter or the sample is transparent. It is invisible to the light but when it absorbs, when it absorbs the energy, part of it can be absorbed, part of it radiated back or reflected/transmitted out or part of it is scattered.

So, now what is that makes a matter or a compound absorb the energy or react to the energy? For this we have to go little bit into what is called quantum mechanics. We say that the matter at a microscopic or an atomic level does not have continuum of energy levels. It has what is called discrete energy levels. This is a called ‘quantized’ system.

So, we have quantized energy levels. And now, the energy which is shown upon this matter/sample will only be absorbed by the matter if the energy matches the difference as shown here, difference between the two energy levels. Let us say that we have this, a very hypothetical system. Let us say we have what is called a ‘ground’ state and we have what is called an ‘excited’ state. So, these basically are different energy levels in a matter, in the sample or in the compound/molecule.

If the energy, which we are supplying, that is $h\nu$, matches this gap between the ground state and excited state, then this energy may be absorbed by the sample and taken up by molecules to go from the ground energy level to the excited energy level. This is the qualitative way of
explaining. For a deeper understanding, I would suggest to also look at the different books which I referred to. But in a qualitative manner, one can understand that when you shine light on this matter, the matter which has different energy levels in it, will absorb that energy if the difference in the energy level matches the energy which we are supplying. This is basically the general idea in spectroscopy. In spectroscopy, you shine light on the matter, the matter either absorbs, reflects or transmits the light and based on that we can find out what is the nature of the matter.

So, the nature of the matter is hidden in the way it responds to light. Based on the response to the light which is shown on the matter, we can figure out what type of compound, what is inside this system and what it consists of. And we can also look at different types of properties such as the structure of the molecule, the dynamics of the molecule, its vibrations, its rotations and so on.
There are different spectroscopic techniques (if you see this slide) depending on the property which you want to study. For example, let us say you are looking at the dynamics of the molecule or rotation of the molecules or vibrations, you can see that there are different energies involved. That means based on what type of energy I apply to the sample, different properties of the sample will respond to that light. This is called the ‘electromagnetic spectrum’ where you can see the whole spectrum is now divided into different regions based on the frequency.

Remember frequency is measured in Hertz. So, we are going to use the Hertz units throughout the course. If you start from the lowest frequency, that is lowest/longest wavelength (remember wavelength and frequency are inversely related) and go to the longest wavelength or shortest or lowest frequency, we come to radio waves. This region is called radio frequency. The next, higher, little higher energy is called microwave. Here, the rotation of the molecules are affected or respond to this particular range of frequency. This is called microwave spectroscopy.

The next higher energy is the infrared, which depends on the vibrations of the molecule. The vibrations in the molecules respond to infrared radiation applied to the sample. Then comes the electronic transitions, that means the electrons in the molecule respond to these energies which lies in the ultra-violet or visible regime. As the word implies, visible essentially is the part of light which you can see with the naked eyes. Then as you go further, very high energy electromagnetic radiation are called X-rays. X-rays are used for different applications and one
of the very popular application is X-ray crystallography where we look at structures of molecules. And further higher in energy is $\gamma$-rays. So, these are the different energy ranges to which the molecule can respond and depending on its property, depending on its composition, constitution and structure.

In NMR spectroscopy we will focus on this lowest energy that is radio waves. The whole of NMR spectroscopy technique is focused on these particular region, the radio waves.

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You can see what is the basic apparatus or setup in any spectroscopic technique in general. Remember NMR spectroscopy is also a spectroscopic technique. So, many of the things are indeed common with other spectroscopic techniques but the details vary. Generally, what you need first is a source of light. You have a source of radiation and this is what you shine on the sample. So, this is a sample, this is a just very schematic qualitative figure.

You take the radiation, put it on the sample and the sample will absorb radiation or transmit and whatever is transmitted is detected physically by a detector which is an electronic component. The signal is analyzed and finally what is displayed is called as a ‘spectrum’.

The Spectrum is essentially a plot of frequencies of light, which are absorbed by the radiation. The radiation basically is a broad source, can be made of different sources of radiations and can be absorbed or transmitted by the sample and that is what we record and display. So, in NMR spectroscopy, this source will basically be a radio wave source, the source of radio waves, as we have seen that NMR relies on radio frequency.
Now let us go to NMR spectroscopy, having understood what spectroscopy in general means. So, let us go to this picture where we see that here is what is called a matter, or in other words a sample or a molecule or a compound. Every molecule, as we know from our basic chemistry and physics, is made up of atoms. So, every molecule consists of atoms.

Now, if you look at an atom more closely, atom consists of nucleus which is at the center of the atom and is surrounded by electron cloud or electrons. If you zoom into this nucleus further, you will see that the nucleus is made up of protons and neutrons and we use the word ‘nucleons’ for both protons and neutrons. Neutrons and protons have two properties associated with them, which we will use in NMR. One of the properties is called charge. All of us know that protons are positively charged and neutrons have a charge zero, they are neutral. But what is one more important is the property they have which is known as ‘spin’.

Spin is essentially an abstract quantity; it is a quantum mechanical quantity. So, although it is not literally that they are spinning, we can always associate all the properties of angular motion with this quantity called spin. So, I would suggest to keep in mind that it is not literally a rotation which is going on; but at the same time we associate a property called spin which will be very useful. The spin value of a proton and neutron are same, both are fermions and equal to $\frac{1}{2}\hbar/2\pi$. This ‘$\hbar$’ is the Planck’s constant.

Both protons and neutrons have spin but the charge is present only for proton, and neutron does not have a charge. But if you see now, the overall nucleus has a positive charge. So, this
whole positively charged nucleus now has a spin associated with it because the protons and neutrons inside the nucleus have spins.

What happens is that the protons and neutrons pair up with each other. Parallel pairing results in addition of the spin values and anti-parallel pairing essentially cancels out the spin of the particles. So, that means, the nucleus, after pairing the protons and neutrons together, has a net spin value. The net spin value is the value which you arrive at after pairing up all the protons and neutrons. This net spin can have any value as we will see shortly and can be determined based on the number of protons and neutrons or basically based on the atomic mass and atomic number. But what is important is that NMR spectroscopy relies on the nucleus having a net spin value which is not zero.

That means if all the protons and neutrons pair with each other then the total spin value of the nucleus will become zero, because the positive spin of a nucleon (proton/neutron) will cancel with a negative spin of another nucleon. And therefore, if the nets total spin is zero NMR cannot be carried out on that particular atom/element. So, this atom or element should have what is called a non-zero spin.

We will see shortly the conditions which are important for getting spin not equal to zero, or total spin of nucleus to be equal to half integer or integer. For this concept that a nucleus or an element can be studied by NMR, we use the word ‘NMR active’. So, we say this particular nucleus is NMR active or this particular nucleus is not active or NMR inactive.

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Let us look at how one can actually qualitatively determine whether a particular nucleus can be studied by NMR or cannot be studied. You can see this table here, which shows how we can estimate the nuclear spin value. We can have an atom having a even atomic mass or it can have an odd atomic mass. Similarly, the atomic number of that atom can be either even or odd depending on the number of protons.

The number of protons decide whether the atomic number is even or odd and the total number nucleons, that is proton plus neutrons, determine whether the atomic mass is even or odd. This is a very basic concept which you learn in chemistry and physics.

Let us say we take a particular nucleus: carbon 12. It has 6 protons and 6 neutrons. This is an element with even atomic number and even atomic mass and therefore, the total spin of its nucleus will be zero. This means all the protons and neutrons pair with each other and the total net value of the spin for that nucleus becomes equal to zero. Therefore, we cannot study carbon-12 by NMR. Similarly, oxygen 16 has 8 protons and 8 neutrons. It also belongs to this even-even category and therefore it cannot be studied by NMR.

Let us look at an example where you have odd atomic number and even atomic mass. This is the case, for example, in Deuterium. Deuterium is an isotope of hydrogen. Another case is Nitrogen-14. Nitrogen-14 has 7 protons, 7 neutrons. It has an even atomic mass but atomic number is odd. For such type of an element, atom or a nucleus, we will have integral value of spin: the spin will be integer multiple of one (i.e., 1, 2 and so on).

Now, let us look at another case where you have even atomic number and odd atomic mass and examples for that include Carbon-13 or Oxygen-17. Carbon-13 is a very popular nucleus for NMR spectroscopy. Here if you notice we have 6 protons, that is even atomic number, but we have odd atomic mass. Therefore, the spin of $^{13}$C is half integer and it could be half, or $3/2$, or $5/2$ and so on; Oxygen 17 is $5/2$ and for $^{13}$C it is $1/2$.

The last case is essentially an odd atomic number-odd mass combination, for example, the most popular nucleus in this category is proton. There are no neutrons in a hydrogen atom, hence, it is called ‘proton’. The nucleus has therefore odd atomic number and also odd atomic weight, mass and that is why its spin is half integer (1/2). A very another important nucleus in this category is N15 (isotope of Nitrogen) which has 7 protons and 8 neutrons. It has as an odd atomic number-odd atomic mass combination and hence its spin value is a half integer (1/2).
So, these are basically the different conditions by which we can study the different NMR properties. You can actually extend this idea to the entire periodic table. Although, the slide is not very clear, the take home from the slide is that the many elements in the periodic table can be studied by NMR spectroscopy, because they have non-zero nuclear spin. So, either the compound, the element itself can be studied in the natural abundance or its isotope, which might be less abundant naturally.

Abundance is something we will be referring to again and again in this course. Abundance basically means that in a given natural state, what is the percentage of a given isotope of the element which is present in that molecule. For example, the natural abundance of Carbon-12 is 99%. This implies 99% of the carbon atoms in a sample have carbon-12. The remaining one percent is mainly Carbon-13. As far the NMR is concerned, only this 1% (13C), which is present, contributes to the signal. The remaining 99% (12C) does not, its just blind or transparent to NMR because it has zero spin.

You can see that the sensitivity of 13C becomes low because on an average out of hundred carbon atoms, only one atom will be C13. We will take up in the in the next class how actually we can look at the spin and how does NMR spectroscopy depend on this particular spin value and take it forward. Thank you.